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Circulating irisin levels are positively associated with endothelium-dependent vasodilation in newly diagnosed type 2 diabetic patients without clinical angiopathy

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ABSTRACT

Objective: Irisin is a newly identified myokine that can promote energy expenditure and alleviate insulin-resistance in animal model. It has been established that insulin resistance is frequently associated with endothelial dysfunction. Therefore, we hypothesize that circulating irisin levels are associated with endothelial dysfunction in type 2 diabetes.

Methods: One hundred and eighty eight patients with newly diagnosed type 2 diabetes and 40 healthy subjects were recruited. Serum irisin concentrations were measured by using enzyme-linked immunosorbent assay, and flow-mediated dilation (FMD) was evaluated by using high-resolution ultrasound.

Results: The mean value of circulating irisin levels in newly diagnosed type 2 diabetes was 13.25 ng/ml, which was significantly lower than that in controls (25.98 ng/ml, $p < 0.001$). By dividing the distribution of FMD levels into quartiles, serum irisin levels were increased gradually with the increase of FMD levels ($p < 0.001$). Multivariate stepwise regression analysis demonstrated that serum irisin levels were independently associated with FMD ($p = 0.009$). By logistic regression analysis the odds ratio for lower FMD levels was reduced by 11.8% per 1 ng/ml increase in serum irisin concentration after adjustment for multivariate metabolic factors [OR (95% CI); 0.882 (0.709–0.969)].

Conclusion: These results demonstrated that circulating irisin levels were decreased in newly diagnosed Chinese type 2 diabetic patients without clinical angiopathy and positively associated with FMD levels.

1. Introduction

Almost 200 million people suffer from type 2 diabetes globally, and the prevalence is rapidly increasing [1]. Endothelial dysfunction is considered as an early marker of cardiovascular disease as well as in type 2 diabetes [2]. Endothelial dysfunction comprises a number of functional alternations in the vascular endothelium, such as impaired endothelium-dependent vasodilatation, impaired barrier function, inflammatory activation [3]. Endothelium-dependent vasodilatation, which is impaired in endothelial dysfunction, can be assessed by measuring responses to reactive hyperemia of the brachial artery [4,5].

Skeletal muscle tissue is an important organ for lipid and glucose metabolism. In addition, it secretes cytokines and peptides that are classified as “myokines” [6], which act as endocrine hormones and regulate whole body metabolism.

Sedentary lifestyle is a major risk factor for cardiovascular complications in type 2 diabetes mellitus [7]. Randomized controlled trials have demonstrated that physical activity interventions improve glucose tolerance and reduce the risk of type 2 diabetes in those with a high risk of the disease, even without obvious body weight change [8,9]. Indeed, our previous study showed that aerobic exercise could improve flow-mediated endothelium-dependent vasodilatation (FMD) in patients with impaired fasting glucose [4]. 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 [10].

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

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Abbreviations list

BMI	body mass index
CRP	C-reactive protein
DBP	diastolic blood pressure
ELISA	enzyme-linked immunosorbent assay
FBG	fasting blood glucose
Fins	fasting serum insulin
FMD	flow-mediated dilation
HbA1c	glycated hemoglobin A1c
GTN	glyceryl trinitrate
HDL-C	high-density lipoprotein cholesterol
Lp(a)	lipoprotein (a)
IR	insulin resistance
LDL-C	low-density lipoprotein cholesterol
FNDC5	membrane protein fibronectin type III domain containing 5
PGC-1α	peroxisome proliferator-activated receptor gamma coactivator-1-alpha
2 h BG	postprandial 2 h glucose
SBP	systolic blood pressure
TC	total cholesterol
UCP1	uncoupling protein 1
UAER	urinary albumin excretion rate

in glucose/fatty acid metabolism, mitochondrial function and mitochondria biogenesis [11]. Actually, sedentary lifestyle and type 2 diabetes are associated with reduced expression of PGC-1α [12]. Moreover, recent study showed that physical exercise induced intramuscular expression of transcriptional coactivator PGC-1 and its downstream molecule, the membrane protein fibronectin type III domain containing 5 (FNDC5), which is proteolytically cleaved to form irisin [13]. The circulating irisin could induce the differentiation of stromal vascular cells isolated from mouse subcutaneous white fat into a brown adipocyte phenotype, a process known as white fat "browning" [14], by stimulating the expression of uncoupling protein 1(UCP1) [15]. Overexpression of this myokine was sufficient to promote energy expenditure and alleviate insulin resistance in diabetic animal model [13]. It has been established that insulin resistance is highly associated with endothelial dysfunction [16]. However, it is currently unknown the relationship between circulating irisin levels and endothelial dysfunction in diabetic subjects. In this study, we investigate the relationship between circulating irisin levels and endothelial dysfunction in newly diagnosed type 2 diabetic patients without clinical angiopathy.

