UDC [616.379-008.64+616.12.331.1]-078:57.083.185'175.8

ROLE OF NESFATIN-1 IN MAINTAINING CARBOHYDRATE HOMEOSTASIS IN HYPERTENSIVE PATIENTS

Vizir M. O., Kovalyova O. M.
Kharkiv national medical university, Kharkiv, Ukraine

The aim of this study is to analyze changes in the nesfatin-1 level in hypertensive patients depending on the carbohydrate profile parameters. 83 hypertensive patients aged 33 to 77 years were examined. Nesfatin-1 levels were determined by enzyme immunoassay method. Hypertensive patients have significantly higher levels of adipocytokine than healthy people. Results of data analyses may indicate a possible antihyperglycemic and insulinotropic effect of nesfatin-1 in hypertensive patients with normoglycemia or prediabetes. Confirmation of these processes requires a special study.

KEY WORDS: nesfatin-1, insulin, hypertension, prediabetes, polymorbidity

РОЛЬ НЕСФАТИНУ-1 У ПІДТРИМЦІ ВУГЛЕВОДНОГО ГОМЕОСТАЗУ У ХВОРИХ НА ГІПЕРТОНІЧНУ ХВОРОБУ

Візір М. О., Ковальова О. М.
Харківський національний медичний університет, Харків, Україна

Метою даного дослідження є аналіз змін рівня несфатину-1 у хворих на гіпертонічну хворобу в залежності від параметрів вуглеводного профілю. Обстежено 83 пацієнта з гіпертонічною хворобою у віці від 33 до 77 років. Рівень несфатину-1 визначали методом імуноферментного аналізу. Хворі на гіпертонічну хворобу мають достовірно вищі рівні адипоцитокіну, ніж здорові люди. Результати аналізу даних можуть вказувати на антигіперглікемічний і інсулінотропний ефект несфатину-1 у хворих на гіпертонічну хворобу з нормоглікемією або прediабетом. Підтвердження цих процесів вимагає окремого дослідження.

КЛЮЧОВІ СЛОВА: несфатин-1, інсулін, гіпертонічна хвороба, прediабет, поліморбідність

РОЛЬ НЕСФАТИНА-1 В ПОДДЕРЖАНИИ УГЛЕВОДНОГО ГОМЕОСТАЗА У БОЛЬНЫХ ГИПЕРТОНИЧЕСКОЙ БОЛЕЗНЬЮ

Визир М. А., Ковалева О. Н.
Харьковский национальный медицинский университет, Харьков, Украина

Целью данного исследования является анализ изменений уровня несфатина-1 у больных гипертонической болезнью в зависимости от параметров углеводного профиля. Обследовано 83 пациента с гипертонической болезнью в возрасте от 33 до 77 лет. Уровень несфатина-1 определяли методом иммуноферментного анализа. Больные гипертонической болезнью имеют достоверно более высокие уровни адипоцитокина, чем здоровые люди. Результаты анализа данных могут указывать на антигипергликемический и инсулинотропный эффект несфатина-1 у больных гипертонической болезнью с нормогликемией или предиабетом. Подтверждение этих процессов требует отдельного исследования.

КЛЮЧЕВЫЕ СЛОВА: несфатин-1, инсулин, гипертоническая болезнь, предиабет, полиморбидность

INTRODUCTION

Cardiovascular diseases remain the leading position in the structure of Ukraine's population mortality for many years. According to the data of the last decade, the proportion of mortality from cardiovascular diseases increased from 62.5 % (2005) to 68.0 % (2015), and mortality rose from 28.9 to 31.8 % respectively among working age people [1]. Hypertension is the most common worldwide factor in the development of cardiovascular events.

Obesity contributes to development of a number of pathological conditions, including essential hypertension (EH), hypercholesterolemia, insulin resistance, and others. The link between these abnormalities exists at the level of etiological factors and pathogenetic mechanisms [2]. The association of hypertension, obesity and metabolic disorders greatly increases the risk of coronary heart disease, type 2 diabetes mellitus (T2DM) and its complications, which directly affects the morbidity and mortality rates. The development of T2DM usually follows a phase of impaired glucose metabolism which is manifested by insulin resistance, elevated fasting glucose and impaired glucose tolerance. Correction of these conditions is complicated partly because of incomplete understanding of the mechanisms by which glucose homeostasis is ensured. Therefore, detection of early predictors of metabolic disorders has important medical and social importance.

