The Association of Irisin with the Progression of Diabetes Mellitus Irisin and Diabetes Progressionⁱ

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Abstract Introduction: There is an ongoing debate over the involvement of irisin in the glucose metabolism. In this study, we aimed to see the association of irisin with the markers of glucose intolerance. Materials and Methods: We analyzed the relation of irisin with metabolic markers such as Homeostatic Model Assessment-Insulin Resistance (HOMA-IR), insulin, and glucose in 152 subjects, subdivided into normal, impaired glucose tolerance and diabetic subgroups. Results: We found that the irisin levels of individuals with different stages of glucose intolerance did not show any significant difference. There was not any correlation between irisin levels with HOMA-IR. However, we observed a positive and significant correlation between circulating irisin concentrations and 120-minute glucose, 120-minute insulin, and HbA1c levels. Conclusion: The conclusive results of this study are that irisin could be somehow associated with insulin resistance but do not show a significant difference with neither diabetes nor different stages of insulin resistance.

Keywords Irisin, Insulin Resistance, Diabetes

1. Introduction

There is increasing evidence about the secretory function of skeletal muscle (1). It releases numerous cytokines or other peptides that exert local or distant specific effects. (2) These cytokines called ‘myokines’ modulate metabolic processes such as metabolic syndrome, obesity, insulin resistance and type 2 diabetes (T2D), through their endocrine effects on other organs (2, 3). Therefore, skeletal muscle is now identified as an endocrine organ.

Irisin is a novel myokine derived from fibronectin type III domain containing 5 (FNDC5, also known FRCP2 and PeP). FNDC5 is a type I transmembrane protein of myocytes. This protein is proteolytically cleaved and secreted as irisin. (4). It is reported that FNDC5 and

consequently irisin secretion is stimulated by peroxisome proliferator-activated receptor- γ coactivator-1 α (PGC1 α) (4). It is previously demonstrated that transgenic mice with mildly elevated muscle PGC1 α , are resistant to age-related obesity and diabetes and have a prolonged lifespan (5). In fact, PGC1 α acts through the irisin like substances released from skeletal muscle. Once secreted, irisin acts on the subcutaneous adipose tissue; induces browning and the expression of mitochondrial uncoupling protein-1 (UCP1), which in turn increases energy expenditure and thermogenesis. It was previously shown that brown adipose tissue modulates peripheral tissue metabolism by thermogenesis and enhance insulin sensitivity; consequently, increased brown adipose tissue (BAT) improves glucose tolerance (6). In their study, Boström and colleagues also showed that exercise caused a mild increase in blood irisin levels which increase the total body energy expenditure and obesity-linked insulin resistance (4). By these findings, it is postulated that irisin is related to exercise and involved in a variety of metabolic processes such as obesity, insulin resistance, and diabetes.

In recent years, several clinical studies, with contradicting results, have investigated the association of irisin with different metabolic situations. One of the most investigated conditions is exercise and irisin relation. However, data obtained from the molecular (mRNA induction of FNDC5/irisin) or clinical (circulation levels of irisin) human studies were controversial. Circulating irisin levels were found increased in some studies (7, 4), transiently increased in some others (8). There were also studies that found an acute increase after exercise but no change after long-term training (9). In their report on FNDC5 mRNA induction after different type of exercise, Pekkala found an increase in only one type of exercise and concluded that neither longer-term nor single exercise markedly increases skeletal muscle FNDC5 expression or serum irisin (10). Other studies did not confirm any difference before or after exercise (11, 12). Another study with gene expression arrays found an exercise-induced increase of muscle FNDC5 mRNA in older subjects but not

in younger ones (13).

The correlation between irisin and obesity is also largely investigated. In fact, recent studies showed that, besides the muscle tissue, irisin is also secreted by white adipose tissue (14, 15). A large number of studies on the relation of irisin and body mass index (BMI) revealed conflicting results; some of them found a positive correlation between irisin and BMI (7, 16-18), while others showed no (19) or even negative correlation (20, 21).

