Circulating irisin levels reflect visceral adiposity in non-diabetic patients undergoing hemodialysis

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ABSTRACT

Background: Recent evidence suggests that increased visceral adiposity is a strong independent risk factor for cardiovascular death and all-cause mortality in hemodialysis (HD) patients. Irisin, which is a novel myokine, can play critical roles in diabetes and adiposity. The purpose of our study was to investigate whether serum irisin levels are associated with body mass index, waist circumference (WC), and total fat mass in non-diabetic patients undergoing maintenance HD.

Methods: This cross-sectional study included 108 non-diabetic HD patients and 40 age- and sex-matched apparently healthy subjects. Serum irisin concentrations were determined using an enzyme-linked immunosorbent assay. Body fat composition (TBF-410 Tanita Body Composition Analyzer) was measured and calculated.

Results: Serum irisin levels did not differ between HD patients and the healthy controls (523.50 ± 229.32 vs. 511.28 ± 259.74, p = 0.782). Serum irisin levels were associated with age (r = 0.314; p = 0.006), HOMA-IR (r = 0.472; p < 0.001), WC (r = 0.862; p < 0.001), and total fat mass (r = 0.614; p < 0.001). In multivariate regression analysis, WC (β = 1.240, p < 0.001) and total fat mass (β = 0.792, p = 0.015) were the variables that were significantly associated with irisin concentrations (R² = 0.684, p < 0.001) after adjusting for confounding factors (age and HOMA-IR).

Conclusions: These results suggest that serum irisin levels are related to visceral adiposity in non-diabetic HD patients.

Introduction

Obesity is currently considered to be a worldwide public health concern because it has been closely associated with a greater risk for cardiovascular disease (CVD) and mortality. However, excess weight is associated with improved survival, better CVD outcomes and mortality in end-stage renal disease (ESRD) patients treated with hemodialysis (HD). Body mass index (BMI) is not a predictor of all-cause and CV death, while waist circumference (WC) and the waist/hip ratio are surrogate measurements for abdominal obesity, which underlies a high risk of all-cause and CV mortality in patients with ESRD. Some studies showed that higher muscle mass appears to be associated with better clinical and survival outcomes in the HD population. It is well known that BMI cannot differentiate skeletal muscle mass and fat mass and that BMI is affected not only by fat and muscle mass but also by fluid status. BMI is not accepted to be a strong indicator of fat mass in body composition.

Irisin is a recently discovered myokine that is the cleavage product of the membrane protein fibronectin type III domain containing (FNDC5). Irisin is directly activated by peroxisome proliferator-activated receptor-γ coactivator-1α, which is a transcriptional coactivator in the muscle that was upregulated during 10 weeks of exercise training in rodent models. Interestingly, this myokine has been proposed to participate in white fat browning and has been reported to be expressed in skeletal muscle. High irisin levels have been associated with improved insulin sensitivity, increased energy expenditure and decreased liver fat content. Circulating irisin levels were observed to correlate with fat mass, BMI, and metabolic syndrome.

The aims of the present work were to evaluate circulating human irisin levels in a group of normal-weight and obese chronic HD patients and to investigate...
whether circulating human irisin levels correlate with direct and indirect markers of adiposity and with hormones that control energy homeostasis.

Materials and methods

The inclusion criteria included patients aged 18 years and older who were undergoing maintenance HD therapy for more than 3 months and who were delivered an adequate dose of dialysis (single-pool Kt/V > 1.2) on a thrice-weekly dialysis program using biocompatible HD membranes. Patients with diabetes mellitus, diabetic nephropathy, cancer, overt infection, vasculitis and liver disease were excluded from this study. Two hundred and sixty-seven consecutive HD patients were evaluated from Hemodialysis unit of Ankara Oncology Training and Research Hospital and Turgut Ozal University Hospital. One hundred and forty-five HD patients met all the inclusion and exclusion criteria. Seventy-five gram oral glucose tolerance test was performed in 145 HD patients. Diabetes was defined as fasting glucose ≥126 mg/dL and/or 2-h post-load glucose ≥200 mg/dL according to the 2006 World Health Organization (WHO) criteria. Thirty-seven established type 2 diabetes. So, the studied subjects included 108 patients (54 males and 54 females, mean age 54.5 ± 3.8 years, mean dialysis history 7.2 ± 0.8 years) who were undergoing HD 3 times per week from December 2012 to February 2015. In addition, 40 age- and sex-matched apparently healthy subjects were included. The study was approved by the Local Ethics Committee (IRB Number: 99950669/343). Written informed consent was obtained from each participant.

