Lower Serum Irisin Levels Are Associated with Increased Abdominal Aortic Calcification in Peritoneal Dialysis Patients

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Keywords

Irisin · Vascular calcification · Cardiovascular disease · Peritoneal dialysis

Abstract

Introduction: Vascular calcification (VC), with the mechanisms remaining unclear, is closely related with dialysis patients' cardiovascular mortality and all-cause mortality. Irisin is a newly identified myokine. This study aims to evaluate the serum irisin levels of peritoneal dialysis (PD) patients and their relationship with VC. **Methods:** This cross-sectional study enrolled stable PD patients in Peking University Third Hospital who were followed for >6 months. We used plain X-ray films of abdomen to quantitatively evaluate VC of abdominal aorta. VC was evaluated by abdominal aortic calcification (AAC) scores, and PD patients were divided into the high AAC score group (AAC score ≥ 4) and the low AAC score group (AAC score <4). Demographic data and laboratory indexes were collected. Serum irisin concentrations were measured by enzyme-linked immunosorbent assay. **Results:** A total of 102 PD patients were enrolled in this study, and 52 patients (51.0%) were found to have a high AAC score of ≥ 4 . Age, diabetic mellitus proportion, pulse pressure, hypercal-

cemia (corrected calcium >2.54 mmol/L) rate, serum ultrasensitive C reactive protein, and AAC scores were significantly higher in the high AAC score group than those of the low AAC score group ($p < 0.05$). The high AAC score group had lower diastolic blood pressure, serum albumin, and serum carbon dioxide combining power compared with the low AAC score group ($p < 0.05$). Serum irisin levels of PD patients with a high AAC score were significantly lower than those of PD patients with a low AAC score (109.7 ± 13.1 ng/mL vs. 115.9 ± 10.1 ng/mL, $p = 0.010$). The multivariate logistic regression analyses showed that serum irisin, diabetic mellitus, serum ultrasensitive C reactive protein, and age were independent factors influencing the occurrence of VC in PD patients. **Conclusion:** Our results are the first to provide a clinical evidence of the association between serum irisin and abdominal aortic calcification in PD patients. Lower irisin levels, diabetic mellitus, higher serum ultrasensitive C reactive protein, and older age could be potential predictive factors for VC in PD patients.

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Introduction

Vascular calcification (VC) is a common complication of patients with end-stage renal disease (ESRD), a predominant cause of cardiovascular disease, and an independent predictor of all-cause death in dialysis patients [1]. The pathogenesis of VC is complex and unclear. Abnormal mineral metabolism of calcium and phosphate, which plays key roles in the progression of VC, is regulated by multiple hormones. It is important to estimate the occurrence of VC in peritoneal dialysis (PD) patients and find out its influencing factors.

Irisin is a novel exercise-induced myokine that is mainly secreted by skeletal muscle cells. Irisin induces browning of beige fat cells and facilitates energy expenditure [2]. Our previous study showed that PD patients, especially patients with protein-energy wasting, had lower irisin levels than normal controls [3]. Several studies have shown inverse correlations between circulating irisin and cardiovascular risk factors. In addition, recent studies have indicated that irisin may play roles in calcium and phosphate homeostasis and promote bone formation [4]. He et al. [5] found that lower irisin concentrations were associated with VC in hemodialysis patients. However, the effect of irisin on VC in PD patients remains unclear and no relative study was reported.

We hypothesize that irisin may play a role in the pathogenesis of VC in PD patients. To test the hypothesis, we investigated the association of serum irisin levels with the presence of abdominal aortic calcification (AAC) in PD patients and then examined the principal influencing factors of VC. Our study would provide more evidence of the interactions between muscles, bones, and vessels in ESRD patients.

Materials and Methods

Subjects

This cross-sectional study screened 160 patients and enrolled 102 maintenance PD patients in Peking University Third Hospital between July and September 2016. Patients were eligible for inclusion if they (1) aged >18 years old and (2) had been on PD for at least 6 months. The exclusion criteria include (1) in the acute phase of infections or some other acute complications and (2) refused to participate in the study. A flowchart of patient recruitment for the study is shown in Figure 1.

The study complied with the principles laid down by Declaration of Helsinki and was approved by the ethics committee of Peking University Third Hospital. Written informed consent was provided by all participants. Authors did not have access to the information that could identify individual participants during or after data collection.

Fig. 1. Flowchart of the study. PD, peritoneal dialysis; AAC, abdominal aortic calcification.

