




Serum Irisin Levels and Its Relationship with Spasticity Severity in Chronic Stroke Patients

Ercan Kaydok¹  Ismail Sari² Serpil Erşan² Esin Benli Küçük¹

¹Department of Physical Medicine and Rehabilitation, Niğde Ömer Halisdemir University Faculty of Medicine, Niğde, Turkey

²Department of Biochemistry, Niğde Ömer Halisdemir University Faculty of Medicine, Niğde, Turkey

Address for correspondence Ercan Kaydok, MD, Faculty of Medicine, Main Campus of Niğde Ömer Halisdemir University, 51100, Niğde, Turkey (e-mail: ercankaydok@ohu.edu.tr).

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Abstract

Objective Irisin is a myokine released from muscles by exercise and it has been shown to be a prognostic indicator in acute stroke patients. However, irisin's relationship with the chronic phase of stroke and spasticity has not been studied yet. We aimed to determine the serum level of irisin to examine its relationship with the functional status and severity of spasticity in patients with chronic stroke, and to compare these with healthy controls.

Materials and Methods A total of 70 (35 chronic stroke and 35 control patients) patients were included in the study. The blood serum irisin levels of the patients and the controls were evaluated using enzyme-linked immunosorbent assay method, their functional status was evaluated with the modified Rankin scale (mRS), and spasticity severity using the modified Ashworth scale (MAS).

Results The mean serum irisin levels of the stroke and the control groups were 6.20 ± 2.2 and 5.45 ± 2.3 , respectively, and there was no statistically significant difference ($p > 0.05$). No significant correlation was found between the serum level of irisin and the severity of spasticity and functional status, assessed by the mRS in stroke patients.

Conclusion These results showed that irisin levels in chronic stroke patients were similar to controls, and there was no relationship between the severity of spasticity and functional status and irisin level.

Keywords

- Irisin
- stroke
- spasticity
- functional outcomes

Introduction

Stroke, which is the development of cerebral dysfunction due to impaired cerebral blood flow due to vascular reasons, is one of the most important causes of morbidity and mortality globally. The incidence of stroke in developed countries is 2%, and it has been determined that one-third of the patients die within a year, and one-third remain disabled.^{1,2} Stroke can

be examined in two main categories, as ischemic and hemorrhagic. Many complications that can negatively affect a person's life such as gait disturbance, speech disorder, balance problems, vision problems, and spasticity may occur because of stroke. The most important risk factors for stroke are age, male gender, hypertension (HT), diabetes mellitus (DM), smoking, and a history of transient ischemic attack.³

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Irisin, which is a proteolytic product of fibronectin type III domain 5 (FNDC5), a membrane protein discovered by Boström et al, is a hormone released from skeletal muscle after regular exercise, with autocrine, paracrine, and endocrine effects. Its function is to enhance energy expenditure by turning white adipose tissue into brown adipose tissue and to reduce the risk of metabolic diseases.⁴

Irisin is also known to be expressed in the brain.⁵ There are different results in studies examining the relationship between stroke and irisin. In a study published in 2017, it was stated that the plasma irisin concentration and the intramuscular FNDC5 protein expression decreased after ischemic stroke.⁶ In the same study, on the other hand, in the stroke model, the plasma irisin levels were negatively correlated with the plasma tumor necrosis factor- α and interleukin-6 levels, and the increased irisin hormone due to exercise was shown to protect neurons against ischemia-related damage by activation of the Akt and ERK1/2 signaling pathways and greatly reduce the cerebral infarction volume through these mechanisms and reduce the neuroinflammation and ischemic oxidative stress. In Wu et al's study in 2018, it was demonstrated that low serum irisin levels in patients with acute ischemic stroke may predict the risk of poor functional outcomes in patients with ischemic stroke.⁷ These data suggest that irisin may be associated with the stroke risk and neuronal recovery after stroke.

