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The normoglycemic first-degree relatives of patients with type 2 diabetes mellitus have low circulating omentin-1 and adiponectin levels

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ABSTRACT

Objective: It has been suggested that adipose-derived cytokines act as insulin sensitizers/insulin-mimetics and some others may induce insulin resistance. In order to elucidate the potential role of novel adipocytokines in the pre-diabetes states, circulating levels of novel adipocytokines were evaluated in first-degree relatives of subjects with type 2 diabetes mellitus (FDRs).
Method: Serum omentin-1, adiponectin and retinol-binding protein 4 (RBP4) levels were measured in 179 subjects (90 glucose tolerant FDRs and 89 age- and sex-matched healthy controls) using enzyme-linked immunosorbent assay (ELISA) methods.
Results: There was no significant difference between the two groups regarding serum RBP4 concentrations. However, serum omentin-1 (median [interquartile range], 6.18 [4.06–11.52] ng/ml versus 10.50 [4.30–20.60] ng/ml, $p = 0.004$) and adiponectin (mean ± SD, 10.07 ± 4.0 μg/ml versus 20.66 ± 8.12 μg/ml, $p < 0.0001$) levels were significantly lower in FDRs when compared with the controls. In multiple logistic regression analysis, FDRs showed a significant association with lower circulating omentin-1 and adiponectin levels, even after adjustments were made for age, sex, body mass index, blood pressure measures, and biochemical parameters including glucose status, lipid profile, insulin levels and HOMA-IR (OR = 0.49, CI [0.30–0.79]; $p = 0.004$ and OR = 0.74, CI [0.67–0.82]; $p < 0.0001$, respectively). However, FDRs did not show a significant association with serum RBP4 levels in different models of regression analyses.
Conclusions: The FDRs showed significant associations with lower omentin-1 and adiponectin levels. A potential role for these adipokines in the FDRs' increased risk of diabetes needs to be further elucidated.

1. Introduction

It has been suggested that adipose-derived cytokines act as a potential link between energy homeostasis, immunity, neuroendocrine, atherosclerosis, type 2 diabetes, and insulin resistance [1–4]. Several of these bioactive mediators that are collectively called 'adipocytokines' may be considered insulin sensitizers/insulin-mimetics, and some others may induce insulin resistance [5].

Omentin-1 is a novel 34 kDa adipokine that is highly and selectively expressed in visceral adipose tissue compared with subcutaneous adipose tissue [6,7]. Furthermore, omentin-1 enhances insulin action and Akt phosphorylation [7]; it is inversely related to obesity [8] and is down regulated by insulin and glucose [9].

Adiponectin is the most abundant adipocytokine with insulin sensitizing, anti-inflammatory, and anti-atherogenic effects [1,4]. Adiponectin levels have inverse correlations with dyslipidemia, atherosclerosis, glucose intolerance, and insulin resistance in diverse patient populations [1,3]. In fact, hypoadiponectinemia has been suggested as one of the most important adipocytokines in the pathogenesis of type 2 diabetes [1].

The serum levels of retinol-binding protein 4 (RBP-4) reflect the risks of impaired glucose tolerance and type 2 diabetes [10]. The link between elevated RBP-4 levels and impaired insulin secretion has been shown in insulin resistant humans with obesity, impaired glucose tolerance and type 2 diabetes, and even in lean

Abbreviations: FDRs, first-degree relatives of subjects with type 2 diabetes mellitus; RBP4, retinol-binding protein 4; ELISA, enzyme-linked immunosorbent assay; BMI, body mass index; OGTT, oral glucose tolerance test; HDL, high-density lipoprotein; LDL, low-density lipoprotein; HOMA-IR, homeostasis model of assessment index; CV, coefficient of variance; WHR, waist to hip ratio.

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normoglycemic subjects with a strong family history of type 2 diabetes [3,4,11,12].

