The official journal of the Japan Atherosclerosis Society and
the Asian Pacific Society of Atherosclerosis and Vascular Diseases

Original Article

J Atheroscler Thromb, 2023; 30: 1045-1056. http://doi.org/10.5551/jat.63824

Relationship between Serum Irisin Levels and MRI-Measured Cerebral Small Vessel Disease in Japanese Men

Mohammad Moniruzzaman[1,2], Aya Kadota[1,2], Takashi Hisamatsu[3], Hiroyoshi Segawa[1], Keiko Kondo[2], Sayuki Torii[2], Naoko Miyagawa[4], Akira Fujiyoshi[5], Yuichiro Yano[1], Yoshiyuki Watanabe[6], Akihiko Shiino[7], Kazuhiko Nozaki[8], Hirotsugu Ueshima[1,2] and Katsuyuki Miura[1,2], on behalf of the SESSA Research Group

[1]NCD Epidemiology Research Center (NERC), Shiga University of Medical Science, Shiga, Japan
[2]Department of Public Health, Shiga University of Medical Science, Shiga, Japan
[3]Department of Public Health, Okayama University, Okayama, Japan
[4]Department of Preventive Medicine and Public Health, Keio University School of Medicine, Tokyo, Japan
[5]Department of Hygiene, School of Medicine, Wakayama Medical University, Wakayama, Japan
[6]Department of Radiology, Shiga University of Medical Science, Shiga, Japan
[7]Molecular Neuroscience Research Center, Shiga University of Medical Science, Shiga, Japan
[8]Department of Neurosurgery, Shiga University of Medical Science, Shiga, Japan

Aim: Irisin, an exercise-induced myokine, is a potential neurotrophic factor; however, its relationship with cerebral small vessel disease (CSVD) remains unknown. Therefore, we investigated whether serum irisin levels are associated with CSVD in healthy Japanese men.

Methods: We analyzed data from 720 men free of stroke and participated in this observational study. Serum irisin levels were measured by enzyme-linked immunosorbent assay. CSVD was assessed on deep and subcortical white matter hyperintensities (DSWMHs), periventricular hyperintensities (PVHs), lacunar infarcts (LIs), and cerebral microbleeds (CMBs) on brain magnetic resonance imaging. We calculated the total CSVD score (ranges 0–4) to express the total CSVD burden. We computed the adjusted odds ratios (ORs), with 95% confidence intervals (CIs), of the total CSVD score and individual CSVD features using logistic regression models according to the quartiles of irisin (reference: Q1).

Results: Serum irisin levels were associated with lower ORs of higher (vs. zero or lower score) total CSVD score, with the lowest risk (OR, 0.63; 95% CI, 0.41–0.97) being observed in Q3 compared to Q1 after adjustment of potential covariates. Similar results were obtained for younger adults (<65 years). Among individual CSVD features, irisin was associated with a reduced risk of LIs in the total sample and PVHs, LIs, and CMBs in younger adults. No relationship was observed in older adults (≥ 65 years).

Conclusions: Serum irisin levels were associated with less burden of total CSVD in healthy Japanese men. Serum irisin levels were also related with a reduced risk of PVHs, LIs, and CMBs, but not DSWMHs.

Key words: Physical activity, Serum irisin levels, Exercise-induced myokine, Cerebral small vessel disease, Aging brain diseases

Introduction

Cerebral small vessel disease (CSVD) is a common, subclinical, and progressive health issue in the general population with aging. CSVD causes severe clinical consequences, such as stroke, cognitive decline, depression, dementia, including Alzheimer's disease, and death[1-5]; however, there are currently no

Address for correspondence: Katsuyuki Miura, NCD Epidemiology Research Center (NERC), Shiga University of Medical Science, Seta Tsukinowa-cho, Otsu, Shiga, 520-2192, Japan E-mail: miura@belle.shiga-med.ac.jp

Received: July 14, 2022 Accepted for publication: September 30, 2022

established therapeutic measures to treat or prevent CSVD[1, 4, 6, 7]. Emerging evidence shows that physical activity (exercise) is neuroprotective for brain health and has become a promising and viable lifestyle intervention[8-10]. We recently demonstrated for the first time that step counts—a direct measure of physical activity—were associated with a reduced risk of CSVD in healthy Japanese men[11]. These findings may be of clinical importance for the attenuation of CSVD. However, the mechanisms by which physical activity reduces the risk of CSVD remain unclear.

Irisin, a newly discovered exercise-induced myokine secreted into the circulation mainly from the skeletal muscle, has been implicated in exercise-mediated decreases in the risk of CSVD. In 2012, a study showed that irisin ameliorated obesity and metabolic disorders by facilitating energy expenditure through the browning of beige fat cells in white adipose tissue[12]. Its role as a neurotrophic factor has recently been attracting increasing attention, and it was shown to exert neuroprotective effects against the pathogenesis of brain diseases, including neurodegenerative diseases such as Alzheimer's disease and stroke[8, 13-19]. However, to the best of our knowledge, the relationship between irisin and the magnetic resonance imaging (MRI)-measured CSVD burden remains uninvestigated.

Therefore, the present study investigated the relationship between serum irisin levels at baseline and the total CSVD burden in a 5-year follow-up in Japanese men and whether this relationship is independent of lifestyle and cardiovascular risk factors. The relationships between serum irisin levels and individual CSVD features (deep and subcortical white matter hyperintensities [DSWMHs], periventricular hyperintensities [PVHs], lacunar infarcts [LIs], and cerebral microbleeds [CMBs]) were also examined.