The role of irisin in diabetes or insulin resistance is also one of the popular topics of the last few years. However, several studies carried on this issue have ended up with discrepant results. Some of them found a positive effect of irisin on glucose homeostasis (16, 20, 22), some negative (13, 17, 23, 24), and some others no effect (25). There are quite a few studies reporting lower insulin concentrations in T2DM patients than those in healthy control subjects (16, 19, 22, 26). In another paper, it is reported that in obese participants (15), FNDC5 gene expression in muscle was significantly decreased in type 2 diabetes. Regarding insulin resistance, some studies reported a negative correlation with HOMA-IR (27), others positive correlation (17, 28-30).

In this present study, we intended to evaluate irisin concentration in different stages of glucose tolerance, from normal to newly diagnosed type 2 diabetes. We aim to see its relation to the markers of glucose intolerance and we expect to contribute to the ongoing debate on its involvement in the glucose metabolism.

2. Material and Methods

The study has included 152 individuals (53 male and 99 female). Patients with any comorbidity such as hepatic, renal, cardiovascular, chronic inflammatory or infectious diseases, gestational diabetes or malignancy were excluded. All participants were questioned about their daily activity and medication used. The study was approved by the ethics committee of our hospital.

A standard 75-gram oral glucose tolerance test (OGTT) was performed, and the total group was divided into five subgroups according to the results of the test. The subgroups were defined based on the American Diabetes Association (ADA) criteria for the diagnosis and classification of diabetes 2010 (31, 32); (1) normal glucose tolerance (NGT): fasting plasma glucose (FPG) < 100 mg/dl and 2 hour postprandial glucose (2hPG) < 140 mg/dl; (2) isolated impaired fasting glucose (IFG): FPG 100-126 mg/dl and 2hPG < 140 mg/dl; (3) isolated impaired glucose tolerance (IGT): FPG < 100 mg/dl and 2hPG 140-199 mg/dl; (4) combined IFG/IGT: FPG 100-125 and 2hPG 140-199 mg/dl; (5) type 2 diabetes mellitus (T2DM): FPG

≥ 126 mg/dl or 2hPG ≥ 200 mg/dl.

Blood samples obtained from the patients were centrifuged, and serum samples were stored at -80 °C until analyzed. Serum glucose values were measured by enzymatic UV test of AU 5800 hexokinase method (Beckman Coulter, Germany), triglycerides, total cholesterol, and HDL cholesterol were measured by AU 5800 enzymatic colorimetric test (Beckman Coulter, Germany). Insulin levels were analyzed by electrochemiluminescence immunoassay method by the Modular E170 analyzer (Roche Diagnostics, Switzerland). HbA1c was analyzed by the cation exchange high-performance chromatography (Variant II Turbo, Biorad, USA). Body mass index (BMI) was calculated body mass divided by the square of the body height (weight/height²) and expressed in units of kg/m². Homeostatic model assessment (HOMA-IR) is used to quantify insulin resistance and calculated as fasting serum glucose (mg/dl) x fasting serum insulin (μU/ml) / 405 and expressed as %. The Irisin assay was performed by Biovendor Elisa kit (Cat. No.: RAG018R, Czech Republic). Sensitivity (limit of detection) of the assay declared by the manufacturer is 1 ng/ml. Assay range is 0.001 μg/ml – 5 μg/ml, intraassay and interassay CV's are 4.86-8.19 μg/ml and 8.27-9.67 μg/ml, respectively.

Descriptive statistics were presented as the mean ± standard deviation (SD) and median (2.5-97.5 %) as required. The comparison of the groups was done with independent samples Student's t or Mann-Whitney U test. Correlations between irisin and the other variables were determined by Pearson's correlation analysis. The normality of the data distribution was evaluated by Kruskal-Wallis test. Multiple regression analysis was performed to determine the relation between irisin and age, gender, body mass index (BMI), fasting glucose, fasting insulin, total cholesterol, HDL cholesterol, triglycerides levels. Values of p < 0.05 were considered significant. The statistical analyses were performed with Medcalc version 15.2.2 statistical software.

3. Results

The total study group included 32 NGT, 29 IFG, 31 IGT, 31 IFG/IGT and 29 T2DM patients (aged from 19 to 78). The demographic and anthropometric characteristics and the results of the biochemical markers are displayed in Table 1. The p values < 0.05 displayed on the table meant that there was a significant difference between the means of at least two of the subgroups, according to one way analysis of variance. There was not a significant difference between groups with regard to age, HOMA-IR, total cholesterol, and HDL levels.

