IRISIN, INTERLEUKIN-33 AND INTERLEUKIN-37 IN PATIENTS WITH ISCHEMIC HEART DISEASE AND OBESITY

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ABSTRACT

Objective. The aim of this work was to investigate the obesity influence on the levels of irisin and interleukins 33 and 37 in patients with coronary heart disease.

Methods. The first group consisted of 36 patients with coronary artery disease (CAD) and obesity. The comparison group consisted of 33 patients with coronary artery disease with normal body weight. The control group included 30 basically healthy persons. The levels of irisin and interleukins were measured by ELISA. Obesity was indicated as increased body mass index (BMI) ≥ 30 kg/m².

Results. The study showed that irisin was reduced to 127.36 (107.52 – 137.88) ng/ml in the group with stable angina pectoris compared with the controls 147.92 (139.04 - 172.55) ng/ml, p = 0.290. Patients with combined coronary artery disease and obesity had significantly increased IL-33 levels (123.56 ± 32.38 pg/ml, p = 0.004) and IL-37 (131.74 ± 24.17 pg/ml, p = 0.004). Multivariate regression analyses showed that BMI positively correlated with the serum irisin in CAD obese patients (β = 0.255, p = 0.039).

Conclusion. The coexistence of obesity in patients with stable angina is suggested to be a significant factor of irisin diminishing and interleukins 33 and 37 levels increasing. Body mass index had direct correlation with irisin concentrations in obese patients with coronary artery disease.

Key words: myocardial ischemia; angina pectoris; obesity; cytokines; interleukins.

INTRODUCTION

The presence of comorbid obesity leads to more severe hyperlipidemia development, increased metabolic acidosis, and in such way contributes to the risk of development of early atherosclerosis with clinical manifestations as pain in the heart/chest region. Large pathogenic changes occur in auto-, endo- and paracrine functions and the production of hormone-like substances, adipokines and various mediators inside adipose tissue (1). Considering that obesity contributes to the progression of vascular changes, significantly worsens the prognosis of cardiovascular diseases (CVD) and is a great risk factor for the development of arterial hypertension (AH) and diabetes mellitus (DM), the determination of its influence on the pathogenesis of inflammation in patients with coronary heart disease (IHD) is of significant importance (2). The role of the inflammation in these processes is evidenced by an increase in immunocompetent cells, chemoattractive molecules, inflammatory mediators, and...
especially interleukins (IL) (3). The last-mentioned are
considered as immune signal peptides which are
responsible for the internal environment body
maintenance, and protection from any genetically foreign
substances.

Interleukin-33 was recently discovered and it belongs
to the IL-1 family. It is released by epithelial, endothelial,
smooth muscle and dendritic cells, as well as by activated
macrophages, keratinocytes, and fibroblasts (4). IL-37 is a
newer cytokine that is considered to be an anti-
inflammatory agent. Modern research has shown that it
can weaken atherosclerosis and changes in the structure of
blood vessels, and may be associated with the
development of atherosclerosis and associated diseases.
IL-37 has a fairly broad spectrum of action, including
several intracellular and extracellular pathways that affect
various aspects of inflammation (5). Irisin is a novel
myokine and was identified in 2012. It is expressed by
myocytes under exercises and participates in energy
regulation, as well as that it works as a mediator between
muscular tissue and other organs and tissues (6).

The relationship between obesity and coronary artery
disease is viewed as a causal relationship through a
potential two-way relationship between vascular changes
and weight gain. It is of a great importance to find a new
way if interrelation between obesity, CAD and
inflammation through determination of irisin, IL-33 and
IL-37 concentrations in such conditions. The purpose of
the work was to evaluate the concomitant obesity
influence on the levels of irisin and interleukins 33 and 37
in patients with coronary artery disease.