Methods

We analyzed data from the Shiga Epidemiological Study of Subclinical Atherosclerosis (SESSA) in the present study. This ongoing population-based observational study conducted in Kusatsu City, Shiga Prefecture, Japan, examines factors affecting subclinical atherosclerosis. Its design and enrollment methods have already been reported[20-23]. The study protocol was approved by the Institutional Review Board of Shiga University of Medical Science (Otsu, Japan). Briefly, at baseline (2006–2008), 2379 general Japanese men aged 40–79 years were randomly selected based on age strata from the Kusatsu City Basic Residents' Register. Among them, 1094 men participated in baseline medical examinations with written consent; 853 subsequently underwent the SESSA follow-up examination (2010–2014). They were reinvited for brain MRI, and 740 underwent 1.5 T MRI on average 2 years later (between 2012 and 2015). We excluded 20 participants with a history of stroke (n=19) at baseline and missing information on serum irisin levels (n=1), leaving 720 men for the final analyses.

To measure serum irisin levels and other laboratory parameters, including lipid and glucose concentrations, we collected blood samples by venipuncture in baseline clinical examinations after an overnight fast of at least 12 h. We separated serum by centrifugation and sent samples to the laboratory for routine tests. Other samples were immediately frozen at −70℃ for later analyses. In 2016, we thawed specimens and measured serum irisin levels in μg/mL at the clinical chemistry laboratory of FALCO Biosystems (Kyoto, Japan) using a commercially available enzyme-linked immunosorbent assay kit (AG-45A-0046EK-k101; Adipogen, Liestal, Switzerland). Detailed procedures were previously reported[21, 22].

We assessed CSVD in participants at the follow-up (2012–2015) using the following neuroimaging markers on brain MRI: DSWMHs, PVHs, LIs, and CMBs[24]. MRI was performed using a 1.5-Tesla MR scanner (Signa HDxt 1.5T ver. 16; GE Healthcare, Milwaukee, WI, USA). Two neurosurgeons, certified by the Japan Neurosurgery Society, independently assessed all MRI images and rated them in duplicate. They were blinded to the clinical information of participants during their evaluations. We dichotomized each form of the four CSVD features as follows: DSWMHs (grade ≥ 3)[25, 26], PVHs (grade ≥ 2)[25], LIs (defined as a lesion number ≥ 1), and CMBs (defined as a lesion number ≥ 1). We also created a "total CSVD score" to express the total CSVD burden[27, 28]. To achieve this, we awarded 1 point to the presence of each MRI CSVD feature and summed them in an ordinal scale ranging between 0 (no outcome) and 4 (all outcomes), which indicated the severity of CSVD[27]. The frequency of participants with each value of the total CSVD score is presented in **Supplemental Table 1**. The assessment of CSVD was described in detail in previous studies[11, 28].

Data on demographic characteristics, lifestyle factors, including the smoking and drinking status, medical history, and the use of medications for hypertension, diabetes, and dyslipidemia were collected from each participant using a self-administered questionnaire at the baseline. We objectively measured step counts for baseline participants on 7 consecutive days using a pedometer

(DIGI-WALKER DW-200; Yamasa Tokei Keiki, Tokyo, Japan) and calculated the 7-day average step counts. Body mass index was defined as weight (kg) divided by height squared (m^2). Systolic blood pressure (SBP) and diastolic blood pressure (DBP) were measured using an automated sphygmomanometer. Fasting blood samples were obtained and centrifuged soon after collection. Total cholesterol and triglyceride concentrations were measured enzymatically and high-density lipoprotein (HDL) cholesterol concentration by a direct method. Low-density lipoprotein (LDL) cholesterol concentration was calculated using the Friedewald equation. Glycated hemoglobin concentration was measured using a latex agglutination immunoassay.

We presented descriptive statistics on participants as percentages for categorical variables and as means (standard deviations) or medians (interquartile ranges) for continuous variables. We performed one-way ANOVA (analysis of variance) to compare continuous variables with normal distribution and the Kruskal–Wallis test for continuous variables that do not follow the normal distribution among the four groups of participants based on the quartile of serum irisin levels. We used the chi-squared test for categorical variables.

We used multivariable ordinal logistic regression analyses to assess the relationship between serum irisin levels and the total CSVD burden because our outcome variable (the total CSVD score) was with five groups on an ordinal scale ranging between 0 (no outcome) and 4 (maximum outcome), and it met the proportional odds assumption. Odds ratios (ORs) were assumed to be the same at each cumulative split of the ordinal outcome variable. Therefore, there was only one OR. We categorized participants into four groups based on the quartiles of serum irisin levels (μg/mL): Q1 (2.67–5.75), Q2 (5.76–7.01), Q3 (7.02–8.33), and Q4 (8.34–24.52). The distribution of serum irisin levels is presented in **Supplemental Table 2 and Supplemental Fig. 1**.

We computed ORs and the corresponding 95% confidence intervals (CIs) for the presence of a higher total CSVD score (vs. zero or lower score) according to the quartiles of serum irisin levels using the lowest quartile (Q1) as the reference group. We ran three models in these analyses: model 1 was adjusted for age (years), model 2 for age and behavioral risk factors (smoking [pack-years] and drinking [ethanol, g/week]), and model 3 for variables in model 2 plus conventional vascular risk factors (body mass index [kg/m^2] and the status of diabetes, dyslipidemia, and hypertension [yes/no]). Adjustment of step counts in the model may affect the precision of estimates and lead to over-adjustment bias because step counts could be an upstream factor in the causal pathway between serum irisin levels and atherosclerosis burden. Therefore, our analyses did not control for daily step counts. Diabetes was defined as a fasting blood glucose level ≥ 126 mg/dL or glycated hemoglobin (National Glycohemoglobin Standardization Program) value ≥ 6.5%, or the use of antidiabetic medication. Dyslipidemia was defined as a triglyceride level ≥ 150 mg/dL or LDL cholesterol level ≥ 140 mg/dL or HDL cholesterol level ≤ 40 mg/dL or the use of lipid-lowering drugs. Hypertension was defined as average SBP ≥ 140 mmHg or DBP ≥ 90 mmHg or the use of antihypertensive medication.