Table 1. Demographic and anthropometric characteristics and biochemical values of the groups. Data are shown as Mean±SD or Median (95% CI for the median) as required, and p values between groups.

	Total Group	NGT	IFG	IGT	IFG/IGT	T2DM	p values
Number	152	32	29	31	31	29	
Age (year)	46.93±11.56	43.5±12.84	47.86±9.77	47.55±12.47	48.42±10.94	47.52±11.41	0.455
BMI (kg/m²)	31.78±5.58	29.46±5.59	31.42±4.67	33.38±6.58	33.82±4.83	30.80±5.07	0.009*
Glucose 0 (mg/dl)	100.87±10.72	90.65±5.98	107.07±6.65	92.13±4.81	108.80±6.49	106.79±10.79	< 0.001*
Glucose 120 (mg/dl)	147 (65.30 to 244.70)	94 (54.90 to 134.10)	110 (61.90 to 136.875)	155 (120.95 to 194.175)	162 (143.55 to 195.725)	218 (197.225 to 279.375)	< 0.001*
Insulin 0 (μU/mL)	14 (5.00 to 36.20)	10.5 (5.30 to 40.70)	16 (6.225 to 26.325)	13 (5.00 to 48.15)	17 (5.275 to 36.80)	14 (4.125 to 30.425)	0.276
Insulin 120 (μU/mL)	84 (22.00 to 304.60)	39 (13.30 to 181.40)	69 (22.90 to 155.00)	118 (42.475 to 351.225)	110 (29.125 to 357.40)	98 (42.275 to 227.125)	< 0.001*
HOMA-IR (%)	3.565 (1.14 to 8.793)	2.33 (1.140 to 9.485)	4.15 (1.629 to 7.511)	3.08 (1.050 to 10.335)	4.52 (1.408 to 9.593)	3.66 (0.920 to 8.437)	0.012*
HbA1c (%)	5.77±0.46	5.56±0.39	5.79±0.42	5.70±0.49	5.83±0.39	5.97±0.51	0.007*
Irisin (μg/ml)	2.27 (0.958 to 5.286)	2.237 (1.173 to 5.331)	1.99 (0.831 to 4.240)	2.49 (1.011 to 4.875)	2.23 (0.738 to 5.669)	2.575 (1.140 to 5.104)	0.466
T. Cholesterol (mg/dl)	212.07±42.46	204.34±49.63	212.24±42.66	218.13±36.62	212.03±43.11	214±40.439	0.786
Triglycerides (mg/dl)	120.5 (46.60 to 390.00)	101.5 (50.80 to 375.20)	131 (38.700 to 300.775)	121 (53.025 to 538.625)	119 (45.225 to 307.275)	138 (52.875 to 745.300)	0.297
HDL (mg/dl)	47 (31.30 to 75.10)	46 (33.00 to 108.10)	47 (34.225 to 63.775)	48 (31.275 to 78.000)	48(35.00 to 69.15)	45 (26.225 to 61.775)	0.561

*p < 0.05

Besides the obvious difference between glucose, insulin, and HbA1c values, the only significant difference was found between the BMI values of the IGT and IFG/IGT groups with the NGT patients (p<0.05). Irisin levels of the five groups did not show any significant difference.

The correlation analysis revealed that irisin negatively correlated with age and positively correlated with HDL (p=0.049 and p=0.021 respectively). Partial correlation with adjustment for age showed a significant positive correlation between irisin and glucose-120, insulin-120 and HbA1c (p=0.026, p=0.35 and p=0.034 respectively) in addition to the positive correlation with HDL which remained significant (p=0.011). The correlation analysis results are given in Table 2. A further adjustment for age and BMI did not present any other differences.

Table 2. Correlation between serum irisin concentrations and the other markers levels, and partial correlation after adjustment for age.