Fig. 2. Abdominal aortic calcification by lateral lumbar radiography in peritoneal dialysis patients.

Biochemical Data Collection and Assessment of Irisin

Data collected included patients' demographics such as age, sex, underlying causes of ESRD, PD vintage, and the comorbidities such as diabetic mellitus. Laboratory data collected at baseline included hemoglobin, serum albumin, serum creatinine, serum corrected calcium, serum phosphorus, serum intact parathyroid hormone (iPTH), serum ultrasensitive C reactive protein (us-CRP), serum carbon dioxide combining power (CO₂CP), and lipid profile. Fractional urea clearance (Kt/V urea) was calculated by the Daugirdas formula.

Table 1. Comparison of irisin levels and other parameters between PD patients with a high AAC score and a low AAC score

Characteristics	AAC score		<i>p</i> value
	≥4 (<i>n</i> = 52)	<4 (<i>n</i> = 50)	
Age, years	61.2±12.8	51.2±12.6	<0.001
Sex, male, <i>n</i> (%)	24 (46)	24 (48)	0.852
Diabetic mellitus, <i>n</i> (%)	21 (40)	11 (22)	0.045
Dialysis vintage, months	50.5 (30.0, 79.8)	43.5 (11.3, 91.0)	0.625
SBP, mm Hg	131.8±16.6	136.1±15.7	0.182
DBP, mm Hg	75.0±12.6	84.5±10.5	<0.001
Pulse pressure, mm Hg	56.9±14.2	51.6±12.1	0.049
BMI, kg/m ²	24.0±3.7	23.1±3.8	0.231
Hemoglobin, g/L	115.7±17.1	117.0±16.5	0.678
Serum creatinine, μmol/L	920.4±212.7	974.6±295.2	0.289
Serum glucose, mmol/L	5.8 (5.1, 7.3)	5.5 (4.7, 5.9)	0.055
Serum albumin, g/L	36.5±4.2	38.7±3.2	0.004
Serum corrected calcium, mmol/L	2.56±0.24	2.49±0.23	0.102
Hypercalcemia, <i>n</i> (%)	31 (59.6)	17 (34.0)	0.010
Serum phosphorous, mmol/L	1.65±0.37	1.66±0.47	0.909
Hyperphosphatemia, <i>n</i> (%)	16 (30.8)	14 (28.0)	0.759
Serum iPTH, pg/mL	152.4 (92.8, 300.5)	157.4 (73.9, 316.6)	0.820
Serum ALP, U/L	71.5 (57.5, 108.0)	69.5 (56.0, 81.3)	0.492
TCHO, mmol/L	4.82±1.28	4.68±0.92	0.533
TG, mmol/L	2.17 (1.35, 3.68)	1.62 (1.33, 2.62)	0.151
HDL-C, mmol/L	0.93 (0.80, 1.11)	0.98 (0.86, 1.16)	0.187
LDL-C, mmol/L	2.71±0.78	2.77±0.77	0.703
Serum us-CRP, mg/L	6.41 (2.10, 13.91)	1.71 (0.59, 4.67)	<0.001
Serum CO ₂ CP, mmol/L	24.9±2.1	26.0±2.8	0.046
Kt/V urea, per week	1.9 (1.7, 2.1)	1.9 (1.7, 2.1)	0.954
Serum irisin, ng/mL	109.7±13.1	115.9±10.1	0.010
AAC score	9.0 (5.5, 14.5)	0.0 (0.0, 2.0)	<0.001

PD, peritoneal dialysis; AAC, abdominal aortic calcification; VC, vascular calcification; SBP, systolic blood pressure; DBP, diastolic blood pressure; BMI, body mass index; iPTH, intact parathyroid hormone; ALP, alkaline phosphatase; TCHO, total cholesterol; TG, triglyceride; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; us-CRP, ultrasensitive C reactive protein; CO₂CP, carbon dioxide combining power; Kt/V urea, fractional urea clearance.

According to 2017 KDIGO guidelines, the target range of serum calcium and phosphorus for CKD stage 5D patients is 2.10–2.54 mmol/L and 1.13–1.78 mmol/L, respectively. Thus, we defined hypercalcemia as serum corrected calcium >2.54 mmol/L and hyperphosphatemia as serum phosphate >1.78 mmol/L.

