IRISIN AS A PREDICTOR OF MICROALBUMINURIA IN OBESE PATIENTS WITH CORONARY ARTERY DISEASE

YULIIA KOVALOVA[1], BORYS SHELEST[2], TETIANA RUDENKO[3], MARIIA KHVYSIUK[3], MARYNA KOLOMIIETS[3]

[1]Department of Internal Medicine No. 2, Clinical Immunology and Allergology named after academician L.T. Malaya, Kharkiv National Medical University, Kharkiv, Ukraine, [2]Department of Internal and Occupational Diseases, Kharkiv National Medical University, Kharkiv, Ukraine, [3]Cardiology, Therapy and Nephrology Department, Kharkiv Medical Academy of Postgraduate Education, Kharkiv, Ukraine

Background. Irisin is a recently discovered protein involved in energy homeostasis and glucose metabolism, and is potentially involved in atherosclerosis, obesity, and cardiovascular diseases. The aim of the study was to investigate the irisin effect on microalbuminuria in obese patients with coronary artery disease (CAD). ***Methods.*** Sixty-four adult subjects with CAD combined with obesity (59.38% of males), mean age 59.43±10.29 years, were enrolled in the study. Control group included 30 sex- and age-matched subjects. Obese patients with CAD were divided into two groups: group 1 (n=31) without microalbuminuria, and group 2 (n=33) with microalbuminuria. The urine albumin to creatinine ratio (ACR, range 30-300 mL/mg) indicated microalbuminuria. Specific enzyme-linked immunosorbent assay was used for serum irisin measurement. ***Results.*** Serum irisin concentrations were significantly different in obese CAD patients with microalbuminuria 121.05 (103.07-133.19) ng/mL and those without it 130.21 (125.21-140.03) ng/mL compared to the control group 147.92 (139.04-172.55) ng/mL (p<0.001), and irisin level was significantly lower in patients with microalbuminuria in comparison with normoalbuminuria (p=0.042). Univariate logistic regression analyses showed irisin to significantly influence microalbuminuria (OR: 0.788, 95% CI 0.589-0.967, p=0.011). Multivariable logistic regression analyses revealed that serum irisin remained a significant predictor of microalbuminuria (OR: 0.857, 95% CI 0.561-0.988, p=0.044). ***Conclusions.*** Lower irisin levels are an independent predictor of microalbuminuria in patients with CAD combined with obesity. Additional larger longitudinal studies are needed to confirm these findings.

Key words: irisin, obesity, coronary artery disease, microalbuminuria, endothelial function

Address for correspondence: Professor Yuliia Kovalova, MD, PhD
Department of Internal Medicine No. 2,
Clinical Immunology and Allergology
named after academician L.T. Malaya
Kharkiv National Medical University,
4 Nauky Avenue
61022, Kharkiv, Ukraine
E-mail: yukovalova28@gmail.com

INTRODUCTION

Currently, cardiovascular diseases (CVD), mainly ischemic heart disease (IHD/coronary artery disease (CAD)), continue to be the leading cause of death in the world and one of the leading causes of disability. At the same time, it is especially frightening that the contemporary situation began to deteriorate in those regions where previously there were favorable tendencies for its improvement (1). In general, the prognoses are such that the spread of inappropriate nutrition and a sedentary lifestyle will only contribute to an increase in the incidence of IHD (2). At the same time, IHD itself is often combined with obesity and both diseases aggravate the course of each other. Since obesity is associated with an earlier development of CVD, with an increase in mortality, it contributes to the progression of coronary heart disease (3,4).

Endothelial dysfunction is an important pathogenetic chain in the progression and development of IHD. Inflammation activation and endothelial dysfunction are the key points in the development of atherosclerosis and are associated with an increased risk of cardio-

vascular events and CAD (5). Adipose tissue through the synthesis of a large number of signal peptides has a significant effect on endothelial dysfunction and atherosclerosis and, accordingly, on CAD (6).

In this regard, great scientific interest arises in order to study the pathophysiological basis of endothelial dysfunction in greater depth, which will give an impetus to the identification of new markers and therapeutic strategies for preventing endothelial dysfunction, and therefore will lead to a decrease in the risk of progression of CAD. Early diagnosis and management of endothelial function seems to be promising for the treatment of patients with CAD and obesity. Thus, identification of new biomarkers is a step towards earlier diagnosis and more effective treatment strategies for individuals from the high-risk group, i.e. obese patients with coronary heart disease.

Irisin is a recently discovered metabolic hormone that is expressed mainly by muscles. It is associated with diseases of the metabolic profile, kidney, insulin resistance, atherosclerosis of carotid arteries (7), ischemic heart disease, stable angina pectoris, acute coronary syndromes (8), and obesity (9). However, the available data are contradictory.

Microalbuminuria is an indicator of the generalized endothelial dysfunction, and is an early and sensitive marker of renal and cardiovascular risk (10-15).

Considering the pathogenetic relationship between endothelial function and ischemic heart disease and obesity, as well as the involvement of irisin in these diseases, it seems important to assess the effect of irisin on microalbuminuria as an indicator of endothelial function in patients with CAD combined with obesity.

