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Association of nesfatin-1 levels with fasting and postload glucose levels in patients with hypothyroidism

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ABSTRACT

Nesfatin-1, which is known as an anorexigenic peptide, has a potential role in the control of glucose/insulin regulation. We aimed to investigate serum nesfatin-1 levels in patients with untreated hypothyroidism due to Hashimoto's thyroiditis and correlate with fasting plasma glucose (FPG) and postload glucose levels. This study included 40 patients with hypothyroidism (mean age; 32.3±12.6 years, body mass index (BMI); 27.8±7.9 kg/m^2) and 54 age and BMI matched healthy control group (mean age; 29.3±8.5 years BMI; 29.7±7kg/m^2). All subjects underwent a 75 gr oral glucose tolerance test. Serum nesfatin 1 levels were similar among the hypothyroid subjects and control group (14.1±13.4 vs 16.7±15.4 ng/ml, respectively) (p=0.152). FPG (95±8 vs 96.6±7.9 mg/dl) and 2 h postload glucose (113.6±26.7 vs 113.1±27.5 mg/dL) levels did not differ between the two groups (p=0.339, p=0.976 (respectively)). There was no significant correlation between nesfatin-1 and free T3, free T4, TSH, anti-thyroglobulin autoantibody (TGAb) and anti-thyroid peroxidase autoantibody (TPOAb) (p>0.05). While nesfatin 1 levels did not correlate with FPG, 2 h postload glucose, The Homeostasis Model Assessment (HOMA-IR), total-cholesterol, HDL-cholesterol and LDL-cholesterol levels, it showed a negative correlation with triglyceride levels (p=0.008). Nesfatin-1 levels were not associated with FPG and postload glucose levels in patients with hypothyroidism.

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1. Introduction

Nesfatin-1, an 82 amino acids peptide, was identified in the hypothalamus as the N-terminal product of the precursor protein nucleobindin 2 protein (NUCB2) (Oh-I et al., 2006). In addition, some studies showed expression of nesfatin-1 in the human and rodent pancreas (Foo et al., 2010). Nesfatin-1 reduced nocturnal food intake after injection into the third cerebral ventricle of rats and induced a concomitant reduction in body weight gain as a result of subchronic administration (Oh-I et al., 2006). The hypothalamic NUCB2 expression in the paraventricular nucleus (PVN), a nucleus that has an association with the control of feeding behavior and metabolism (Shimizu et al., 2009; Stengel and Taché, 2011), varies with alterations of energy status being reduced after short or chronic fasting and conversely activated after refeeding (Foo et al., 2010).

Immunoreactivity of nesfatin in pancreatic beta cells and co-localization with insulin, suggest a potential role in the control of glucose/insulin regulation (Su et al., 2010; Gonzalez et al., 2011). In experimental studies, it has been shown that nesfatin-1 increases glucose-induced insulin release from pancreatic beta cells by promoting calcium influx through L-type calcium channels (Nakata et al., 2011). In human studies, nesfatin-1 showed an inverse relationship with circulating glucose levels in patients with type 2 diabetes mellitus (Li et al., 2010). Similarly, nesfatin-1 levels were lower in obese patients (Abaci et al., 2013) and subjects with non-alcoholic fatty liver disease (Başar et al., 2012).

Table 1. The clinical, biochemical and hormonal results in hypothyroid patients and healthy controls

Variable	Hypothyroid group (n=40)	Control group (n=54)	p
Age (years)	32.3±12.6	29.3±8.5	0.484
Gender (% female)	90% (36)	81.5% (44)	0.251
BMI (kg/m^2)	27.8±7.9	29.7±7	0.131
WC (cm)	90±15.6	94.9±14.9	0.122
SBP (mmHg)	125.6±17.9	122.4±10.5	0.519
DBP (mmHg)	75.4±13.4	74.4±10.2	0.642
FT3 (pg/mL)	3.1±0.3	3.2±0.3	0.093
FT4 (ng/dL)	1±0.2	1.1±0.1	0.002
TSH (μIU/mL)	11±15.1	2±1.1	<0.001
TGAb (uIU/mL)	243±302.2	1.8±1.7	<0.001
TPOAb (uIU/mL)	456.3±407.2	1.4±5.6	<0.001
Fasting plasma glucose (mg/dL)	95±8	96.6±7.9	0.339
2 h-PG (mg/dL)	113.6±26.7	113.1±27.5	0.976
Fasting insulin (μ IU/mL)	8±4.7	9±4.6	0.167
HOMA-IR	1.8±1	2.2±1.1	0.121
T-Cholesterol (mg/dL)	197.3±43	186.9±36.3	0.208
Triglyceride (mg/dL)	112.1±75	100.3±50	0.622
HDL (mg/dL)	51.5±10.7	47.7±11.3	0.056
LDL (mg/dL)	123.3 (38.2)	119.9 (30.4)	0.632
hsCRP (mg/dL)	0.2±0.3	0.3±0.6	0.292
Nesfatin-1 (ng/mL)	14.1±13.4	16.7±15.4	0.152