Participants' blood samples were collected after fasting for 12 h. For patients with dialysate dwelled overnight, the dialysis was not disturbed. We collected the biochemical data in the subsequent week. Parts of the serum and plasma were stored at –80°C and were not thawed until analysis. Serum irisin concentrations were measured using enzyme-linked immunosorbent assay kits (Phoenix Pharmaceuticals, Burlingame, CA, USA) in accordance with the manufacturer's instructions. The sensitivity of the assay was 0.1 ng/mL and the linear range of the standard was 0.1–1,000 ng/mL. The intra- and interassay coefficients of variation were 4.5 and 8%, respectively.

Assessment of Vascular Calcification

VC was imaged within 2 weeks of enrollment: AAC by lateral lumbar radiography (shown in Fig. 2) with Kauppila scoring [6]. Patients were allocated to the high AAC score group if they had an AAC score of ≥4 (moderate or heavy calcification) and the low AAC score group if they had an AAC score of <4 (no or minor calcification) [7].

Statistical Analysis

Data were expressed as mean ± SD for normal distribution data, and independent Student's *t* test was used to compare the differences of these variables between 2 groups. Median and percentile (p₂₅, p₇₅) were used to describe continuous variables that did not follow normal distribution, and Mann-Whitney U tests were applied to examine the differences in these variables between 2 groups. The frequency and percentage were used to de-

scribe categorical variables. The χ^2 test was used to compare categorical variables. Correlations were expressed as Pearson's correlation coefficients for 2 normally distributed variables, and Spearman rank correlations were used for nonnormally distributed variables. Univariate linear regression analyses were performed to evaluate the determinants of serum irisin levels. The multivariate stepwise linear regression model was employed to select variables independently related to serum irisin. Binary logistic analysis was performed to determine the independent influencing factors of VC (using back LR). All statistical analyses were performed using the statistical package 22.0 (IBM, Armonk, NY, USA).

Results

Comparison of Serum Irisin Levels and Other Established Parameters between the High AAC Score Group and the Low AAC Score Group

102 PD patients (48 male patients, mean age 58.3 ± 13.4 years, average PD duration 54.6 ± 40.7 months) were enrolled in the study. In 52 patients (51.0%), the X-ray showed a high AAC score of ≥ 4 (shown in Fig. 2). The median calcification score was 4.0 (interquartile range 0.0–10.5).

Compared with the low AAC score group, the high AAC score group had significantly lower serum irisin concentrations (109.7 ± 13.1 vs. 115.9 ± 10.1 ng/mL, $p = 0.010$). PD patients with a high AAC score had lower diastolic blood pressure, serum albumin, and serum CO₂CP levels compared with those of patients with a low AAC score ($p < 0.05$). However, age, diabetic mellitus proportion, pulse pressure, hypercalcemia proportion, serum us-CRP, and AAC scores were significantly higher in PD patients with a high AAC score than those of patients with a low AAC score ($p < 0.05$). In addition, there was no difference in systolic blood pressure, BMI, hemoglobin, serum creatinine, serum glucose, serum phosphorus, lipid levels, serum iPTH, serum alkaline phosphatase, and Kt/V urea between the high AAC score group and the low AAC score group; see Table 1.

In addition, serum irisin levels were lower in PD patients with hypercalcemia, which was defined as serum corrected calcium > 2.54 mmol/L, than those of patients without hypercalcemia (111.1 ± 10.1 ng/mL vs. 115.1 ± 13.0 ng/mL, $p = 0.033$). However, there was no significant difference in serum irisin levels between PD patients with hyperphosphatemia (serum phosphorus > 1.78 mmol/L) and patients without hyperphosphatemia.

Table 2. Correlation coefficients of serum irisin and other clinical parameters in PD patients

Variables	<i>r</i>	<i>p</i> value
Age (years)	0.073	0.367
Sex (male = 1, female = 2)	0.037 [#]	0.644
Diabetic mellitus (no = 0, yes = 1)	−0.026 [#]	0.746
Dialysis vintage (months)	0.072	0.372
SBP (mm Hg)	0.089	0.270
DBP (mm Hg)	−0.057	0.478
Pulse pressure (mm Hg)	0.192	0.017
Hemoglobin (g/L)	−0.065	0.418
Kt/V urea (per week)	−0.113 [#]	0.160
BMI (kg/m ²)	0.090	0.266
Serum albumin (g/L)	−0.083	0.303
Serum creatinine (μmol/L)	0.010	0.896
Serum corrected calcium (mmol/L)	−0.038	0.636
Hypercalcemia (no = 0, yes = 1)	−0.147 [#]	0.067
Serum phosphorus (mmol/L)	−0.023	0.774
Hyperphosphatemia (no = 0, yes = 1)	−0.021 [#]	0.793
Serum glucose (mmol/L)	0.005 [#]	0.952
Serum us-CRP (mg/L)	0.031 [#]	0.762
TG (mmol/L)	0.026 [#]	0.752
HDL-C (mmol/L)	−0.045 [#]	0.579
LDL-C (mmol/L)	−0.008	0.923
Serum CO ₂ CP (mmol/L)	0.169	0.039
Serum ALP (U/L)	0.139 [#]	0.126
Serum iPTH (pg/mL)	0.018 [#]	0.827
AAC score	−0.202 [#]	0.046
AAC score ≥ 4 (no = 0, yes = 1)	−0.383 [#]	<0.001