We used multivariable binary logistic regression analyses to assess the relationships between serum irisin levels and individual MRI CSVD features (DSWMHs, PVHs, LIs, and CMBs [yes/no]). We reproduced the same models of the ordinal logistic regression and computed adjusted ORs with CIs for the presence of each form of CSVD according to the quartiles of serum irisin levels using the lowest quartile (Q1) as the reference group.

Statistical analyses were performed using SAS software version 9.4 (SAS Institute, Cary, NC, USA). We presented results for overall and age-stratified groups (<65 and ≥ 65 years). A p-value less than the two-sided significance level of 0.05 was significant for all analyses.

Results

Table 1 shows the participant characteristics. The mean (SD) age and educational attainment of all participants were 63.6 (9.4) and 12.7 (3.0) years, respectively. Participants in the lower two quartiles of serum irisin levels were younger, had higher education, and consumed less alcohol. Participants in the upper two quartiles had lower body mass index, a better lipid profile (higher HDL cholesterol and lower LDL cholesterol and triglycerides, whereas total cholesterol did not differ among the quartiles), and required less medication for dyslipidemia.

Table 2 shows the multivariable-adjusted ORs, using ordinal logistic regression, for the total CSVD score by the quartiles of serum irisin levels, with Q1 as the reference group. The number and percentage of participants with each CSVD score are shown in **Supplemental Table 1**. Serum irisin levels were associated with lower ORs of higher (vs. zero or lower score) total CSVD score, with the lowest risk (OR, 0.63; 95% CI, 0.41–0.97) being observed at Q3 (7.02–8.33 μg/mL) relative to Q1 (2.67–5.75 μg/mL) in model 3, which was adjusted for age, behavioral

Table 1. Participant Characteristics at Baseline (n=720): SESSA, Men Aged 40–79 Years (2006–2008)

Characteristics	Overall (n=720)	Quartiles of serum irisin levels in μg/mL (n=180 in each quartile)				p-value
		Q1 (2.67–5.75)	Q2 (5.76–7.01)	Q3 (7.02–8.33)	Q4 (8.34–24.52)	
Age, y	63.6 (9.4)	60.4 (9.9)	63.6 (9.2)	64.7 (9.3)	65.7 (8.3)	<0.001
Years of education	12.6 (3.0)	12.9 (3.0)	12.8 (3.0)	12.6 (2.6)	12.3 (3.1)	0.042
7-d average step counts	8516.4 (3540.1)	8885.6 (3619.8)	8761.4 (3453.0)	8090.6 (3564.6)	8339.6 (3496.5)	0.246
Smoking status, %						0.445
Current	29.4	32.2	27.8	31.1	26.7	
Past	51.0	47.8	50.5	47.8	57.8	
Never	19.6	20	21.7	21.1	15.5	
Smoking, pack-years	23.1 (4.0–42.0)	21.0 (2.2–38.7)	24.0 (2.5–44.3)	23.0 (1.3–45.3)	24.2 (8.5–42.0)	0.308*
Drinking status, %						0.596
Current	79.3	75.0	79.4	83.3	79.4	
Past	5.1	6.1	6.1	3.3	5.0	
Never	15.6	18.9	14.5	13.4	15.6	
Alcohol consumption (ethanol), g/wk	98.0 (9.6–259.3)	80.6 (0.0–235.2)	93.3 (7.0–259.3)	106.0 (14.0–268.8)	153.1 (12.3–301.3)	0.032*
Body mass index, kg/m^2	23.6 (2.9)	24.0 (2.7)	23.7 (2.8)	23.6 (3.0)	23.1 (3.0)	0.006
HbA1c, %	5.6 (0.8)	5.6 (0.7)	5.7 (0.8)	5.7 (0.8)	5.6 (0.7)	0.808
Medication for diabetes mellitus, %	10.0	10.6	12.8	8.3	8.3	0.438
Diabetes mellitus, %	22.4	21.1	26.7	22.8	18.9	0.341
Total cholesterol, mg/dL	210.3 (32.5)	213.8 (31.4)	207.0 (36.6)	211.2 (31.2)	209.4 (30.2)	0.403
HDL cholesterol, mg/dL	59.3 (17.0)	55.2 (13.9)	55.8 (15.8)	60.5 (16.5)	65.9 (19.2)	<0.001
LDL cholesterol, mg/dL	126.2 (30.7)	132.1 (29.2)	126.5 (34.4)	124.6 (31.0)	121.6 (27.0)	0.001
Triglycerides, mg/dL	105.0 (76.0–150.0)	119.5 (85.5–159.0)	104.0 (75.5–149.5)	104.0 (78.0–157.5)	90.0 (71.5–128.0)	0.001*
Medication for dyslipidemia, %	14.4	15.0	15.6	12.8	14.4	0.890
Dyslipidemia, %	55.8	66.7	57.2	52.8	46.7	0.001
Systolic blood pressure, mmHg	135.8 (18.0)	135.7 (17.7)	135.6 (17.9)	133.7 (18.1)	137.4 (18.2)	0.612
Medication for hypertension, %	29.2	27.2	32.2	26.1	31.1	0.515
Hypertension, %	53.2	47.2	56.7	50.0	58.9	0.088

All continuous variables are presented as means (SDs), except for pack-years smoking, alcohol consumption, and triglycerides (median [interquartile range]); other values are percentages. P values are calculated by one-way ANOVA (analysis of variance) for all continuous variables, unless otherwise indicated, and the chi-squared test for categorical variables. *The Kruskal-Wallis test. Diabetes was defined as a fasting blood glucose level ≥ 126 mg/dL or HbA1c (National Glycohemoglobin Standardization Program) value ≥ 6.5% or the use of antidiabetic medication. Dyslipidemia was defined as a triglyceride level ≥ 150 mg/dL or LDL cholesterol level ≥ 140 mg/dL or HDL cholesterol level ≤ 40 mg/dL or the use of lipid-lowering drugs. Hypertension was defined as average systolic blood pressure ≥ 140 mm Hg or diastolic blood pressure ≥ 90 mm Hg or the use of antihypertensive medication. HbA1c, glycated hemoglobin; HDL, high-density lipoprotein; LDL, low-density lipoprotein; and SESSA, Shiga Epidemiological Study of Subclinical Atherosclerosis.

