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Relationship of serum irisin levels to prevalence and progression of coronary artery calcification: A prospective, population-based study☆

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ABSTRACT

Background: The mechanisms by which exercise reduces the risk of coronary heart disease remain poorly understood. Irisin, an exercise-induced polypeptide secreted from skeletal muscles, is proposed to potentially mediate beneficial effects of exercise, especially in metabolic regulation and development of atherosclerosis. We examined whether higher serum irisin levels are associated with lower prevalence and progression of coronary atherosclerosis.

Methods and results: We performed a prospective, population-based study of Japanese men aged 40–79 years without known coronary heart disease. We measured baseline serum irisin levels using an enzyme-linked immunosorbent assay and quantified coronary artery calcification (CAC) from serial computed tomography scans. Of 1038 participants (mean age, 63.9 years) at baseline, 670 (64.6%) had prevalent CAC. Of 810 participants at follow-up (median, 5.1 years), 407 (50.3%) experienced CAC progression. In Poisson regression with robust error variance adjusted for age and behavioral factors, serum irisin levels were inversely associated with CAC prevalence (relative risk [RR] of 4th versus 1st quartiles [95% confidence interval], 0.88 [0.78–0.99]; trend $P = 0.016$) and CAC progression (RR, 0.76 [0.63–0.91]; trend $P = 0.002$). After further adjustment for cardiometabolic risk factors, the inverse association with CAC prevalence disappeared (RR, 0.95 [0.84–1.08]; trend $P = 0.319$), but that with CAC progression persisted (RR, 0.77 [0.64–0.93]; trend $P = 0.003$). These associations were consistent when we applied ordinal logistic regression and across subgroups by cardiometabolic risk factor status.

Conclusions: Higher serum irisin levels were associated with less burden of coronary atherosclerosis. This association would be mediated through and beyond traditional cardiometabolic pathways.

1. Introduction

Cardiovascular disease, particularly coronary heart disease (CHD), continues to be the leading cause of death worldwide. Exercise is one of the mainstay interventions for preventing and treating CHD [1], and regular physical exercise is highly recommended by the current guidelines [2,3]. However, because no >40% of the risk reduction related to exercise can be explained by favorable changes in cardiovascular risk factors, the underlying mechanisms by which exercise reduces the risk of CHD have not been fully determined [1].

Contractile activity of skeletal muscle affects its secretory function of a variety of factors, which may be associated with the health-promoting effects of exercise [4]. Irisin is a novel exercise-induced polypeptide that is mainly secreted by skeletal muscle [5]. Irisin is proposed to promote browning of beige fat cells in white adipose tissue and this results in facilitating energy expenditure [5]. This may potentially have profound benefits for obesity and metabolic disorders [5]. Notably, several epidemiological studies have shown inverse relations between circulating levels of irisin and distributions of cardiometabolic risk factors [6–8]. Additionally, recent animal and human experimental studies have indicated that irisin can directly contribute to structural stabilization and functional improvement in vascular endothelium [9–11], which would

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be critical for preventing development of atherosclerosis. However, few studies have examined the possible association between circulating irisin levels and development of atherosclerosis and the possible roles of cardiometabolic risk factors as mediators in such an association.

We hypothesized that higher irisin levels are associated with a lower atherosclerotic burden and that this association is attenuated, but still present, after accounting for cardiometabolic risk factor profiles. To test these hypotheses, we investigated the association of serum irisin levels with the prevalence and progression of coronary artery calcification (CAC) which is a surrogate marker of subclinical atherosclerosis in a community-based population.

2. Methods

2.1. Study participants

Between all recruitments and baseline examinations from 2006 to 2008, 2379 eligible male candidates aged 40–79 years were identified on the basis of a random sample from Kusatsu City (Shiga, Japan) Basic Residents' Register [12]. This register includes the name, age, and sex of all city residents. Among them, 1094 men (participation rate, 46.0%) agreed to participate in the examinations with written informed consent. After excluding those with a history of myocardial infarction ($n = 29$), triglycerides levels ≥400 mg/dL ($n = 16$; we used Friedewald's formula to estimate low-density lipoprotein cholesterol levels), and missing information on computed tomography (CT) data, serum irisin, and other covariates ($n = 11$), a total of 1038 men were analyzed for the prevalence of CAC. Participants of the baseline survey were invited for a follow-up examination between 2010 and 2014. After excluding those who died or were lost to follow-up ($n = 228$), a total of 810 men were analyzed for the progression of CAC. The study complies with the Declaration of Helsinki and was approved by the Institutional Review Board of Shiga University of Medical Science, Otsu, Japan.

