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Serum levels of irisin predict short-term outcomes in ischemic stroke

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ABSTRACT

Objective: Irisin is a 112-amino acid peptide found in rat and human skeletal muscle after exercise. Previous studies had suggested that higher circulating irisin levels were associated with an increased risk of vascular atherosclerosis and cardiovascular diseases. In this study, we determined irisin levels in serum, and investigated their associations with functional outcomes in a 3-month follow-up study in Chinese patients with first-ever acute ischemic stroke (AIS).

Methods: From September 2015 to December 2016, consecutive first-ever AIS patients admitted to the Department of Emergency of our hospital were identified. Serum irisin levels were measured at admission. Functional impairment was evaluated at discharge using the modified Rankin scale. The levels of irisin were expressed as median and interquartile ranges [IQR].

Results: The irisin level was obtained in 324 patients (97.6%) with a median value of 291.2 ng/ml (IQR: 214.1–404.2 ng/ml). There were significantly negative correlations between levels of irisin and NHISS ($r = -0.272$; $P < 0.001$) and BMI ($r = -0.193$; $P = 0.003$). A poor functional outcome was found in 99 patients (30.6%; 95%CI: 25.5–35.6%). The poor functional outcome distribution across the irisin quartiles ranged between 51.9% (first quartile: Q1) to 12.4% (fourth quartile: Q4). In a multivariate model using the Q1 of irisin vs. Q2-4 together with the clinical variables, the marker displayed prognostic information and increased risk of poor outcomes by 94% (OR for Q1, 1.94 [95% CI, 1.19–3.42]; $P = 0.018$) and mortality 66% (OR for Q1, 1.66 [95% CI, 1.11–3.07]; $P = 0.009$). In addition, a model containing known risk factors plus irisin compared with a model containing known risk factors without irisin showed a greater discriminatory ability to predict poor outcomes ($P = 0.01$) and mortality ($P = 0.02$).

Conclusions: A low serum irisin level is a predictor of poor early functional outcome in ischemic stroke patients. The underlying mechanisms of these associations remain to be investigated.

1. Introduction

Stroke is a medical emergency caused by interrupted blood supply to the brain that further leads to rapid loss of brain functions. In China, the annual stroke mortality rate is approximately 1.6 million [1], which has exceeded ischemic heart disease to become the leading cause of death and adult long-term disability [2]. Rapidly measurable biomarkers to predict stroke functional outcome and mortality are useful for optimized care and allocation of healthcare resources [1].

Boström et al. [3] discovered irisin in 2012, a 112-amino acid peptide found in rat and human skeletal muscle after exercise. This hormone is released upon cleavage of the plasma membrane protein, fibronectin type III domain-containing protein 5 (FNDC5). The FNDC5 gene is located on chromosome 1p35.1 encoding a 203-amino acid protein [4]. The highest basal levels of FNDC5 expression are seen in brain and heart [5].

Irisin has been found to be not only a myokine but also an adipokine [6]. Accumulating evidence has demonstrated that irisin contributes to the regulation of glucose and lipid metabolism in skeletal muscle and adipose tissue [7,8]. In addition, previous investigations have subsequently confirmed that it plays substantial roles in the pathophysiology of obesity [9], insulin resistance [10], type 2 diabetes [11] or metabolic diseases [12]. Due to the crosstalk between metabolic dysfunction and cardio-cerebrovascular diseases, a role for irisin in the cardio-cerebrovascular system is also expected.

A previous study suggested that the plasma irisin concentration and intramuscular FNDC5 protein expression decreased after ischemic stroke [13], while another study linked higher circulating irisin levels with signs of vascular atherosclerosis [14]. Furthermore, Park et al. [15] found that circulating irisin levels were associated with an

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increased 10-year risk of cardiovascular diseases. These data suggest that irisin might be associated with the risk of stroke. However, the association between irisin and stroke functional outcomes has not yet been evaluated. In this study, we determined irisin levels in serum, and investigated their associations with functional outcomes in a 3-month follow-up study in Chinese patients with first-ever acute ischemic stroke (AIS).

