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Increased Serum Nesfatin-1 Levels in Patients with Impaired Glucose Tolerance

Bozulmuş Glukoz Toleransı Olanlarda Artmış Serum Nesfatin-1 Düzeyleri

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Abstract

Purpose: Nesfatin-1 is a recently discovered energy-regulating peptide, widely expressed in both central and peripheral tissues. It is involved in various functions, such as the stimulation of hypothalamic-pituitary-adrenal axis and sympathetic nervous system, influencing visceral functions, water intake, and regulation of temperature and emotions. It exerts a direct glucose-dependent insulinotropic action on the beta cells of pancreatic islets. The current study evaluated nesfatin-1 levels and insulin response to glucose load in patients with impaired glucose tolerance (IGT) and in healthy subjects.

Material and Method: Of those patients who underwent the oral glucose tolerance test (OGTT), 14 with IGT and 13 body mass index- (BMI) and age-matched healthy subjects as controls were included in the study. Blood samples were taken at 0, 60 and 120 min, and the glucose, insulin, and nesfatin-1 levels were measured.

Results: The basal levels of glucose, insulin, and nesfatin–1 were significantly higher in the patients with IGT than in controls. Two-way repeated measures ANOVA revealed that change in time (CIT) for glucose and insulin during an OGTT was significant ($p<0.001$ and $p<0.001$, respectively). CIT for glucose and insulin was significantly different between the IGT patients and the controls ($p<0.001$ and $p=0.003$, respectively). CIT for nesfatin-1 was not significant ($p=0.406$) and did not differ significantly between the two groups ($p=0.331$).

Discussion: The elevated levels of basal nesfatin-1 were observed in the patients with IGT. There was no change in the absolute nesfatin–1 levels in response to glucose load in either group. The increase in the levels of basal nesfatin-1 may reflect a compensatory mechanism to regulate the impaired glucose metabolism in the IGT patients, which is later underwhelmed with the onset of diabetes.

Keywords: Impaired glucose tolerance; insulin; nesfatin-1

Özet

Amaç: Nesfatin-1 enerji düzenleyici bir peptid olarak yakın zamanda keşfedilmiştir ve hem merkezi hem de periferal dokularda mevcuttur. Hipotalamik-hipofizer-adrenal aks ve sempatik sinir sisteminin uyarılması, viseral fonksiyonların devamı, su alımı ile ısı ve duyguların düzenlenmesi gibi birçok farklı fonksiyonla ilişkili olduğu düşünülmektedir. Pankreas adacıkların beta hücreleri üzerine direkt glukoz bağımlı insulinotropik etkisi vardır. Çalışmada sağlıklı ve bozulmuş glukoz toleranslı (IGT) kişilerde glukoz yüklemesine insülin ve nesfatin-1 cevapları incelenmiştir.

Gereç ve Yöntem: Oral glukoz tolerans testi (OGTT) yapılan kişilerin 14 tanesi IGT tanılı 13 tanesi de vücud kütle indeksi (BMI) ve yaş açısından eşleşmiş sağlıklı kontroller çalışmaya dahil edilmiştir.Kan örnekleri 0., 60. ve 120. dakikalarda alınmış, glukoz, insülin ve nesfatin-1 düzeyleri ölçülmüştür.

Bulgular: Glukoz, insülin ve nesfatin-1 bazal düzeyleri kontroller ile karşılaştırıldığında IGT'li kişilerde daha yüksek saptanmıştır. İki yönlü tekrarlanmış ölçümlerin ANOVA'sı , OGTT sırasında glukoz ve insülin için zaman içindeki değişimlerin (change in time;CIT) anlamlı olduğunu göstermiştir. IGT ve sağlıklı kontroller arasında glukoz ve insülin için CIT anlamlı farklılığa sahiptir (sırasıyla, $p<0.001$ ve $p= 0.003$). Nesfatin-1 için CIT anlamlı bulunmamış ($p=0.406$) ve gruplar arasında fark izlenmemiştir ($p=0.311$).

Tartışma: IGT'li kişilerde artmış bazal nesfatin-1 düzeyleri olduğu görülmüştür. Her iki grupta da glukoz yüklemesine cevap olarak mutlak nesfatin-1 düzeylerinde bir değişiklik izlenmemiştir. Bazal nesfatin-1 düzeylerindeki artış IGT'li hastalarda bozulmuş glukoz metabolizmasını düzenleyen kompenzatuar bir mekanizmayı yansıtıyor olabilir. Bu, daha sonra diyabet başlangıcı ile etkisiz hale gelmektedir.