PD, peritoneal dialysis; SBP, systolic blood pressure; DBP, diastolic blood pressure; Kt/V urea, fractional urea clearance; BMI, body mass index; us-CRP, ultrasensitive C reactive protein; TG, triglyceride; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; CO₂CP, carbon dioxide combining power; ALP, alkaline phosphatase; iPTH, intact parathyroid hormone; AAC, abdominal aortic calcification. [#] Spearman rank correlations were performed; others were expressed as Pearson's correlation coefficients.

Correlation Analysis of Irisin with Other Parameters in PD Patients

Bivariate correlation analysis revealed that in PD patients, serum irisin was positively correlated with pulse pressure (Pearson $r = 0.192$, $p = 0.017$) and serum CO₂CP (Pearson $r = 0.169$, $p = 0.039$), while tended to be negatively correlated with hypercalcemia (Spearman $r = -0.147$, $p = 0.067$). Furthermore, circulating irisin was also negatively correlated with a high AAC score (Spearman $r = -0.383$, $p < 0.001$) and AAC scores (Spearman $r = -0.202$, $p = 0.046$); see Table 2.

Univariate linear regression analyses revealed that pulse pressure (β : 0.173; 95% CI: 0.031 to 0.315; $p = 0.017$),

Table 3. Univariate and multivariate linear regression analysis* (outcome: serum irisin levels)

Variables	Univariate linear regression		Multivariate linear regression	
	B (95% CI)	p value	B (95% CI)*	p value
Age (years)	0.064 (−0.076 to 0.205)	0.367	Not selected	
Sex (male = 1, female = 2)	−0.045 (−3.780 to 3.689)	0.981	Not selected	
Diabetic mellitus (no = 0, yes = 1)	0.205 (−3.722 to 4.132)	0.918		
Dialysis vintage (months)	0.021 (−0.025 to 0.067)	0.372		
SBP (mm Hg)	0.077 (−0.032 to 0.186)	0.166		
DBP (mm Hg)	−0.043 (−0.193 to 0.107)	0.571		
Pulse pressure (mm Hg)	0.173 (0.031 to 0.315)	0.017	0.269 (0.097 to 0.440)	0.002
Hemoglobin (g/L)	−0.046 (−0.158 to 0.066)	0.418		
Kt/V urea (per week)	−3.369 (−7.776 to 1.039)	0.133		
BMI (kg/m ²)	0.297 (−0.228 to 0.822)	0.266		
Serum albumin (g/L)	−0.256 (−0.745 to 0.233)	0.303		
Serum creatinine (μmol/L)	0.001 (−0.007 to 0.008)	0.896		
Serum corrected calcium (mmol/L)	−1.918 (−9.916 to 6.080)	0.636		
Hypercalcemia (no = 0, yes = 1)	−4.012 (−7.700 to −0.324)	0.033	Not selected	
Serum phosphorus (mmol/L)	−0.653 (−5.149 to 3.843)	0.774		
Hyperphosphatemia (no = 0, yes = 1)	−1.313 (−5.433 to 2.807)	0.530	Not selected	
Serum glucose (mmol/L)	0.029 (−0.565 to 0.623)	0.923		
Serum us-CRP (mg/L)	−0.014 (−0.241 to 0.213)	0.903		
TG (mmol/L)	0.182 (−0.777 to 1.141)	0.708		
HDL-C (mmol/L)	−3.290 (−10.264 to 3.684)	0.353		
LDL-C (mmol/L)	−0.122 (−2.601 to 2.357)	0.923		
Serum CO ₂ CP (mmol/L)	0.743 (0.038 to 1.448)	0.039	Not selected	
Serum ALP (U/L)	−0.003 (−0.042 to 0.035)	0.859		
Serum iPTH (pg/mL)	0.004 (−0.005 to 0.013)	0.397		
AAC score ≥4 (no = 0, yes = 1)	−6.153 (−10.823 to −1.482)	0.010	−7.394 (−11.879 to −2.908)	0.002