The aim of the study was to elicit the effect of irisin on microalbuminuria as an endothelial dysfunction indicator in obese patients with CAD.

PATIENTS AND METHODS

A total of 64 patients with CAD combined with obesity were enrolled in the study. There were 38 males (59.38%), mean age 59.43±10.29 years. The diagnosis of CAD was made in accordance to the recommendations of the 2019 Guidelines on Chronic Coronary Syndromes (16). The obesity diagnosis was based on body mass index (BMI) over 30 kg/m^2 in accordance with the World Health Organization criteria.

All subjects were divided into two groups according to the urine albumin to creatinine ratio (ACR): group 1 with albuminuria (30≤ACR≤300 mg/g; n=31), and group 2 without microalbuminuria (ACR <30 mg/g; n=33). Control group included 30 apparently healthy subjects without a history of CAD, obesity or overweight, and renal abnormalities.

All participants were examined in the Cardiology Department of the Kharkiv City Clinical Hospital No. 27, which is a clinical base of the Kharkiv National Medical University. The study groups were matched by age, sex, severity of clinical condition, and comorbidity.

Exclusion criteria were: severe obesity (BMI >35 kg/m^2), angina pectoris III and IV FC, acute inflammatory, infectious, oncologic, immune and rheumatic diseases, secondary hypertension, ejection fraction (EF) <45%, anemia, estimated glomerular filtration rate (eGFR) <60 mL/min/1.73 m^2, acute coronary syndrome within the previous 3 months, rhythm and conduction disturbances, chronic obstructive pulmonary diseases, and patient discontinuation at any phase of the study.

Inclusion criteria were: patients with chronic CAD, angina pectoris not more severe than II functional class, obesity I class, age ≥35≤75 years, and signed informed consent to participate in the study.

Ethics procedure. The study was performed in accordance with the provisions of the Declaration of Helsinki. The study protocol No. 7, November 6, 2019, was approved by the Ethics and Deontology Commission of the Kharkiv National Medical University. All enrolled subjects signed an informed consent for participation in the study.

Measurements. Serum samples were obtained from blood in a fasting state by centrifugation. Serum level of irisin was determined by the enzyme immunoassay assay kits (Cusabio, PR China), according to the manufacturer's instructions. Intra-assay precision coefficient of variation (CV) was <8% and inter-assay CV was <10%. Immunoassay studies were carried out on a Labline-90 enzyme immunoassay analyzer (Austria). Routine enzymatic methods were used to determine triglycerides (TG), total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C) and serum creatinine (Scr). Fasting glucose (FG) was measured by a glucose oxidase procedure. eGFR was calculated by CKD-EPI (Chronic Kidney Disease Epidemiology Collaboration) equation. Echocardiographic examination was carried out using the Philips HD11XE (USA), according to the generally recognized pulse echo method with an ultrasound frequency of 7.5 MHz.

Statistical analysis. Sample size was determined through power analysis taking into account the preliminary means with the following assumptions: α 0.05, β

0.05, and power of 95%. A minimum of 26 subjects in the two groups (total 52 patients) are needed to detect significant difference in serum irisin levels. Testing of normality of distribution was done by Shapiro-Wilk test. In normal distribution, variables were presented as mean ± standard deviation (SD), and in not normal distribution as median (interquartile range). Categorical variables were given as percentages. The χ^2-test was used for comparison of categorical variables. Analysis of variance (ANOVA) or Kruskal-Wallis test was used to compare more than two groups for normal and not normal distribution, respectively. To compare two groups, Student's t test and Mann-Whitney U test were used for normally distributed variables and not normally distributed variables, respectively. In order to elicit independent predictors of microalbuminuria, logistic regression analysis was used. The possible confounding factors were analyzed in univariate regression analysis and confounders with a p value of <0.1 were tested in multivariate logistic regression analysis. The regression analyses results were indicated as odd ratios (OR) and 95% confidence intervals (CI). A p value of <0.05 was accepted as statistically significant. Statistical analyses of the data obtained was carried out using Statistica 10.0 statistical software (StatSoft Inc., USA).

RESULTS

The baseline clinical characteristics of CAD patients with concomitant obesity and control group are presented in Table 1 according to the presence/absence of microalbuminuria.

The ANOVA or Kruskal-Wallis test, when appropriate, showed significantly different levels of systolic blood pressure (SBP) (129.76±9.41 mm Hg; 134.97±9.11 mm Hg; 129.02±8.95 mmHg, p=0.023), diastolic blood pressure (DBP) (81.34±6.47 mm Hg; 85.95±5.57 mm Hg, 83.01±6.12 mm Hg, p=0.013), BMI (22.32±2.63 kg/m^2, 33.75±2.98 kg/m^2,32.59±3.05 kg/m^2, p<0.001), and low density lipoprotein cholesterol (LDL-C) (2.25 (1.92-2.57) mmol/L, 2.63 (2.13-3.05) mmol/L, 2.47 (2.09-2.95) mmol/L, p<0.001), as well as decreased concentrations of irisin (147.92 (139.04-172.55) ng/mL, 121.05 (103.07-133.19) ng/mL, 130.21 (125.21-140.03) ng/mL, p<0.001) among the study subjects comparing the three groups, i.e. controls, obese CAD with microalbuminuria and obese CAD without microalbuminuria. It is important that all enrolled subjects from both experimental groups and control group were matched by all other examined parameters, i.e., gender, age, smoking, EF, fasting glucose, TG, TC, and HDL-C.