Anahtar kelimeler: Bozulmuş glukoz tolerans; insülin; nesfatin-1

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Introduction

Nesfatin-1 is a novel appetite-regulating, hypothalamic, NUCB2-derived peptide, which has been found associated with melanocortin signaling pathway in a rat model (1). Nesfatin-1 is expressed in several tissues including the nervous tissue (hypothalamic paraventricular nucleus, supraoptic nucleus, arcuate nucleus, lateral hypothalamic area, nucleus tractus solitarius, and spinal cord) and peripheral tissues (pancreas, liver, subcutaneous and visceral fat tissues, brown adipose tissue, and skeletal muscles) (1-6).
Recent reports have demonstrated that the effect of nesfatin-1 on insulin secretion is dependent on blood glucose concentrations (7, 8). Foo et al. (9) demonstrated that the release of NUCB2/Nesfatin-1 from the isolated rat pancreatic islets significantly increased in response to glucose. Nesfatin-1 contributes to the improvement in insulin sensitivity in the hyperglycemic state. An increase in the levels of circulating nesfatin-1 may shift the glucose uptake toward peripheral organs (10). The changes in the nesfatin-1 levels (increase, decrease or no change) have been reported in different disease states (11). The current study aimed to investigate the insulin and nesfatin-1 response to glucose load in patients with IGT and in healthy subjects.

Materials and Methods

Study population

Fourteen subjects were diagnosed with IGT using a 75 g OGTT, in accordance with the World Health Organization criteria. These fourteen IGT patients (mean age=45.9±8.8 years) and thirteen age- and BMI-matched healthy subjects as controls (mean age=43.4 ±7.1 years) were included in the study (Table 1). All the subjects were examined using a standardized form that included a medical history and physical examination findings. Weight, height, waist, and hip circumferences (waist: midway between the lower rib margin and the iliac crest, hip: widest circumference over the greater trochanter) were measured. The BMI [weight in kilograms/(height in meters)^2] was calculated for each subject. The patients with type 1 and type 2 diabetes mellitus (DM), acute and chronic infectious diseases, heart failure, hypertension, liver and kidney diseases, cancer, and pregnancy were excluded from the study. This study was approved by the Ethics Committee of Faculty of Medicine, Hacettepe University.

Assays

Basal blood samples of the subjects were tested for fasting glucose levels, fasting insulin levels, total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), and triglycerides (TG). The blood samples were then taken at 0, 60 and 120 min of OGTT for the measurement of glucose, insulin, and nesfatin-1 levels. These blood samples were centrifuged, within 30 min of collection, at 4 °C for 20 min at 4000 rpm. The supernatant plasma samples obtained were transferred into polypropylene tubes and stored at -80 °C, until assayed.
Glucose levels were measured by a spectrophotometric assay (Human Gesellschaft, Wiesbaden, Germany) and insulin levels were measured by an immunoradiometric assay (Immunotech IRMA, Prague, Czech Republic). Triglycerides, total cholesterol, and HDL cholesterol were measured by enzymatic colorimetric test (Roche Diagnostics GmbH, Mannheim, Germany). LDL cholesterol level was calculated using the Friedewald formula. The fasting plasma level of nesfatin-1 was measured using a commercially available enzyme-linked immunosorbent (ELIA) assay kit, in accordance with the manufacturer's instructions (Phoenix Pharmaceuticals, Belmont, CA).

Statistical analysis

All statistical analyses were performed using SPSS 15.0 software (SPSS Inc. Chicago, IL, USA), and a value of $p<0.05$ was considered statistically significant. Descriptive statistics were expressed as mean±standard deviation. As the relationship between the two variables was not linear, it was evaluated using Spearman's rank correlation coefficient. Two-way repeated measures ANOVA, with a correction for Greenhouse-Geisser epsilon, was used to assess the significance of glucose, insulin, and nesfatin-1 data among the two groups, and their change in time (0, 60, and 120 min). In addition, the interaction between time and the groups was also examined.

Results

The characteristics of the patients and the controls are given in Table 1. Baseline glucose, insulin, and nesfatin–1 levels were significantly higher in the patients with IGT than in controls (Table 1). Two-way repeated measures ANOVA revealed that CIT for glucose and insulin during the OGTT was significant ($p<0.001$ and $p<0.001$, respectively). The change in time for glucose and insulin was significantly different between the IGT patients and controls ($p<0.001$ and $p=0.003$, respectively). The change in time for nesfatin-1 was not significant ($p=0.406$); also, it was not significantly different between the two groups ($p=0.331$) (Table 2).
Fasting plasma nesfatin-1 levels correlated negatively with age in the IGT patients ($r=-0.548$, $p=0.042$). In the controls, nesfatin-1 levels correlated positively with LDL-C ($r=0.736$, $p=0.004$). However, no relation was observed between the CIT for nesfatin-1, and glucose, and insulin.