PD, peritoneal dialysis; SBP, systolic blood pressure; DBP, diastolic blood pressure; Kt/V urea, fractional urea clearance; BMI, body mass index; us-CRP, ultrasensitive C reactive protein; TG, triglyceride; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; CO₂CP, carbon dioxide combining power; ALP, alkaline phosphatase; iPTH, intact parathyroid hormone.

* Variables were tested by the multiple stepwise linear regression analysis to determine predictors of serum irisin levels of PD patients. Age, sex, hypercalcemia, hyperphosphatemia, serum CO₂CP, serum iPTH, and a high AAC score of ≥4 were tested.

hypercalcemia (β : −4.012; 95% CI: −7.700 to −0.324; p = 0.033), serum CO₂CP (β : 0.743; 95% CI: 0.038 to 1.448; p = 0.039), and a high AAC score (β : −6.153; 95% CI: −10.823 to −1.482; p = 0.010) were associated with serum irisin levels. When age, sex, hypercalcemia, hyperphosphatemia, serum CO₂CP, serum iPTH, and a high AAC score (≥4) were included as candidate variables, the multivariate stepwise linear regression analysis showed that pulse pressure (β : 0.269; 95% CI: 0.097–0.440; p = 0.002) and a high AAC score (β : −7.394; 95% CI: −11.879 to −2.908; p = 0.002) were independent variables related to serum irisin levels in PD patients; see Table 3.

Independent Influencing Factors of VC by Binary Logistic Analysis in PD Patients

A logistic regression model was performed to estimate the independent influencing factors of VC in PD patients.

The variables that significantly related to a high AAC score of ≥4 in Table 1 (age, diabetic mellitus, pulse pressure, serum albumin, serum us-CRP, hypercalcemia, serum CO₂CP, and serum irisin) and some previously reported variables such as serum phosphorus, iPTH, and dialysis vintage entered the analysis as candidate variables. Finally, age, serum irisin, serum us-CRP, and diabetic mellitus were independently associated with a high AAC score in PD patients (p < 0.05); see Table 4.

Discussion

We found that 51.0% PD patients suffered from moderate or heavy abdominal aortic calcification using X-ray plain films. We demonstrated for the first time that serum irisin, a novel myokine, was significantly lower in

Table 4. Independent influencing factors of a high AAC score by multiple regression analysis* in PD patients

Variables	Exp(B)	p value	95% CI for Exp(B)
Age (years)	1.102	<0.001	1.046–1.160
Diabetic mellitus (no = 0, yes = 1)	4.471	0.023	1.234–16.196
Serum us-CRP (mg/L)	1.166	0.003	1.053–1.291
Serum irisin (ng/mL)	0.919	0.004	0.868–0.973

AAC, abdominal aortic calcification; PD, peritoneal dialysis; CI, confidence interval; us-CRP, ultrasensitive C reactive protein; CO₂CP, carbon dioxide combining power; iPTH, intact parathyroid hormone. * Age, dialysis vintage, diabetic mellitus, pulse pressure, serum albumin, serum us-CRP, hypercalcemia, serum CO₂CP, hyperphosphatemia, serum iPTH, and serum irisin were included in the multivariate logistic regression model.

PD patients with a high AAC score compared with PD patients with a low AAC score; lower serum irisin was an independent influencing factor of VC in PD patients.

Niu et al. [8] reported that AAC could predict all-cause mortality and cardiovascular mortality in PD patients. Plain radiography is reliable in diagnosing AAC with 100% specificity. Calcification of large arteries observed in plain films includes both intimal and medial calcification; the former is associated with atherosclerosis and the latter with aging, diabetes, and ESRD. Although patients with ESRD can develop the 2 types of VC, medial calcification is the more specific one. The reported incidence of large arterial calcification in PD patients varied from 57.3 to 79.7% [9, 10]. The incidence of AAC in our PD patients is lower than the reported data in whites, but nearing to the reported incidence in Chinese PD patients. Race, sample sizes, and the methodologies used to evaluate arterial calcification may partially explain the differences.