Table 2. Multivariable Ordinal Logistic Regression Models for Relationships (Odds Ratio with 95% CI) between Serum Irisin Levels at Baseline and the Total Cerebral Small Vessel Disease Score in the 5-year Follow-up (n=720)

	Quartiles of serum irisin levels in μg/mL (n=180 in each quartile)			
	Q1 (2.67–5.75)	Q2 (5.76–7.01)	Q3 (7.02–8.33)	Q4 (8.34–24.52)
TCSVD Score				
Model 1	1.00	0.70 (0.46–1.05)	0.65 (0.43–0.99)	0.71 (0.47–1.07)
Model 2	1.00	0.69 (0.45–1.05)	0.63 (0.41–0.96)	0.67 (0.44–1.02)
Model 3	1.00	0.66 (0.43–1.00)	0.63 (0.41–0.97)	0.65 (0.42–0.99)

Model 1 was adjusted for age.
Model 2 was adjusted for variables in model 1 plus alcohol intake (ethanol) and smoking (pack-years).
Model 3 was adjusted for variables in model 2 plus body mass index, diabetes, dyslipidemia, and hypertension.
CI indicates confidence interval; and TCSVD, total cerebral small vessel disease.

Table 3. Multivariable Ordinal Logistic Regression Models for Relationships (Odds Ratio with 95% CI) between Serum Irisin Levels at Baseline and the Total Cerebral Small Vessel Disease Score in the 5-year Follow-up (n=720)

	Quartiles of serum irisin levels in μg/mL							
	<65 years (n=386)				≥ 65 years (n=334)			
	Q1 (2.67–5.74) n=119	Q2 (5.75–7.01) n=97	Q3 (7.02–8.33) n=88	Q4 (8.34–19.67) n=82	Q1 (3.69–5.75) n=61	Q2 (5.76–7.01) n=83	Q3 (7.02–8.30) n=92	Q4 (8.31–24.52) n=98
TCSVD Score								
Model 1	1.00	0.56 (0.31–1.00)	0.48 (0.26–0.89)	0.71 (0.39–1.27)	1.00	0.87 (0.48–1.59)	0.89 (0.50–1.61)	0.75 (0.42–1.35)
Model 2	1.00	0.53 (0.29–0.97)	0.45 (0.24–0.85)	0.69 (0.38–1.27)	1.00	0.86 (0.47–1.57)	0.87 (0.48–1.57)	0.69 (0.38–1.25)
Model 3	1.00	0.47 (0.25–0.86)	0.42 (0.22–0.80)	0.67 (0.36–1.23)	1.00	0.86 (0.47–1.58)	0.88 (0.48–1.62)	0.68 (0.37–1.26)

Model 1 was adjusted for age.
Model 2 was adjusted for variables in model 1 plus alcohol intake (ethanol) and smoking (pack-years).
Model 3 was adjusted for variables in model 2 plus body mass index, diabetes, dyslipidemia, and hypertension.
CI indicates confidence interval; and TCSVD, total cerebral small vessel disease.

Table 4. Multivariable Logistic Regression Models for Relationships (Odds Ratio with 95% CI) between Serum Irisin Levels at Baseline and Cerebral Small Vessel Disease in the 5-year Follow-up (n=720)

	Quartiles of serum irisin levels in μg/mL (n=180 in each quartile)			
	Q1 (2.67–5.75)	Q2 (5.76–7.01)	Q3 (7.02–8.33)	Q4 (8.34–24.52)
DSWMHs, n (%)	40 (22.2)	37 (20.6)	42 (23.3)	37 (20.6)
Model 1	1.00	0.82 (0.49–1.36)	0.92 (0.56–1.53)	0.76 (0.45–1.28)
Model 2	1.00	0.83 (0.49–1.39)	0.92 (0.55–1.54)	0.70 (0.41–1.20)
Model 3	1.00	0.80 (0.47–1.36)	0.94 (0.56–1.59)	0.70 (0.40–1.20)
PVHs, n (%)	45 (25.0)	47 (26.1)	43 (23.9)	48 (26.7)
Model 1	1.00	0.84 (0.51–1.39)	0.67 (0.40–1.12)	0.74 (0.45–1.23)
Model 2	1.00	0.87 (0.53–1.45)	0.67 (0.40–1.13)	0.76 (0.46–1.27)
Model 3	1.00	0.85 (0.51–1.41)	0.67 (0.39–1.13)	0.73 (0.43–1.23)
LIs, n (%)	38 (21.1)	31 (17.2)	32 (17.8)	42 (23.3)
Model 1	1.00	0.61 (0.35–1.06)	0.58 (0.33–1.00)	0.79 (0.47–1.33)
Model 2	1.00	0.55 (0.32–0.96)	0.53 (0.30–0.93)	0.69 (0.40–1.18)
Model 3	1.00	0.53 (0.30–0.93)	0.55 (0.31–0.98)	0.69 (0.39–1.20)
CMBs, n (%)	27 (15.0)	18 (10.0)	26 (14.4)	27 (15.0)
Model 1	1.00	0.54 (0.28–1.04)	0.78 (0.43–1.43)	0.79 (0.44–1.44)
Model 2	1.00	0.54 (0.28–1.03)	0.75 (0.41–1.38)	0.77 (0.42–1.42)
Model 3	1.00	0.54 (0.28–1.04)	0.77 (0.42–1.43)	0.77 (0.41–1.43)

Model 1 was adjusted for age.
Model 2 was adjusted for variables in model 1 plus alcohol intake (ethanol) and smoking (pack-years).
Model 3 was adjusted for variables in model 2 plus body mass index, diabetes, dyslipidemia, and hypertension.
CI indicates confidence interval; CMBs, cerebral microbleeds; DSWMHs, deep and subcortical white matter hyperintensities; LIs, lacunar infarcts; and PVHs, periventricular hyperintensities.

factors (alcohol consumption and smoking), and vascular risk factors (body mass index, diabetes, dyslipidemia, and hypertension). Since SBP is a strong risk factor for CSVD, instead of hypertension in model 3, we also adjusted for SBP and medication use for hypertension, and the results were similar (results are not shown). Similar results were obtained for younger adults (<65 years) as shown in **Table 3**.