2.2. Exposure and covariate measurement

Venipuncture was performed early in the clinic visit after an overnight fast of at least 12 h. We separated serum by centrifugation (3000 rpm, for 15 min) at 4 °C within 90 min. Samples for routine tests were sent to the laboratory, and other samples were immediately frozen at −70 °C until analysis. In 2016, specimens were thawed and serum irisin levels were measured 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). Specimens were run in random order without reference to the prevalence of CAC. The lower limit of detection of the assay is 0.001 μg/ml, and the intra-assay and inter-assay coefficients of variation were 6.9 ± 1.3% and 9.0 ± 0.8%, respectively. Plasma glucose levels were determined from NaF-treated plasma using a hexokinase glucose-6 phosphate-dehydrogenase enzymatic assay. Lipid measurements were standardized according to the protocol of the Centers for Disease Control and Prevention/Cholesterol Reference Method Laboratory Network. Total cholesterol and triglycerides levels were measured using enzymatic assays, and high-density lipoprotein cholesterol levels were determined using a direct method. Low-density lipoprotein cholesterol levels were estimated using Friedewald's formula.

A self-administered questionnaire was used to obtain information on demography, smoking habits, alcohol drinking, and medication use and history. After the participants completed the questionnaires, trained nurses confirmed them with the participants. The body mass index was calculated as weight (kg) divided by height squared (m^2). Cumulative pack-year smoking was estimated by multiplying the average number of packs smoked daily by the number of smoking years. Using an automated sphygmomanometer (BP-8800; Omron Health Care, Kyoto, Japan), the mean of 2 consecutive measurements on the right arm with participants in a seated position after a strict 5-min rest period was used to determine blood pressure. Step counts were recorded over 7 consecutive days by a pedometer (DIGI-WALKER DW-200; Yamasa Tokei Keiki, Tokyo, Japan), and then the daily average steps were calculated. Obesity was defined as body mass index ≥25 kg/m^2. Hypertension was defined as systolic blood pressure ≥ 140 mm Hg, diastolic blood pressure ≥ 90 mm Hg, or anti-hypertensive medication use. Dyslipidemia was defined as low-density lipoprotein cholesterol levels ≥160 mg/dl, high-density lipoprotein cholesterol levels <40 mg/dl, triglycerides levels ≥150 mg/dl, or lipid-lowering medication use. Diabetes mellitus was defined as fasting blood glucose levels ≥126 mg/dl or antidiabetic medication use.

2.3. Assessment of CAC

The details for assessment of CAC have been published elsewhere [12]. Briefly, CAC at baseline was measured by either electron-beam computed tomography (EBCT) using a C-150 scanner (Imatron, South San Francisco, CA, USA) or by 16-channel multidetector row computed tomography (MDCT) using an Aquilion scanner (Toshiba Medical Systems, Tokyo, Japan), and CAC at follow-up was measured by 16-channel MDCT. Images were obtained from the level of the aortic root through the heart at every 3-mm slice, with a scan time of 100 ms (EBCT) or 320 ms (MDCT). The presence of CAC was defined as a minimum of 3 contiguous pixels (area = 1 mm^2) with a density of ≥130 Hounsfield units (HU) using AccuImage software (AccuImage Diagnostics, South San Francisco, CA, USA). The software program implements the widely-accepted Agatston method [13]. The total CAC score was obtained by multiplying the pixel area (mm^2) by a density score (1, 130–199 HU; 2, 200–299 HU; 3, 300–399 HU; and 4, ≥400 HU) depending on the highest density measurement (HU) anywhere in the plaque and summing all lesion scores. All images were evaluated by 1 trained physician who was blinded to the participants' characteristics. The standardized protocol for CAC assessment was established in a separate cohort study by our research group in which the intra-reader reproducibility of a non-zero CAC score had an intra-class correlation of 0.98 [14]. Because a stratified analysis by CT type showed similar results, the definitions of CAC by EBCT and MDCT were considered equivalent.