Nesfatin-1 was found by Oh-I and his colleagues in 2006 [3]. They showed that nesfatin-1 is released by neurons of nuclei of the hypothalamus responsible for appetite control, and identified it as a satiety molecule. Some studies have found that nesfatin-1 is secreted by peripheral tissues such as adipose tissue, the mucosa of the stomach, testis, and others in addition to some structures of the central nervous system [4]. In experimental studies nesfatin-1 production was found in beta-cells of the pancreatic islets. It was shown that nesfatin-1 potentiates glucose-induced insulin secretion by activating the transmembrane transport of Ca^{2+} ions through L-type calcium channels [5]. Unlike anorexigenic effect with CNS-mediated mechanism, peripheral nesfatin-1 improves glucose metabolism (shown in rodents, both non-obese and obese) by direct action on insulin targeted organs: skeletal muscle, liver and adipose tissue [6].

During 10 years studying by different authors, it has been identified both positive and negative properties of nesfatin-1. For example, due to its anorectic effect, the administration of nesfatin-1 is regarded as a potential method of obesity correction [7]. The increase in insulin secretion by nesfatin-1 administration gives hope for the possible using of it for the correction of hyperglycemia in patients with diabetes [5]. At the same time, other researchers identified some adverse effects of increasing its level. Tanida et al. showed that intracerebroventricular injection of nesfatin-1 stimulates the activity of the sympathetic nervous system, and the blood pressure rises significantly through the central melanocortin system [8]. Recently nesfatin-1 was presented as a factor regulating thyroid function in patients with T2DM and the aging process, due to its significant role in energy balance and glucose metabolism [9, 10].

Given the ambiguity of the clinical manifestations of nesfatin-1 plasma level changes, it is required further study of its relationship with the development of comorbid metabolic disorders in humans.

OBJECTIVE

The aim of this study is to analyze changes in the nesfatin-1 level in hypertensive patients depending on the carbohydrate profile parameters.

MATERIALS AND METHODS

83 hypertensive patients aged 33 to 77 years were examined. All patients were divided into 3 groups by clinical characteristics: Group 1 – 50 (60 %) patients with hypertension and normoglycemia, Group 2 – 15 (18 %) patients with hypertension and prediabetes, Group 3 – 18 (22 %) patients with hypertension and T2DM. The control group consisted of 12 healthy individuals.

Patients with next pathology were excluded from the study: secondary hypertension; heart rhythm or AV conduction disturbances; presence of chronic heart failure stage IIB-III (NYHA classification); acute myocardial infarction or stroke in the past, acute left- or right ventricular failure; traumatic lesions of the central nervous system; comorbid psychiatric disorders, alcoholism; decompensated liver disease (increased AST, ALT more than 3 times); diffuse connective tissue diseases; infectious diseases or cancer.

Blood pressure was measured in the sitting position of the patient after 5 min of rest. EH verification was based on the revision of the European Society of Hypertension recommendations (ESH, 2013). T2DM has been verified on the basis of recommendations of the American Diabetes Association (ADA, 2014).

The blood sample for the biochemical and enzyme immunoassay researches was taken from the ulnar vein in the morning after 6–12-hours starvation. Anthropometric measurements included height (cm), weight (kg), waist

circumference (WC, cm) and hips circumference (HC, cm). It was followed by calculation of body mass index (BMI, kg/m^2) according to the formula BMI = body weight / height^2 as well as calculation of the waist to hip ratio (WHR).

Fasting glucose of serum was taken and determined by the glucose oxidase method for the control of carbohydrate metabolism. In case of the diabetes absence and with patient's agreement the glucose tolerance test was conducted: during 5 minutes after taking of fasting blood sample, patient drinks a glass of warm water with glucose solution (75 g), followed by blood sampling in 2 hours. According to the ADA recommendations (2014), prediabetes has been established in patients with impaired fasting glucose (5.6–6.9 mmol/l) and postprandial hyperglycemia (7.8–11.0 mmol/L).

Insulin levels (mkIE/ml) were determined by enzyme immunoassay method using «DRG Instruments GmbH» kit of reagents (Germany). Insulin resistance (IR) was assessed using HOMA-IR (Homeostasis Model Assessment Insulin Resistance) = concentration of insulin (mkIE/ml) × fasting glucose (mmol/L) / 22.5. Caro indexes were calculated additionally as a ratio of fasting glucose to insulin levels. Nesfatin-1 levels (ng/ml) were determined by enzyme immunoassay method using Kono Biotech® Human Nesfatin-1 ELISA Kit reagents.