There are conflicting results in studies examining the relationship between exercise and release of the irisin from the muscles. Boström et al reported that FNDC5 mRNA increased in skeletal muscle after exercise in humans and mice.⁴ Again, Timmons et al found that FNDC5 mRNA increased by 30% in the elderly as a result of a vigorous exercise program.⁸ In an in vitro study with human primary muscle cells, it was reported that PGC1 α mRNA doubled, FNDC5 mRNA increased by 18% and that the irisin level increased by 20% in forskolin- and ionomycin-treated muscle tissue to simulate exercise.⁹ In contrast to these studies, as a result of their study with adults with anorexia nervosa, Hofmann et al found that there was no correlation between exercise and irisin levels,¹⁰ while Timmons did not find a significant change in FNDC5 mRNA levels of 43 participants who received regular and controlled exercise for 20 weeks.⁸

Spasticity is a motor disease characterized by a speed-dependent increase in tonic stretch reflexes (muscle tone), which results from overstimulation of the stretch reflex. It is a component of the upper motor neuron syndrome, and its pathophysiology can be considered simply as an imbalance of excitatory and inhibitory mechanisms.¹¹ The most common causes in its etiology are cerebrovascular diseases, cerebral palsy, traumatic brain injury, spinal cord injury, multiple sclerosis, and anoxic brain damage.¹²

There are no studies examining the relationship of irisin, which has contradictory exercise-related results, to spasticity and which is the situation of involuntary contraction of the affected extremity muscles and increased muscle tone. In this study, it was aimed to examine the relationship between

the blood level of irisin in chronic stroke patients, the increase in the release of which has been shown from muscles with exercise and can be a prognostic marker in patients with acute stroke, and the severity of spasticity and functional outcomes, and to compare it with healthy controls.

Materials and Methods

Study Design and Participants

This study was designed as a crossover study. Thirty-five chronic (> 3 months) stroke patients and thirty-five control patients between the ages of 18 and 75 years with similar sociodemographic characteristics, who were followed up in the local physical medicine and rehabilitation clinic, were included in the study after obtaining their written consents.

The inclusion criteria for the stroke group comprised being in the chronic stroke period (> 3 months) due to hemorrhage or ischemia, to be aged between 18 and 75 years, and to have spasticity of at least one limb with a magnitude of one or more according to the modified Ashworth scale (MAS). The inclusion criteria for the control group included being between the ages of 18 and 75 years. The exclusion criteria for the stroke and the control groups were as follows: having dementia or moderate-to-severe cognitive dysfunction, being very weak or obese (body mass index [BMI] < 15 or > 40), having advanced kidney-liver failure, history of malignancy, and chronic rheumatic disease. In addition to these criteria, it was paid attention that patients in the control group did not have a history of neurological disease.

A semistructured sociodemographic information form prepared by us, taking into account the aims of the study, was used for all of the participants. Age, gender, BMI, and a history of accompanying systemic diseases (HT, DM, coronary artery disease [CAD], hyperlipidemia) were questioned and recorded. In addition, data such as the time elapsed after the event, stroke etiology, and the affected side were noted in the stroke group.

Clinical Assessment Scales

The spasticity and functional status of all stroke patients included in the study were evaluated by a physician in charge and recorded in the information form. The spasticity of the patients was evaluated using the MAS, which has been used in many studies with well-known validity and reliability. The MAS is a clinical scale that evaluates the spasticity severity of the muscles in the affected area of the patient on a scale of 0-4.¹³ The independence and the functional status of the patients were evaluated using the modified Rankin scale (mRS). The mRS is a clinical scale with proven validity and reliability, specifically designed for stroke. In this scale, the functional status of the patients is scored between 0 and 6. The higher the score the patients get from the scale, the more dependent the patients are. Scoring less than 3 on the scale means being independent, 3 points and above means being dependent, and 6 points means death.¹⁴

Collection of Blood Samples and Determination of Serum Irisin Levels

After 12 hours of fasting, 5 mL of venous blood were collected in the morning hours from both controls and patients into empty tubes containing sterile gel. The collected blood samples were immediately centrifuged at 3,000g for 10 minutes and the serum samples obtained were taken into a different tube and kept at -80°C until the analysis day. In the serum samples, the irisin serum levels were determined following the instructions of the enzyme-linked immunosorbent assay (ELISA) kit (Yehua, Shanghai, China; Cat No: YHB1765Hu) and using the ELISA reader (GF-M3000, China). The range of the assay was 0.05 to 15 $\mu\text{g/mL}$ for irisin.