Systolic blood pressure and diastolic blood pressure were assessed using a digital monitor (Medisana AG, MTC, Neuss, Germany) according to the WHO criteria. Venous blood was sampled in the morning before dialysis after an overnight fast of more than 8 h. Serum total cholesterol (TC), high-density lipoprotein-cholesterol (HDL-c), triglycerides (TG) C-reactive protein (CRP), creatinine and glucose concentrations were measured using an autoanalyzer (Cobas Integra 800, Roche Diagnostics GmbH, Manheim, Germany) with specific kits. Low-density lipoprotein-cholesterol (LDL-c) levels were calculated using the Friedewald formula, as follows: LDL-c = TC – HDL-c – TG/5. Plasma glucose was measured using the glucose oxidase method. Serum insulin was measured using an enzyme immunoassay kit (SRL, Inc., Tokyo, Japan). Insulin resistance was calculated using the homeostasis model assessment (HOMA-IR) method.12

Body weight measurements were performed using a digital balance accurate to 0.1 kg (Tanita bioelectrical impedance (BIA); TBF-410, Tanita, Tokyo, Japan), and height was measured using a wall-mounted stadiometer (Seca 220; Vogel and Halke, Hamburg, Germany). BMI was calculated as the body weight divided by the height squared (kg/m²). WC was measured at the site of the smallest circumference between the rib cage and the iliac crest. Body fat was quantified using the TBF-410 Tanita Body Composition Analyzer (Tokyo, Japan). All measurements were collected with the subjects in underwear and after an overnight fast. Anthropometric and body composition measurements were performed after the completion of dialysis on the last day of the week.

Serum irisin concentrations were analyzed using enzyme-linked immunosorbent assay (ELISA) kits (catalog no. EK-067–52, Aviscera Biosciences, Santa Clara, CA), according to the manufacturer’s protocol. The assay was proven to be highly sensitive for detecting human irisin. All samples were analyzed in duplicate. The sensitivity of the assay was 4.15 ng/mL, and the detectable range of the kit was 0.066–1024 ng/mL.24,25 The intra- and inter-assay variations were both less than 10%.

All analyses were performed using the SPSS statistical package (version 17; SPSS, Inc., Chicago, IL). Continuous data were presented as the mean ± SD or the median (interquartile range). Categorical variables were summarized as percentages and comparison of categorical variables was evaluated by the chi-square test. The distribution of variables was assessed for normality using the Kolmogorov–Smirnov test. The Spearman correlation was used to examine the association of irisin with other variables for univariate analysis. Multivariate regression analysis was performed with irisin as the dependent variable and the variables of interest as independent variables that were found to approach significance (p < 0.1) in the univariate analysis.

Results

The clinical, laboratory, and demographic characteristics of the study participants are presented in Table 1. Serum irisin levels were similar in HD patients and the healthy controls (523.5 ± 229.3 ng/mL vs. 511.2 ± 259.7 ng/mL, p = 0.782). No significant differences in age and gender were observed between the HD patients and the control group. Serum irisin levels were significantly and positively correlated with age (r = 0.314; p = 0.006), HOMA-IR (r = 0.472; p = 0.003), WC (r = 0.862; p < 0.001) (Figure 1), and total fat mass (r = 0.614; p < 0.001) (Figure 2), but not other metabolic parameters (Table 2). Conversely, irisin serum levels were not correlated with two parameters of adiposity: body weight and BMI.
Multivariate regression analysis showed that WC ($\beta = 1.240$, $p < 0.001$) and total fat mass ($\beta = 0.792$, $p = 0.015$) were the variables that were significantly associated with irisin concentrations ($R^2 = 0.684$, $p < 0.001$), whereas age and HOMA-IR did not correlate significantly with irisin concentrations (Table 2).