Although adipokines may be involved in the pathogenesis of diabetes mellitus and insulin resistance, the contributions of individual adipokines to the pathophysiological features of insulin resistance and/or insulin sensitivity must still be fully clarified.

First-degree relatives of subjects with type 2 diabetes mellitus (FDRs) frequently have insulin resistance [13]. The results of a large population study [14] showed that β cell impairment exists in the offspring of type 2 diabetes patients, even in the absence of insulin resistance. Thus, β cell dysfunction is the outstanding determinant for the development of diabetes mellitus in this group.

FDRs bear a 40% lifetime risk for developing type 2 diabetes mellitus [15]. The background of insulin resistance/β cell dysfunction and glucose metabolism in FDRs used to assess the circulating levels of novel adipocytokines such as omentin, RBP-4 and adiponectin may be helpful in illustrating some pathophysiological aspects of adipose tissue involvement in the development of insulin resistance in this group. In the present study, we performed the first evaluation of serum omentin in addition to assessing RBP-4 and adiponectin levels in FDRs, matching control subjects without a family history of type 2 diabetes mellitus.

2. Methods

2.1. Subjects and physical measurements

Ninety normoglycemic subjects (mean age ± SD 40.55 ± 9.36 years: 41 men and 49 women) who were first-degree relatives of patients with type 2 diabetes mellitus (FDRs) and who consecutively visited the Endocrine Clinic at Fatemeh-Zahra University Hospital were enrolled in this study. The age and sex-matched controls (41.375 ± 9.47 years: 49 men, 40 women) were selected from a cohort of healthy subjects recruited by the Persian Gulf Healthy Heart Study at Bushehr University of Medical Sciences [16] for evaluation of cardiovascular risk factors in the Persian Gulf region. The following criteria were used for the healthy controls: (1) they had no family history of diabetes mellitus, type 2 diabetes or endocrine disorders, cardiovascular diseases, or hepatic or renal dysfunction; (2) they had no history of taking antidiabetic and antiobesity drugs, glucocorticoids, anti-inflammatory agents, estrogenic or androgenic medications.

The participants in the control group had anthropometric measurements comparable to those of the FDRs (Table 1). All subjects were asked to fast and to present to the Persian Gulf Health Research Center between 7:30 and 9:30 a.m. They underwent a 75-g oral glucose tolerance test (OGTT). The definition of glucose tolerance was a subject who had a fasting plasma glucose level lower than 110 mg/dl and a second-hour blood sugar level after oral glucose load (2 h-OGTT) lower than 140 mg/dl [17].

Blood pressure was assessed twice at the right arm after a 15 min rest in the sitting position, using a standard mercury sphygmomanometer. Height and weight were measured using a stadiometer. Heavy outer garments and shoes were removed before height and weight were measured. Body mass index (BMI) was calculated. Waist circumference was defined at the midway level between the costal margins and the iliac crests. Hip circumference was measured at the level of the greater trochanters. Waist-to-hip ratio was calculated for all participants.

The study was approved by the Medical Ethics Committee of Bushehr University of Medical Sciences.

2.2. Laboratory measurements

A fasting blood sample was taken, all samples were promptly centrifuged, and sera were separated and kept frozen at −70 °C until they were used. Analyses for biochemical parameters (blood glucose, triglyceride, and cholesterol levels) were carried out at the Persian Gulf Health Research Center on the day of blood collection using a Selectra 2 autoanalyzer (Vital Scientific, Spankeren, The Netherlands). Glucose was assayed with the enzymatic (glucose oxidase) colorimetric method using a commercial kit (Pars Azmun Inc., Tehran, Iran). Serum total cholesterol and HDL (high-density lipoprotein) cholesterol were measured using cholesterol oxidase phenol aminoantipyrine and triglycerides using the glycerol-3 phosphate oxidase phenol aminoantipyrine enzymatic method. Serum LDL (low-density lipoprotein) cholesterol was calculated using the Friedwald formula. LDL cholesterol was not calculated when the triglycerides concentration was >400 mg/dl.