2.4. Statistical analysis

Participants' characteristics were compared after adjustment for age across quartiles of serum irisin levels using linear regression for continuous variables with an approximately normal distribution, median regression for those with a skewed distribution, and logistic regression for categorical variables. Analyses were performed using STATA 14.0 (StataCorp LP, College Station, TX, USA). Two-tailed P values <0.05 were considered statistically significant.

We confirmed a skewed distribution and a large number of zeros for baseline CAC score and changes in CAC score which was defined as the numerical difference in CAC scores between CT scans. The prevalence of CAC at baseline was defined as CAC score > 0. There are no standard guidelines for assessing the progression of CAC. Therefore, we used the square root-transformed difference method in which CAC progression was measured as the difference in the square root-transformed CAC scores between CT scans [15,16]. The cutoff point for the presence of CAC progression was defined as the difference > 2.5 according to the clinical significance and consistency with previous studies [15,17,18]. For these dichotomous outcomes, we used Poisson regression with robust error variance [19] to estimate relative risks (RRs) and 95% confidence intervals (CIs) according to quartiles of serum irisin levels. Because the prevalence or progression of CAC was >10% in the cohort, odds ratios could not be interpreted as RRs. We tested for trends across quartiles based on assigning a median value for each category and modeling this variable as a continuous variable. In Model 1, we adjusted for age. In Model 2, we adjusted for age and behavioral characteristics (smoking status [former, current], pack-year smoking, alcohol intake [g/week]). In Model 3, we adjusted for Model 2 plus cardiometabolic risk factors, such as body mass index, systolic blood pressure, anti-hypertensive medication use (yes/no), low- and high-density lipoprotein cholesterol, triglycerides, lipid-lowering medication use (yes/no), fasting glucose, and antidiabetic medication use (yes/no). We also included a term for scanner pairs in all models to consider CT scanner changes [12]. For analyzing CAC progression, we further adjusted for duration between CT scans and baseline CAC score. Incorporating daily step counts as an index of exercise into the model may introduce over-adjustment because this variable could be an upstream factor in the causal pathway between serum irisin levels and atherosclerotic burden [4]. Therefore, our primary analyses did not control for daily step counts. As an exploratory model, we took this variable into consideration. We repeated analysis for the presence of CAC progression using another definition as follows: CAC score > 0 at follow-up if baseline CAC score = 0; annualized change in CAC score ≥ 10 at follow-up if baseline CAC score > 0 to <100; and annualized percentage change in CAC score ≥ 10% at follow-up if baseline CAC score ≥ 100 [20]. For sensitivity analysis, we examined the categories of baseline CAC scores (0, >0 to <100, ≥100 to <300, and ≥ 300) [21] and those of changes in CAC score (≤0, >0 to <100, ≥100 to <300, and ≥300) as dependent variables in ordinal logistic regression. Finally, we assessed the associations of serum irisin levels with the prevalence and progression of CAC across subgroups based on age, behavioral and cardiometabolic risk factors, and the presence of CAC (only for CAC progression) at baseline using a fully adjusted model; we further tested for multiplicative interactions between these groups.

3. Results

3.1. Cross-sectional association of serum irisin levels with CAC at baseline

The age-adjusted baseline characteristics according to quartiles of serum irisin levels are shown in Table 1. Participants with higher serum irisin levels were older, consumed more alcohol, and had a lower body mass index, lower low-density lipoprotein cholesterol levels, lower triglycerides levels, higher high-density lipoprotein cholesterol levels, and lower prevalence of obesity, dyslipidemia, and antidiabetic medication use. There was no significant association between serum irisin levels and daily step counts. Similar distributions of cardiometabolic risk factors across serum irisin levels were observed after further adjustment for behavioral factors, including smoking and alcohol drinking (data not shown).

The association of serum irisin levels with the prevalence of CAC at baseline is shown in Table 2. CAC was prevalent in 670 (64.6%) participants, and CAC scores of >0 to <100, ≥100 to <300, and ≥300 were observed in 418 (40.3%), 132 (12.7%), and 120 (11.6%) participants,

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