Table 4 shows the multivariable-adjusted ORs, using binary logistic regression, for the prevalent individual CSVD features by quartiles of serum irisin levels, with Q1 as the reference group. The prevalence of CSVD features (DSWMHs, PVHs, LIs, and CMBs) decreased in Q2 and Q3 of serum irisin levels, except for DSWMHs in Q3 and PVHs in Q2, in which their prevalence was slightly higher than in Q1 and/or Q4. Approximately 25% of participants had DSWMHs and PVHs, approximately 20% had LIs,

and approximately 15.0% had CMBs across the quartiles of serum irisin levels. Serum irisin levels generally had slightly lower OR for each form of CSVD (DSWMHs, PVHs, LIs, and CMBs). However, serum irisin levels in Q2 and Q3 were significantly associated with a reduced risk of LIs (OR, 0.53; 95% CI, 0.30–0.93 and OR, 0.55; 95% CI, 0.31–0.98, respectively) in the total sample, those in Q3 with PVHs (OR, 0.27; 95% CI, 0.10–0.74), and those in Q2 with LIs (OR, 0.37; 95% CI, 0.15–0.92) and CMBs (OR, 0.25; 95% CI, 0.08–0.83) in younger adults **(Table 4 and Supplemental Table 3)**. No relationships were observed in older adults (≥ 65 years).

Discussion

In this population-based observational study on Japanese men who were free from stroke and cognitive decline, serum irisin levels at baseline were associated with lower total CSVD score, that is, burden in the 5-year follow-up. Baseline serum irisin levels were also associated with a reduced risk of individual MRI CSVD features (PVHs, LIs, and CMBs), except for DSWMHs. These associations were independent of age, behavioral (the smoking and drinking status), and vascular (body mass index, diabetes, dyslipidemia, and hypertension) risk factors. To the best of our knowledge, this is the first study to investigate the relationship between serum irisin levels and the MRI-measured CSVD burden. Therefore, we were unable to compare the results obtained with previous findings; more evidence is awaited in this field. However, since some studies used physical activity (typically measured with a questionnaire), it was reasonable to compare our results with their findings.

In the present study, baseline serum irisin levels were associated with lower total CSVD score (burden) in the 5-year follow-up. A single study recently investigated cross-sectional and longitudinal relationships between self-reported physical activity and the total CSVD score (ranging between 0 and 3, generated from white matter hyperintensities, LIs, and CMBs) among 503 patients with CSVD[29]. Cross-sectionally, the active physical activity group had a significantly lower CSVD score in a zero-order relationship. However, it was no longer significant after adjustments for confounders, and no relationship was observed between physical activity and the CSVD score in longitudinal analyses[29]. It is important to note that they used patients with CSVD who were older than those in the present study and analyzed self-reported physical activity, which may explain the discrepancy between the present results and their findings.

Of note, we found a J-shaped relationship between serum irisin levels and the total CSVD burden; Q3 (serum irisin levels of 7.02–8.33 μg/mL) had lower OR than Q1 **(Tables 2 and 3)**. We previously reported a similar relationship between step counts and CSVD[11]. However, the underlying mechanisms have not yet been elucidated. Recent systematic reviews and meta-analyses confirmed that relationships between physical activity/step counts and health outcomes, including all-cause mortality, are generally curvilinear or nonlinear[30, 31]. The threshold effects of physical activity/irisin levels may have contributed to these relationships. The beneficial effects of physical activity on CSVD warrant further studies using exercise-induced biomarkers, such as irisin, including randomized controlled trials to eliminate alternative causal mechanisms.

In the present study, serum irisin levels generally tended to have lower OR for prevalent subclinical PVHs; however, a significant association was observed in younger adults. We previously reported, using the same samples, that step counts significantly reduced the risk of prevalent PVHs[11]. This result is also consistent with the findings of other studies in which increasing self-reported physical activity levels at baseline were associated with a combined[32] and separate[33] rating of deep and periventricular white matter hyperintensities in 3- and 5-year follow-ups, respectively. In contrast, we did not find statistically significant relationships between serum irisin levels and DSWMHs in overall and age-stratified analyses. This discrepancy may be explained by differences in the functional, microstructural, and etiological features between DSWMHs and PVHs[34]. However, the underlying mechanisms of such differential relationships of serum irisin levels with location-specific white matter hyperintensities (PVHs and DSWMHs) are unclear and unknown. Additionally, the number of prevalent PVHs and DSWMHs in the present study, especially in younger adults, may not be sufficient to detect small differences or find statistically significant results in DSWMHs, or the observed significant results in PHVs might have occurred by chance. These relationships need to be examined in more detail in longitudinal studies and clinical trials.

In the present study, serum irisin levels were associated with a reduced risk of LIs, reaffirming the relationship between step counts and the prevalent subclinical LIs observed in our previous study[11]. Two other studies reported similar findings showing that higher self-reported physical activity was associated with a reduced risk of subclinical cerebral infarct on MRI[35, 36]. A recent study also found a similar cross-sectional relationship between baseline physical

activity (self-reported) and LIs among patients with CSVD[29]. However, this relationship disappeared in the longitudinal analysis because patients with CSVD who were older and medically less fit were more likely to drop out[29].

We also observed a significant association of serum irisin levels with prevalent CMBs. In a single cohort study targeting healthy adults, the weekly frequency of physical activity significantly mitigated the risk of CMBs[37]. Another cohort study that targeted a patient population (mild cognitive impairment with CSVD) reported that low physical activity (self-reported) was associated with lobar CMBs[38]. In contrast, a recent study with a similar patient population reported a null association[29]. In the present study, we used irisin, an exercise-induced myokine/biomarker, which provided a more reliable relationship.