Statistical Analysis

Whether the continuous variables fit the normal distribution or not was evaluated using the Kolmogorov-Smirnov test, and it was determined that all data were normally distributed. The descriptive data were presented as the mean \pm standard deviation. The Student's *t*-test was used for comparing the differences between groups of continuous data with normal distribution, and the chi-squared test was used to compare the frequency of categorical data such as gender, age, and comorbidities between groups. The Spearman's rank correlation was used for the bivariate correlations. A *p*-value of less than 0.05 was determined as statistical significance for all tests.

Results

The initial sociodemographic and clinical characteristics of the patients have been presented in (►Table 1). A total of 70 participants (35 hemiplegia-35 controls) were included in the study. No statistically significant difference was found between the patient and the control groups included in the study in terms of demographic data such as gender, age, occupation, and BMI. Forty percent of the patients and 42% of the controls were female. The frequency of HT in the patient group was significantly higher than that of the control group (71.4%, 37.1%, respectively, $p = 0.004$). However, there was no

significant difference between the two groups in terms of the frequency of other additional problems (►Table 1).

The serum irisin levels measured by ELISA of the control group and patients were 6.20 ± 2.2 and 5.45 ± 2.3 , respectively, and there was no statistically significant difference between the patient and the control groups (►Table 1, $p = 0.177$). The mean serum irisin level was 6.03 ± 3.04 in men and 5.39 ± 2.4 in women, and there was no statistically significant difference ($p = 0.349$).

The mean time elapsed after the event was 30.2 months for stroke patients, ranging from 3 to 120 months. About 74.3% of the patients had a stroke due to infarction (►Fig. 1). In 15 (42.9%) patients with stroke, the affected side was the right side and there was no patient affected bilaterally. The mean spasticity measured according to the MAS of the patients was 2.08 ± 0.6 for the upper extremity and 2.34 ± 0.7 for the lower extremity (►Table 2). The mean functional status score of the patients measured using MRS was 3.20 ± 1.1 (►Table 2).

When the patients who obtained 0, 1, and 2 scores from the MRS were evaluated independently, it was seen that 26 (74.3%) of the patients were dependent on their daily activities (►Fig. 2).

In the evaluation performed to examine the effect of various diseases (such as HT, DM, CAD, and hyperlipidemia),

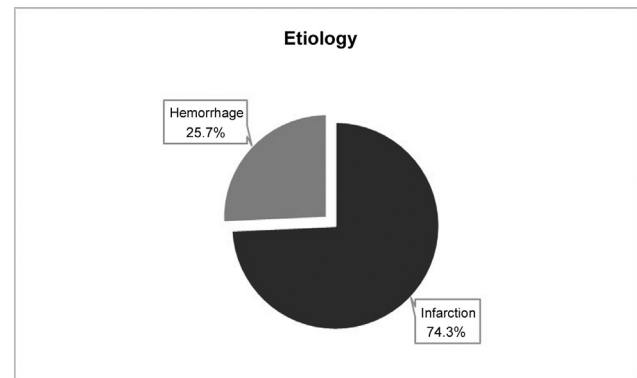


Fig. 1 Stroke etiology of patients.

Table 1 Baseline demographic and laboratory data of all groups

	Stroke (N = 35)		Control (N = 35)		p-Value
	n	Mean \pm SD	n	Mean \pm SD	
Age (years)		54.88 ± 12.4		50.34 ± 9.3	0.089
BMI (kg/m^2)		27.61 ± 4.4		25.64 ± 4.4	0.062
Gender (female/male)	14/21		15/20		0.808
Hypertension (yes/no)	25/10		13/22		0.004 ^a
Diabetes mellitus (yes/no)	8/27		7/28		0.771
Coronary arterial disease	12/23		6/29		0.081
Hyperlipidemia	9/26		5/30		0.232
Irisin ($\mu\text{g/mL}$)		6.20 ± 2.2		5.45 ± 2.3	0.177