**PATIENTS AND METHODS**

Two groups were created for the purpose of the study.
The first one included 36 patients with coronary artery
disease [stable angina pectoris, II functional class (FC)]
and obesity. The second one (comparison group) consisted
of 33 patients with coronary artery disease (stable angina
pectoris, II FC) with normal body weight. The average age
of the subjects was 62.7 ± 6.4 years (from 45 up to 74
years old). Chronic heart failure functional class was not
more severe than II FC. The control group included 30
apparently healthy individuals. The subjects were
examined in the cardiology departments of the Kharkiv
City Clinical HZospital No. 27, which is the clinical base
of Kharkiv National Medical University.

The clinical diagnosis was established in accordance
with the criteria of the Orders of the Ministry of Health of
Ukraine, on the basis of the patients’ complaints, medical
history, and objective examination data. The diagnosis
was verified using laboratory and instrumental methods
according to the recommendations of the 2019 Guidelines
on Chronic Coronary Syndromes (7).

The 12-lead electrocardiography in the supine position
after 5 minutes of rest; transthoracic echocardiography (apparatus "Philips HD11XE", USA, according to the
generally recognized method by the pulse echo method
with an ultrasound frequency of 7.5 MHz) were applied
among instrumental methods.

The patients with concomitant acute inflammatory,
infectious, oncological, immune and rheumatic diseases,
patients with secondary hypertension, with EF < 45%,
aemia, renal failure, episodes of acute heart failure, acute
coronary syndrome within the previous 3 months, rhythm
and conduction disturbances, chronic obstructive
pulmonary diseases, occlusive vascular diseases of the
lower extremities were excluded from the study.

Laboratory measurements. To study serum
concentrations of interleukins (IL-33 - ELISA Kit R&D
Systems Minneapolis, MN and IL-37 - Adipogen AG,
Liestal, Switzerland), enzyme immunoassay kits were
used. The level of irisin serum was determined by the
enzyme immunoassay using a set of ELISA reagents on an
enzyme immunoassay analyzer "Labline-90" (Austria).

Body weight was assessed by the body mass index
(BMI) recommended by the World Health Organization
(WHO). BMI in the range of 18.5-24.9 kg / m² was
considered optimal. The criterion for overweight was BMI
25-29.9 kg / m² and obesity - more than 30.0 kg / m².

The work was carried out in accordance with the
requirements of the provisions of the Helsinki Declaration
of the World Medical Association, the charter of the
Ukrainian Association for Bioethics and the Good Clinical
Practice (GCP) standards (1992), according to the
requirements and norms of the ICH GCP, model
provisions on ethics of the Ministry of Health of Ukraine
No. 66 dated 13/02/2006. All patients expressed their
informed consent to participate in the study and were fully
aware of the methods and scope of the study.

Statistical analysis. The distribution of quantitative
indicators in all compared groups was close to normal,
assessed by Kolmogorov-Smirnov test. The differences
between the two groups were analyzed by using a relevant
variant of the Student's test or Mann-Whitney U test.
Kruskal-Wallis test was used for not normally distributed
variables for more than 2 groups comparison while
ANOVA was used for normally distributed ones. Chi-
square test was used for categorical variables comparison.
During the sampling analysis, qualitative and quantitative
indicators were assessed using absolute and relative (in
percent) frequencies. The results were presented as the
expression: mean (M) ± standard deviation (SD) when the
distribution was normal, or presented as median
(interquartile range) as non-parametric method. The
correlations analyses between irisin and other parameters
were done by Pearson test. For determining the
contribution of various factors to serum irisin levels
multiple linear regression analysis was used. The critical value of the significance level \( p \) was chosen to be 0.05. Mathematical calculations were performed in Statsoft Statistica 10.0.

**RESULTS**

The baseline characteristics of the enrolled subjects

The Table 1 indicates the baseline clinical characteristics of the enrolled subjects. There were no significant differences between the examined groups in age, gender, smoking state, systolic blood pressure (SBP), diastolic blood pressure (DBP), heart failure (HF), cholesterol, high-density lipoprotein (HDL-C), triglycerides (TG) and fasting glucose levels, but the concentrations of C-reactive protein, Homeostasis Model Assessment of Insulin Resistance (HOMA-IR), and BMI were significantly increased in the patients with combined course of CAD with obesity compared to control: \( p = 0.022; p = 0.014; p < 0.001; p < 0.001 \), respectively. Both experimental groups with CAD differed significantly only by BMI, \( p < 0.001 \).