When comparing the two main groups depending on microalbuminuria, it was found that there were no statistically significant differences in almost all parameters. As it follows from the group definition, ACR levels were significantly higher in group 1 (187.29 (151.75-203.91) mg/g) *versus* group 2 (19.01 (11.27-25.83) mg/g, p<0.001), and *versus* control group (14.18 (9.21-21.56) mg/g, p<0.001). Significantly elevated concentrations of creatinine (Cr) were found in group 1 patients with microalbuminuria (73.69 (64.71-82.92) μmol/L) compared to controls (65.80 (59.09-73.23) μmol/L, p=0.041), but did not differ significantly from those without microalbuminuria (69.11 (63.25-79.67) μmol/L, p=0.057). However, SBP (134.97±9.11 mm Hg *versus* 129.02±8.95 mm Hg, p = 0.011) and DBP (85.95±5.57 mm Hg *versus* 83.01±6.12 mm Hg, p=0.049) were significantly higher in those patients with microalbuminuria. On the contrary, irisin concentrations were significantly reduced in obese CAD patients with normoalbuminuria compared to group 1 (121.05 (103.07-133.19) ng/mL *versus* 130.21 (125.21-140.03) ng/mL, p=0.042).

Table 1.
Clinical characteristics of study patients and controls.

Parameter	Control group, n=30	Group 1 (with micro albuminuria), n=31	Group 2 (without micro albuminuria), n=33	p	p1	p2	p3
Age (yrs)	57.21±7.34	59.01±9.87	59.95±10.71	0.512	0.423	0.246	0.717
Gender, males, n (%)	17 (56.66)	18 (58.06)	20 (60.60)	0.950	0.934	0.811	0.875
Smoking, n (%)	7 (23.33)	9 (29.03)	8 (24.24)	0.859	0.804	0.968	0.829
BMI, kg/m^2	22.32±2.63	33.75±2.98	32.59±3.05	<0.001	<0.001	<0.001	0.129
EF, %	52.39±3.01	52.03±2.98	51.04±3.57	0.226	0.641	0.112	0.235
SBP, mm Hg	129.76±9.41	134.97±9.11	129.02±8.95	0.023	0.032	0.750	0.011
DBP, mm Hg	81.34±6.47	85.95±5.57	83.01±6.12	0.013	0.004	0.297	0.049
Fasting glucose, mmol/L	4.87±1.33	5.19±1.63	4.91±1.73	0.686	0.405	0.919	0.508
TG, mmol/L	1.58 (1.12-1.79)	1.65 (1.15-1.93)	1.59 (1.20-2.03)	0.626	0.587	0.703	0.619

TC, mmol/L	4.32 ± 0.85	4.71±0.93	4.47±0.89	0.230	0.093	0.498	0.296
HDL-C, mmol/L	1.39±0.35 (1.18-1.80)	1.22±0.3 (1.03-1.37)	1.23±0.39 (1.07-1.54)	0.120	0.107	0.232	0.385
LDL-C, mmol/L	2.25 (1.92-2.57)	2.63 (2.13-3.05)	2.47 (2.09-2.95)	<0.001	<0.001	0.003	0.465
Cr, µmol/L	65.80 (59.09-73.23)	73.69 (64.71-82.92)	69.11 (63.25-79.67)	0.047	0.041	0.272	0.057
ACR, mg/g	14.18 (9.21-21.56)	187.29 (151.75-203.91)	19.01 (11.27-25.83)	<0.001	<0.001	0.117	<0.001
Irisin, ng/mL	147.92 (139.04-172.55)	121.05 (103.07-133.19)	130.21 (125.21-140.03)	<0.001	<0.001	<0.001	0.042

Values are presented as mean ± standard deviation, n (%), mean (interquartile range); p – difference among 3 groups; p1 – difference between group 1 and control; p2 – difference between group 2 and control; p3 – difference between groups 1 and 2; NS – nonsignificant (p<0.05); SBP – systolic blood pressure; DBP – diastolic blood pressure; BMI – body mass index; TG – triglycerides; TC – total cholesterol; HDL-C – high-density lipoprotein cholesterol; LDL-C – low-density lipoprotein cholesterol; EF – ejection fraction; Cr – creatinine; ACR – urine albumin to creatinine ratio.

