Clin. Lab. 2017;63:XXX-XXX

ORIGINAL ARTICLE

The Levels of Serum Irisin as a Predictor of Insulin Resistance in Han Chinese Adults with Metabolically Healthy Obesity

Bo-Wei Liu [1], Fu-Zai Yin [1], Xi-Ming Qi [2], Dong-Mei Fan [1], Yue Zhang [1]

[1] *Department of Endocrinology, The First Hospital of Qinhuangdao, Hebei Medical University, Hebei Province, China*
[2] *Central Laboratory, The First Hospital of Qinhuangdao, Hebei Medical University, Hebei Province, China*

SUMMARY

Background: **The aim of this study was to evaluate serum irisin levels and analyze its related factors in Han adults with metabolically healthy obesity.**
Methods: **This cross-sectional study included 75 metabolically healthy, non-obese adults and 51 metabolically healthy, obese adults. Anthropometric measurements, including height, weight, waist circumference (WC), and blood pressure, were performed. All patients underwent an oral glucose tolerance test (OGTT) after 8 hours of fasting, and the levels of glucose, insulin, lipids, and serum irisin were measured.**
Results: **The levels of serum irisin (5.40 ± 1.69 vs. 6.46 ± 1.37 μg/mL) were significantly lower in the metabolically healthy obese group ($p < 0.05$). Irisin correlated positively with high density lipoprotein cholesterol (HDL-C) ($r = 0.303$) and correlated negatively with body mass index (BMI) ($r = -0.389$), WC ($r = -0.324$), fasting plasma glucose (FPG) ($r = -0.441$), HOMA-IR ($r = -0.429$), triglycerides (TG) ($r = -0.185$), total cholesterol (TC) ($r = -0.209$), low density lipoprotein cholesterol (LDL-C) ($r = -0.157$) ($p < 0.05$). Multiple regression analysis revealed that FPG ($\beta = -1.720$, $p = 0.001$) and HOMA-IR ($\beta = -0.399$, $p = 0.006$) were still significantly associated with irisin. Serum irisin ($\beta = -0.246$, $p = 0.005$) and BMI ($\beta = 0.078$, $p = 0.043$) were significant independent predictors for HOMA-IR.**
Conclusions: **Serum irisin levels were reduced in metabolically healthy, obese Han adults. Irisin reduction appears to be associated with elevated FPG and insulin resistance but not obesity. In additional, falling irisin may increase the occurrence of insulin resistance in metabolically healthy Han adults and should be examined in future studies.**
(Clin. Lab. 2017;63:xx-xx. DOI: 10.7754/Clin.Lab.2016.160805)

Correspondence:
Fu-Zai Yin
Department of Endocrinology
The First Hospital of Qinhuangdao
Hebei Medical University
No. 258 Wenhua Road
Qinhuangdao, 066000, Hebei Province
China
Phone: +86 335-5908604
Fax: +86 335-3032042
Email: yinfu_zai@163.com

Manuscript accepted December 19, 2016

KEY WORDS

irisin, metabolically healthy obesity, insulin resistance, body mass index

INTRODUCTION

Obesity is closely associated with cardiovascular diseases (CVD) and type 2 diabetes mellitus (T2DM) [1, 2]. Both obesity and T2DM share common pathogenetic mechanisms as components of insulin resistance (IR) syndrome. In this regard, obesity is a risk factor for T2DM and CVD. However, some obese individuals, despite having excessive body fat, exhibit metabolic health that is comparable with that of lean individuals. The 'healthy obese' phenotype was described in 1982

[3]. Recently, research has shown that metabolically healthy obese adults show a substantially increased risk of developing T2DM compared with metabolically healthy normal-weight adults [4]. Prospective evidence does not indicate that healthy obesity is a harmless condition.