Levels of irisin and interleukin-33, and interleukin-37 in CAD patients compared to obese CAD patients and controls

The study showed that irisin was reduced 127.36 (107.52 – 137.88 ng / ml) with stable angina pectoris II FC (Table 2) compared to the control group 147.92 (139.04-172.55) ng / ml, \( p = 0.290 \). At the same time, in case of obesity coexistence with the addition to CAD, irisin level became significantly (\( p = 0.015 \)) lower 124.91 (106.71 – 132.77) ng / ml compared to the control group.

Our study found that the level of pro-inflammatory IL-33 (115.36±29.62 pg / ml) and anti-inflammatory IL-37 (123.87±19.42 pg / ml) turned out not to be significantly higher in patients with stable angina pectoris with the II functional class than in the control group: 101.25±28.14 pg / ml \( (p = 0.058) \) and 112.36±28.47 pg / ml \( (p = 0.064) \), respectively. There were no significant differences between the studied interleukins 33, IL-37 and irisin between the two experimental groups with coronary heart disease: \( p = 0.278; p = 0.143; p = 0.129 \); respectively. Hence, the increase in both interleukins was also observed in patients with a combination of coronary heart disease with obesity. However, in the group of patients with a combination of coronary artery disease and obesity, the in IL-33 (123.56±32.38 pg / ml, \( p = 0.004 \)) and IL-37 (131.74±24.17 pg / ml, \( p = 0.004 \)) was significant.

The association of irisin concentrations with examined parameters

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control (n=30)</th>
<th>CAD (n=33)</th>
<th>CAD and obesity (n=36)</th>
<th>P1</th>
<th>P2</th>
<th>P3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>57.21 ± 7.34</td>
<td>61.08 ± 8.73</td>
<td>60.53 ± 8.62</td>
<td>0.063</td>
<td>0.101</td>
<td>0.793</td>
</tr>
<tr>
<td>Males, n, (%)</td>
<td>17, (56.66)</td>
<td>18, (54.54)</td>
<td>21, (58.33)</td>
<td>0.867</td>
<td>0.892</td>
<td>0.753</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>22.32 ± 2.63</td>
<td>23.52 ± 2.39</td>
<td>32.61 ± 3.07</td>
<td>0.063</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Smoking, n, %</td>
<td>7, (23.33)</td>
<td>9, (27.27)</td>
<td>10, (27.77)</td>
<td>0.722</td>
<td>0.684</td>
<td>0.963</td>
</tr>
<tr>
<td>HF, %</td>
<td>52.39 ± 3.01</td>
<td>51.18 ± 2.79</td>
<td>50.85 ± 3.44</td>
<td>0.103</td>
<td>0.060</td>
<td>0.665</td>
</tr>
<tr>
<td>SBP (mmHg)</td>
<td>129.76±9.41</td>
<td>128.37±8.51</td>
<td>131.46 ± 11.87</td>
<td>0.540</td>
<td>0.528</td>
<td>0.222</td>
</tr>
<tr>
<td>DBP (mmHg)</td>
<td>81.34 ± 6.47</td>
<td>82.45 ± 7.56</td>
<td>83.53 ± 8.63</td>
<td>0.536</td>
<td>0.256</td>
<td>0.584</td>
</tr>
<tr>
<td>HOMA-IR</td>
<td>2.41 ± 0.59</td>
<td>2.97 ± 1.26</td>
<td>3.01 ± 1.18</td>
<td>0.030</td>
<td>0.014</td>
<td>0.892</td>
</tr>
<tr>
<td>Cholesterol (mmol/L)</td>
<td>4.32 ± 0.85</td>
<td>4.57 ± 0.83</td>
<td>4.75 ± 0.92</td>
<td>0.242</td>
<td>0.055</td>
<td>0.398</td>
</tr>
<tr>
<td>HDL-C (mmol/L)</td>
<td>1.39±0.35</td>
<td>1.51±0.43</td>
<td>1.55±0.47</td>
<td>0.232</td>
<td>0.128</td>
<td>0.714</td>
</tr>
<tr>
<td>TG (mmol/L)</td>
<td>1.58 ± 0.26</td>
<td>1.67 ± 0.34</td>
<td>1.69 ± 0.51</td>
<td>0.246</td>
<td>0.289</td>
<td>0.850</td>
</tr>
<tr>
<td>Glucose (mmol/L)</td>
<td>4.87±1.33</td>
<td>4.99±1.41</td>
<td>5.21±1.57</td>
<td>0.730</td>
<td>0.352</td>
<td>0.544</td>
</tr>
<tr>
<td>CRP (ng/ml)</td>
<td>4.63 ± 1.26</td>
<td>5.01 ± 1.37</td>
<td>5.43 ± 1.47</td>
<td>0.258</td>
<td>0.022</td>
<td>0.225</td>
</tr>
</tbody>
</table>