2. Patients and methods

From September 2015 to December 2016, consecutive first-ever AIS patients without any pre-morbid handicap who were admitted to the Department of Emergency of the Zhongnan Hospital of Wuhan University China, were identified. This was a prospective cohort study. AIS was defined according to World Health Organization recommendations (defined stroke as a "neurological deficit of cerebrovascular cause that persists beyond 24 h or is interrupted by death within 24 h") [16]. The clinical diagnoses were validated on the basis of computed tomography (CT) and/or magnetic resonance imaging (MRI). The exclusion criteria were (1) intracerebral hemorrhage, malignant tumor, sarcopenia, liver insufficiency and renal insufficiency (creatinine > 1.5 mg/dl), febrile disorders and autoimmune diseases; intolerance or allergy to alcohol consumption, regular use of sedative/hypnotics (2) without informed consents, lost blood samples, or current use of metronidazole or warfarin, and psychiatric illness; (3) lost or give up during the follow-up. The present study has been approved by the ethics committee of the Zhongnan Hospital of Wuhan University (No. 2015-ZWU-A18). All participants or their relatives were informed of the study protocol, and their written informed consents were obtained.

At admission, sex, age and body mass index (BMI) were collected. The following vascular risk factors were collected: hypertension, diabetes mellitus, hypercholesterolemia, atrial fibrillation, smoking, previous myocardial infarction, and a history of transient ischemic attack (TIA). Pre-stroke therapy, including oral anticoagulants, antiplatelet agents, antihypertensive treatment, and statins, as well as acute treatment (IV thrombolysis and/or mechanical thrombectomy), were recorded.

Patients were evaluated with the National Institute of Health Stroke Scale (NIHSS) [17] score at their admission, performed by a stroke neurologist certified in the use of this scale. Strokes were classified according to the criteria of the TOAST (Trial of Org 10172 in Acute Stroke Treatment) classification [18]. The clinical stroke syndrome was determined by applying the criteria of the Oxfordshire Community Stroke Project (OCSP). MRI with diffusion-weighted imaging (DWI) was available for some patients. The infarct volume was calculated by using the formula $0.5 \times a \times b \times c$ (where a is the maximal longitudinal diameter, b is the maximal transverse diameter perpendicular to a, and c is the number of 10-mm slices containing infarct) [19].

Functional outcome was obtained on month 3 after admission according to the modified Rankin Scale (mRS) [20] blinded to irisin levels. The primary end point of this study was good functional outcome of stroke patients, defined as a mRS score of 0 to 2 points. Secondary end point in stroke patients was death from any cause within a 3-month follow-up. Outcome assessment was performed by one trained medical staff blinded to irisin levels with a structured follow-up telephone interview with the patient or, if not possible, with the relative.

For all patients, blood samples were drawn on the first morning (07:00) after admission under fasting state and within 48 h of onset of stroke symptoms/signs (within 0–6 h [n = 88], 6–12 h [n = 105], 12–24 h [n = 54], and 24–48 h [n = 77] from the symptom onset). Within 30 min, aliquots of plasma and serum were separated and stored at − 80 °C until further analysis. Serum irisin concentrations were measured in duplicate by using the enzyme-linked immunosorbent assay (ELISA) kits (Aviscera Biosciences, Santa Clara, CA, USA), in accordance with the manufacturer's instructions. The sensitivity of the assay was 1.0 ng/ml and the linear range of the standard was 1.0–500 ng/ ml. The intra-and inter-assay coefficients of variation (CV) were 3.5%-6.0% and 5.0%-7.0%, respectively. Serum interleukin-6 (IL-6) was determined by ELISA (Human IL-6 Quantikine ELISA Kit, R&D Systems, Inc., Minneapolis, MN, USA). The levels of serum total cholesterol (TC), triglycerides (TG), low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C) and high sensitivity C-reactive protein (Hs-CRP) were measured by enzymatic assays. Homocysteine(HCY) was measured by immuno-chemiluminescence medthod. Fasting serum glucose(FSG) levels were measured using the hexokinase method (Olympus AU2700; Olympus, Hamburg, Germany). Fasting insulin (FINS) was measured by electrochemiluminescence immunoassay (BECK, Germany).

3. Statistical analysis

The results were expressed as percentages for categorical variables and as medians (interquartile ranges [IQR]) for continuous variables. The Mann-Whitney *U* test and chi-square test were used to compare the two groups. Spearman's rank correlation was used for bivariate correlations.

