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Lower circulating irisin is associated with type 2 diabetes mellitus ☆,☆☆

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ABSTRACT

Aims: Irisin is a novel myokine secreted in response to PPAR-γ co-activator-1α (PGC-1α) activation. Earlier studies suggested that PGC-1α expression and activity were lower in myocytes in type 2 diabetes mellitus (T2DM). Therefore, we hypothesize that circulating irisin levels are lower in T2DM patients.
Methods: In this observational study, we recruited 96 T2DM subjects and 60 non-diabetic control subjects. Among T2DM subjects, 38% were on insulin treatment, 78% were taking statins and 72% were taking renin-angiotensin system antagonists. Circulating irisin was quantified by ELISA and its association with markers of metabolic phenotype was analyzed by Pearson bivariate correlation and multiple linear regression.
Results: Circulating irisin was significantly lower in individuals with T2DM compared with non-diabetic controls (T2DM 204 ± 72 ng/ml vs. non-diabetic control 257 ± 24 ng/ml, $p < 0.0001$). In non-diabetic subjects, circulating irisin was correlated with age ($r = 0.398$, $p < 0.01$), BMI ($r = 0.387$, $p < 0.01$), total cholesterol ($r = 0.341$, $p < 0.01$), total triglycerides ($r = 0.299$, $p < 0.05$), fasting blood glucose ($r = 0.430$, $p < 0.01$) and diastolic blood pressure ($r = 0.306$, $p < 0.05$). Multiple linear regression model revealed that BMI ($\beta = 0.407$, $p = 0.012$) and FBG ($\beta = 0.315$, $p = 0.034$) were associated with irisin in non-diabetic subjects after adjusting for multiple co-variates. However, similar analysis in T2DM subjects didn't reveal significant association between circulating irisin and major markers of metabolic phenotype.
Conclusions: Circulating irisin is lower in T2DM compared with non-diabetic controls. Plasma irisin levels appear to be associated with important metabolic factors in non-diabetic subjects but not in individuals with type 2 diabetes.

1. Introduction

Muscle has been thought to be an important secretory organ since many years. Cytokines and other peptides produced and secreted by myocytes are classified as "myokines" (Pedersen et al., 2003). Bioinformatics modeling and proteomics studies suggest that myocytes may secrete up to hundreds of putative myokines (Bortoluzzi, Scannapieco, Cestaro, Danieli, & Schiaffino, 2006; Henningsen, Rigbolt, Blagoev, Pedersen, & Kratchmarova, 2010). These myokines work as endocrine hormones and regulate functions of distant organs.

Sedentary lifestyle is a major risk factor for type 2 diabetes mellitus (T2DM). Randomized controlled trials have demonstrated that physical activity interventions improve glucose tolerance and reduce the risk of T2DM in those with a high risk of the disease, even without obvious body weight change (Gillies et al., 2007; Laaksonen et al., 2005). Therefore, it has been speculated for a long time that physical exercise may exert its beneficial effects on energy metabolism through secreted factors from myocytes (Pedersen & Febbraio, 2012).

Peroxisome proliferator-activated receptor gamma coactivator-1-alpha (PGC-1α) is a versatile transcription cofactor which can be induced by various nutritional and physiological cues and involves in glucose/fatty acid metabolism, mitochondrial function and mitochondria biogenesis (Charos et al., 2012). Exercise, especially chronic exercise, increases PGC-1α expression in muscles while sedentary life style and T2DM are associated with reduced expression of PGC-1α (Handschin & Spiegelman, 2008). Prompted by the finding that transgenic mice expressing PGC-1α selectively in muscle showed remarkable resistance to age-related obesity and metabolic disorders, Bostrom et al. recently reported that membrane protein fibronectin type III domain containing 5 (FNDC5) is stimulated by PGC-1α

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expression in myocytes (Bostrom et al., 2012; Wenz, Rossi, Rotundo, Spiegelman, & Moraes, 2009). FNDC5 expressed on plasma membrane of myocytes can be cleaved and secreted as a novel myokine termed as irisin. Further studies revealed that irisin induced browning of subcutaneous adipocytes in vitro and in vivo and protected diet-induced obesity and diabetes in mouse models (Bostrom et al., 2012). Whether or not these findings can be translated into humans has important implications in prevention and treatment of metabolic disorders including T2DM (Cunha, 2012; Kelly, 2012; Sanchis-Gomar, Lippi, Mayero, Perez-Quilis, & Garcia-Gimenez, 2012; Timmons, Baar, Davidsen, & Atherton, 2012; Villarroya, 2012).

PGC-1α is strongly expressed in human skeletal muscles and can be induced by endurance training. Weighted evidences support that dysregulation of PGC-1α involves in pathogenesis of type 2 diabetes (Russell et al., 2003; Soyal, Krempler, Oberkofler, & Patsch, 2006). Earlier studies found that expression of PGC-1α and its activities were reduced in skeletal muscles in T2DM (Mootha et al., 2003; Patti et al., 2003; Petersen, Dufour, Befroy, Garcia, & Shulman, 2004). These findings prompt us to hypothesize that circulating irisin may be lower in T2DM patients, presumably due to lower PGC-1α activity in skeletal muscles.