Relationship of serum irisin concentrations with microalbuminuria

Microalbuminuria was considered as an indicator of endothelial dysfunction in obese patients with CAD. It was found that serum concentrations of irisin in group 1 (with microalbuminuria) were significantly reduced compared to control group (121.05 (103.07-133.19) ng/mL *versus* 147.92 (139.04-172.55) ng mL, p<0.001). Moreover, in group 2 (without microalbuminuria) there was a significant decrease in this peptide also compared to control group (130.21 (125.21-140.03) ng/mL *versus* 147.92 (139.04-172.55) ng/mL, p<0.001).

The association of serum irisin with microalbuminuria was analyzed among all enrolled subjects (n=64) using logistic regression; the presence of microalbuminuria was coded as 1 (yes) and absence as 0 (no). At the first stage, univariate logistic analysis was performed to find independent influencers on endothelial dysfunction indicated as microalbuminuria. Confounders with a significance of p<0.1 in univariate regression analysis were considered as needed for further multivariate logistic regression analysis. Table 2 shows that serum irisin level (OR: 0.788, 95% CI 0.589-0.967, p=0.011); SBP (OR: 1.048, 95% CI 1.019-1.082, p<0.001); DBP (OR: 1.039, 95% CI 1.015-1.061, p=0.026); BMI (OR: 1.031, 95% CI 1.003-1.099, p=0.027); LDL-C (OR: 1.019, 95% CI 1.005-1.511, p=0.036), and Cr (OR: 1.016, 95% CI 1.005-1.028, p=0.004) were independent confounding factors for the presence of microalbuminuria in obese patients with CAD (Table 2).

Table 2.
Logistic regression analysis to determine risk factors for developing microalbuminuria.

Parameter	Univariate logistic regression		Multivariate logistic regression	
	OR (95% CI)	p	OR (95% CI)	p
Age, years	1.017 (0.951-1.069)	0.401		
Gender,	1.088 (0.781-1.824)	0.782		
BMI, kg/m^2	1.031 (1.003-1.099)	0.027	1.027 (0.978-1.102)	0.071
SBP, mmHg	1.048 (1.019-1.082)	<0.001	1.025 (1.009-1.047)	0.012
DBP, mmHg	1.039 (1.015-1.061	0.026	1.018 (0.976-1.056)	0.739
Fasting glucose, mmol/L	1.154 (0.909-1.438)	0.793		
TG, mmol/L	1.231 (0.893-1.289)	0.591		
TC, mmol/L	1.356 (0.899-1.478)	0.784		
HDL-C, mmol/L	1.275 (0.781-1.889)	0.833		
LDL-C, mmol/L	1.019 (1.005-1.511)	0.036	1.010 (0.688-1.853)	0.431
EF, %	0.946 (0.312-2.239)	0.735		
Cr (µmol/L)	1.012 (1.009-1.031	0.004	1.003 (1.002–1.097)	0.039
Irisin, ng/mL	0.788 (0.589-0.967)	0.011	0.857 (0.561–0.988)	0.044

Data are presented as odds ratio - 95% confidence interval (OR 95% CI); SBP – systolic blood pressure; DBP – diastolic blood pressure; BMI – body mass index; TG – triglycerides; TC – total cholesterol; HDL-C – high-density lipoprotein cholesterol; LDL-C – low-density lipoprotein cholesterol; EF – ejection fraction; Cr – creatinine; ACR – urine albumin to creatinine ratio.

In the next step, these were analyzed in a multivariate logistic regression model. Serum irisin concentration remained a significant predictor of microalbuminuria (endothelial dysfunction) after multivariate logistic regression (OR: 0.857, 95% CI 0.561-0.988, p=0.044). Furthermore, SBP (OR: 1.025, 95% CI 1.009-1.047,

p=0.012) and Cr (OR: 1.003, 95% CI 1.002-1.097, p=0.039) also remained significant predictors of microalbuminuria, in contrast to BMI (OR: 1.027, 95 CI 0.978-1.102, p=0.071); DBP (OR: 1.018, CI 95% 0.976-1.056, p=0.739); and LDL-C (OR: 1.010, 95% CI 0.688-1.853, p=0.431), which became nonsignificant in multivariate logistic regression analyses.

DISCUSSION

The key results of our study were that, firstly, the new peptide irisin turned out to be an active independent factor in the development of microalbuminuria in patients with CAD and obesity. This result indicates the important place of the studied peptide in the pathogenetic process of the development of endothelial dysfunction. And the latter, in turn, is one of the key points in the development of ischemic heart disease. This gives great prospects for further in-depth understanding of the physiology of the development and progression of CAD in patients with obesity.

Second, the present study demonstrated that serum irisin may correlate with DBP and SBP in the studied patients, since these parameters, along with irisin, were significantly higher in those subjects with microalbuminuria compared to normoalbuminuric ones. Possibly, higher levels of irisin may be considered as a factor in reducing the risk of microalbuminuria in patients with CAD and obesity.

It is important that the study by Wang *et al*. (17) showed definitely similar results close in significance. They pointed out the relationship between the decrease in irisin with albumin excretion and dilation of arteries. However, it is worth noting that in this study, there were only patients with type 2 diabetes mellitus (T2DM), while our study patients were without it.