**Table 2.** Baseline characteristics of HD patients and healthy controls.

| Patients (M/F) | 108 (54/54) | 40 (20/20) |
|---------------|-------------|------------|
| Age (years)   | 52.48 ± 12.14 | 52.85 ± 12.81 | 0.872 |
| Dialysis duration (years) | 3.25 ± 1.85 | — | — |
| Systolic pressure (mmHg) | 134 ± 12 | 128 ± 10 | 0.219 |
| Diastolic pressure (mmHg) | 83 ± 9 | 79 ± 8 | 0.305 |
| Body weight (kg) | 91.38 ± 11.67 | 90.94 ± 11.77 | 0.837 |
| BMI (kg/m²) | 27.78 ± 2.96 | 27.59 ± 2.94 | 0.875 |
| WC (cm) | 86.29 ± 11.02 | 85.87 ± 11.12 | 0.784 |
| Total body fat (kg) | 24.52 ± 6.00 | 24.30 ± 5.84 | 0.848 |
| Percentage of body fat (%) | 26.52 ± 3.80 | 26.44 ± 3.67 | 0.904 |
| Smoker (n, %) | 30, 27.7 | 11, 27.5 | 0.857 |
| Fasting serum glucose (mg/dL) | 95.6 ± 12.8 | 93.7 ± 11.9 | 0.496 |
| Creatinine (mg/dL) | 9.35 ± 2.27 | 0.64 ± 0.23 | <0.001 |
| Urea nitrogen (BUN) (mg/dL) | 85.6 ± 22.7 | 16.2 ± 4.5 | <0.001 |
| Uric acid (mg/dL) | 7.5 ± 2.1 | 4.8 ± 1.9 | <0.001 |
| Hemoglobin (g/dL) | 11.1 ± 2.7 | 14.7 ± 1.8 | 0.045 |
| TC (mg/dl) | 221 ± 32 | 220 ± 30 | 0.746 |
| HDL-cholesterol (mg/dl) | 38 ± 11 | 39 ± 13 | 0.537 |
| LDL-cholesterol (mg/dl) | 142 ± 13 | 140 ± 15 | 0.691 |
| TG (mg/dl) | 205 ± 45 | 207 ± 48 | 0.786 |
| C-reactive protein (mg/L) | 5.5 ± 3.78 | 1.45 ± 1.05 | 0.025 |
| Albumin (g/dL) | 4.05 ± 0.65 | 4.42 ± 0.74 | 0.127 |
| HOMA-IR | 3.29 ± 0.47 | 3.28 ± 0.45 | 0.896 |
| Irisin (ng/mL) | 523.50 ± 229.32 | 511.28 ± 259.74 | 0.782 |

Notes: TG: triglyceride; TC: total cholesterol; HDL-C: high-density lipoprotein; LDL-C: low-density lipoprotein; HOMA-IR: Homeostasis Model Assessment of Insulin Resistance.

**Discussion**

This study is the first study to evaluate the association between circulating irisin levels and adiposity in non-diabetic patients undergoing maintenance HD. In this study, we demonstrated that circulating irisin levels are related to adiposity in non-diabetic patients undergoing maintenance HD.

According to our results, circulating irisin levels correlate with WC and total fat mass in non-diabetic patients undergoing maintenance HD. Some evidence showed that circulating irisin levels are elevated in obese individuals.\textsuperscript{20–22,26} Likewise, a correlation was observed between circulating irisin levels and BMI.\textsuperscript{20–22,27} Furthermore, clinical and experimental studies revealed that irisin correlates with fat mass.\textsuperscript{20,22,26,28} However, there were some reports of negative correlations,\textsuperscript{23,29} despite those reports of positive correlations.\textsuperscript{20,22,26,28} Thus, contradictory data concerning the relationship between irisin and adiposity have been reported in the literature. In contrast to these studies, we observed no correlation of BMI with circulating irisin levels. We explained this unexpected result as follows: WC may be an optimal surrogate of visceral obesity in comparison to BMI, which poorly reflects intra-abdominal (truncal) fat. Indeed, Postorino et al. showed that WC was a stronger predictor of all-cause and CV death than BMI among 537 patients with ESRD. Although current guidelines and most large epidemiologic studies...

![Figure 1](image-url) There was a strong association between serum irisin levels and WC.
use BMI to define obesity, BMI has a limited ability to
differentiate between muscle mass and fat mass. In
non-diabetic patients undergoing maintenance HD, we
suggest that circulating irisin levels reflect net body adi-
posity and visceral adiposity due to the strong associ-
ation between serum irisin levels and both WC and total
fat mass. Our explanation for this finding is outlined
below. First, irisin was initially identified as a myokine
that is secreted by muscles after aerobic exercise training;
it might be feasible that the overexpression and
increased release of muscle irisin is stimulated by signals
that originate in adipose tissue, contributing to the
increased levels of circulating irisin that were observed
in obese individuals. Second, an adaptive mechanism
for responding to decreased insulin sensitivity and other
metabolic disturbances that are associated with obesity
can lead to increased circulating irisin levels. Third,
FNDC5/irisin is also an adipokine that is expressed and
secreted mainly by adipose tissue. Thus, this report
led us to speculate that increased adipose tissue can
play a role in determining circulating FNDC5/irisin levels.