The relation of irisin with two end points (outcome and mortality) was investigated using logistic regression models in multivariate adjustment with possible confounders—i.e., age, sex, BMI, infarct volume, NIHSS score, time from onset to blood collection, pre-stroke and acute treatment, stroke syndrome, stroke etiology, vascular risk factors and serum levels of FSG, Hs-CRP, HCY, IL-6, TG, TC, HDL, LDL and insulin. We used crude models and multivariate models adjusted for all significant predictors and reported odds ratios (ORs). Furthermore, receiver operating characteristic (ROC) curve was used to test the overall accuracy of the NIHSS and serum biomarkers to predict outcomes, and results were reported as area under the curve (AUC). All statistical analyses were performed with SPSS for Windows, version 20.0 (SPSS Inc., Chicago, IL, USA) and the ROCR package (version 1.0–2), which is available from CRAN repository (http://cran.r-project.org/). Statistical significance was defined as $P < 0.05$.

4. Results

Of a total of 407 eligible patients, the study could not be performed in 47 patients (4 patients died before the samples collection, 25 were discharged to another institution and 18 refused to included). Of the original 360 S patients, 332 completed the 3-month follow-up and were available for analysis (20 lost follow-up and 8 withdrawals). However, these included patients were similar in terms of baseline characteristics [age ($P = 0.28$), sex ($P = 0.75$) and NIHSS ($P = 0.14$)] compared to the overall cohort. The irisin level was obtained in 324 patients (97.6%) with a median value of 291.2 ng/ml (IQR: 214.1–404.2 ng/ml). The baseline characteristics of the patients presenting with ischemic stroke are described in Table 1. The overall median age was 65 (IQR, 57–78) years, and 175 (54.0%) were male. The median NIHSS score on admission was 9 (IQR, 5–15).

Irisin levels were lower in patients with diabetes, coronary artery disease, with no differences between ages of patients, sex, other risk factors, pre-stroke treatment and stroke subtype distribution. Furthermore, there were significantly negative correlations between levels of irisin and NHISS ($r = -0.272$; $P < 0.001$) and BMI ($r = -0.193$; $P = 0.003$). In patients for whom MRI data were available ($n = 180$), there was a negative correlation between levels of irisin and the infarct volume ($r = -0.389$, $P < 0.001$). In addition, irisin levels were negatively correlated with Hs-CRP, HCY, IL-6 and LDL, while were positively correlated with HDL ($P < 0.05$, all).

During the 3-month follow-up period, a poor functional outcome (defined as a mRS score > 2) was found in 99 patients (30.6%; 95%CI: 25.5–35.6%). Fifty-one patients died, and the mortality rate was thus 15.7% (95%CI: 11.8–19.7%). Serum irisin levels in patients with poor outcomes were significantly lower as compared with those in patients

Table 1
Baseline characteristics of patients with stroke.

	N = 324
Male, N (%)	175(54.0)
Age, years medians (IQR)	65(57–78)
BMI, kg/m2, medians (IQR)	27.7(25.4–29.6)
Vascular risk factors, N (%)	
Hypertension	195(60.2)
Diabetes	113(34.9)
Hypercholesterolemia	134(41.4)
Atrial fibrillation	46(14.2)
Coronary artery disease	91(28.1)
Previous TIA	41(12.7)
Smoking	56(17.3)
Pre-stroke treatment, n (%)	
Antihypertensive treatment	151(46.6)
Antiplatelet agents	102(31.5)
Anticoagulants	29(9.0)
Hypoglycemic drugs	66(20.4)
Acute treatment, TPA-T No. (%)	37(11.4)
NIHSS at admission, medians (IQR)	9(5–15)
Lesion volumes (N = 180, ml), median (IQR)	24(6–41)
Stroke syndrome no. (%)	
TACS	91(28.1)
PACS	95(29.3)
LACS	65(20.1)
POCS	73(22.5)
Stroke etiology no. (%)	
Small-vessel occlusive	70(21.6)
Large-vessel occlusive	78(24.1)
Cardioembolic	112(34.6)
Other	43(13.3)
Unknown	21(6.5)
Laboratory findings, medians (IQR)	
Hs-CRP, mg/dl	0.51(0.33–1.05)
HCY, umol/l	19.2(13.1–24.3)
Irisin, ng/ml	291.2(214.1–404.2).
Glucose, mmol/l	5.86(5.12–6.38)
IL-6, pg/ml	133.5(112.1–158.9)
Insulin, mIU/ml	9.3(8.2–10.4)
TC, mmol/l	4.82(4.55–5.02)
TG, mmol/l	1.83(1.70–1.95)
HDL, mmol/l	1.09(0.93–1.42)
LDL, mmol/l	2.49(2.28–2.61)