An important recent review work (18) demonstrated irisin as a potential treatment agent for vascular dysfunction in individuals with hypertension and atherosclerosis. At the same time, the focus was on the fact that irisin can partially improve vascular function caused by obesity and T2DM. It is also noted that today, there are many gaps in understanding the role of irisin in endothelial regulation. Our work was also aimed at reducing the volume of such gaps, especially with CVD.

Mageswari *et al*. (19) showed an interrelation of irisin with renal pathology. However, their work had rather contradictory results, since according to their data, eGFR was negatively correlated with irisin (r=-0.324, p=0.034), however, irisin concentrations were significantly increased with nephropathy in comparison with those without it. This inconsistency makes it difficult to interpret such results and compare them with others. In addition, they only examined diabetics, as opposed to the current study.

The study by Hu *et al*. (20) demonstrated a significantly reduced concentration of irisin in macroalbuminuria than in normoalbuminuria and microalbuminuria. In contrast to our work, only diabetics were included in the study. However, it was shown that irisin in patients with T2DM negatively correlated with fasting plasma glucose and creatinine, and positively correlated with creatinine clearance. The association of irisin with vascular lesions also suggested that serum irisin was significantly reduced in patients with retinopathy compared to patients without diabetic retinopathy. In general, the results of their study can be interpreted as the fact that the concentration of irisin is associated with the presence of vascular and endothelial pathology through the presence of diabetic nephropathy and diabetic retinopathy.

Shelbaya *et al*. (21) found significantly lower irisin levels in diabetics compared to controls (p<0.001). They also found a significant negative correlation between irisin and ACR, serum creatinine, SBP and DBP, which can be compared with the data found in our study. At the same time, they also showed a connection between the discussed protein and duration of diabetes, BMI and HbA1c. However, on multivariate analysis, they found that duration of diabetes was the only independent factor associated with irisin. Their study enrolled only T2DM patients. Overall, this work confirmed our hypothesis that patients have a more significant decrease in irisin levels when renal dysfunction is present.

In line with our findings are data from the study conducted by Yang *et al*. They showed that high serum irisin levels were associated with a reduced risk of CKD, while they mostly underlined a significant effect of the percentage of fat on the relation with albumin and renal pathology (22).

A meta-analysis (23) of 1735 patients with T2DM showed that patients with microalbuminuria had significantly lower serum irisin levels compared with diabetics with normoalbuminuria. This review also indicated that irisin was significantly reduced with macroalbuminuria compared to microalbuminuria, and those with eGFR <60 mL min 1.73 m^2 had significantly reduced irisin levels compared with eGFR ≥60 mL min 1.73 m^2 . In general, this work also supports our hypothesis, however, only diabetics were taken into account in that work, while our study included patients with CAD and obesity.

Sadeghi Shad *et al*. (24) also confirmed irisin to be involved in the regulation of the progression of CKD from stage 2 to stage 4. Irisin levels decreased significantly according to the progression of CKD.

It is worth considering the work that linked irisin and cardiovascular pathology. For example, a recent study by Khorasani *et al.* (25) showed that the level of irisin in blood serum was significantly reduced in the presence of CAD in diabetics compared with the group of diabetic patients without CAD. It should be noted that after adjustment for the potential confounding factors, irisin levels remained associated with the presence of CAD in diabetes. This is consistent with our results.

Anastasilakis *et al.* (26) showed the presence of myocardial infarction and CAD to be associated with a decrease in irisin. Irisin was not inferior to CK-MB in predicting myocardial infarction. They summarize that irisin production is associated with myocardial blood supply. Weng (27) indicated the same conclusion in his study. Irisin levels were a predictor of CAD, and low concentrations of irisin were potentially associated with the presence and severity of CAD. Efe *et al.* (28) also conclude that irisin is an independent indicator of the severity of stable CAD. Similarly, the findings reported by Pan *et al*. (29) suggest that at higher irisin levels, a higher survival rate is observed in patients with stable CAD, and irisin is significantly reduced in patients with CAD.

Our results also indicate a link between coronary heart disease and a significantly lower level of irisin, as in the studies presented above.

A prospective population-based study by Hisamatsu *et al*. (30) found higher levels of irisin to be associated with a lower chance of developing coronary atherosclerosis, and irisin was also recognized as an obligatory prognostic marker for coronary heart disease, as well as a therapeutic strategy for CVD. Such work is of interest, but the study had important limitations as only Japanese men were included.

It is also worth noting in terms of discussion the study by Bi *et al*. (31). They showed that irisin strengthened the function of endothelial barrier and was a factor that favorably influenced the course of diseases associated with microvascular leakage. Microvascular permeability is a key sign of cardiovascular and renal diseases. In addition, irisin has the ability to suppress inflammation and to increase survival.

It is also important to compare our results on irisin and microalbuminuria link with data describing the relationship of irisin and other markers of endothelial dysfunction. At the same time, there is still a very limited amount of such kind of works.