Irisin, a myokine secreted by skeletal muscle after exercise, improves adipose tissue browning and glucose homeostasis. Multiple studies have evaluated the effects of irisin on atherosclerotic cardiovascular disease (CVD) but yielded conflicting results [11, 12]. One possible explanation for the conflicting results is the reverse causality. Elevated irisin may compensate for irisin resistance and maximize cardiovascular protective effects among patients with high-CVD risk. In our study, we speculate that the reduced serum irisin may affect PD patients' VC progression.

The traditional risk factors of medial calcification in dialysis patients include age, hypercalcemia or increased calcium load, hyperphosphatemia, prolonged dialysis vintage, and inflammation. Consistently, our results showed that older age and higher serum us-CRP were independent risk factors of AAC in PD patients. Recent

studies have explored more mechanisms of VC in CKD patients: (1) endothelial dysfunction: in vitro experiments, uremic toxins and inorganic phosphate induced endothelial cells to express interleukin-8 and aggravated human aortic smooth muscle cell calcification [13]. Vasodilators from endothelia, such as nitric oxide, inhibit VC, whereas vasoconstrictors, such as endothelin, accelerate VC [14]. Vascular endothelial growth factors [15] may also promote VC in CKD. (2) Fibroblast growth factor 23 (FGF23) and klotho: osteocytes and osteoblasts produce FGF23, one earliest biomarker of CKD-mineral bone disorder, inducing reduced 1,25(OH)₂D and renal phosphate reabsorption. α -Klotho, an enzyme expressed mainly in kidneys, is the coreceptor of FGF23. FGF23 levels increase while α -klotho decreases in CKD patients. Most studies suggested that α -klotho is protective against VC [16]; however, whether FGF23 has a protective or harmful effect on VC is controversial now. (3) Activin receptor: Agapova et al. [17] reported that the activin receptor type IIA (ActRIIA) ligand trap increased ActRIIA signaling in the aorta of CKD mice and inhibited the osteoblastic transition of vascular smooth muscle cells.

Our study is the first to identify that lower serum irisin significantly correlated with VC in PD patients. Consistently, He et al. [5] reported that hemodialysis patients with VC had lower serum irisin levels than patients without VC. Several mechanisms could explain our findings. The lower irisin in PD patients with VC may be partially attributed to the reduction in its muscle source. Irisin is exercise-induced and mainly secreted from skeletal muscle; however, PD patients with VC generally suffer from sarcopenia and limited physical activity. In our study, the proportion of protein-energy wasting (a more comprehensive concept than malnutrition) in the high AAC score group was significantly

higher than that of the low AAC score group ($\chi^2 = 11.580$, $p = 0.001$). Serum irisin positively correlated with serum CO₂CP, and CO₂CP was lower in patients with a high AAC score compared with patients with a low AAC score in the current study. Metabolic acidosis in CKD patients has adverse effects on muscle and bone, leading to muscle protein degradation [18] and the reduction in irisin concentrations.

Besides, irisin may alleviate the risk factors of VC mentioned above in CKD patients: (1) irisin could reduce inflammatory reactions. Serum irisin inversely correlated with us-CRP and interleukin-6 in acute stroke patients [19]. Irisin ameliorated the inflammatory status of bone and promoted bone anabolism in a rat model of inflammatory bowel disease [20]. We found no significant correlation between serum irisin and us-CRP in PD patients, possibly due to the relatively small sample size. (2) Irisin could alleviate endothelial dysfunction by reducing oxidative stresses [21] and inhibiting glucose-induced apoptosis [22].

Both pulse pressure and pulse wave velocity are indicators of arterial stiffness, which is closely related with VC in CKD patients. Interestingly, our study indicated that serum irisin positively correlated with pulse pressure, which was significantly higher in the high AAC score group than the low AAC score group. In a study of obese adults, regular exercise induced increased circulating irisin and decreased pulse wave velocity [23], with the two negatively correlated with each other. The possible vasodilation effect of irisin may be through activating the AMPK-Akt-eNOS signaling pathway. We infer that increased irisin in PD patients with higher pulse pressure might be a compensatory adaption to VC. Clearly, these hypotheses need to be tested in future studies.