Irisin is an exercise-induced myokine possibly leading to the browning of white adipose tissue, thereby increasing energy expenditure and improving systemic metabolism [5]. Circulating irisin was found to be significantly reduced in long-term [6], new onset [7], and undefined [8] T2DM patients compared with nondiabetic controls, which suggested either the diabetic state itself or the metabolic condition that caused progression to T2DM is accompanied by lower circulating irisin [9]. Recently, available evidence about the effect of adiposity on circulating irisin has been controversial [6,8,10]. Therefore, further studies are warranted to address this discrepancy of the effect of obesity on circulating irisin. In this study, we aimed to evaluate the circulating irisin levels and examine the independent effect of serum irisin on metabolically healthy adults.

MATERIALS AND METHODS

Study design

We performed a cross-sectional study in Chinese adults with metabolically healthy obesity. All subjects were of the Han ethnicity, and each underwent an oral glucose tolerance test (OGTT) with 75 g of oral anhydrous glucose. The inclusion criteria included the following: 1) subjects were clinically stable with no previous medical history of diabetes, hypertension, dyslipidemia, coronary artery diseases, or cerebral stroke; 2) subjects without clinical evidence of endocrinopathy, 3) subjects were not taking medications known to affect glucose and lipid metabolism, such as statins, glucocorticoids, thyroid hormones, and thiazide diuretics, and 4) subjects did light manual labor, for nearly three months without active weight loss exercise. The exclusion criteria included the following: 1) subjects with hepatic or renal dysfunction (> 1.5-fold elevation of alanine aminotransferase, aspartate aminotransferase, or serum creatinine > 115 μmol/L) and 2) subjects with acute and chronic inflammation. This study was approved by the ethics committee of the First Hospital of Qinhuangdao. All subjects provided written informed consent before study initiation.

Cases and controls

We enrolled 51 metabolically healthy, obese adults and 75 metabolically healthy, non-obese adults with who had gone to the First Hospital of Qinhuangdao for health examinations during 2014. Obesity was defined as waist circumference (WC) ≥ 90 cm in men and ≥ 85 cm in women. Metabolically healthy status was defined as none of the following metabolic risk factors: 1) hypertension (clinic BP > 130/85 mmHg, or hypertension diagnosis); 2) impaired glycaemic control (fasting plasma glucose (FPG) levels that were ≥ 6.1 mmol/L and/or 2-h plasma glucose (2-h PG) levels that were ≥ 7.8 mmol/L after a 75-g OGTT); 3) adverse high density lipoprotein cholesterol (HDL-C) < 1.03 mmol/L in men and < 1.30 mmol/L in women) and adverse triglycerides (TG) (≥ 1.7 mmol/L) [11].

Anthropometric measurements

Anthropometric measurements, including height, weight, WC, and blood pressure, were obtained while the subjects were in light clothing and not wearing shoes. Body mass index (BMI) was calculated by dividing weight (kg) by height squared (m^2). Blood pressure was measured twice with a mercury sphygmomanometer after 10 minutes of rest while the subjects were seated, and the average of the two measurements was used for analysis.

Laboratory examinations

All subjects underwent OGTT with 75 g of oral anhydrous glucose at 8:00 AM after 8 hours of fasting. 75 g anhydrous glucose was dissolved in 250 mL water. Peripheral venous blood samples were taken at 0 and 120 minutes after glucose loading. Plasma glucose concentration was measured using the glucose oxidase method and serum lipids were measured using enzymatic procedures with an autoanalyzer (Hitachi, Tokyo, Japan). C-reactive protein (CRP) was measured using scattering turbidimetry with IMMAGE (USA). Serum irisin levels were determined using a commercially available human ELISA kit (Bio Vision, Milpitas, CA 95035 USA). The sensitivity of the assay was 0.2 μg/mL. The intra and inter-assay variations were both less than 10%. The ELISA kits of insulin were purchased from USCNLIFE company, USA. Insulin and serum irisin were measured using an enzyme linked immunosorbent assay (ELISA) with a model 680 microplate reader (BIO-RAD, USA). The following equation for homeostasis model assessment of insulin resistance (HOMA-IR) was used: fasting insulin level (μU/mL) x fasting glucose level (mmol/L)/22.5.