	Correlation		Partial correlation	
	Correlation coefficient	Significance Level P	Correlation coefficient	Significance Level P
Age (year)	-0.160	0.0493*		
BMI (kg/m²)	0.011	0.892	0.021	0.802
Glucose 0 (mg/dl)	0.010	0.900	0.020	0.811
Glucose 120 (mg/dl)	0.151	0.064	0.181	0.0258*
Insulin 0 (μU/mL)	0.027	0.739	-0.009	0.908
Insulin 120 (μU/mL)	0.098	0.232	0.171	0.0353*
HOMA-IR (%)	0.031	0.706	-0.005	0.952
HbA1c (%)	0.110	0.176	0.173	0.0337*
T. Cholesterol (mg/dl)	-0.008	0.926	0.084	0.304
Triglycerides (mg/dl)	-0.097	0.234	-0.069	0.403
HDL (mg/dl)	0.187	0.0208*	0.207	0.0107*

*p < 0.05

Regression analysis revealed that only age and triglycerides (p=0.004 and p=0.015) contributed significantly to the prediction of irisin. The other variables were not found to be related to irisin according to multiple correlation coefficient or p-values (Table 3).

Table 3. Multiple regression analysis of serum irisin concentrations with the other markers levels.

Independent variables	Coefficient	Std. Error	r_{partial}	t	P
(Constant)	1.139				
Age (year)	-0.025	0.009	-0.238	-2.900	0.0043*
BMI (kg/m ²)	-0.002	0.019	-0.009	-0.104	0.917
Glucose 0 (mg/dl)	-0.011	0.019	-0.047	-0.551	0.583
Glucose 120 (mg/dl)	0.003	0.002	0.116	1.383	0.169
Insulin 0 (μU/mL)	-0.063	0.108	-0.050	-0.587	0.559
Insulin 120 (μU/mL)	0.002	0.002	0.086	1.024	0.308
HOMA-IR (%)	0.258	0.443	0.049	0.582	0.562
HbA1c (%)	0.386	0.218	0.148	1.772	0.079
T. Cholesterol (mg/dl)	-0.002	0.003	-0.058	-0.691	0.491
Triglycerides (mg/dl)	0.000	0.001	-0.014	-0.170	0.866
HDL (mg/dl)	0.024	0.010	0.204	2.466	0.0149*

4. Discussion

Even though it is a newly discovered biomarker, irisin has been extensively studied during the recent years. The evidence on the relation of the circulating irisin levels and exercise or BMI (4, 33) arose plenty of expectations. Irisin was thought to be a beneficial tool not only in diagnosis but also in the treatment of various metabolic problems. There are a quite large number of clinical researches performed on the concentration of irisin in several metabolic conditions, such as obesity, insulin resistance, or diabetes. Unfortunately, these studies did not end up in consistent results. They revealed negative, positive or null associations with age (16, 25, 28, 29), BMI (16, 17, 27,30), HOMA (17, 29,30), or T2DM (16, 20, 25, 26, 29).

The main output of this present study was that the irisin levels of individuals with different stages of glucose intolerance showed no significant difference. We did not find a difference between the irisin results of NGT and diabetes patients. There was not any correlation between irisin levels and insulin resistance index, namely HOMA-IR. On the other hand, we observed a positive and significant correlation between circulating irisin concentrations and the levels of 120-minute glucose, 120-minute insulin, and HbA1c.

Some studies performed on various populations in the recent few years claimed that irisin levels were lower in T2DM patients than normal subjects (16, 20, 26). Furthermore, very recently, Duran et al., have observed lower circulating irisin levels in type 2 diabetes patients. They also have found that a significant decrease in irisin levels begins at the level of both IFG + IGT and gradually decrease with progression of glucose intolerance (19). Our data did not support these reports. However, the results of our study were consistent with other studies claiming that irisin levels were not associated with T2DM (25, 29, 34). We suppose that these discrepancies were partly due to the

diverse study populations concerning the age, gender, body composition, activity, etc. Additionally, the effect of methodological factors should be kept in mind, because of the not fully validated enzyme immunoassays kits, the poor standardization of the methods, varying target epitopes among manufacturers and, possible presence cross-reacting proteins. (35, 36) The method problems also could explain the marked difference between the circulating irisin levels in the mentioned reports, from 0.204 μg/ml to 2.83 μg/mL (16, 19).