Huerta-Delgado *et al.* (32) found negative correlations between irisin and triglycerides, soluble neural cell adhesion molecule, intercellular adhesion molecule-2, vascular cell adhesion molecule-1 and monocyte chemoattractant protein-1, and positive correlations with TC, HDL-C and LDL-C. Decrease in irisin leads to inadequate suppression of oxidative stress and inflammation. However, this work was carried out in patients under 16 years of age. In general, their results confirm the presence of a connection between irisin and endothelial function.

The relationship between irisin and endothelial dysfunction biomarkers in models is also evidenced in the work by Fu *et al*. (33). They found that irisin enhanced vasorelaxation in spontaneously hypertensive rats, which could indicate a role for irisin in increasing nitric oxide expression and phosphorylation of endothelial nitrate oxide synthase (eNOS) in endothelial cells. Hou *et al.* (34) found that irisin had a beneficial effect on endothelial function in obese mouse models. Most likely, irisin can stop endothelial dysfunction in obesity through exposure and regulation of processes in the perivascular adipose tissue. Inoue *et al.* (35) showed in humans that aerobic exercise increased irisin levels and this, in turn, led to a decrease in arterial stiffness in obese adults. In general, most of the data available to date are consistent with our results. Consequently, serum irisin is promising for use as a biomarker for assessing the risk of developing microalbuminuria and endothelial dysfunction.

The present study showed that serum irisin correlated with the parameters of endothelial, vascular and renal function. Irisin may be involved in the pathogenesis of ischemic heart disease, obesity and renal problems, not just diabetes. Additional extensive studies are required to accurately explain and elucidate the role of irisin in the progression of CAD, obesity, and the most important chains of these diseases such as atherosclerosis, vascular and endothelial dysfunction, and inflammation. In addition, we did not find a correlation between serum irisin and lipoprotein fractions, which are the key in understanding the pathogenesis of CAD.

There are few works aiming at studying the role of irisin in stable and acute forms of ischemic heart disease. There are many gaps in identifying the links between irisin and various instrumental and laboratory markers of endothelial dysfunction and atherosclerosis. There is a lack of evidence for the role of irisin in atherosclerosis, its relationship with lipoproteins, triglycerides, and, for example, with the intima-media thickness.

The presented study showed that a decrease in the concentration of irisin in blood serum correlated with

the development and progression of renal pathology, more precisely, microalbuminuria as its incipient marker. We assume that irisin can be considered as an important participant in the formation of endothelial dysfunction in patients with CAD associated with obesity. We associated a decrease in irisin concentrations with the cumulative effect of the pathogenetic factors of ischemic heart disease and obesity, namely, that progressive atherosclerotic changes, together with low-gradient inflammation in obesity, suppressed the production of irisin. We suggest that low physical activity was probably the general rule for patients with microalbuminuria and low irisin levels. This association has been shown in earlier studies. Subjects with greater physical activity were less likely to have microalbuminuria and renal pathology (36,37).

Our work had some limitations. It should be stated that the sample size was in some degree limited for definitive conclusions. Furthermore, a possible limitation can be attributed to the absence of patients with normal BMI and severe obesity, although this is what made it possible to single out a definite cohort of patients. Thus, further studies with large populations will provide great opportunities and certainties. In addition, a cross-sectional design was used in our study. Therefore, the causal relationship could not be accurately confirmed. This will be explored in future longitudinal studies.

CONCLUSION

A decreased level of irisin in serum was found in obese patients with CAD compared with healthy controls. The reduced levels of serum irisin are an independent predictor of microalbuminuria in patients with combined CAD and obesity. Irisin can be suggested as an important player in the pathogenesis of renal and endothelial function lesions in obese patients with CAD.

REFERENCES

1. Roth GA, Mensah GA, Johnson CO *et al.* GBD-NHL-BI-JACC Global Burden of Cardiovascular Diseases Writing Group (2020). Global burden of cardiovascular diseases and risk factors, 1990-2019: update from the GBD 2019 study. J Am Coll Cardiol 2020; 76(25): 2982-3021.

2. Ralapanawa U, Sivakanesan R. Epidemiology and the magnitude of coronary artery disease and acute coronary syndrome: a narrative review. J Epidemiol Glob Health 2021; 11(2): 169-77. doi: 10.2991/jegh.k.201217.001

3. Virani SS, Alonso A, Benjamin EJ *et al.* Heart Disease and Stroke Statistics – 2020 Update: A Report From the American Heart Association. Circulation 2020; 141(9): e139-e596.

4. Katta N, Loethen T, Lavie CJ, Alpert MA. Obesity and coronary heart disease: epidemiology, pathology, and coronary artery imaging. Curr Probl Cardiol 2021; 46(3):100655.

5. Medina-Leyte DJ, Zepeda-García O, Domínguez-Pérez M *et al.* Endothelial dysfunction, inflammation and coronary artery disease: potential biomarkers and promising therapeutical approaches. Int J Mol Sci 2021; 22(8): 3850.

6. Kwaifa IK, Bahari H, Yong YK, Noor SM. Endothelial dysfunction in obesity-induced inflammation: molecular mechanisms and clinical implications. Biomolecules 2020; 10(2): 291.