Statistical analysis

Data are expressed as mean ± standard deviation (SD) or medians with interquartile ranges (IQR). When data was not normally distributed, they were log transformed for analysis. Comparisons were conducted between groups using the *t*-test. The χ^2 test was used to test for differences in proportions. To measure the strength of association between 2 variables, Pearson's correlation coefficient was used. To examine the association between irisin and other variables, multiple linear regression analysis was used. Analyses were performed with the computer software SPSS version 11.5 software (SPSS Inc., Chicago, IL, U.S.A.). Statistical significance was established at $p < 0.05$.

Table 1. Clinical and laboratory characteristics of the subjects in different groups.

Variable	Obesity group (n = 51)	Non-obese group (n = 75)	*t* or χ^2	p
Age (years) mean (SD)	35.20 (6.51)	35.01 (7.09)	-0.147	0.883
Gender (male/female)	26/25	34/41	8.034	0.067
BMI (kg/m^2) mean (SD)	27.80 (3.04)	22.62 (2.52)	-10.419	0.000
WC (cm) mean (SD)	93.95 (8.02)	76.24 (5.67)	-11.987	0.000
SBP (mmHg) mean (SD)	116.37 (8.82)	111.24 (9.97)	-2.969	0.004
DBP (mmHg) mean (SD)	76.20 (6.04)	72.69 (7.31)	-2.931	0.004
FPG (mmol/L) mean (SD)	5.38 (0.27)	5.12 (0.32)	-3.344	0.001
PPG-2h (mmol/L) mean (SD)	6.67 (2.37)	5.79 (1.38)	-1.999	0.049
lgINS (μIU/mL) (SD)	1.02 (0.15)	0.93 (0.13)	-3.310	0.001
TG (mmol/L) median (IQR)	1.08 (0.37)	0.83 (0.27)	-3.645	0.001
TC (mmol/L) mean (SD)	5.11 (0.79)	4.73 (0.62)	-3.092	0.003
HDL-C (mmol/L) mean (SD)	1.37 (0.26)	1.57 (0.23)	4.651	0.000
LDL-C (mmol/L) mean (SD)	3.22 (0.87)	2.60 (0.67)	-4.512	0.000
HOMA-IR mean (SD)	2.65 (1.06)	2.10 (0.93)	-3.074	0.003
CRP (mg/dL) mean (SD)	0.41(0.12)	0.38 (0.14)	-0.379	0.772
Muscle content (g/cm^2) mean (SD)	49171.94 (10842.51)	39370.40 (7326.97)	5.981	0.016
Fat content (g/cm^2) mean (SD)	29006.72 (5511.59)	20431.16 (5699.45)	0.010	0.919
Irisin mean (SD)	5.40 (1.69)	6.46 (1.37)	3.732	0.000

Data are expressed as mean ± standard deviation (SD) or medians with interquartile ranges (IQR). When data was not normally distributed, it was log transformed for analysis. BMI - body mass index, WC - waist circumference, SBP - systolic blood press, DBP - diastolic blood press, FPG - fasting plasma glucose, PPG-2h - postprandial plasma glucose, INS - fasting insulin, TG - triglycerides, TC - cholesterol, HDL-C - high density lipoprotein cholesterol, LDL-C - low density lipoprotein cholesterol, HOMA-IR - homeostasis model assessment of insulin resistance, CRP - C-reactive protein, SD - standard deviation, IQR - indicates interquartile range.

Table 2. Simple correlations between the serum irisin and other variables in the study subjects.

Variable	r	p
Age (years)	-0.013	0.881
BMI (kg/m^2)	-0.389	0.000
WC (cm)	-0.324	0.000
SBP (mmHg)	-0.151	0.091
DBP (mmHg)	0.037	0.679
FPG (mmol/L)	-0.441	0.000
PPG-2h	-0.073	0.539
IgINS (μIU/mL)	-0.376	0.000
TG (mmol/L)	-0.185	0.038
TC (mmol/L)	-0.209	0.019
HDL-C (mmol/L)	0.303	0.001
LDL-C (mmol/L)	-0.157	0.078
HOMA-IR	-0.429	0.000

BMI - body mass index, WC - waist circumference, SBP - systolic blood press, DBP - diastolic blood press, FPG - fasting plasma glucose, PPG-2h - postprandial plasma glucose, INS - fasting insulin, TG - triglycerides, TC - total cholesterol, HDL-C - high density lipoprotein cholesterol, LDL-C - low density lipoprotein cholesterol, HOMA-IR - homeostasis model assessment of insulin resistance.