IQR, interquartile range; NIHSS, National Institutes of Health Stroke Scale; LACS, lacunar syndrome; PACS, partial anterior circulation syndrome; POCS, posterior circulation syndrome; TACS, total anterior circulation syndrome; TPA-T: Tissue plasminogen activator-treated; Hs-CRP, High-sensitivity C-reactive protein; HCY, homocysteine; BMI, body mass index; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; TG, triglyceride; TC, total cholesterol; IL-6, interleukin-6.

with good outcomes [257.1 (IQR, 119.6–305.8) ng/mL vs. 315.5 (IQR, 260.6–429.7) ng/mL; Z = 6.179, P < 0.001; Fig. 1). The poor functional outcome distribution across the irisin quartiles ranged between 51.9% (first quartile) to 12.4% (fourth quartile), P for trend < 0.001; Fig. 2. In univariate logistic regression analysis, we calculated the odds ratio (OR) of irisin levels as compared with the NIHSS score and other risk factors. With an unadjusted OR of 0.993 (95% confidence interval [CI], 0.991–0.996, P < 0.001), irisin had a strong association with poor outcomes. After adjusting for all other significant outcome predictors, irisin remained an independent predictor with an adjusted OR of 0.996 (95% CI, 0.0.992–0.999, P = 0.001), table 2. In the subgroup of patients (N = 180) in whom MRI evaluations were performed, irisin was an independent predictor with an OR of 0.995 (95% CI, 0.993–0.998; P < 0.001) after adjusting for both lesion size and the NIHSS score.

For a more detailed exploration of the irisin concentration-functional outcome relationship, we also used multivariate analysis models to estimate adjusted OR and 95% CIs of poor functional outcome for irisin quartiles (with fourth quartile of irisin as reference). In multivariate models comparing the first, second and third quartiles against the fourth quartile of the irisin (Table 3), levels of irisin were associated with poor functional outcome, and increased risk of poor outcomes by 215% (OR for Q1: 3.15; 95%CI: 1.76–6.03; P < 0.001) and 108% (OR for Q2: 2.08; 95%CI: 1.28–4.16; P = 0.012). The independent association of irisin with poor outcomes was confirmed using the likelihood ratio test (P = 0.019). In a multivariate model using the Q1 of irisin vs. Q2-4 together with the clinical variables, the marker displayed prognostic information and increased risk of poor outcomes by 94% (OR for Q1, 1.94 [95% CI, 1.19–3.42]; P = 0.018).

In addition, with an AUC of 0.72 (95% CI, 0.66–0.78), irisin showed a significantly greater discriminatory ability to predict poor outcomes as compared with Hs-CRP (AUC, 0.64; 95% CI, 0.57–0.73; P < 0.001), HCY (AUC, 0.61; 95% CI, 0.55–0.68; P < 0.001), IL-6 (AUC, 0.67; 95% CI, 0.61–0.72; P = 0.001), while was in the range of NIHSS score (AUC, 0.74; 95% CI, 0.68–0.80; P = 0.15). Irisin improved the NIHSS score (AUC of the combined model, 0.76; 95% CI, 0.72–0.82; P = 0.02). In addition, a model containing known risk factors plus irisin compared with a model containing known risk factors without irisin showed a greater discriminatory ability (P = 0.01), the area under the curve (AUC) increased from 0.78 to 0.83 (95% confidence interval [CI], 0.76–0.86). A significant difference in the AUC between the clinical variables alone and the addition of irisin level was observed (difference, 0.05 [95% CI, 0.03–0.07]; P = 0.01).