Insulin was measured using a commercially available enzyme-linked immunosorbent assay kit (Insulin ELISA, DRG Diagnostics, Marburg, Germany). The assay sensitivity was 1.76 μIU/ml; the intra- and inter-assay coefficients of variance were 1.79–2.6% and 2.88–5.99%, respectively.

Insulin resistance was assessed by calculating the homeostasis model of assessment index (HOMA-IR) using the following equation: fasting insulin (μIU/ml) × fasting glucose (mg/dl)/405.

Serum omentin-1 concentrations were measured using manual omentin-1 (human) detection (ELISA kit [intelectin-1 (human) ELISA kit, Apotech Corporation, Switzerland]). The detection limit of the assay was 0.4 ng/ml (range 0.5–32 ng/ml). The mean intra-assay and inter-assay CVs of the omentin-1 assay were 4.51–7.4% and 4.19–9.27%, respectively. The antibodies used in this kit are specific to the measurement of natural and recombinant human omentin-1.

To detect adiponectin in the serum samples, commercially (Cat. No. AG-45A-0001EK-KI01) available enzyme-linked immunosorbent assay kits (AdipoGen, Incheon, Korea) were used according to the manufacturer's instructions. The limit of detection of the assay was 100 pg/ml; the intra- and inter-assay coefficients of variance were 2.9–3.8% and 2.8–5.5%, respectively.

For the detection of RBP4 in the serum samples, commercially available ELISA (AdipoGen, Incheon, Korea) kits were used according to the manufacturer's instructions. The assay sensitivity was

Table 1
The general characteristics, including blood pressure and anthropometric measurements, and the biochemical parameters of first-degree relatives of subjects with type 2 diabetes mellitus (FDRs) and healthy controls.

	FDRs (n = 90)	Control (n = 89)	p Value
Female/male ratio	49/41	40/49	0.233
Age (years)	40.55 ± 9.63	41.37 ± 9.47	0.575
Systolic BP (mmHg)	115 ± 13.8	115.82 ± 12.84	0.687
Diastolic BP (mmHg)	74.72 ± 9.95	74.45 ± 10.47	0.864
WHR	0.90 ± 0.14	0.91 ± 0.17	0.511
BMI (kg/m^2)	27.57 ± 4.25	26.52 ± 4.24	0.104
Fasting glucose (mg/dl)	80.5 ± 8.56	79.77 ± 10.46	0.612
2 h-OGTT (mg/dl)	92.17 ± 15.68	92.03 ± 16.65	0.956
Total cholesterol (mg/dl)	198.54 ± 36.85	196.92 ± 37.24	0.770
Triglyceride (mg/dl)	155.72 ± 82.3	155.52 ± 75	0.279
HDL-cholesterol (mg/dl)	43 ± 10.38	45.44 ± 11.65	0.140
LDL-cholesterol (mg/dl)	121.01 ± 31.55	120.04 ± 32.44	0.840
Insulin (μIU/ml)	8.75 (5.25–16.68)	6.56 (5.03–10.18)*	0.027
HOMA-IR	1.82 (1.05–3.06)	1.29 (0.98–1.93)*	0.010
Omentin-1 (ng/ml)	6.18 (4.06–11.52)	10.50 (4.30–20.60)	0.004
RBP4 (μg/ml)	100.0 ± 42.73	95.28 ± 55.34	0.535
Adiponectin (μg/ml)	10.07 ± 4.0	20.66 ± 8.12	<0.0001

BMI, body mass index; WHR, waist to hip ratio; BP, blood pressure; 2 h-OGTT, second-hour oral glucose tolerance test; HOMA-IR, homeostasis model of assessment index; RBP4, retinol-binding protein 4.
Data are means (SD), except for insulin and HOMA-IR, and omentin-1 which are medians (interquartile ranges).
* p values <0.05 (the FDRs in comparison to the controls).