Table 3. Multiple linear regression analyses for serum irisin (Stepwise Method).

Model	Unstandardized coefficients B	Std. error	Standardized coefficients B	t	p	95% CI	R^2
(Constant)	16.148	2.510		6.433	0.000	11.142 to 21.155	
FPG	-1.720	0.493	-0.368	-3.490	0.001	-2.702 to -0.737	0.206
HOMA-IR	-0.399	0.141	-0.299	-2.836	0.006	-0.679 to -0.118	0.287

Dependent variable: serum irisin.
Independent variables: Gender, Age, BMI, WC, SBP, DBP, FPG, PPG-2h, TG, HDL-C, HOMA-IR.

Table 4. Multiple linear regression analyses for homeostasis model assessment of insulin resistance (HOMA-IR) (Stepwise Method).

Model	Unstandardized coefficients B	Std. error	Standardized coefficients B	t	p	95% CI	R^2
(Constant)	1.939	1.214		1.597	0.115	-0.483 to 4.360	
Irisin	-0.246	0.084	-0.329	-2.927	0.005	-0.414 to -0.078	0.163
BMI	0.078	0.038	0.232	2.064	0.043	0.003 to 0.154	0.211

Dependent variable: HOMA-IR.
Independent variables: Gender, Age, BMI, WC, SBP, DBP, FPG, PPG-2h, TG, HDL-C, serum irisin.

RESULTS

The age, gender, C-reactive protein (CRP), and fat content were similar in the two groups ($p > 0.05$). Table 1 showed clinical and laboratory characteristics in the study subjects. Subjects in the obesity group had higher BMI, WC, systolic blood press (SBP), diastolic blood press (DBP), FPG, 2-h PG, TG, cholesterol (TC), low density lipoprotein cholesterol (LDL-C), HOMA-IR, muscle content, and lower HDL-C than subjects in the non-obese group ($p < 0.05$). The levels of serum irisin (5.40 ± 1.69 vs. 6.46 ± 1.37 μg/mL) were significantly lower in the metabolically healthy, obese group ($p < 0.05$).

Serum irisin levels positively correlated with HDL-C ($r = 0.303$) and negatively correlated with BMI ($r = -0.389$), WC ($r = -0.324$), FPG ($r = -0.441$), HOMA-IR ($r = -0.429$), TG ($r = -0.185$), TC ($r = -0.209$), LDL-C ($r = -0.157$) ($p < 0.05$) (Table 2). When irisin was considered as the dependent variable in a multiple regression analysis with age, gender, BMI, WC, SBP, DBP, TG, TC, HDL-C, FPG, and 2-h PG and HOMA-IR as independent variables, FPG ($\beta = -1.720$, $p = 0.001$) and HOMA-IR ($\beta = -0.399$, $p = 0.006$) were still significantly associated with irisin (Table 3). When HOMA-IR was considered as the dependent variable in a multiple regression analysis with age, gender, BMI, WC, SBP, DBP, TG, HDL-C, FPG, and 2-h PG and irisin as the independent variable, irisin ($\beta = -0.246$, $p = 0.005$) and BMI ($\beta = 0.078$, $p = 0.043$) maintained an independent association with HOMA-IR (Table 4).

