Low levels of spexin and adiponectin may predict insulin resistance in patients with non-alcoholic fatty liver

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ABSTRACT

Adipose tissue is endocrine organ that responds by secreting numerous hormones that regulate metabolism in skeletal muscle and the liver. The aim of this study was to compare the levels of spexin and adiponectin in patients with non-alcoholic fatty liver and evaluate the relationship between circulating adipocytokines and insulin resistance. Two groups of subjects were evaluated: 41 non-alcoholic fatty liver subjects (age 35.17 ± 12.29 year, BMI 30.97 ± 2.75 kg/m2) and 38 normal controls (age 38.47 ± 11.63 year, BMI 22.83 ± 3.00 kg/m2). Plasma concentrations of spexin and adiponectin were determined using immunosorbent assay kits. Insulin resistance was assessed using the homeostasis model assessment (HOMA-IR) formula derived from fasting insulin and glucose levels. Compared to normal controls, plasma concentrations of spexin and adiponectin were significantly lower in patients with non-alcoholic fatty liver ($P < 0.001$). Spexin did not correlate with BMI but did significantly correlate with HOMA-IR ($r = -0.368$; $P = 0.018$) and adiponectin ($r = 0.378$; $P = 0.043$), and this correlation remained significant after adjustment for gender and BMI. In this small group of patients with non-alcoholic fatty liver we demonstrated that insulin resistance correlated strongly with spexin and adiponectin levels.

1. Introduction

Nonalcoholic fatty liver disease (NAFLD), characterized by the accumulation of large droplets of triglycerides within hepatocytes in the absence of chronic alcohol consumption, is closely related to obesity and has become an important health problem because of its high prevalence and association with chronic liver diseases [19]. It was estimated to affect 12–24% of the general population in Asians, and 20–30% in Western countries [19]. Despite extensive investigations into NAFLD entity, the mechanisms underlying its development largely remain to be defined and its treatment is currently an unmet medical need [23].

Adipose tissue accounts for only 10% of insulin-stimulated glucose uptake, while this process is important for controlling whole-body energy homeostasis. The adipocytes serve as endocrine organ that responds by secreting numerous cytokines that regulate metabolism in skeletal muscle and the liver [7]. Several biomarkers of cytokines involved in the physiology of the adipose tissue have been identified as potential candidates for obesity [5]. Of cytokines recently identified, spexin appears to be important in regulating insulin sensitivity

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[8]. Spexin, a 14-amino-acid neuropeptide, was first discovered in 2007 by bioinformatics from the data mining of human proteome [21]. It distributes widely throughout the central and peripheral nervous system as well as other tissues, such as visceral fat, liver, kidney, thyroid and pancreatic islets [8]. Clinical studies indicated that the lower plasma spexin levels were found in patients with type 1 and type 2 diabetes and metabolic syndrome [3,12], but higher in the women with gestational diabetes mellitus, which was characterized by hyperglycemia and insulin resistance [1,4]. The spexin levels were negatively correlated with the fasting blood glucose, BMI, HOMA-IR, HbAlc and triglyceride contents in obese female volunteers and women with type 2 diabetes mellitus [10,15,18], whereas spexin levels were positively correlated with high molecular weight adiponectin [16]. Furthermore, spexin treatment not only led to weight loss but also improved glucose tolerance and insulin sensitivity in diabetic male mice [9]. Finally, it was well established that the spexin suppressed lipogenesis and hepatic fat accumulation in mice with hepatic steatosis/nonalcoholic fatty liver disease [9,14]. These results suggest that spexin plays a major role in glucose homeostasis. The aim of this study was to compare the levels of spexin and adiponectin in patients with non-alcoholic fatty liver and evaluate the relationship between circulating spexin and adiponectin and insulin resistance.

2. Materials and methods

The present study consisted of 41 NAFLD volunteers and 38 age- and gender-matched healthy volunteers according to a physical examination and routine laboratory tests. According to the criteria of nonalcoholic fatty liver disease (NAFLD) [13], inclusion criteria for the NAFLD patients were: 1) age > 18 years; 2) bright liver on ultrasound imaging and increased liver function tests for at least 6 months before liver biopsy; and 3) patient's consent for liver biopsy. NAFLD patients were subdivided into those with NAFL or NASH according to the criteria of NAFLD Activity Score (NAS) [13]. NASH patients were not included in this study. Inclusion criteria for the controls were: 1) age >18 years; 2) no history of abnormal liver ultrasound imaging or abnormal liver function tests; 3) currently normal liver function tests. Individuals with hepatitis carrier, diabetes, hypertension, chronic renal failure on hemodialysis, congestive heart failure or other known major disease were excluded from the study. Written informed consent of all participants was obtained from the study subjects or their relatives, and the protocol of the study was approved by the Ethics Committee of Clinical Medical College, Yangzhou University.