2. Subjects and methods

2.1. Study subjects

From March 2012 to December 2012, a total of 188 newly diagnosed Chinese type 2 diabetic patients (101 men and 87 women, aged 36–66 years, mean 51.5 ± 8.2), were recruited from 1179 type 2 diabetes, who referred to our hospital. Patients with clinical angiopathy including micro- and macroangiopathy as well as hypertension were excluded from this study. Microangiopathy included nephropathy [urinary albumin excretion rate (UAER) > 20 μg/min], retinopathy (at least one microaneurysm or hemorrhage or exudates in either eye), neuropathy (pain in extremities, paresthesias, and absent tendon reflexes and/or absent vibration sense), and macroangiopathy included coronary artery disease (myocardial infarction, ischemia electrocardiogram changes, and angina), cerebrovascular disease (transient ischemic attack or stroke), and peripheral vascular disease (the abolition of one or more peripheral arterial pulse and/or intermittent claudication and/or a past history of revascularization of the lower limbs). The recruited patients were all required to have an office BP < 130/80 mmHg (Blood pressure was measured by a trained nurse. After at least 5-min rest, two successive readings were taken from the right arm using a mercury manometer with a 12-cm by 33.5-cm cuff). During the same period, 40 healthy subjects (all from medical staff in our hospital) were selected as control subjects. Each subject was asked details of smoking history and physical exercise as well as family history of premature vascular disease. Cigarette smoker was defined as subjects who had smoked at least one cigarette daily for 1 year. Physical exercise was defined as subjects who walked at least 25–30 min a day and at least 3–4 days a week for at least half a year. Family history was considered positive if a first-degree relative had clinical evidence of coronary artery disease (angina, myocardial infarction, or bypass surgery) at age ≤ 55 years. Subjects who were obese (BMI > 30 kg/m^2) and those with malignant neoplasms, renal or liver diseases, or endocrinological disease other than diabetes were excluded from the study. Also, no patient was taking any drugs, such as anti-hypertensive drugs (including β-blockers), diabetes medications, estrogen supplements, thyroxine, diuretics, hypolipidemic drugs. All subjects enrolled in the study gave informed consent. The study protocol was in agreement with the guidelines of the ethics committee at our hospital and an approval from the ethics committee of Wuhan General Hospital was obtained.

2.2. Biochemical measurements

Blood samples were obtained from participants after a 12-h fast. Aliquots of serum and plasma were stored at −80 °C and were not thawed until analyzed. Serum irisin concentrations were measured in duplicate by using the enzyme-linked immunosorbent assay (ELISA) kits (Aviscera Biosciences, Santa Clara, CA), in accordance with the manufacturer's instructions. The sensitivity of the assay was 0.2 ng/ml and the linear range of the standard was 5–500 ng/ml. The intra- and inter-assay coefficients of variation (CV) were 4.5% and 8%, respectively. Serum total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C), triglyceride, and high-density lipoprotein cholesterol (HDL-C) were measured enzymatically. Serum lipoprotein(a) [Lp(a)] concentration was measured by an ELISA. Blood glucose including fasting blood glucose (FBG) and postprandial 2 h glucose (after 75-g glucose loading, 2 h BG) was measured by a glucose oxidase procedure. Hemoglobin A1c (HbA1c) was measured by high-performance chromatography. Ultrasensitive C-reactive protein (CRP) was measured by particle enhanced immunoturbidimetric assay. Serum fasting insulin concentration was measured by electrochemiluminescence immunoassay. HOMA insulin resistance (HOMA-IR) was calculated by fasting serum insulin (Fins, mU/ml) × FBG (mmol/l)/22.5. UAER was measured by radioimmunoassay. Coefficients of variation for these assays were 1–2% (blood glucose, TC, HDL-C, and HbA1c), 2–3% (triglyceride, LDL-C, Fins and CRP), 2–4% (UAER), and 4–7% [Lp(a)].

2.3. Ultrasound study of the brachial artery

The vascular studies of the brachial artery were performed non-invasively in the AM following a controlled diet, as described in our previous publications [4,5,17,18]. High resolution ultrasound was used to measure changes in arterial diameter in response to reactive hyperemia (with increased flow producing an endothelium-dependent stimulus to vasodilation, FMD) and to glyceryl