DISCUSSION

In the present study, we choose to use waist circumference (WC) as the obesity group standard. Because WC reflects a measure of central fat distribution, while BMI reflects a combination of both fat mass and lean mass, some researchers have argued that WC is a better indicator of an adverse metabolic profile than BMI [12,13]. We found that the serum irisin level was reduced in Han young adults with metabolically healthy obesity. In addition, the subjects in the obesity group had higher BMI, SBP, DBP, FPG, 2-h PG, TG, TC, LDL-C, HOMA-IR, muscle content, and lower HDL-C than subjects in the non-obese group. Although we ruled out the factor of metabolic abnormalities, obesity still plays an important role in metabolic disorder. It is well known that not all obese individuals develop metabolic disease. In fact, the presence of obesity-related metabolic disturbances varies widely among obese individuals. We were trying to determine whether serum irisin levels correlate with other commonly used biochemical parameters in clinical medicine. In our study, there was a negative correlation between serum irisin levels and BMI, WC, FPG, HOMA-IR, TG, TC, LDL-C, and a positive correlation with HDL-C.

Multiple linear regression analysis showed that FPG and HOMA-IR were significant independent predictors for serum irisin levels. In additional, irisin maintained an independent association with HOMA-IR, which explained 16.3% of the total variance. Our results further illustrated that serum irisin reduction appears to be associated with elevated FPG and insulin resistance but not obesity. Reduced serum irisin levels may increase the occurrence of insulin resistance in metabolically healthy Han adults. Our findings provided us with new evidence that serum irisin plays an important role in the pathogenesis of insulin resistance; it may predict the occurrence of insulin resistance in metabolically healthy Chinese adults.

Bostrom et al. reported that the expression of the exercise- and PGC1-a-induced myokine, irisin, drives brown fat-like development of white fat and protects diet-induced obesity and diabetes in mouse models. They also reported that irisin increased total energy expenditure, improved glucose tolerance, and reduced fasting insulin in animal models [14]. Circulating irisin has been found to be reduced in T2DM patients compared with non-diabetic controls [6-8]. Because T2DM and obesity share the same pathology of insulin resistance, it is thus reasonable to speculate that obese patients have lower serum irisin. The present study found that serum irisin levels were significantly decreased in adults with metabolically healthy obesity. At the same time, we also found that the levels of serum irisin were connected with insulin resistance. Of course, the exact mechanism of the association between serum irisin and insulin resistance remains to be clarified. A recent study reported that an increase of serum irisin was associated with reduced risks of raised FPG and metabolic syndrome (MetS) in Chinese population [15]. It implies that irisin may play an important role in insulin resistance and MetS.

Available evidence about the effects of adiposity and muscle mass on circulating irisin has been controversial. Several studies have shown that irisin was not related to BMI and WC [16,17]. The correlation of irisin and BMI were not clear in different studies [6-8,10]. Yan B et al. reported that WC was negatively associated with serum irisin with marginal statistical significance after multiple regression analysis, while the negative association of circulating irisin with BMI was not statistically significant [15]. Reinehr T et al. reported that irisin levels are related to pubertal stage and insulin resistance but not to weight status in childhood [18]. However, our results were different from the above research. We found that BMI and WC were negatively associated with serum irisin; it was not statistically significant after multiple regression analysis.

There were some limitations in our study. First, it only included adults of the Han ethnicity, limiting the ability to apply to other ethnic groups. The second concerns the cross-sectional design, which precludes the establishment of a causation between events. The third was the low number of subjects included on each group that can decrease the power of the statistical analysis performed. The fourth, our CRP test was not high-sensitive method, it has the potential to interfere with the definition of metabolically healthy. In addition, data on other confounding factors such as diet and physical activity were not considered in this study.

CONCLUSION

The present study demonstrates that serum irisin levels were reduced in Chinese adults with metabolically healthy obesity. Serum irisin reduction appears to be associated with elevated FPG and insulin resistance but not obesity. In addition, falling irisin may increase the occurrence of insulin resistance in metabolically healthy Han Chinese adults. It suggested that irisin may predict the occurrence of insulin resistance and should be examined in future studies.

Acknowledgement:
This study was self-financed.

Declaration of Interest:
The authors state that there are no conflicts of interest in the publication of this article.

References:

1. Vazquez G, Duval S, Jacobs DR Jr, Silventoinen K. Comparison of body mass index, waist circumference, and waist/hip ratio in predicting incident diabetes: a meta-analysis. Epidemiol Rev 2007;29:115-28 (PMID: 17494056).