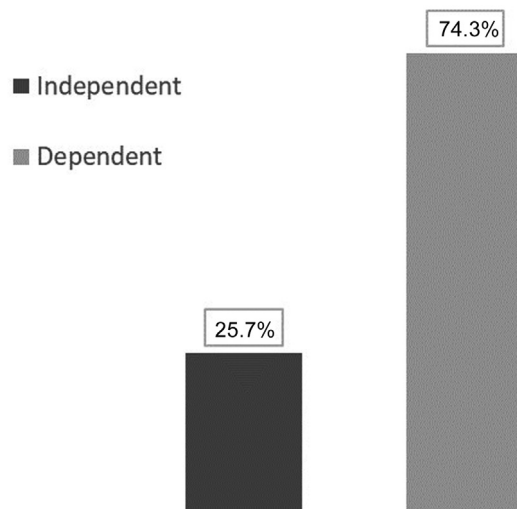
Values are presented as mean \pm standard deviation (SD) or n (%). BMI, body mass index.

^aChi-squared test $p < 0.05$.

Table 2 Spasticity and modified Rankin scale of stroke patients

	Mean \pm SD	Range
Upper extremity spasticity (N = 35)	2.08 \pm 0.6	(1–3)
Lower extremity spasticity (N = 35)	2.34 \pm 0.7	(1–4)
Modified Rankin scale (N = 35)	3.20 \pm 1.1	(1–5)

Abbreviation: SD, standard deviation.

**Fig. 2** Final functional outcomes of patients. Patients with modified Rankin scale ≥ 3 were considered dependent.

which are accused in the etiology of stroke and have been reported to affect the level of irisin in previous studies, we found that there was no significant difference between stroke patients with and without these diseases (**Table 3**, $p < 0.05$).

Again, in our study, the 70 of upper-lower extremity spasticity, the mRS and the disease duration in the stroke group, and the BMI, age, and the serum irisin levels in both stroke and control groups were investigated, and no significant correlation was found (**Table 4**). When the relationship between severity of spasticity and functional dependence was examined, a strong relationship was found between the severity of both upper and lower extremity spasticity and dependence (mRS 3–5) ($p = 0.01$).

Discussion

This study is the first to examine the relationship between the plasma irisin level and the functional outcomes and the spasticity severity of chronic stroke patients. The first aim of the study was to determine whether the serum level of irisin, the release of which from muscles increases with exercise,^{4,8} and the neuroprotective effects of which have been demonstrated in stroke¹⁵ and Alzheimer's disease,¹⁶ is related to poststroke spasticity, which is the involuntary increase in muscle tone, and to examine whether spasticity has effects on neuroplasticity and neuroprotection or not. The findings

Table 3 The relationship of comorbid conditions with irisin level in stroke patients

	No	Yes	p-Value
Hypertension	6.32 \pm 2.3	6.44 \pm 2.2	0.911
Diabetes mellitus	6.66 \pm 3.3	5.57 \pm 1.9	0.385
Coronary artery disease	6.40 \pm 3.02	6.42 \pm 3.2	0.986
Hyperlipidemia	6.34 \pm 2.8	6.61 \pm 3.8	0.819

Table 4 Correlations between irisin levels and clinical data

	Stroke group		Control group	
	R	P	R	P
Upper extremity spasticity	0.187	0.283	¥	¥
Lower extremity spasticity	0.196	0.258	¥	¥
Modified Rankin scale	0.068	0.696	¥	¥
Duration of disease	–0.248	0.151	¥	¥
Body mass index	–0.117	0.505	–0.225	0.194
Age	0.123	0.481	–0.079	0.651

Pearson's correlation.

¥ Spasticity, duration of stroke, and modified Rankin scale were not included in the evaluation in the control group.

we obtained in our study indicate that the severity of either upper or lower extremity spasticity was not related to the level of the irisin in chronic stroke patients (**Table 4**).