Discussion

In this study, it was observed that the baseline nesfatin–1 levels were significantly higher in patients with IGT than in controls. Absolute CIT for nesfatin-1 was non-significant, and it was similar in both of the groups. Moreover, it was observed that the percentage CIT was not different for nesfatin-1. Li et al. (12) demonstrated that the mean fasting plasma nesfatin-1 levels were slightly, but not significantly, higher in the patients with type 1 DM compared to the healthy subjects. However, they demonstrated that the fasting plasma nesfatin-1 levels were significantly lower in the patients with type 2 DM, compared to the healthy subjects as well as the patients with type 1 DM. Zhang et al. (13) reported that when compared to control subjects, the plasma nesfatin-1 levels were elevated in the patients with newly diagnosed type 2 DM as well as in the patients with IGT. They demonstrated that the plasma nesfatin-1 levels also correlated positively with HbA1c, fasting blood glucose, blood glucose level 2 h after a glucose load, fasting plasma insulin, and the homeostatic model assessment of insulin resistance (HOMA-IR). They explained that insulin resistance might be the possible reason for the increased levels of nesfatin-1 in newly diagnosed type 2 diabetic patients.
Riva et al. (14) reported that nesfatin-1 increased the glucagon secretion *in vitro* and insulin secretion *in vivo* in mice. They demonstrated that NUCB2 is expressed in human and rodent beta cells and that

Table 1. Clinical characteristics of the study groups.

Variables	IGT groups (n=14)	Control groups (n=13)	P
Age (years)	45.9±8.8	43.4±7.1	0.627
Gender (F/M)	11/3	9/4	0.678
BMI (kg/m^2)	29.0±4.3	29.0±3.8	0.996
Waist (cm)	96.0±13.6	93.1±12.7	0.579
Hip (cm)	104.5±9.2	105.7±6.7	0.706
SBP (mmHg)	121.1±15.5	126.8±11.3	0.296
DBP (mmHg)	76.2±8.9	82.7±8.6	0.066
Total cholesterol (Normal range: 0-199 mg/dL)	196.2±23.2	188.2±36.7	0.502
Triglyceride (Normal range: 0-149 mg/dL)	143.1±76.4	123.9±31.8	0.408
LDL-K (Normal range: 0-130 mg/dL)	133.6±27.7	115.9±16.0	0.059
HDL-K (Normal range: 35-70 mg/dL)	56.0±24.2	54.4±12.1	0.830
Fasting blood glucose (Normal range: 70-100 mg/dL)	103.7±12.6	91.1±8.1	**0.005**
Fasting insulin (Normal range 2-25 µIU/mL)	15.2±6.1	10.4±3.5	0.018
Fasting nesfatin-1 (ng/mL)	2.1±2.6	0.5±0.2	**0.033**

Table 2. Change in time for glucose, insulin, and nesfatin-1 levels.

	IGT			Control			Time	Time×group
Variable	**0.min**	**60.min**	**120.min**	**0.min**	**60.min**	**120.min**	**P value**	**P value**
Glucose (mg/dL)	103.7±12.5	175.3±45.4	145.7±25.1	91.0±8.0	111.8±31.6	96.3±19.0	<0.001	0.001
Insulin (µIU/mL)	15.2±6.0	106.4±52.9	54.5±25.3	10.4±3.5	43.8±29.3	27.2±13.8	<0.001	0.003
Nesfatin (ng/mL)	2.2±2.6	2.0±2.4	1.9±2.1	0.5±0.2	0.5±0.2	0.5±0.2	0.406	0.331

NUCB2 gene expression is reduced in the islets of type 2 DM patients. Their data suggested that nesfatin-1 is a novel glucagon-stimulatory factor in the beta cells and that its expression is decreased in the islets of type 2 DM patients (14). The results of our study reflect a possible defensive increase in the plasma nesfatin-1 concentration to regulate the impaired glucose metabolism in IGT patients, which is later corrupted by the development of diabetes. Our study was, however, limited by its cross-sectional design and a small sample size.

In conclusion, the baseline plasma nesfatin-1 levels were significantly higher in IGT patients compared to healthy controls. This contradictory finding can be explained as a defensive increase of nesfatin-1 to regulate the impaired glucose metabolism in IGT patients, which is later corrupted by the development of diabetes. Our study is a pioneer in demonstrating that the CIT for nesfatin-1 was not different between IGT patients and controls. Further studies are required to assess the impact of plasma nesfatin-1 on glucose metabolism.

Author Contributions

Ethics: Yes.

Patient approval: Yes.

Concept: Safak Akin, Nese Ersoz Gulcelik, Duygu Yazgan Aksoy, Design: Safak Akin, Nese Ersoz Gulcelik, Duygu Yazgan Aksoy, Data Collection or Processing: Safak Akin, Nese Ersoz Gulcelik, Duygu Yazgan Aksoy, Analysis or Interpretation: Safak Akin, Nese Ersoz Gulcelik, Duygu Yazgan Aksoy, Jale Karakaya, Literature Search: Safak Akin, Writing: Safak Akin.

Conflict of Interest: The authors declare that they have no conflict of interest.

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