Values are expressed as mean±SD. **BMI:** Body mass index; **WC:** Waist circumference; **SBP:** Systolic blood pressure; **DBP:** Diastolic blood pressure; **FT3 free T3, FT4 free T4, TSH:** Thyroid stimulating hormone; **TGAb**: Anti-thyroglobulin autoantibody; **TPOAb**: Anti-thyroid peroxidase autoantibody; 2h-PG 2 hour plasma glucose after a 75 g glucose load; **HOMA-IR;** Homeostasis model assessment insulin resistance index; **T-C:** Total cholesterol; **HDL-C:** High density lipoprotein cholesterol; **LDL-C**: Low density lipoprotein cholesterol; **hs-CRP:** High-sensitive C-reactive protein.

Hypothyroidism is associated with changes in energy metabolism. Hypothyroid patients usually gain weight in spite of decrease in their appetite (Iglesias and Diez, 2007). It is not clear whether the circulating nesfatin-1 levels contribute to these changes in hypothyroid patients, and the present data regarding the relationship between nesfatin-1 levels and hypothyroidism are limited. Recently, one study has demonstrated that nesfatin-1 levels were lower in children with untreated subclinical hypothyroidism, however they did not observe a relationship between nesfatin-1 and thyroid hormones (Sawicka and Bossowski, 2013).

As nesfatin-1 may play an important role in the regulation of body weight and insulin secretion, we hypothesized that plasma nesfatin-1 levels might be affected by thyroid hormone levels and insulin resistance in subjects with hypothyroidism. We, therefore, investigated serum nesfatin-1 levels in untreated patients with hypothyroidism due to Hashimoto's thyroiditis and correlated them with Homeostasis Model Assessment-Insulin Resistance (HOMA-IR), fasting plasma glucose (FPG) and two hour plasma glucose levels after a 75 gr glucose load.

2. Materials and methods

Study population

This study included 40 patients with hypothyroidism and age-body mass index (BMI) matched 54 healthy controls. Most of the patients (72.5%) were diagnosed with subclinical hypothyroidism. Each participant gave a written consent. None of the patients were receiving levothyroxine (LT4) or antithyroid drugs. Subjects with a history of diabetes mellitus, renal or hepatic dysfunction, acute or chronic inflammatory disease, cancer and infectious diseases were excluded from the study. The other exclusion criteria were; drug use affecting thyroid function tests and glucose metabolism such as antilipidemic and antihypertensive drugs, insulin-sensitizing drugs and glucocorticoids. The control subjects were not suffering from any health problems and were not receiving any medications.

Clinical, biochemical and hormonal measurements

The patients having serum thyrotropin (TSH) (N: 0.35-4.94 uIU/mL) levels over 4.9 IU/mL and serum free T4 (fT4) (N: 0.7-1.48 ng/dL) levels below 0.7 ng/dL were classified as hypothyroid group, the patients having TSH levels over 4.9 IU/mL and serum fT4 levels 0.7-1.48 ng/dL were classified as subclinical hypothyroid. We measured serum levels of anti-thyroglobulin autoantibody (TGAb), anti-thyroid peroxidase autoantibody (TPOAb) in all patients and thyroid ultrasonography was performed in all patients. The patients with Hashimoto's thyroiditis (confirmation with antibody positivity or sonographic appearance of thyroiditis) were included in the study.

All the subjects underwent a physical examination and weight, height, waist circumference (WC) and systolic and diastolic blood pressure were measured. The waist circumference was measured at the high point of the iliac crest at minimal respiration to the nearest 0.1 cm. BMI was calculated by dividing the body weight in kilograms by the square of the height in meters.