7. Gouveia MC, Vella JP, Cafeo FR, Affonso Fonseca FL, Bacci MR. Association between irisin and major chronic diseases: a review. Eur Rev Med Pharmacol Sci 2016; 20(19): 4072-7.

8. Ou-Yang WL, Guo B, Xu F *et al.* The controversial role of irisin in clinical management of coronary heart disease. Front Endocrinol (Lausanne) 2021; 12: 678309.

9. Jia J, Yu F, Wei WP *et al.* Relationship between circulating irisin levels and overweight/obesity: a meta-analysis. World J Clin Cases 2019; 7(12): 1444-55.

10. Ochodnicky P, Henning H, van Dokkum RPE, de Zeeuw D. Microalbuminuria and endothelial dysfunction: emerging targets for primary prevention of end-organ damage. J Cardiovasc Pharmacol 2006; 47: S151-S162.

11. Winocour P, Marshall S. Microalbuminuria as a marker of endothelial dysfunction. In: Microalbuminuria: Biochemistry, Epidemiology and Clinical Practice. Cambridge: Cambridge University Press, 1998; p. 97-115.

12. Huang M.J, Wei RB, Zhao J *et al.* Albuminuria and endothelial dysfunction in patients with non-diabetic chronic kidney disease. Med Sci Monit 2017; 23: 4447-53.

13. Sun HJ, Hou B, Wang X *et al.* Endothelial dysfunction and cardiometabolic diseases: role of long non-coding RNAs. Life Sci 2016; 167: 6-11.

14. Sun HJ, Zhu X.X., Cai WW, Qiu LY. Functional roles of exosomes in cardiovascular disorders: a systematic review. Eur Rev Med Pharmacol Sci 2017; 21: 5197-206.

15. Prasad RM, Tikaria R. Microalbuminuria. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2021.

16. Knuuti J, Wijns W, Saraste A *et al.* ESC Scientific Document Group, 2019 ESC Guidelines for the diagnosis and management of chronic coronary syndromes: the Task Force for the diagnosis and management of chronic coronary syndromes of the European Society of Cardiology (ESC). Eur Heart J 2020; 41(3): 407-77.

17. Wang HH, Zhang XW, Chen WK, Huang Q, Chen, QQ. Relationship between serum irisin levels and urinary albumin excretion in patients with type 2 diabetes. J Diabetes Complications 2015; 29(3): 384-9.

18. Byun K, Lee S. The potential role of irisin in vascular function and atherosclerosis: a review. Int J Mol Sci 2020; 21(19): 7184.

19. Mageswari R, Sridhar MG, Nandeesha H, Parameshwaran S, Vinod KV. Irisin and visfatin predict severity of diabetic nephropathy. Indian J Clin Biochem 2019; 34(3): 342-6.

20. Hu W, Wang R, Li J, Zhang J, Wang W. Association of irisin concentrations with the presence of diabetic nephropathy and retinopathy. Ann Clin Biochem 2016;53(Pt 1):67-74.

21. Shelbaya S, Abu Shady MM., Nasr MS *et al.* Study of irisin hormone level in type 2 diabetic patients and patients with diabetic nephropathy. Curr Diabetes Rev 2018; 14(5): 481-6.

22. Yang S, Xiao, Pan L *et al*. Association of serum irisin and body composition with chronic kidney disease in obese Chinese adults: a cross-sectional study. BMC Nephrol 2015; 16: 16.

23. Wang R, Liu H. Association between serum irisin and diabetic nephropathy in patients with type 2 diabetes mellitus: a meta-analysis. Horm Metab Res 2021; 53(5): 293-300.

24. Sadeghi Shad J, Akbari R, Qujeq D, Hajian-Tilaki K. Measurement of serum irisin in the different stages of chronic kidney disease. Caspian J Intern Med 2019; 10(3): 314-9.

25. Khorasani ZM, Bagheri RK, Yaghoubi MA *et al*. The association between serum irisin levels and cardiovascular disease in diabetic patients. Diabetol Metab Syndr 2019; 13(1): 786-90.

26. Anastasilakis A, Koulaxis D, Kefala N *et al*. Circulating irisin levels are lower in patients with either stable coronary artery disease (CAD) or myocardial infarction (MI) *versus* healthy controls, whereas follistatin and activin A levels are higher and can discriminate MI from CAD with similar to CK-MB accuracy. Metabolism 2017; 73: 1-8.

27. Deng W. Association of serum irisin concentrations with presence and severity of coronary artery disease. Med Sci Monit 2016; 22: 4193-7.

28. Efe TH, Açar B, Ertem AG *et al*. Serum irisin level can predict the severity of coronary artery disease in patients with stable angina. Korean Circ J 2017; 47(1): 44-9.

29. Pan JA, Zhang H, Yu Q *et al*. Association of circulating irisin levels and the characteristics and prognosis of coronary artery disease. Am J Med Sci 2021; 362(1): 63-71.