Analysis of the data was carried out by methods of nonparametric statistics. In samples with the non-parametric data distribution the results are presented as Me (Q25–Q75), where Me – median (the 50th percentile), Q25 and Q75 – the 25th and 75th percentiles respectively (the upper and lower quartiles). The Mann-Whitney test, ANOVA rank Kruskal-Wallis test, the median test were used for comparison of the results between groups. Spearman's rank correlation coefficient was used for estimation of the relationship between two variables. The null hypothesis is excluded at the level of $p < 0.05$ significance.

RESULTS AND DISCUSSION

The data of patients groups are shown in Table.

The analysis of these parameters showed that insulin levels were significantly higher in all hypertensive patients compared with control subjects. First of all, this may be due to the fact that most of the patients have varying degrees of obesity, which is often characterized by insulin resistance. In hypertensive non-obese patients peripheral insulin resistance manifests in more than half of the cases [11, 12]. Insulin levels in Groups 2 and 3 were significantly higher than in Group 1. However, difference wasn't established in comparison of the levels of insulin in prediabetes and T2DM patients.

HOMA-IR index in hypertensive patients with dysglycemia was also higher than in Group 1 or control group. Differences between HOMA-IR indexes in prediabetes and T2DM patients were not significant.

Caro index among all patients was significantly lower in Groups 1 and 2. In hypertensive patients with T2DM, this parameter is somewhat higher, that is probably due to higher levels of fasting blood glucose.

Nesfatin-1 levels in all patients (7.63 (6.91–8.43) ng/ml) were significantly higher than in the control group, regardless of the carbohydrate status. It can indicate possible prohypertensive effect of this adipocytokine. According to some studies, intracerebro-ventricular injection of nesfatin-1 increased blood pressure in rats due to stimulation the sympathetic nervous system by acting on the central melanocortin receptors [13]. It has also been shown that intravenous injections of nesfatin-1 to rats cause vasoconstriction by inhibiting the synthesis of nitric oxide, thereby increasing blood pressure [14].

No significant differences have been identified in comparing of nesfatin-1 levels of the groups. This was probably due to the fact, that some of patients in each group had concomitant obesity, which can be accompanied by changes in adipocytokine level. So, given the fact that initially nesfatin-1 has been identified as a satiety molecule, scientists have shown its ability to reduce the consumption of food by rodents [7]. It is also necessary to consider that the production of nesfatin-1 by adipose tissue is increasing in obesity and varies depending on the type of food [15].