Increased circulating calcium load in dialysis patients is associated with increased VC and CVD risk. In our study, the incidence of hypercalcemia (serum corrected calcium >2.54 mmol/L) was significantly higher in the high AAC score group than the low AAC score group; serum irisin tended to negatively correlate with hypercalcemia. Multiple studies suggested that irisin may promote bone formation [24]. We speculate that irisin may correct the imbalance between bone formation and resorption, thus inhibiting the promoters of VC. To maintain calcium balance, restricting the dose of calcium-based phosphate binder and using the dialysate with a calcium concentration of 1.25 mmol/L [25] may be helpful, especially in the presence of persistent hypercalcemia and arterial calcification.

We found no significant correlation between VC, iPTH, and serum phosphorus, possibly due to the relatively small sample size, the generally well-controlled serum phosphorus and iPTH, and the single serum phosphorus data used in our study. Dialysis vintage did not significantly correlate with VC in our study. We are unsure about the exact reasons. The generally high Kt/V levels (the index of dialysis adequacy) in our PD patients showed a satisfactory dialysis quality, which may attenuate the impact of prolonged dialysis vintage on VC in our study.

Our study has several limitations. First, the analysis included only a limited number of serum samples. Second, because it is a cross-sectional study, the cause-effect relationship between irisin and VC in PD patients could not be determined. Third, participants that failed to finish the X-ray examination in our PD center may have difficulties in physical activity and compliance, thus are likely to have greater VC risk. Considering this, we may have underestimated the association between serum irisin levels and VC in PD patients. Fourth, residual confounding factors remain possible, thus more study is needed to explore the relationship between FGF23, α -Klotho, activin receptor, and irisin.

In conclusion, our study is the first to provide a clinical evidence of the association between serum irisin and AAC in PD patients. Lower irisin levels, higher serum us-CRP, diabetic mellitus, and older age are independent influencing factors of VC in PD patients. These findings indicate that myokines may be involved in the pathophysiology of VC for further basic and clinical investigation.

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Statement of Ethics

This research was conducted ethically in accordance with the World Medical Association Declaration of Helsinki. The subjects in the study have given their written informed consent, and the study protocol was approved by the Peking University (2015-0043) Third Hospital ethical committee on human research.

Conflict of Interest Statement

The authors have no conflicts of interest to declare.

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Author Contributions

Si-Jia Zhou collected the data, interpreted the results, and wrote the article. Wen Tang and Qing-Feng Han contributed to the data collection. Xiao-Xiao Wang contributed to the data analysis. Ai-Hua Zhang and Lian He conceptualized the idea and were involved in writing the article. All authors have read and approved the final article.