Serum irisin levels in patients who died were significantly lower as compared with patients who survived [255.7 (IQR, 107.9–295.8) ng/mL vs. 309.6 (IQR, 256.1–422.5) ng/mL; Z = 4.675, P < 0.001). The mortality distribution across the irisin quartiles ranged between 28.4% (first quartile) to 6.2% (fourth quartile), P for trend < 0.001. In a multivariate model using the Q1 of irisin vs. Q2-4 together with the clinical variables, the marker displayed prognostic information and increased risk of mortality by 66% (OR for Q1, 1.66 [95% CI, 1.11–3.07]; P = 0.009). Furthermore, a model containing known risk factors plus irisin compared with a model containing known risk factors without irisin showed a greater discriminatory ability to predict mortality (P = 0.02), the area under the curve (AUC) increased from 0.77 to 0.81 (95% confidence interval [CI], 0.75–0.85). A significant difference in the AUC between the clinical variables alone and the addition of irisin level was observed (difference, 0.04 [95% CI, 0.02–0.06]; P = 0.02).

5. Discussion

The present study was the first report to investigate the prognostic potential of Irisin in a substantial cohort of Chinese AIS patients. The finding suggested that low circulating irisin level was a strong and independent prognostic marker of functional outcome and mortality in Chinese patients with AIS and added significant additional predictive information to the clinical score of the NIHSS. Consistent with our finding, a study identified serum irisin as a predictive biomarker for 1-year all-cause mortality in Acute Heart Failure patients [21].

Furthermore, low irisin levels were associated with the neurological deficit score (defined by NIHSS score and infarct volume) at admission. Consistent with our results, another study found that plasma irisin levels were negatively associated with brain infarct volume, the neurological deficit score, and plasma IL-6 concentrations [13]. Efe et al. [22] showed that serum irisin level was an independent predictor of coronary artery severity in patients with stable coronary artery disease.

A growing body of evidence suggests that irisin may influence lipid levels. Park et al. showed that circulating irisin levels were negatively associated with HDL cholesterol and positively associated with metabolic syndrome [15]. Plasma irisin concentrations have also been correlated with LDL receptor mRNA expression and overall cholesterol homeostasis [23]. An association between the reduction in plasma irisin levels and the depletion of apolipoprotein(Apo) B level was observed in another study [24]. However, in this study, we found that irisin levels were negatively correlated with lipid levels. Furthermore, previous study suggested that irisin-encoding gene (FNDC5) variant can change

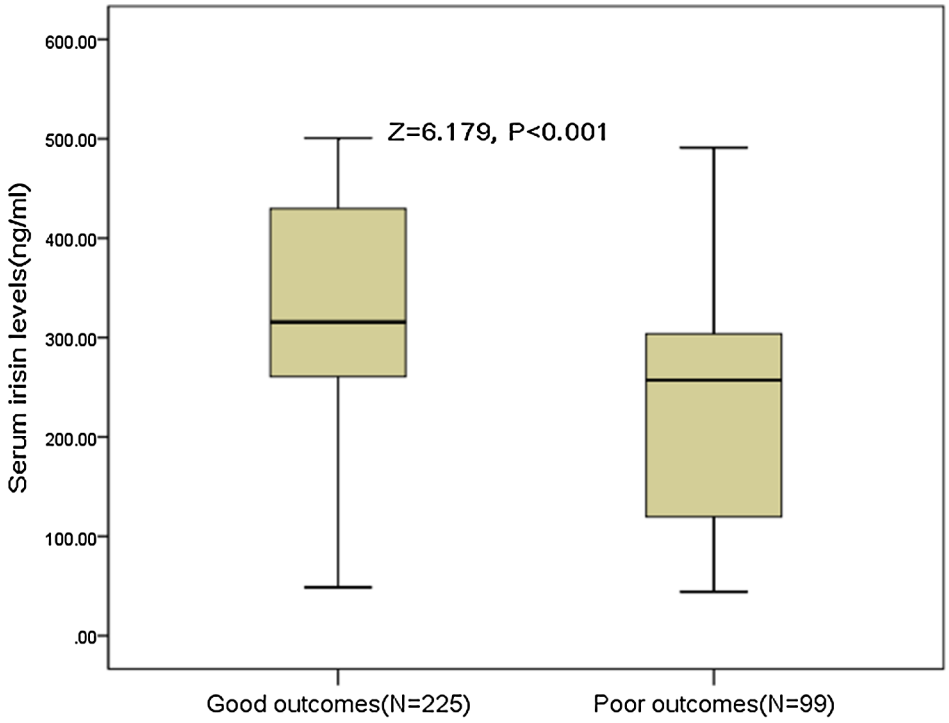

Fig. 1. Distribution of serum Irisin levels in stroke patients with good outcomes and poor outcomes. All data are medians and in-terquartile ranges (IQR). P values refer to Mann-Whitney U tests for differences between groups. Poor outcome was defined as mRS in 3–6.