Venous blood samples were obtained from all subjects following 12 h overnight fasting. The levels of fasting glucose, insulin, total cholesterol (T-C), triglyceride (TG), high-density lipoprotein cholesterol (HDL), low-density lipoprotein cholesterol (LDL-C) and high-sensitive C-reactive protein (hs-CRP) were measured. All the subjects underwent a 75 g oral glucose tolerance test (OGTT), with venous glucose sampling at 0 min and 120 min. Categories of glucose tolerance were defined according to 2006 WHO criteria (WHO report, 2006). Impaired fasting glucose (IFG) is defined by an elevated fasting plasma glucose (FPG) concentration (≥100 and<126 mg/dL). Impaired glucose tolerance (IGT) is defined by an elevated 2-hour plasma glucose concentration (≥140 and<200 mg/dL) after a 75-g glucose load on the OGTT in the presence of a FPG concentration <126 mg/dL. An insulin resistance score HOMA-IR was computed by the following formula (18): HOMA-IR=fasting plasma glucose (mmol/L)×fasting serum insulin (mU/mL)/22.5. The cut off value was taken 2.7 for HOMA-IR (Gokcel et al., 2003).

The hexokinase method was used to measure glucose levels, and photometric method (Abbott Architect c16000

Table 2. The results of OGTT in hypothyroid and control group

OGTT	Hypothyroid group	Control group	p
Normal	65% (26)	59.3% (32)	
IFG	20% (8)	29.6% (16)	
IGT	12.5% (5)	9.3% (5)	0.403
IFG+IGT	2.5% (1)	-	
Diabetes	-	1.9% (1)	

OGTT: Oral glucose tolerance test; **IFG:** Impaired fasting glucose; **IGT:** Impaired glucose tolerance

autoanalyzer) was used to measure the total cholesterol, TG, HDL, and LDL levels. Insulin was measured using Chemiluminescent Microparticle Immunoassay (CMIA) (Abbott, Architect system, USA). The concentration of hsCRP was measured using immunotubidimetric method with Abbott Architect C16000 autoanalyzer (Abbott Diagnostic, USA). The cut off for hsCRP was taken <0.5 mg/dL.

TSH, free T3 (fT3), free T4, TGAb and TPOAb concentrations were measured using CMIA method with Abbott Architect i2000 (Abbott Diagnostic, USA).

Measurement of nesfatin-1

The concentration of nesfatin-1 was measured using enzyme-linked immunosorbent assay (ELISA) method. We used commercially available human nesfatin-1 ELISA kit (MyBiosource, USA). The procedure for the ELISA method was according to the instructions provided by the manufacturer. Absorbance was measured at a wavelength of 450 ηm using ELISA reader. The levels of nesfatin-1 are presented as ng/mL. The intra-assay and inter-assay coefficient of variation were <10% and <12%, respectively. The limit of detection (LOD) for the nesfatin-1 assay was 0.15 ng/mL.

Statistical analysis

Data were analyzed using SPSS Software (Version 17, SPSS, Inc., Chicago, IL, USA). Results were expressed as mean± standard deviation. The Mann-Whitney U test was used to compare the continuous variables and the Chi-square test was used to compare categorical variables. Spearman's rank correlation test was used for calculation of associations between variables. Chi-square test test was used to compare the results of OGTT between the patient and control groups. A p value of less than 0.05 was considered to be statistically significant.

3. Results

We enrolled 40 patients with hypothyroidism (mean age; 32.3±12.6 years, BMI; 27.8±7.9 kg/m^2) and 54 age and BMI matched healthy control subjects (mean age; 29.3±8.5 years, BMI; 29.7±7 kg/m^2) in the current study. The clinical and biochemical results of the study population are shown in (Table 1).

FPG (95±8 vs 96.6±7.9 mg/d<l) and 2 h postload glucose (113.6±26.7 vs 113.1±27.5 mg/dL) levels did not differ between the hypothyroid and control groups respectively (p=0.339, p=0.976) (Table 1). According to the OGTT; 20% of the hypothyroid patients were diagnosed with IFG, 12.5% IGT and 2.5% IFG+IGT; and these ratios were similar in the control group (Table 2). The values of HOMA-IR were not different between the two groups (1.8±1 vs 2.2±1.1, p=0.121). Lipid parameters and hsCRP levels were also similar between groups (Table 1).

Serum nesfatin-1 levels were not different among the hypothyroid subjects and control group (14.1±13.4 vs 16.7±15.4 ng/mL, respectively, p=0.152). Also, nesfatin-1 levels were not correlated with TSH, FT3, FT4, TGAb and TPOAb levels (p>0.05) (Table 3). There was no significant correlation between nesfatin-1 levels and BMI, WC, FPG, fasting insulin and HOMA-IR values (Table 3). Nesfatin-1 levels did not correlate with the results of OGTT (p=0.231) and also did not change according to the presence of prediabetes (0.671). Serum levels of nesfatin-1 showed a significant negative correlation with triglyceride levels (Table 3, p= 0.008), however there was not a relation with the other lipid parameters.