For each case body mass index (BMI) was calculated at the time of blood collection as weight in kilograms divided by height in meters squared. HOMA-IR index was calculated for each patient using the formula [fasting glucose (mmol/L) × fasting insulin (mIU/L)/ 22.5] [20,24].

Blood samples were collected from each study participant at 08:00 a.m., 12 h after an overnight fast, taken on ice, immediately centrifuged in cool conditions (4 °C) as described previously [6]. In brief, the blood samples (2 mL) were collected in prechilled EDTA tubes containing 100 μl aprotinin (1 μg/mL) and were immediately centrifuged for 15 min at $1000 \times g$, 4 °C within 30 min of collection. Plasma was separated into vials and stored at -80 °C until measurement. Plasma insulin levels were measured by radioimmunoassay (the average sensitivity was 2 μIU/mL. Intra-assay precision CV%<10% and inter-assay precision CV%<15%). Regular biochemical tests included glucose, triglyceride (TG), total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C) and low-density lipoprotein cholesterol (LDL-C) were performed on the Olympus AU2700 automatic chemistry analyzer (Olympus Corporation, Tokyo, Japan). After routine analysis, left over plasma aliquots were immediately stored at −80 °C for spexin and adiponectin determination.

Plasma concentration of spexin was measured using an enzyme-linked immunosorbent assay (Phoenix Pharmaceuticals, Burlingame, California, USA). According to the manufacturer's specification, the lower limit of the assay was 0.11 ng/mL, and the average sensitivity was 0.11 ng/mL. Intra-assay precision CV%<10% and inter-assay precision CV%<15%. Plasma concentration of adiponectin was measured using an enzyme-linked immunosorbent assay (CUSABIO, Inc. Wuhan, China). According to the manufacturer's specification,

Table 1
Biochemical and demographic characteristics of two groups.

	Lean Control	Obese Subject	p value
N	38 (male, 16; female 22)	41 (male, 18; female 23)	
Age (years)	38.47 ± 11.63	35.17 ± 12.29	0.224
Body weight (kg)	62.16 ± 8.90	81.91 ± 9.90	<0.001
BMI (kg/m2)	22.83 ± 3.00	30.97 ± 2.75	<0.001
Fasting glucose (mmol/L)	4.84 ± 0.50	5.77 ± 1.20	<0.001
Fasting Insulin (mIU/L)	6.75 ± 2.80	11.14 ± 3.47	<0.001
ALT (U/L)	20.21 ± 15.65	39.13 ± 18.73	<0.001
AST(U/L)	19.12 ± 6.97	25.65 ± 9.45	0.031
Bilirubin(umol/L)	17.11 ± 4.45	18.43 ± 5.32	0.312
TG (mmol/L)	1.54 ± 0.82	2.36 ± 0.89	<0.001
TC (mmol/L)	4.70 ± 0.97	5.47 ± 0.97	0.001
HDL-C (mmol/L)	1.37 ± 0.23	1.26 ± 0.20	0.030
LDL-C (mmol/L)	2.67 ± 0.54	3.06 ± 0.53	0.002
HOMA-IR	1.48 ± 0.72	2.83 ± 0.90	<0.001
Spexin (ng/mL)	3.68 ± 0.69	2.75 ± 0.70	<0.001
Adiponectin (ng/mL)	61.69 ± 23.90	46.12 ± 15.68	<0.001

Results are shown as means ± SD; N, number of cases; Statistical significance p < 0.05.

the lower limit of the assay was 1.562 ng/mL, and the average sensitivity was 1.102 ng/mL. Intra-assay precision CV%<8% and inter-assay precision CV%<10%. All measurements were performed in duplicate, and the mean of the two measurements was considered.

2.1. Statistical analysis

All statistical analyses were performed with SPSS 17.0 for Windows (SPSS, Chicago, IL). Data for each respective study were presented as mean ± SD. The differences between the groups were analyzed with independent t-test. Correlations were evaluated using the multiple linear regression method. Statistical significance was considered to be P < 0.05.