In our study, we observed a significant positive cor-
relation between circulating irisin levels and insulin
resistance, as indicated by HOMA-IR; this finding is simi-
lar to the findings reported in the study of Park et al. However, we found no association between circulating
irisin levels and HOMA-IR after multivariate analysis.
Irisin was identified as a potential factor associated with
the progression of insulin resistance during the weight
maintenance period after a dietary weight-lowering pro-
gram in obese patients. Previous reports demonstrated
the existence of positive associations between
circulating irisin levels or FNDC5 expression in human
myotubes and both fasting insulin concentrations and
HOMA-IR. Irisin is likely to be involved in glucose
metabolism and may thus prevent the development of

| Univariate | Multivariate |
|------------|--------------|
| r          | p Values     | Beta (standardized coefficients) | p Values |
| Age (years) | 0.314        | 0.006                  | 0.208     | 0.529     |
| Gender*    | 0.085        | 0.871                  | —         | —         |
| Dialysis duration (years) | 0.063 | 0.906                  | —         | —         |
| Systolic pressure (mmHg) | 0.102 | 0.647                  | —         | —         |
| Diastolic pressure (mmHg) | 0.098 | 0.725                  | —         | —         |
| Body weight (kg) | 0.136 | 0.289                  | —         | —         |
| BMI (kg/m²) | 0.148        | 0.272                  | —         | —         |
| WC (cm)    | 0.862        | <0.001                 | 1.240     | <0.001    |
| Total body fat (kg) | 0.614 | <0.001                 | 0.792     | 0.015     |
| Percentage of body fat (%) | 0.119 | 0.547                  | —         | —         |
| TC (mg/dl) | 0.051        | 0.932                  | —         | —         |
| HDL-cholesterol (mg/dl) | -0.049 | 0.948                  | —         | —         |
| LDL-cholesterol (mg/dl) | 0.071 | 0.893                  | —         | —         |
| TG (mg/dl) | 0.062        | 0.910                  | —         | —         |
| C-reactive protein (mg/L) | 0.031 | 0.952                  | —         | —         |
| Albumin (g/dL) | 0.159 | 0.196                  | —         | —         |
| HOMA-IR    | 0.472        | 0.003                  | 0.556     | 0.331     |

Notes: TG: triglyceride; TC: total cholesterol; HDL-C: high-density lipoprotein; LDL-C: low-density lipoprotein; HOMA-IR: Homeostasis Model Assessment of Insulin Resistance.

*Male, 0; female, 1.
insulin resistance.

Some evidence has addressed the relationship between decreased serum irisin levels and insulin resistance or diabetes. This effect could be explained by the fact that central obesity correlates strongly with insulin resistance. Thus, the association of irisin with HOMA-IR may be subsequently reflected in central obesity.

Some limitations of our study should be noted. First, the cross-sectional design provides associative rather than causal evidence. Second, anthropometric measures and bioelectrical impedance analysis (BIA) are accessible, safe, and cost-efficient methods that avoid exposure to radiation and have been widely used to measure body composition in clinical populations; however, visceral adipose tissue (VAT) is only an indirect measure when using these approaches. Only CT and MRI can provide direct volumetric measures of VAT. Third, the physical activity status of the participants is unknown, although numerous approaches have been used to assess physical activity and demonstrate that irisin is augmented with activity or exercise training. Fourth, irisin has a positive effect on glucose homeostasis. HD patients with diabetes mellitus were not included to eliminate this interaction. Fourth, the measurement of plasma or serum irisin levels by ELISA is easily available and may provide remarkable information for a wide range of pathological conditions, therapeutics and clinical practice, but the heterogeneous and often discrepant results were reported. In addition, a normal range of circulating irisin levels has not yet been determined. The inconsistency in circulating irisin levels observed only reflect inter-population or methodological variations, assay discrepancies and/or preanalytical variability (i.e., blood collection, handling, storage, repeated melting, etc.).

In summary, our results suggest that serum irisin levels correlate positively with total fat mass and WC and represent a surrogate method for measuring visceral adiposity in non-diabetic HD patients. Prospective studies with larger sample sizes are needed to clarify the physiological role of irisin in obesity in the general population and in HD patients.

Disclosure statement

All authors declare that they have no conflict of interest. Ethical approval: All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards (IRB Number: 99950669/343). Informed consent: Informed consent was obtained from all individual participants included in the study.

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