We reported no significant associations (for all analyses) in older adults aged ≥ 65 years in our sample. Although serum irisin levels are associated with age, the co-existence of higher comorbidities, such as diabetes and hypertension, in older adults than in younger adults (data not shown) may have contributed to this result even after appropriate adjustments. It is also possible that age is not only a strong confounder but also an effect modifier to these relationships. Evidence shows that age is strongly associated with CSVD in Asian populations[5]. Our study also showed no association between serum irisin levels and step counts **(Supplemental Fig. 2)**. Various factors, including age, cardiometabolic state, and exercise habits/types or duration, may influence the regulation of irisin in humans. For instance, a recent review report suggests that acute exercise can promote irisin secretion in humans. However, the effects of chronic exercise remain under debate[39], which may support our study finding on "no association" between serum irisin levels and step counts. Research in this field is still in the infancy stage; therefore, more evidence is needed from clinical trials to clarify these relationships.

The mechanisms underlying the protective effects of serum irisin levels on CSVD remain unclear. However, mounting evidence revealed that irisin crosses the blood-brain barrier through a peroxisome proliferator-activated receptor gamma coactivator 1-alpha/fibronectin type III domain-containing protein 5 pathway to exert its neuroprotective effects in animal models and humans[13, 17-19]. It has been shown to upregulate brain-derived neurotrophic factor, a neurotrophin, in the brain, which protects neurons from injury. Irisin also influenced mitochondrial dynamics, which are essential for normal neuronal functions[13, 17-19]. Notably, serum irisin was recently posited as a potential biomarker/mediator for cognitive decline and neurodegenerative diseases, including Alzheimer's disease[18, 19, 40, 41], and a new protagonist to treat and prevent acute brain injury, including stroke[42].

The use of serum irisin levels is one of the strengths of the present study, which may help clarify, for the first time, the mechanisms underlying the relationship between physical activity and CSVD, thereby supporting physical activity, including step counts, as a neuroprotective lifestyle factor. A better understanding of how physical activity exerts neuroprotective effects may be a potential motivational factor for individuals and facilitate behavioral changes. The present results provide novel insights that may provide the general population with a better understanding of the brain health implications of physical activity. Moreover, serum irisin levels were independently related with the reduced risk of CSVD and, thus, have potential as a new biomarker and therapeutic target for CSVD.

There are several limitations that need to be addressed. CSVD features on MRI (DSWMHs, PVHs, LIs, and CMBs) were not assessed at baseline; therefore, we were unable to confirm the onset of CSVD during the follow-up. Furthermore, these features may have been misclassified because of their visual ratings, which cannot be overruled for certainty. In addition, the present study only included Japanese men; therefore, the results obtained cannot be applied to other populations. Another limitation is the cross-sectional nature of the study, which prevents a cause–effect relationship being established. The relationships of serum irisin levels with individual CSVD features in younger adults should be interpreted cautiously considering the number of prevalent cases. Furthermore, other unknown or unmeasured factors that influence serum irisin levels and CSVD may have been residual confounders. Due to the paucity of information on the role of irisin in subclinical CSVD, further studies need to be conducted on different populations to elucidate the mechanisms by which irisin mediates the neuroprotective effects of physical activity (exercise) on CSVD.

Conclusions

In general Japanese men, serum irisin levels were associated with less burden of total CSVD, with the lowest risk being observed among participants with serum irisin levels of approximately 7.0–8.5 μg/mL. Serum irisin levels were also related to a reduced risk of subclinical PVHs, LIs, and CMBs, but not

DSWMHs. Physical activity (exercise) as a neuroprotective lifestyle factor may contribute to the prevention of CSVD.

Acknowledgements

The authors sincerely appreciate the members of the SESSA Research Group, who approved the current study.

Notice of Grant Support

The SESSA has been supported by the JSPS KAKENHI Grant Numbers (A) 13307016, (A) 17209023, (A) 21249043, (A) 23249036, (A) 25253046, (A) 15H02528, (A) 18H04074, (B) 26293140 (B) 24790616, (B) 21790579, (B) 18H03048, (B) 15K19224, (B) 15K19225, and (C) 23590790 from the Ministry of Education, Culture, Sports, Science, and Technology Japan, and by the grant R01HL068200 from Glaxo-Smith Kline GB. JSPS has also supported the present study for Postdoctoral Fellowships for Research in Japan. The funding agencies listed above had no role in the study design, data collection, analyses, interpretation of results, or writing of the manuscript.

Conflicts of Interest

The authors do not have any conflicts of interest to disclose.

Author Contributions

MM: conceptualized and designed the study, analyzed data, interpreted results, and wrote the manuscript. AK and YY: conceptualized and designed the study, guided the manuscript writing, critically interpreted results, and revised the manuscript. YW, AS, KN, and AF: acquisition of MRI-related data, and reviewed the manuscript. NM and TH: coordinated and supervised data collection, controlled quality at all stages, and reviewed the manuscript. HS, KK, and ST: collected and managed data, and reviewed the manuscript. HU and KM: oversaw the quality control measures, provided overall guidance for manuscript writing, and critically reviewed the manuscript. All authors read and approved the final manuscript.

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Members of the SESSA Research Group:

Co-principal investigators: Hirotsugu Ueshima, Katsuyuki Miura (Shiga University of Medical Science, Otsu, Shiga).