The association of irisin secretion with insulin resistance has also been examined by various researchers last years. Most of the studies reported a positive correlation between irisin and HOMA values as an insulin resistance marker (17, 29, 30). In this present study, we could not find an association between irisin and HOMA-IR values. However, we observed a positive correlation between irisin concentrations and 120-minute insulin and 120-minute glucose. There are also controversial results on this subject. The data of Boström et al. on high-fat fed mice, illustrated that even moderately increased levels of circulating irisin potentially increases energy expenditure, and improves diet-induced insulin resistance (4). On the other hand, in their study of single nucleotide polymorphisms (SNPs), Staiger et al. reported that a common genetic variation in the human FNDC5 locus, encoding the irisin precursor, determines insulin sensitivity and revealed a negative association between FNDC5 expression and in vivo measures of insulin sensitivity (24). They postulated that the biological functions of irisin might differ in mice and humans. Our data support the findings of the latter and some other clinical reports that claimed a positive correlation between irisin and insulin levels (17, 18, 28-30). Further studies are recommended to elucidate whether irisin is an adaptive response to counteract the disturbances in metabolic homeostasis or irisin levels represent a promotor of such disturbances (35). The potential

resistance to irisin in obesity leading to hyperinsulinemia and insulin resistance should also be considered on this issue.

Our study did not reveal a significant correlation between irisin and BMI, in concordance with some authors (25, 29). However, there is a pretty much evidence of the positive correlation between irisin and BMI (16, 17, 27, 30). Crujeiras et al. hypothesized that adipose tissue may play a role in determining circulating FNDC5/irisin levels in co-operation with muscle and that the muscle/adipose secretion ratio is affected by the pathophysiological situation (37). This could be an explanation for the inconsistent results. On this subject, Sanchis et al. suggested further studies to enlighten the issues such as; [1] irisin levels associated with BMI, reflecting primarily muscle or fat mass, [2] irisin levels are related to other biological variations, [3] the differences observed only reflect inter-population or methodological variations and/or assay discrepancies (38).

The irisin levels of our population showed a negative correlation with age. This result was consistent with Ebert's report (29) but not with some others which found no correlation (25, 28) or a positive correlation (16). We speculate that this discrepancy could be attributed to population or method variations.

The association of irisin values with lipid profile was studied in different populations, but the results were controversial. Some studies found a positive association between irisin levels and beneficial lipid profile. As an example, in one of the largest studies, Oelmann et al. observed a significantly inverse association between irisin concentration and total and LDL cholesterol in male subjects (40). Also, most of the studies have found positive correlation between irisin and HDL (29, 41, 42, 43, 44). However, not all studies gave consistent results with total and LDL cholesterol and triglycerides. The relation of irisin with total and LDL cholesterol was positive in some studies (16, 29, 42) and negative in some others (40, 43, 44). Similarly, some studies showed a positive correlation (16, 42) between irisin and triglycerides, some others showed a negative one (29, 43, 44). We suggest that these discrepancies arise from the size and the type of the populations selected, the comorbidities which affects the results. As seen in Table 2, we found a weak negative correlation between irisin and total cholesterol and triglycerides, and a weak positive correlation between irisin and HDL, which supported positive association between irisin levels and beneficial lipid profile. However, the significance level was very low. As the study population was neither large enough, nor was selected for lipid profile evaluation, we presumed that our results could not be a contribution to the literature.

5. Study Limitations

The study had a couple limitations. First of all, it could

be based on a larger population that would render the statistical analysis more reliable. Second, the activity status was based on the statements of the subjects instead of a measurable tool.

6. Conclusions

The conclusive results of this study are that irisin is somehow associated with insulin resistance but does not show a significant difference with diabetes or different stages of insulin resistance. We suppose that the association of irisin with the metabolic status, although considerably examined so far, needs further researches since the results are still conflicting. As proposed by Novelle et al., more studies are necessary on the precise roles of different forms of FNDC5/irisin and its receptor and the signaling pathway which will allow a better understanding of irisin function (39). We modestly propose that the elimination of the methodological problems, the definition of the biological variation affecting circulating irisin levels, large population-based molecular or clinical studies on irisin function and its association with metabolic processes could help to solve the issues. Irisin, which is a promising molecule, would then be a diagnostic or even therapeutic tool in metabolic disturbances.

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i The authors have no conflict of interest to declare.