2. Subjects and methods

2.1. Subjects

Diagnosis of T2DM was based on ADA (2006). T2DM subjects were recruited consecutively from the diabetes clinic in a single secondary hospital in Singapore. Non-diabetic controls were recruited from a health screening program among healthcare workers from the same hospital based on strict exclusion criteria. Subjects with active cancer, acute or chronic infection, laboratory evidence of kidney or liver disease, chronic vascular diseases (stroke, coronary artery disease and peripheral arterial diseases) and those on anti-hyperglycemia, anti-hypertension and lipids-lowering medications were all excluded from the control group. In consideration that the relationship between impaired fasting glucose (IFG) and circulating irisin was unknown, we did not exclude subjects with IFG from our non-diabetic control group. The study complied with principles laid down by Declaration of Helsinki and it was approved by our institution's domain specific ethics review board. Written informed consent was obtained from all participants.

2.2. Biochemical measurements

Fasting plasma glucose (FBG), total cholesterol (TC), high density lipoprotein cholesterol (HDL-C) and triglyceride (TG) were measured by enzymatic methods. Plasma insulin was measured by electro-chemiluminescence immunoassay (Roche Cobas C system). Total adiponectin was quantified by ELISA (R&D Systems, Minneapolis, MN) and high sensitivity C-reactive protein (hsCRP) was assayed by particle enhanced immuno-turbidimetric assay.

Circulating irisin was quantified using a commercial ELISA kit (USCN Life Science Inc, Wuhan, China). The assay is based on a rabbit anti-human irisin polyclonal antibody conjugated with biotin. Circulating irisin in the plasma is incubated with the antibody pre-coated at the bottom of ELISA plates. Plasma irisin bound with irisin polyclonal antibody is detected by secondary antibody conjugated with horseradish peroxidase. The polyclonal antibody used in this assay is generated against a fragment of human FNDC5 protein expressed in E. Coli (amino acid 30 to 150). The amino acid sequence used for antibody generation aligns with the full length of human endogenous irisin. Plasma was diluted 1:10,000 as recommended by the manufacturer immediately before the assay. Assessment performed in our own lab showed that the recovery rate was 103% at the recommended plasma dilution and the linearity-of-dilution evaluation showed good linearity in serial plasma dilution (1:5000, 1:10,000; 1:20,000 and 1:40,000 dilution in duplicates, $R^2 = 0.969$). The intra-assay coefficients of variability (CV) and inter-assay CVs reported by the manufacturer were less than 10% and 12%, respectively. The sensitivity of the assay kit reported by the manufacturer was 6.36 pg/ml. We calculated the sensitivity of the assay ourselves by the formula: sensitivity = background mean/(low standard mean − background mean) × low standard mean + 2 × standard deviation. The sensitivity of the assay kit used in this study was 13.61 pg/ml. All the plasma samples were coded. All ELISAs were performed by one researcher to ensure technical consistency. The researcher who performed the ELISA assays did not know the identity and grouping of the subjects.

2.3. Statistical analysis

Continuous variables were expressed as mean ± SD. Urine albumin creatinine ratio (ACR) was log-transformed before data analysis. Student's t test was employed to compare the means of normally distributed continuous variables between two groups. Categorical data were expressed as proportion and compared by χ^2 test. General linear model was also used to adjust for age and gender when we compared the difference in plasma irisin levels between T2DM and non-diabetic controls. We also employed general linear model to further address the potential confounding of BMI in comparison of irisin levels between diabetic subjects and non-diabetic controls. In this model, plasma irisin was entered as dependent variable and both BMI and grouping of subjects (non-diabetic = 0 and type 2 diabetes = 1) were entered as covariates. Pearson bivariate correlation analysis was performed to study the correlation between two continuous variables. Multiple linear regression models were employed to identify variables which were independently associated with variations in irisin concentrations. $p < 0.05$ was considered as statistically significant.

3. Results

The baseline characteristics and biochemical profiles of the subjects were described in Table 1. Subjects with T2DM were older, had higher BMI, higher blood pressure, higher total plasma

Table 1
Baseline characteristics and biochemical profiles of subjects.

	Non-diabetic control (n = 60)	Type 2 diabetes (n = 96)	p value
Age (years)	40.6 ± 12.7	58.7 ± 9.3	<0.001
Male	25 (42%)	58 (60%)	0.022
BMI (Kg/m2)	25.0 ± 4.8	27.6 ± 4.6	0.001
SBP (mmHg)	128.1 ± 17.1	142.6 ± 21.7	<0.001
DBP (mmHg)	78.5 ± 10.3	82.4 ± 12.1	0.038
TC (mM)	5.56 ± 1.16	4.61 ± 1.07	<0.001
TG (mM)	1.17 ± 0.69	2.10 ± 2.49	0.005
HDL-C (mM)	1.57 ± 0.38	1.20 ± 0.36	<0.001
LDL-C (mM)	3.46 ± 1.06	2.72 ± 0.97	<0.001
urine ACR (inter-quartile)	13.1 (6–15.3)	606.9 (12.9–254.4)	<0.001
FBG(mM)	5.3 ± 0.8	–	–
Adiponectin (μg/ml)	6.4 ± 4.8	–	–
Insulin (μU)	11.9 ± 10.6	–	–
Diabetes duration (years)	–	13.1 ± 9.3	–
HbA1c (%)	–	8.3 ± 1.9	–
eGFR (ml/min per 1.73 m^2)	–	77.8 ± 41.7	–

Continuous variables were presented as Mean ± SD or Mean (inter-quartile) and male gender was expressed as percentages.
BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; TC, total cholesterol; TG, total triglyceride; HDL-C, high density lipoprotein-cholesterol; LDL-C, low density lipoprotein-cholesterol; FBG, fasting blood glucose; eGFR, estimated glomerular filtration rate.