3. Results

The main characteristics of two groups were listed in Table 1. In group of demographic data, statistically significant differences between the tested groups were found for body weight (81.91 ± 9.90 vs. 62.16 ± 8.90 kg, P < 0.001), BMI (30.97 ± 2.75 vs. 22.83 ± 3.00 kg/m2, P < 0.001), TG (2.36 ± 0.89 vs. 1.54 ± 0.82 mmol/L, P < 0.001), TC (5.47 ± 0.97 vs. 4.70 ± 0.97 mmol/L, P = 0.001), HDL-C (1.26 ± 0.20 vs. 1.37 ± 0.23 mmol/L, P = 0.030), LDL-C (3.06 ± 0.53 vs. 2.67 ± 0.54 mmol/L, P = 0.002), ALT (39.13 ± 18.73 vs. 20.21 ± 15.65 U/L, P < 0.001) and AST (25.65 ± 9.45 vs. 19.12 ± 6.97 U/L, P = 0.031) (see Table 1). The mean fasting plasma glucose level was significantly higher in patients with non-alcoholic fatty liver than in normal subjects (5.77 ± 1.20 vs. 4.84 ± 0.50 mmol/L, P < 0.001) but still within the impaired fasting glycaemia range (5.6–6.1 mmol/L) [2]. Insulin resistance, assessed with the HOMA-IR, was significantly increased in patients with non-alcoholic fatty liver compared with normal controls (2.83 ± 0.90 vs. 1.48 ± 0.72, P < 0.001). Interestingly, plasma concentrations of spexin and adiponectin were significantly lower in patients with non-alcoholic fatty liver compared with age- and gender-matched normal controls (spexin, 2.75 ± 0.70 vs. 3.68 ± 0.69 ng/mL, P < 0.001; adiponectin, 46.12 ± 15.68 vs. 61.69 ± 23.90 ng/mL, P < 0.001) (see Table 1).

The possible correlations were assessed by multiple linear regression analyses. Spexin did not correlate with BMI (r = 0.181, P = 0.558) but did significantly correlate with HOMA-IR (r = -0.368; P = 0.018) and adiponectin (r = 0.378; P = 0.043). Besides, there is negatively significant correlation between adiponectin and BMI (r = -0.526; P < 0.001) or HOMA-IR (r = -0.412; P = 0.008). Since HOMA-IR is not a particularly precise measure of insulin resistance, we also analyzed the correlations between the spexin and fasting glucose, spexin and insulin. Besides, the correlations between adiponectin and fasting glucose, adiponectin and insulin has been studied. The significantly negative correlations were found between spexin and fasting insulin levels (r = -0.379; P = 0.042) as well as glucose (r = -0.416; P = 0.025).

4. Discussion

Despite some previous encouraging reports, the effect of spexin in the actual pathology in obesity and T2DM is still controversial. Recent studies have shown that spexin levels were found lower in obese and type diabetic individuals compare with control subjects [10, 15,18]. Besides, a positive correlation between circulating spexin levels and BMI has been reported in obese and type diabetic individuals [10,15,18]. However, the correlation of serum spexin with diabetes or with circulating parameters for insulin sensitivity cannot be demonstrated in young adolescent with type 2 diabetes mellitus [11]. In present study, circulating spexin levels were significantly decreased in patients with non-alcoholic fatty liver. Interestingly, spexin levels showed a negative correlation with HOMA-IR index in this study. Collectively, these results indicated that the lower plasma spexin levels were found in patients with NAFLD, and spexin levels were negatively correlated with insulin resistance in patients with NAFLD.

As previously reported by others, adiponectin levels were reduced in obese subjects [25,26]. The plasma adiponectin level is positively correlative to the high-density lipoprotein levels [22], but negatively correlative to body weight and TG levels [17]. Again, the present study showed that circulating adiponectin levels were significantly reduced in patients with NAFLD. Besides, the significant negative correlations were found between adiponectin and BMI, HOMA-IR, glucose and TC. These results further supported that the low level of plasma adiponectin could participate in the etiology of insulin resistance in patients with NAFLD.

Free fatty acid and hepatic triglyceride accumulation is a cardinal feature of NAFLD, and commonly occurs in the setting of insulin resistance and obesity. Spexin and adiponectin have been identified to have beneficial effects on insulin sensitivity. As to our expectation, significant correlations between spexin and adiponectin as well as spexin and HOMA-IR were observed in patients with NAFLD group. In addition, we analyzed the correlations between the plasma spexin levels and fasting glucose or insulin separately. There was an obvious negative correlation between spexin and fasting insulin levels as well as glucose in patients with NAFLD group. These results provided evidence that the spexin levels were negatively correlated with the insulin resistance in patients with NAFLD.

In conclusion, plasma concentrations of spexin and adiponectin were significantly lower in patients with NAFLD. The spexin levels were strongly correlated with fasting insulin and HOMA-IR as well as glucose in patients with NAFLD. Besides, the significant negative correlations were found between adiponectin and BMI, HOMA-IR and glucose. Thus, in this small group of patients with non-alcoholic fatty liver we demonstrated that insulin resistance correlated strongly with spexin and adiponectin levels.

Declaration of competing interest

The authors declare that there are no conflicts of interest.

Acknowledgments

Ethical statement

The authors declare that there are no conflicts of interest. This article does not contain any study data with animals performed by any of the authors. Informed consent was obtained from all individuals recruited in the study.

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