Research members: Yoshihisa Nakagawa, Yasutaka Nakano, Emiko Ogawa, Hiroshi Maegawa, Katsutaro Morino, Itsuko Miyazawa, Yoshiyuki Watanabe, Kazuhiko Nozaki, Ikuo Tooyama, Akihiko Shiino, Akira Andoh, Teruhiko Tsuru, Hisakazu Ogita, Naomi Miyamatsu, Yasuyuki Nakamura, Yuichiro Yano, Aya Kadota, Keiko Kondo, Sayuki Torii, Takashi Kadowaki, Sayaka Kadowaki, Takahiro Ito, Ayako Kunimura, Hiroyoshi Segawa, Yukiko Okami (Shiga University of Medical Science, Otsu, Shiga), Akira Fujiyoshi, Aya Higashiyama (Wakayama Medical University, Wakayama), Tomonori Okamura, Naoko Miyagawa (Keio University, Tokyo), Tatsuya Sawamura (Shinshu University, Matsumoto, Nagano), Michiya Igase (Ehime University, Toon, Ehime), Yasuharu Tabara (Shizuoka Graduate University of Public Health, Shizuoka), Akira Sekikawa, Emma JM Barinas-Mitchell (University of Pittsburgh, Pittsburgh, PA, USA),Daniel Edmundowicz (Temple University, Philadelphia, PA, USA), Takayoshi Ohkubo (Teikyo University, Tokyo), Atsushi Hozawa (Tohoku University, Sendai, Miyagi), Yoshitaka Murakami (Toho University, Tokyo), Nagako Okuda (Kyoto Prefectural University), Hisatomi Arima, Atsushi Satoh (Fukuoka University, Fukuoka), Yoshikuni Kita (Tsuruga Nursing University, Tsuruga, Fukui), Takashi Hisamatsu (Okayama University, Okayama), Masahiko Yanagita (Doshisha University, Kyotanabe, Kyoto), Seiko Ohno (National Cerebral and Cardiovascular Center, Suita, Osaka), Naoyuki Takashima (Kindai University, Osakasayama, Osaka), Takashi Yamamoto (Kohka Public Hospital, Shiga), Koichiro Azuma (Nerima General Hospital, Tokyo), Maryam Zaid (Fudan University Shanghai, China), Yoshino Saito (Aino University, Ibaraki, Osaka).

Supplemental Table 1. Frequency of Participants with Each Value of the Total Cerebral Small Vessel Disease Score (n=720)

TCSVD score	Overall (n=720)	<65 years (n=386)	≥ 65 years (n=334)
0	395 (54.9)	257 (66.6)	138 (41.3)
1	158 (21.9)	71 (18.4)	87 (26.1)
2	98 (13.6)	36 (9.3)	62 (18.6)
3	50 (6.9)	15 (3.9)	35 (10.5)
4	19 (2.6)	7 (1.8)	12 (3.6)

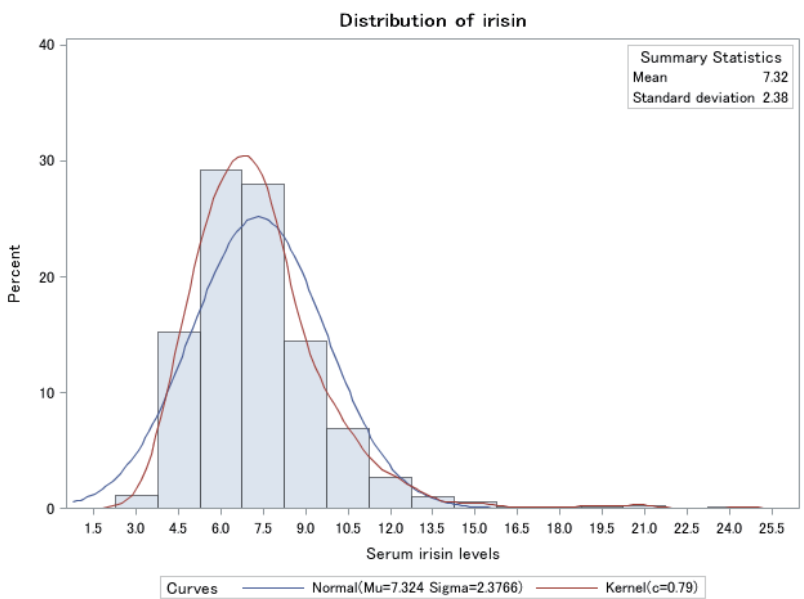
Values are presented as numbers (percentages).
TCSVD indicates total cerebral small vessel disease.

Supplemental Table 2. The Distribution of Serum Irisin Levels (n=720): SESSA, Men Aged 40–79 Years (2006–2008)

		Quartiles of serum irisin levels in μg/mL (n=180 in each quartile)				
Parameter	Overall (n=720)	Q1 (2.67–5.75)	Q2 (5.76–7.01)	Q3 (7.02–8.33)	Q4 (8.34–24.52)	p-value
Serum irisin levels, μg/mL	7.0 (5.8–8.3)	5.0 (4.5 –5.4)	6.4 (6.1–6.7)	7.6 (7.3–7.9)	9.7 (8.9–10.9)	<0.001

Results are presented as median (interquartile range), and the p-value is calculated by the Kruskal-Wallis test.

In this study participants, the serum irisin levels are skewed to the right. The distribution of serum irisin levels is given shown below;



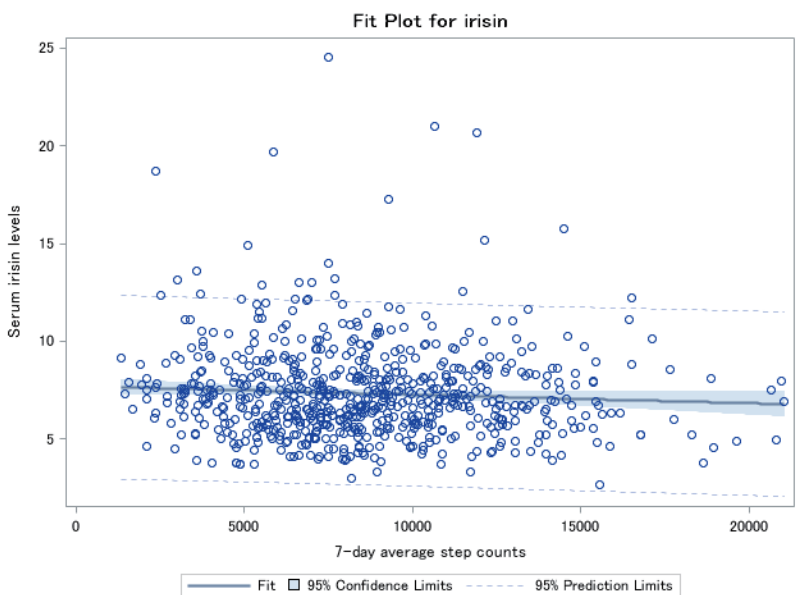
Supplemental Fig. 1. The Distribution of Serum Irisin Levels in All Study Participants (n=720)
The above Supplemental Figure 1 (histogram) shows the distribution of serum irisin levels in all study participants (n=720).