Spasticity is common after stroke and there are studies reporting its prevalence ranging from 30 to 80%. The incidence of spasticity gradually increases in stroke patients. Its incidence was reported to be 27% at the first month, 43% at 6 months, and 34% at 18 months after stroke.^{17,18} Although spasticity prevents early muscle atrophy, chronic spasticity has been found to reduce the number of sarcomeres, cause atrophy, and increase the rate of connective tissue in the muscle. These changes in soft tissue can cause pulling forces to be more easily transmitted to the muscle spindles, which can increase sensory input from the muscle spindles. In addition, these changes may increase the spasticity, leading to a vicious circle and eventually contractures.¹⁹ In our study, the mean duration of the disease was longer than 30 months and the majority of patients had chronic spasticity. The severity of spasticity and plasma irisin levels may not be correlated with the above-mentioned sarcopenia (decrease in the number of sarcomeres) and muscle atrophy in patients with chronic stroke and elderly patients.

In two recent acute stroke studies, it has been demonstrated that a low plasma irisin level is a poor prognostic marker for stroke and is associated with poor functional status in the future.^{7,20} In our study, no correlation was found between the patients' functional status evaluated with the mRS and the irisin level (**Table 4**). This finding may have several reasons. First, as shown in previous studies, the irisin

level may have increased for the neuroprotective effect in the acute phase of stroke but returned to normal again as a result of the slowing of the neuronal recovery process in the chronic phase. The second reason may be that the patients we included in the study were generally individuals in need of rehabilitation and with limited daily activities. As it is known from previous studies, the plasma levels of the irisin and its precursor FDNC5 mRNA increase especially with regular aerobic exercise and vigorous exercise.^{4,8} About 74.3% of our patients were dependent patients in their daily activities with a high mRS (►Fig. 2). It is inevitable for these patients to do less exercise and daily activities than healthy people.

In our study, when we compared the plasma irisin level of the patients with the controls, the serum irisin level of the patients was slightly higher than the controls, but this difference was not statistically significant (►Table 1, $p > 0.05$). This finding is interesting, because previous studies have shown that irisin plays a role in the pathogenesis of diseases known to be important risk factors for cerebrovascular events such as HT,^{21,22} insulin resistance,²³ metabolic syndrome,²⁴ and type 2 diabetes,²⁵ and has been confirmed to be significantly lower in these patients in many studies. Furthermore, Park et al found that circulating irisin levels were associated with a 10-year increased risk of cardiovascular disease.²⁶ In our study, the frequency of HT, which is the most important risk factor for stroke, was significantly higher in the stroke group compared to the control group ($p = 0.04$), but there was no significant difference in the frequency of other risk factors (such as DM, CAD, and hyperlipidemia). However, the irisin level was not found to be low in patients with HT, as found in previous studies.^{21,22} This may be due to the fact that patients regularly used antihypertensive medication. Çelik et al²² showed that plasma irisin levels in newly diagnosed HT patients were lower than controls, but the irisin levels increased significantly after 12 weeks of treatment with antihypertensives (amlodipine or valsartan).

Our study has some important limitations. The first is that the study had a crossover design. This prevented us from understanding how serum irisin level changed with disease duration, patients' functional status improvements (such as mobility and upper extremity function), and the severity of spasticity. The second limitation is that the condition of the diseases known to affect the irisin level at that time was not known due to the lack of measurable parameters such as arterial blood pressure, homeostasis model of assessment-insulin resistance in terms of insulin resistance, hemoglobin A1C, and the blood lipid panel (high-density lipoprotein, low-density lipoprotein, total cholesterol and triglyceride levels) values of the patients. The third limitation is that the data on potential confounding factors that may affect the irisin level, such as physical activity levels of patients and controls, were not obtained. The fourth important limitation is that stroke patients without spasticity were not included in the study. Due to this limitation, it is difficult to understand whether spasticity or general changes due to stroke affect the level of irisin. However, as a result of the analysis of the data in the

study, we think that the lack of correlation between the level of irisin and the severity of spasticity of the patients (►Table 4) reduces the importance of this limitation. The last important limitation of our study is the small number of participants.

Conclusion

The findings of our study suggest that chronic stroke patients were similar to the control group, and there was no significant relationship between the severity of spasticity and the functional status of the patients and serum irisin levels. However, prospective studies with larger populations are needed to examine the relationship between irisin, which is known to have neuroprotective properties, with spasticity, and the change in its level in stroke patients during surveillance.

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Conflicts of Interest

None declared.

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