Notes: P1 = CAD group compared to control group. P2 = CAD with obesity compared to control group. P3 = CAD with obesity compared to CAD group; CAD = coronary artery disease; BMI = body mass index; HF = heart failure; SBP = systolic blood pressure; DBP = diastolic blood pressure; HOMA-IR = Homeostasis Model Assessment of Insulin Resistance; HDL-C = high-density lipoprotein cholesterol; TG = triglycerides; CRP = C-reactive protein; *fasting
Spearman correlation analysis was done to find the indices correlated with irisin levels in the presented cohort of patients with CAD and obesity. As presented in Table 3, serum irisin correlated with BMI ($r = 0.399, p = 0.001$), SBP ($r = 0.199, p = 0.023$), HOMA-IR ($r = 0.509, p = 0.001$), HDL-C ($r = 0.187, p = 0.029$), TG ($r = 0.329, p = 0.041$), fasting glucose ($r = -0.390, p = 0.034$) CRP ($r = 0.323, p = 0.001$), and IL-37 ($r = 0.433, p = 0.021$). It worth to indicate the absence of significant links between irisin levels and age, gender, smoking state, HF, DBP, TC, or IL-33.

The next step was to observe the correlations after adjusting for age and gender. It was found that serum irisin levels were significantly associated with BMI ($r = 0.273, p = 0.015$), SBP ($r = 0.201, p = 0.033$), HOMA-IR ($r = 0.399, p = 0.030$), TG ($r = 0.287, p = 0.045$), fasting glucose ($r = -0.350, p = 0.039$) and CRP ($r = 0.301, p = 0.028$). IL-37 and HDL-C lose its significance of association with irisin concentration.

At last, a multiple linear regression analysis was performed with significant correlation parameters for determining possible characteristics that may have an effect on the irisin concentrations in patients with CAD. It showed that BMI ($β = 0.255, p = 0.039$), SBP ($β = 0.151, p = 0.048$), and CRP ($β = 0.290, p = 0.036$) remained positively correlated with serum irisin with negative correlation of TG ($β = -0.023, p = 0.045$) and fasting glucose ($β = -0.029, p = 0.032$). The results of our work showed that obesity presented as BMI, SBP and inflammatory markers as CRP positively affect the irisin concentrations in the presented patients with coronary artery disease, and fasting glucose with TG remained conversely associated with irisin.