References

- Okuno S, Ishimura E, Kitatani K, Fujino Y, Kohno K, Maeno Y, et al. Presence of abdominal aortic calcification is significantly associated with all-cause and cardiovascular mortality in maintenance hemodialysis patients. *Am J Kidney Dis*. 2007 Mar;49(3):417–25.
- Bostrom P, Wu J, Jedrychowski MP, Korde A, Ye L, Lo JC, et al. A PGC1- α -dependent myokine that drives brown-fat-like development of white fat and thermogenesis. *Nature*. 2012 Jan 11;481(7382):463–8.
- Zhou SJ, Han QF, Zhang AH, Tang W, Sun LH. Irisin and volume overload are associated with protein energy wasting in peritoneal dialysis patients. *Kidney Blood Press Res*. 2017 Jan 20;42(6):1216–24.
- Colaizzi G, Mongelli T, Colucci S, Cinti S, Grano M. Crosstalk between muscle and bone via the muscle-myokine irisin. *Curr Osteoporos Rep*. 2016 Aug;14(4):132–7.
- He L, He WY, A LT, Yang WL, Zhang AH. Lower serum irisin levels are associated with increased vascular calcification in hemodialysis patients. *Kidney Blood Press Res*. 2018; 43(1):287–95.
- Wilson PWF. New indices to classify location, severity and progression of calcific lesions in the abdominal aorta: a 25-year follow-up study. *Atherosclerosis*. 1997 Jul 25;132(2):245–50.
- Peeters MJ, van den Brand JA, van Zuijlen AD, Koster Y, Bots ML, Vervloet MG, et al. Abdominal aortic calcification in patients with CKD. *J Nephrol*. 2017 Feb;30(1):109–18.
- Niu Q, Zhao H, Wu B, Tsai S, Wu J, Zhang M, et al. Abdominal aortic calcification is superior to other arteries calcification in predicting the mortality in peritoneal dialysis patients: a 8 years cohort study. *BMC Nephrol*. 2019 Dec 2;20(1):439.
- Martino F, Di Loreto P, Giacomini D, Kaushik M, Rodighiero MP, Crepaldi C, et al. Abdominal aortic calcification is an independent predictor of cardiovascular events in peritoneal dialysis patients. *Ther Apher Dial*. 2013 Aug; 17(4):448–53.
- Niu Q, Zhao H, Wu B, Tsai S, Wu J, Zhang M, et al. Study on the prevalence of vascular calcification in different types of arteries and influencing factors in maintenance peritoneal dialysis patients. *Blood Purif*. 2019;47(Suppl 1):8–16.
- Efe TH, Açar B, Ertem AG, Yayla KG, Algül E, Yayla Ç, et al. Serum irisin level can predict the severity of coronary artery disease in patients with stable angina. *Korean Circ J*. 2017 Jan;47(1):44–9.
- Mantzoros CS. Circulating irisin in relation to insulin resistance and the metabolic syndrome. *J Clin Endocrinol Metab*. 2013;98(12):4899–907.
- Bouabdallah J, Zibara K, Issa H, Lenglet G, Kchour G, Caus T, et al. Endothelial cells exposed to phosphate and indoxyl sulphate promote vascular calcification through interleukin-8 secretion. *Nephrol Dial Transplant*. 2019 Jul 1;34(7):1125–34.
- Wu SY, Zhang BH, Pan CS, Jiang HF, Pang YZ, Tang CS, et al. Endothelin-1 is a potent regulator in vivo in vascular calcification and in vitro in calcification of vascular smooth muscle cells. *Peptides*. 2003 Aug;24(8):1149–56.
- Mikhaylova L, Malmquist J, Nurminkaya M. Regulation of in vitro vascular calcification by BMP4, VEGF and Wnt3a. *Calcif Tissue Int*. 2007 Nov;81(5):372–81.
- Chang JR, Guo J, Wang Y, Hou YL, Lu WW, Zhang JS, et al. Intermedin-1-53 attenuates vascular calcification in rats with chronic kidney disease by upregulation of α -Klotho. *Kidney Int*. 2016 Mar;89(3):586–600.
- Agapova OA, Fang Y, Sugatani T, Seifert ME, Hruska KA. Ligand trap for the activin type IIA receptor protects against vascular disease and renal fibrosis in mice with chronic kidney disease. *Kidney Int*. 2016 Jun;89(6):1231–43.
- Kittiskulnam P, Srijarunruang S, Chulakadabba A, Thokanit NS, Praditpornsilpa K, Tungsanga K, et al. Impact of serum bicarbonate levels on muscle mass and kidney function in pre-dialysis chronic kidney disease patients. *Am J Nephrol*. 2020;51(1):24–34.
- Tu WJ, Qiu HC, Cao JL, Liu Q, Zeng XW, Zhao JZ. Decreased concentration of irisin is associated with poor functional outcome in ischemic stroke. *Neurotherapeutics*. 2018 Oct;15(4):1158–67.
- Metzger CE, Narayanan SA, Elizondo JP, Carter AM, Zawieja DC, Hogan HA, et al. DSS-induced colitis produces inflammation-induced bone loss while irisin treatment mitigates the inflammatory state in both gut and bone. *Sci Rep*. 2019 Oct;9(1):15144.
- Zhu D, Wang H, Zhang J, Zhang X, Xin C, Zhang F, et al. Irisin improves endothelial function in type 2 diabetes through reducing oxidative/nitritative stresses. *J Mol Cell Cardiol*. 2015 Oct;87:138–47.
- Lu J, Xiang G, Liu M, Mei W, Xiang L, Dong J. Irisin protects against endothelial injury and ameliorates atherosclerosis in apolipoprotein E-null diabetic mice. *Atherosclerosis*. 2015 Dec;243(2):438–48.
- Inoue K, Fujie S, Hasegawa N, Horii N, Uchida M, Iemitsu K, et al. Aerobic exercise training-induced irisin secretion is associated with the reduction of arterial stiffness via nitric oxide production in adults with obesity. *Appl Physiol Nutr Metab*. 2020 Jul;45(7):715–22.
- Qiao X, Yong Qiao X, Nie Y, Ma Y, Xian Ma Y, Chen Y, et al. Irisin promotes osteoblast proliferation and differentiation via activating the MAP kinase signaling pathways. *Sci Rep*. 2016 Jan 7;6:18732.
- Wang AY. Calcium balance and negative impact of calcium load in peritoneal dialysis patients. *Perit Dial Int*. 2014 Jun;34(4):345–52.