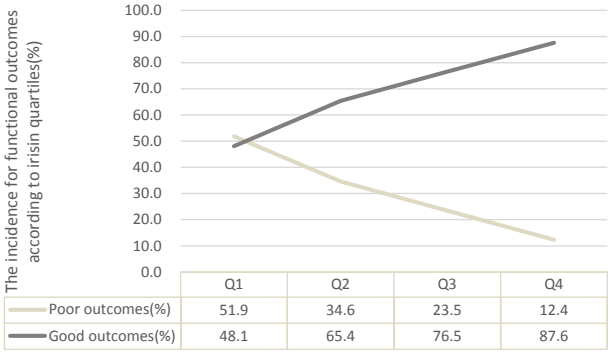

Fig. 2. The incidence for functional outcomes according to the baseline irisin quartiles. The irisin level was obtained in 324 patients with a median value of 291.2 ng/ml (IQR: 214.1–404.2 ng/ml). Poor outcome was defined as mRS in 3–6.

Table 2
Multivariate analysis of predictors of poor outcome.[a]

Predictors	OR	95% CI	P
Irisin (per unit increase)	0.996	0.992–0.999	0.001
Age (per unit increase)	1.185	1.048–1.669	0.030
NIHSS (per unit increase)	1.277	1.136–1.408	0.003
Other stroke etiology[b]	0.122	0.021–0.447	0.007
Acute treatment, TPA-T (yes vs. no)	0.209	0.118–0.349	< 0.001
Infarct volume (per unit increase)[c]	1.173	1.108–1.405	0.013

OR, odds ratio; CI, confidence interval; Hs-CRP, High-sensitivity C-reactive protein; HCY, homocysteine; BMI, body mass index; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; TG, triglyceride; TC, total cholesterol; IL-6, interleukin-6; TPA-T: Tissue plasminogen activator-treated.

[a] Multivariable model included all of the following variables: age, sex, BMI, stroke etiology, stroke syndrome, the NIHSS score, pre-stroke and acute treatment, vascular risk factors, blood levels of glucose, Hs-CRP, HCY, IL-6, TG, TC, HDL, LDL and Irisin quartiles. Poor outcome was defined as mRS in 3–6.

[b] Large artery ischemic stroke used as the reference.

[c] N = 180 were included in the multivariable model.

blood pressure in men with type 2 diabetes [25], while irisin improves endothelial function in type 2 diabetes [26]. Those evidence suggested that irisin might play a protective role in AIS. Further studies are needed to assess the specific mechanisms.

Furthermore, some possible mechanisms that low irisin involved in poor stroke outcomes can be considered. Irisin at baseline was related to stroke size, and severity suggests that it could be a marker of the severity of the initial insult rather than specific for recurrence per se. However, in our study, irisin was still related with recurrence event after adjusting for stroke size and severity (NIHSS score) in the multivariable regression analysis. In addition, systemic inflammation was associated with poor outcomes [27], however, irisin remained significantly associated with poor outcomes even after adjustment for Hs-CRP or IL-6, suggesting that the effect of irisin on prognosis was independent of inflammation. Other possible mechanisms should also be considered. First, a previous study provided evidence of an association between irisin and HCY, which may be due to nicotinamide metabolism [28]. Elevated total HCY levels in acute ischemic stroke were associated with long-term mortality [29]. Second, circulating irisin levels were positively associated with flow-mediated dilation (FMD) levels [30]. Brachial flow-mediated dilation (FMD) is a predictor of incident cardiovascular events in population-based adults [31], and impaired FMD in patients with acute ischemic stroke is associated with poor outcome [32]. Third, lower levels of irisin are independently associated with endothelial dysfunction [33]. A previous study showed that circulating endothelial progenitor cells were associated with stroke outcome [34]. In addition, irisin protects against endothelial injury and ameliorates atherosclerosis in Apo-E knockout mice [35]. Irisin reduces ischemia-induced neuronal injury via activation of the Akt and ERK1/2 signaling pathways and contributes to the neuroprotective effect of physical exercise against cerebral ischemia [13]. Lastly, Irisin and omentin-1 are stable within-person, inversely associated with each other, and closely related to lipoprotein profile [36]. Circulating omentin-1 level was independently correlated with stroke [37]. Hence, further studies should explore the mechanism to elucidate direct irisin effects on stroke prognosis.