2. Bouchi R, Minami I, Ohara N, et al. Impact of increased visceral adiposity with normal weight on the progression of arterial stiffness in Japanese patients with type 2 diabetes. BMJ Open Diabetes Res Care 2015 Mar 10;3(1):e000081 (PMID: 25806115).

3. Sims EAH. Characterization of the syndromes of obesity. In: Brodoff BN, Bleicher SJ (eds). Diabetes Mellitus and Obesity. Williams & Wilkins: Baltimore, MD, 1982, pp. 219-26.

4. Bell JA, Kivimaki M, Hamer M. Metabolically healthy obesity and risk of incident type 2 diabetes: a meta-analysis of prospective cohort studies. Obes Rev 2014;15:504-15 (PMID: 24661566).

5. Polyzos SA, Kountouras J, Shields K, Mantzoros CS. Irisin: a renaissance in metabolism? Metabolism 2013;62:1037-44 (PMID: 23664085).

6. Liu JJ, Wong MD, Toy WC, et al. Lower circulating irisin is associated with type 2 diabetes mellitus. J Diabetes Complications 2013;27:365-9 (PMID: 23619195).

7. Choi YK, Kim MK, Bae KH, et al. Serum irisin levels in new-onset type 2 diabetes. Diabetes Res Clin Pract 2013;100:96-101 (PMID: 23369227).

8. Moreno-Navarrete JM, Ortega F, Serrano M, et al. Irisin is expressed and produced by human muscle and adipose tissue in association with obesity and insulin resistance. J Clin Endocrinol Metab 2013;98:E769-78 (PMID: 23436919).

9. Hojlund K, Bostrom P. Irisin in obesity and type 2 diabetes. J Diabetes Complications 2013;27:303-4 (PMID: 23659776).

10. Stengel A, Hofmann T, Goebel-Stengel M, Elbelt U, Kobelt P, Klapp BF. Circulating levels of irisin in patients with anorexia nervosa and different stages of obesity-correlation with body mass index. Peptides 2013;39:125-30 (PMID: 23219488).

11. Bell JA, Kivimaki M, Hamer M. Hamer. Metabolically healthy obesity and risk of incident type 2 diabetes: a meta-analysis of prospective cohort studies. Obes Rev 2014;15:504-515 (PMID: 24661566).

12. Klein S, Allison DB, Heymsfield SB, et al. Waist circumference and cardiometabolic risk: a consensus statement from Shaping America's Health: Association for Weight Management and Obesity Prevention; NAASO, The Obesity Society; the American Society for Nutrition; and the American Diabetes Association. Obesity (Silver Spring) 2007 May;15(5):1061-7 (PMID: 17495180).

13. Czernichow S, Kengne AP, Stamatakis E, Hamer M, Batty GD. Body mass index, waist circumference and waist-hip ratio: which is the better discriminator of cardiovascular disease mortality risk? Evidence from an individual-participant meta-analysis of 82 864 participants from nine cohort studies. Obes Rev 2011;12: 680-7 (PMID: 21521449).

14. Bostrom P, Wu J, Jedrychowski MP, et al. A PGC1-adependent myokine that drives brown-fat-like development of white fat and thermogenesis. Nature 2012;481:463-8 (PMID: 22237023).

15. Yan B, Shi X, Zhang H, et al. Association of serum irisin with metabolic syndrome in obese Chinese adults. PLoS One 2014; 9:e94235 (PMID: 24709991).

16. Timmons JA, Baar K, Davidsen PK, Atherton PJ. Is irisin a human exercise gene? Nature 2012;488:E9- E10 (PMID: 22932392).

17. Sanchis-Gomar F, Alis R, Pareja-Galeano H, et al. Circulating irisin levels are not correlated with BMI, age, and other biological parameters in obese and diabetic patients. Endocrine 2014;46: 674-7 (PMID: 24510629).

18. Reinehr T, Elfers C, Lass N, Roth CL. Irisin and its relation to insulin resistance and puberty in obese children: a longitudinal analysis. J Clin Endocrinol Metab 2015;100:2123-30 (PMID: 25781361).