**Table 2. Irisin and interleukins (IL-33, IL-37) concentrations in examined groups and controls**

<table>
<thead>
<tr>
<th>Cytokine</th>
<th>Controls (n=30)</th>
<th>CAD (n=33)</th>
<th>CAD and obesity (n=36)</th>
<th>P1</th>
<th>P2</th>
<th>P3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Irisin, ng/mL</td>
<td>147.92 (139.04 - 172.55)</td>
<td>127.36 (107.52 - 137.88)</td>
<td>124.91 (106.71 - 132.77)</td>
<td>0.290</td>
<td>0.015</td>
<td>0.129</td>
</tr>
<tr>
<td>IL-33, pg/mL</td>
<td>101.25 ± 28.14</td>
<td>115.36 ± 29.62</td>
<td>123.56 ± 32.38</td>
<td>0.058</td>
<td>0.040</td>
<td>0.278</td>
</tr>
<tr>
<td>IL-37 pg/mL</td>
<td>112.36 ± 28.47</td>
<td>123.87 ± 19.42</td>
<td>131.74 ± 24.17</td>
<td>0.064</td>
<td>0.004</td>
<td>0.143</td>
</tr>
</tbody>
</table>

**DISCUSSION**

The performed work found that serum irisin was correlated with the presence of obesity after the multivariate regression analysis. Thus, irisin concentrations can be suggested to play a great role in the risk of progressing obesity in CAD patients. The study indicated diminished serum irisin levels in the patients with just CAD and also with combined CAD with obesity.
Furthermore, coexistence of obesity lead to significantly dropped irisin concentrations compared to only CAD and control patients.

Previous studies determined the role of irisin in obese and diabetic patients, but there were limited number of investigations focused on the relations of serum irisin with coronary artery disease. Thus, the reasons of low serum irisin levels in CAD and obesity remained unknown. And further larger investigations need to be done for explanation such results. In addition, the data presented found that obese tissue can affect irisin production as well as muscle tissue in CAD patients.

It is worth pointing out that our results are to some extent comparable to some previously conducted studies that examined the relationship between obesity and irisin. These existing studies have predominantly shown that irisin is directly associated with the level of BMI (8-10). It is interesting to note that irisin has been shown to be associated with a percentage of fat mass with muscle mass and waist circumference (11, 12). At the same time, the studied adipokine is very closely related to the dynamics of the weight of patients (13). However, it should be borne in mind that there are other results on the relationship between irisin and obesity, where a negative correlation was noted between irisin and the level of body weight (14-16). Actually, such previously found contradictions inspired us to work on studying the correlation between irisin and obesity, especially among patients with concomitant cardiovascular pathology.

A feature of the work was the study of the effect of irisin in patients with high cardiovascular risk, while in previous studies the emphasis was simply on people with obesity or diabetes (17-19). At the same time, we also introduced into the studied parameters and markers of inflammation both the well-known CRP and IL33 and IL37. At the same time, only one serious study was found, a meta-analysis of data on the association of irisin with CRP (20), which showed contradictory results, but in general, more inclined and similar to our results about a weak direct connection of irisin with CRP. At the same time, no studies of the relations of IL 33 and IL 37 with irisin were found.

We observed the obesity actual effect on the serum levels of all studied cytokines, as irisin, IL-37, IL-37 in patients with coronary heart disease. Our consideration was based on the baseline clinical characteristic of the examined patients and suggested that low-gradient inflammation simultaneously with compromised carbohydrate metabolism was associated with the irisin, IL-33 and IL-37 levels.

Obesity and its general effects are the main risk factors for cardiovascular diseases, which are characterized by chronic systemic and vascular inflammation (21). Adipose tissue when obesity is present is characterized by an inflamed immune environment consisting of classically activated macrophages, cytotoxic T cells, and Th1 proinflammatory cytokines (such as tumor necrosis factor-α and interferon-γ) (22). In contrast, lean adipose tissue is characterized by an anti-inflammatory environment of alternatively activated macrophages, eosinophils, Th2 cells, and anti-inflammatory Th2-type cytokines (such as IL-4, IL-5, IL-9, and IL-13) (23-25).

Changes in levels of interleukins that were found in the presented study confirmed indication of the presence of subclinical inflammation in these patients with stable angina pectoris, as well as in combination with obesity. Obviously, such a process can be considered as a consequence of further deterioration of the process in concomitant pathology, accompanied by the action of intermediate toxic substances, products of fatty acid metabolism and proliferation of macrophage-type cells in adipose tissue (26). Recently, IL-33 has been shown to regulate white adipose tissue homeostasis (WAT), a process when deregulated, progressively leads to pro-inflammatory conditions, obesity, insulin resistance, and metabolic syndrom (4). Serum IL-33 levels are lower in lean people than in non-lean people and are negatively correlated with BMI and weight in lean and overweight, but that was not observed in obese people (27).