Table 3. The correlation between nesfatin 1 levels and metabolic parameters and thyroid hormone levels in hypothyroid patients

Parameter	r	p
Age	-0.272	0.090
SBP	-0.066	0.687
DBP	0.056	0.733
FT3	0.039	0.809
FT4	0.023	0.887
TSH	0.009	0.957
TGAb	-0.174	0.282
TPOAb	0.037	0.823
Fasting plasma glucose	0.022	0.893
Insulin	0.052	0.749
2-hour plasma glucose of OGTT	0.047	0.773
T-C	-0.173	0.285
Triglyceride	-0.412	0.008
HDL-C	0.205	0.205
LDL-C	-0.166	0.305
BMI	-0.080	0.623
HOMA-IR	0.065	0.692

SBP: Systolic blood pressure; **DBP:** Diastolic blood pressure; **FT3 free T3, FT4 free T4, TSH:** Thyroid stimulating hormone; **TGAb:** Anti-thyroglobulin autoantibody; **TPOAb:** Anti-thyroid peroxidase autoantibody; **T-C:** Total cholesterol; **HDL-C:** High density lipoprotein cholesterol; **LDL-C:** Low density lipoprotein cholesterol; **BMI:** Body mass index; **HOMA-IR:** Homeostasis model assessment insulin resistance index

4. Discussion

In the present study, we showed that serum levels of nesfatin-1, which is known as an anorexigenic peptide, did not change in response to hypothyroidism, and were not correlated with BMI, HOMA-IR, fasting and two hour plasma glucose levels after a 75 g glucose load. We demonstrated a significant negative correlation between nesfatin-1 and triglyceride levels.

Patients with thyroid dysfunction frequently show changes in metabolic parameters that affect fat mass. Hypothyroidism is usually associated with decreased appetite with a slight weight gain, decreased thermogenesis and metabolic rate. In our study, both of the two groups comprised overweight subjects. BMI of the patients were not different in hypothyroid and healthy subjects. Some studies investigated the relationship between hypothyroidism and peptides which have effects on appetite and energy metabolism. Altinova et al. (2006) showed decreased serum levels of ghrelin in hypothyroid patients. In another study, levels of obestatin, which decrease the appetite, were lower in children with untreated subclinical hypothyroidism (Sawicka et al., 2009).

Nesfatin-1, which inhibits nocturnal food intake, may have influence on metabolic parameters in patients with hypothyroidism. There is one study in the literature that investigates circulating nesfatin-1 levels in patients with thyroid hypofunction and they demonstrated that nesfatin-1 levels were lower in children with untreated subclinical

hypothyroidism in Hashimoto´s thyroiditis compared to the healthy subjects (Sawicka and Bossowski, 2013). In our study, nesfatin-1 levels were similar in hypothyroid and healthy individuals.

Nesfatin-1 increases glucose-stimulated insulin release from pancreatic beta cells and recently it has been demonstrated that nesfatin-1 also affects glucose metabolism by a direct peripheral mechanism to increase insulin secretion and insulin sensitivity in the skeletal muscle, adipose tissue and liver (Li et al., 2013). In human studies, there are conflicting results on nesfatin-1 levels in patients with impaired glucose intolerance and type 2 diabetes. Li et al. (2010) have reported that levels of fasting plasma nesfatin-1 are lower in type 2 diabetes mellitus than in normal controls. However, Zhang et al. (2012) reported a positive relationship between plasma nesfatin-1 levels and impaired glucose tolerance. In our study, we did not find a relationship between nesfatin-1 levels and parameters associated with glucose metabolism, such as fasting plasma glucose, HOMA-IR and 2 hour plasma glucose levels after a 75 g glucose load.

Hypothyroidism is known to be associated with lipid abnormalities. Lipolysis is reduced and the serum concentrations of triglycerides and cholesterol are elevated (Pucci et al., 2000). However, there was not a difference in lipid levels between hypothyroid and control group in the present study. This may be due to the low ratio of overt hypothyroidism in the study population. Our study demonstrated a negative correlation between nesfatin-1 and triglyceride levels. This may be explained by the anorexigenic effects of nesfatin-1.

In conclusion, hypothyroidism did not seem to be associated with metabolic parameters, such as obesity, abnormal glucose metabolism, dyslipidemia and insulin resistance. Nesfatin-1 levels were not related with TSH, fT3, fT4 and autoantibody levels and also did not have an impact on FPG, 2 h postload glucose levels and HOMA-IR. We showed a negative correlation between nesfatin-1 and triglyceride levels in hypothyroid patients.

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