Table 3
Odds ratios for poor outcomes according to Irisin quarters at admission.[a]

Irisin quarters	Poor, N	Unadjusted OR (95%CI)[c], P	Adjusted OR (95%CI)[b,c], P
Q1, N = 81	42	7.65(3.46–16.89), < 0.001	3.15(1.76–6.03), < 0.001
Q2, N = 81	28	3.75(1.68–8.39), 0.001	2.08(1.28–4.16), 0.012
Q3, N = 81	19	2.18(0.94–5.03), 0.065	1.32(0.76–3.89), 0.305
Q4, N = 81	10	References	References
Q1 VS. Q2-4	–	3.51(2.08–5.95), 0.003	1.94(1.19–3.42), 0.018

OR: odds ratio; CI: confidence interval; NIHSS, National Institutes of Health Stroke Scale; mRS, Modified Rankin Scale; Hs-CRP, High-sensitivity C-reactive protein; HCY, homocysteine; BMI, body mass index; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; TG, triglyceride; TC, total cholesterol; IL-6, interleukin-6.

[a] The irisin level was obtained in 324 patients (97.9%) with a median value of 291.2 ng/ml (IQR: 214.1–404.2 ng/ml). Poor outcome was defined as mRS in 3–6.

[b] Adjusted for age, sex, BMI, stroke etiology, stroke syndrome, the NIHSS score, pre-stroke and acute treatment, vascular risk factors, blood levels of glucose, Hs-CRP, HCY, IL-6, TG, TC, HDL, LDL and Irisin quartiles.

[c] P value for the trend < 0.001.

Interestingly, two previous reports challenged the putative presence of irisin in human plasma [38]. Furthermore, the irisin levels in different population had a huge range, from 0.58 to 457.2 ng/ml [39,40]. Interestingly, Alis et al. [28] found that mean serum levels of Irisin in type 2 diabetes were 263 ng/ml, while another study reported that mean serum levels of Irisin in newly diagnosed type 2 diabetes and controls were 13.25 ng/ml and 25.98 ng/ml [30]. Irisin is usually measured by ELISA, but the quantification varies greatly between the kits. These differences probably come from the variety in the irisin epitopes being targeted for measurement by the manufacturing companies. In this study, we selected a previously validated ELISA kit that was commercially available [41–43], and our results corroborated the existence of irisin in human circulation.

Furthermore, several other limitations of this study should be noted. First, the cross-sectional nature of the study precludes us to draw any conclusion on the role of irisin in the development of functional outcomes and therefore, no conclusion regarding cause–effect relationships can be made. Second, we adjusted for many risk factors in the multivariate analysis, but the possibility of residual confounding remains. We cannot exclude the possibility that residual confounding factors, including exercise or a poorer health status, may have been missed. In fact, concentrations of irisin increased significantly after endurance exercise training in both mice and humans [44]. In addition, some other cytokines, such as omentin-1 and IL-6 were not tested in this study. So, we could not determine the association of those factors with serum irisin levels and outcomes of Chinese patients with ischemic stroke. Future studies should consider those factors. Third, our data came from a hospital-based registry, which could have hospital selection bias. Finally, all participants to the present study were Chinese, and whether these observations can also be extended to other ethnic groups with different body composition remains to be determined.

6. Conclusions

In conclusion, our findings suggest that reduced serum levels of irisin can predict the risk of poor functional outcomes in Chinese patients with ischemic stroke. Further studies are needed to confirm our findings and to determine whether irisin supplementation can reduce the risk of poor outcomes in stroke patients.

7. Contributors

Ke had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

Study concept and design: Wu, Guo, Jin, Li, Yang, Tang, Wang, Ke.

Acquisition of data: Wu, Guo, Ke.

Analysis and interpretation of data: Jin, Li, Yang, Tang, Wang.

Drafting of the manuscript: Wu, Guo, Jin, Li.

Critical revision of the manuscript for important intellectual content: Yang, Tang, Wang, Ke.

Administrative, technical, or material support: Wu, Guo, Jin, Tang, Wang,

Obtain funding. Ke.

Study supervision: Ke.

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Conflict of interest

All authors have no conflicts of interest to disclose.

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