In addition, IL-33 has been found to have a negative effect on the body and is associated with a protective lipid profile. On the other hand, severe obesity is associated with increased expression of IL-33 in adipose tissue endothelial cells in both humans and mice, although the significance of this observation for endothelial function or inflammation is unclear (28). Also, IL-33 promotes adhesion of human leukocytes to human endothelial cells and induces vascular cell adhesion molecule-1, intercellular adhesion molecule-1, endothelial selectin and protein expression in endothelial cells of coronary arteries and umbilical vein and atherosclerotic plaques (29).

According to Meng Chai et al. (2017), their study did not establish significant difference in IL-37 concentrations between mild and moderate coronary artery calcification (CCA). A significant increase in the level of IL-37 was observed only in the group with severe CCA. Although the sample size was relatively small and the consequences are not yet clear, these interesting results may suggest that increased expression of IL-37 may be a result of activation of inflammation (30).

In some tissues, irisin probably acts through integrins, which are widely expressed transmembrane receptors. Irisin is a myokine that increases energy expenditure by stimulating "darkening" of white adipose tissue and plays an important physiological role in modulating energy homeostasis. The majority of contemporary investigations found a direct correlation between irisin and obesity, while, on the other hand, there are limited contrary results.
(31). Studies Korta and co-workers (32) and Perakakis and associates (8) have proved the versatile irisin functions and its favourable effects on organism homeostasis. Irisin decreases inflammatory processes, stores a balance between resorption, and modulates metabolic processes. It suppresses the expression and release of proinflammatory cytokines in obese people and reduces inflammation in adipose tissue. The levels of irisin, IL-33, and IL-37 will presumably provide new opportunities for assessing the severity of coronary heart disease with comorbid pathology, and, therefore future studies are needed for deeper analysis of these markers as criteria for the progression of cardiovascular diseases.

We indicate that the study has some limitations. The study population belongs to a group of patients limited by territory and ethnicity, and therefore our results cannot be extrapolated to all other groups of the population. The cross-sectional design did not have a possibility to establish a causal relationship between irisin and obesity and CAD. The dynamics of irisin after exercise was not assessed, but only at rest in the morning. Thus, we were unable to observe a causal relationship between irisin and physical activity. The only obese indicator BMI was measured, but without waist circumference (WC) and waist-to-hip ratio (W-t-H). Glycated hemoglobin (HbA1c) was not used in analyses. Further studies with included HbA1c, WC, and W-t-H are expected to be conducted. A small sample can also be attributed to limitations, while we consider it sufficient for statistically relevant conclusions. However, several strengths stand out. This appears to be the first study to establish irisin levels along with interleukins 33 and 37 in patients with coronary artery disease and in comorbidity with obesity. We want to emphasize the possible significance of changes in the concentration of irisin in coronary artery disease in combination with obesity. More indepth further studies are needed to find the role of irisin in the pathogenesis of obesity influence in patients with coronary artery disease.

In conclusion, in patients with ischemic heart disease without concomitant obesity, the level of irisin was reduced in comparison to the control group, but only under the influence of coexistent obesity, this diminished level was significant. The levels of interleukins 33 and 37 were increased in patients with coronary heart disease and in combination with obesity in comparison with the control group, but only in comorbidity with obesity such an increase was significant. Thus, the coexistent obesity effect has a significant influence on the levels of irisin, IL33, and IL37 concentrations in patients with ischemic heart disease. Obesity positively correlated with the serum irisin in the presented patients with coronary artery disease. The study of irisin and other myokines together with markers of inflammation will help improve the diagnosis of coronary heart disease with frequently occurred comorbid pathology - obesity.

There is no conflict of interest.

REFERENCES