30. Hisamatsu T, Miura K, Arima H *et al*. Relationship of serum irisin levels to prevalence and progression of coronary artery calcification: a prospective, population-based study. Int J Cardiol 2018; 267: 177-82.

31. Bi J, Zhang J, Ren Y *et al*. Exercise hormone irisin mitigates endothelial barrier dysfunction and microvascular leakage-related diseases. JCI Insight 2020; 5(13): e136277.

32. Huerta-Delgado AS, Roffe-Vazquez DN, Gonzalez-Gil AM *et al*. Serum irisin levels, endothelial dysfunction, and inflammation in pediatric patients with type 2 diabetes mellitus and metabolic syndrome. J Diabetes Res 2020; 1949415.

33. Fu J, Han Y, Wang J *et al*. Irisin lowers blood pressure by improvement of endothelial dysfunction *via* AMPK-Akt-eNOS-NO pathway in the spontaneously hypertensive rat. J Am Heart Assoc 2016; 5(11): e003433.

34. Hou N, Du G, Han F *et al*. Irisin regulates heme oxygenase-1/adiponectin axis in perivascular adipose tissue and improves endothelial dysfunction in diet-induced obese mice. Cell Physiol Biochem 2017; 42(2): 603-14.

35. Inoue K, Fujie S, Hasegawa N *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; 45(7): 715-22.

36. Wadén J, Forsblom C, Thorn LM *et al*. Physical activity and diabetes complications in patients with type 1 diabetes: the Finnish Diabetic Nephropathy (FinnDiane) study. Diabetes Care 2008; 31(2): 230-2.

37. Pongrac Barlovic D, Tikkanen-Dolenc H, Groop PH. Physical activity in the prevention of development and progression of kidney disease in type 1 diabetes. Curr Diab Rep 2019; 19(7): 41.

S A Ž E T A K

IRISIN KAO PREDIKTOR MIKROALBUMINURIJE U PRETILIH BOLESNIKA S KORONARNOM BOLEŠĆU

Y. KOVALOVA[1], B. SHELEST[2], T. RUDENKO[3], M. KHVYSIUK[3], M. KOLOMIIETS[3]

[1]Odjel interne medicine broj 2, Klinička imunologija i alergologija po imenu akademika L.T. Malaya, Harkovsko nacionalno medicinsko sveučilište, Harkov; [2]Odjel za unutarnje i profesionalne bolesti, Harkovsko nacionalno medicinsko sveučilište, Harkov; [3]Odjel za terapiju, nefrologiju i unutarnju medicinu, Harkovska medicinska akademija za poslijediplomsko obrazovanje, Harkov, Ukrajina

Pozadina: Irisin je nedavno otkriveni protein koji sudjeluje u energetskoj homeostazi i metabolizmu glukoze i potencijalno je uključen u aterosklerozu, pretilost, kardiovaskularne bolesti. Cilj studije bio je istražiti učinak irisina na mikroalbuminuriju u pretilih bolesnika s ishemijskom bolesti srca (IBS). ***Uzorak i metode:*** 64 odrasla ispitanika s koronarnom bolešću u kombinaciji s pretilošću (59,38 % muškaraca), prosječne dobi 59,43±10,29 godina; 30 ispitanika sastojalo se od kontrola usklađenih po spolu, dobi. Pregledani pretili bolesnici s IBS-om podijeljeni su u dvije skupine. Prva skupina (n=31) bila je bez mikroalbuminurije, a u drugoj su skupini (n=33) bili bolesnici s mikroalbuminurijom. Omjer albumina i kreatinina u mokraći (ACR u rasponu od 30-300 mL/mg) bio je pokazatelj mikroalbuminurije. Za mjerenje irisina u serumu korišten je enzimski imunosorbentni test. ***Rezultati:*** Utvrđeno je da se koncentracije irisina u serumu značajno razlikuju u pretilih bolesnika s IBS s mikroalbuminurijom 121.05 (103,07-133,19) ng/mL i bez mikroalbuminurije 130,21 (125,21-140,03) ng/mL u usporedbi s kontrolnom skupinom 147,92 (139,04-172,55) ng/mL, p<0,001, a razina irisina značajno je smanjena u bolesnika s mikroalbuminurijom u usporedbi s normoalbuminurijom, p=0,042. Univarijatne logističke regresijske analize pokazale su da je irisin značajno utjecao na mikroalbuminuriju (OR: 0,788, 95 % CI 0,589-0,967, p=0,011). Više varijabilne logističke regresijske analize otkrile su da je irisin u serumu ostao značajan prediktor mikroalbuminurije (OR: 0,857, 95 % CI 0,561-0,988, p=0,044). ***Zaključak:*** Niže razine irisina neovisni su prediktor mikroalbuminurije u bolesnika s koronarnom bolešću u kombinaciji s pretilošću, ali potrebne su daljnje veće longitudinalne studije kako bi se potvrdili ti nalazi.

Ključne riječi: irisin, pretilost, ishemijska bolest srca, mikroalbuminurija, endotelna funkcija