Supplemental Table 3. Multivariable Logistic Regression Models for Relationships (Odds Ratio with 95% CI) between Serum Irisin Levels at Baseline and Cerebral Small Vessel Disease in the 5-year Follow-up (n=720)

	Quartiles of serum irisin levels in μg/mL							
	<65 years (n=386)				≥ 65 years (n=334)			
	Q1 (2.67–5.74) n=119	Q2 (5.75–7.01) n=97	Q3 (7.02–8.33) n=88	Q4 (8.34–19.67) n=82	Q1 (3.69–5.75) n=61	Q2 (5.76–7.01) n=83	Q3 (7.02–8.30) n=92	Q4 (8.31–24.52) n=98
DSWMHs, n (%)	24 (20.2)	14 (14.4)	14 (15.9)	19 (23.2)	16 (26.2)	23 (27.7)	28 (30.6)	18 (18.4)
Model 1	1.00	0.59 (0.29–1.24)	0.66 (0.31–1.38)	0.99 (0.49–1.98)	1.00	1.09 (0.52–2.31)	1.26 (0.61–2.62)	0.65 (0.30–1.40)
Model 2	1.00	0.58 (0.27–1.24)	0.66 (0.31–1.41)	0.94 (0.46–1.95)	1.00	1.08 (0.51–2.27)	1.24 (0.59–2.58)	0.56 (0.25–1.25)
Model 3	1.00	0.50 (0.23–1.10)	0.61 (0.28–1.33)	0.97 (0.46–2.04)	1.00	1.08 (0.50–2.30)	1.24 (0.59–2.62)	0.55 (0.24–1.24)
PVHs, n (%)	21 (17.7)	15 (15.5)	7 (8.0)	14 (17.1)	24 (39.3)	32 (38.6)	36 (39.1)	34 (34.7)
Model 1	1.00	0.70 (0.33–1.49)	0.31 (0.12–0.78)	0.68 (0.31–1.47)	1.00	0.99 (0.50–1.96)	1.04 (0.53–2.03)	0.85 (0.44–1.66)
Model 2	1.00	0.75 (0.35–1.64)	0.29 (0.11–0.78)	0.71 (0.32–1.58)	1.00	0.98 (0.50–1.94)	1.04 (0.53–2.03)	0.84 (0.43–1.65)
Model 3	1.00	0.69 (0.31–1.54)	0.27 (0.10–0.74)	0.69 (0.31–1.56)	1.00	0.98 (0.49–1.95)	1.05 (0.53–2.08)	0.81 (0.41–1.64)
LIs, n (%)	18 (15.1)	10 (10.3)	9 (10.2)	11 (13.4)	20 (32.8)	21 (25.3)	23 (25.0)	31 (31.6)
Model 1	1.00	0.56 (0.24–1.29)	0.54 (0.23–1.29)	0.68 (0.30–1.56)	1.00	0.66 (0.32–1.38)	0.62 (0.30–1.28)	0.88 (0.44–1.75)
Model 2	1.00	0.42 (0.17–1.03)	0.46 (0.19–1.12)	0.60 (0.26–1.41)	1.00	0.66 (0.32–1.38)	0.58 (0.28–1.22)	0.79 (0.39–1.61)
Model 3	1.00	0.37 (0.15–0.92)	0.48 (0.19–1.18)	0.55 (0.23–1.32)	1.00	0.67 (0.31–1.42)	0.61 (0.29–1.31)	0.84 (0.40–1.76)
CMBs, n (%)	15 (12.6)	4 (4.1)	9 (10.2)	12 (14.6)	12 (19.7)	14 (16.9)	17 (18.5)	15 (15.3)
Model 1	1.00	0.25 (0.08–0.80)	0.67 (0.27–1.63)	0.93 (0.40–2.15)	1.00	0.83 (0.35–1.95)	0.98 (0.41–2.13)	0.74 (0.32–1.72)
Model 2	1.00	0.25 (0.08–0.79)	0.59 (0.23–1.52)	0.87 (0.37–2.06)	1.00	0.81 (0.34–1.91)	0.95 (0.41–2.19)	0.73 (0.31–1.70)
Model 3	1.00	0.25 (0.08–0.83)	0.60 (0.23–1.55)	0.89 (0.37–2.13)	1.00	0.81 (0.34–1.93)	0.97 (0.42–2.27)	0.70 (0.29–1.68)

Model 1 was adjusted for age.
Model 2 was adjusted for variables in model 1 plus alcohol intake (ethanol) and smoking (pack-years).
Model 3 was adjusted for variables in model 2 plus body mass index, diabetes, dyslipidemia, and hypertension.
CI indicates confidence interval; CMBs, cerebral microbleeds; DSWMHs, deep and subcortical white matter hyperintensities; LIs, lacunar infarcts; and PVHs, periventricular hyperintensities.

In this study participants, we found no association between serum irisin levels and step counts in the zero-order relationship (p=0.09) and also the age-adjusted model (p=0.360).



Supplemental Fig. 2. The Relationship between Serum Irisin Levels and Step Counts (n=720)

The above Supplemental Figure 2 shows the unadjusted relationship between serum irisin levels and 7-day average step counts in overall study participants (n=720).