Table

Clinical characteristics, anthropometric and laboratory indicators of patients

Index	Index value, Me (Q25-Q75)			
	Group 1	**Group 2**	**Group 3**	**Control group**
Age, years	60.5 (52.0–64.0) $p_2 > 0.05$ $p_3 > 0.05$ $p_0=0.05$	61.0 (55.0–66.0) $p_1 > 0.05$ $p_3 > 0.05$ $p_0=0.05$	65.0 (58.0–69.0) $p_1 > 0.05$ $p_2 > 0.05$ $p_0=0.05$	53.0 (49.5–55.0)
Height, cm	165.0 (160.0–172.0) $p_2 > 0.05$ $p_3 > 0.05$ $p_0 > 0.05$	168.0 (159.0–175.0) $p_1 > 0.05$ $p_3 > 0.05$ $p_0 > 0.05$	165.0 (160.0–170.0) $p_1 > 0.05$ $p_2 > 0.05$ $p_0 > 0.05$	170.0 (164.0–177.0)
Weight, kg	88.0 (80.0–98.0) **$p_2 < 0.001$** $p_3 > 0.05$ **$p_0 < 0.001$**	103.0 (91.0–118.0) **$p_1 < 0.001$** **$p_3 < 0.05$** **$p_0 < 0.001$**	89.0 (75.0–112.0) $p_1 > 0.05$ **$p_2 < 0.05$** **$p_0 < 0.001$**	63.5 (59.0–70.5)
BMI, kg/m^2	32.36 (28.84-36.84) **$p_2 < 0.001$** $p_3 > 0.05$ **$p_0 < 0.001$**	38.83 (33.57-44.58) **$p_1 < 0.001$** **$p_3 < 0.05$** **$p_0 < 0.001$**	31.92 (28.35-40.90) $p_1 > 0.05$ **$p_2 < 0.05$** **$p_0 < 0.001$**	22.47 (21.47–23.09)
WC, cm	104,0 (94,0–116,0) **$p_2 < 0,001$** $p_3 > 0,05$ **$p_0 < 0,001$**	120.0 (111.0–130.0) **$p_1 < 0.001$** **$p_3 < 0.05$** **$p_0 < 0.001$**	102.0 (94.0–125.0) $p_1 > 0.05$ **$p_2 < 0.05$** **$p_0 < 0.001$**	73.5 (71.0–80.0)
HC, cm	117.0 (103.0-122.0) **$p_2 < 0.01$** $p_3 > 0.05$ **$p_0 < 0.001$**	128.0 (111.0-139.0) **$p_1 < 0.01$** $p_3 > 0.05$ **$p_0 < 0.001$**	115.5 (102.0-139.0) $p_1 > 0.05$ $p_2 > 0.05$ **$p_0 < 0.001$**	95.0 (94.0–98.0)
WHR	0.90 (0.87-0.95) **$p_2 < 0.01$** $p_3 > 0.05$ **$p_0 < 0.001$**	0.94 (0.88-0.98) **$p_1 < 0.01$** **$p_3 < 0.01$** **$p_0 < 0.001$**	0.91 (0.83-0.94) $p_1 > 0.05$ **$p_2 < 0.01$** **$p_0 < 0.001$**	0.76 (0.74–0.85)
Fasting glucose, mmol/L	4.52 (4.18–5.00) **$p_2 < 0.001$** **$p_3 < 0.001$** $p_0 > 0.05$	6.63 (5.91–6.78) **$p_1 < 0.001$** $p_3 > 0.05$ **$p_0 < 0.001$**	7.45 (5.00–8.23) **$p_1 < 0.001$** $p_2 > 0.05$ **$p_0 < 0.001$**	4.62 (4.30-4.93)
Postprandial glucose, mmol/L	5.09 (3.63–6.88) **$p_2 < 0.001$**	8.52 (7.79–9.22) **$p_1 < 0.001$**	-	-
Insulin, mkIE/ml	29.90 (20.93–39.24) **$p_2 < 0.001$** **$p_3 < 0.05$** **$p_0 < 0.001$**	38.58 (27.16–58.62) **$p_1 < 0.001$** $p_3 > 0.05$ **$p_0 < 0.001$**	40.57 (20.03–48.80) **$p_1 < 0.05$** $p_2 > 0.05$ **$p_0 < 0.001$**	13.15 (11.26–15.01)
HOMA-IR	5.54 (4.04–8.33) **$p_2 < 0.001$** **$p_3 < 0.001$** **$p_0 < 0.001$**	11.42 (7.25–18.05) **$p_1 < 0.001$** $p_3 > 0.05$ **$p_0 < 0.001$**	9.22 (7.30–14.97) **$p_1 < 0.001$** $p_2 > 0.05$ **$p_0 < 0.001$**	2.61 (2.30–3.13)
Caro index	0.15 (0.120.21) $p_2 > 0.05$ **$p_3 < 0.05$** **$p_0 < 0.001$**	0.16 (0.12–0.22) $p_1 > 0.05$ **$p_3 < 0.05$** **$p_0 < 0.001$**	0.19 (0.13–0.33) **$p_1 < 0.05$** **$p_2 < 0.05$** **$p_0 < 0.01$**	0.34 (0.29–0.41)
Nesfatin-1, ng/ml	7.62 (6.94–8.43) $p_2 > 0.05$ $p_3 > 0.05$ **$p_0 < 0.001$**	7.21 (6.79–8.27) $p_1 > 0.05$ $p_3 > 0.05$ **$p_0 < 0.001$**	7.76 (6.60–8.47) $p_1 > 0.05$ $p_2 > 0.05$ **$p_0 < 0.001$**	4.53 (4.23–4.87)

where p_1 - confidence level in comparison with parameters of Group 1, p_2 - confidence level in comparison with parameters of Group 2, p_3 - confidence level in comparison with parameters of Group 3, p_0 - confidence level in comparison with parameters of the control group.

A negative correlation of nesfatin-1 levels with BMI ($r = -0.164$, $p < 0.05$) in hypertensive patients with normoglycemia could be evidence of its anorexigenic effect [3, 7], preventing the development of obesity. Positive correlations of nesfatin-1 levels were detected with the levels of fasting glucose ($r = 0{,}198$, $p < 0{,}05$), insulin ($r = 0{,}180$, $p < 0{,}05$) and HOMA-IR index ($r = 0{,}205$, $p < 0{,}05$). These data may be a confirmation of previous studies, which have shown glucose-induced insulinotropic action of nesfatin-1 [5].

Nesfatin-1 levels were positively correlated with WHR ($r = 0.529$, $p < 0.001$) in the group of patients with hypertension and diagnosed prediabetes. By detailed examination of the characteristics of Group 2 it become apparent significantly higher levels of body weight, BMI, WC, HC, WHR in comparison with other groups. This can be explained by the fact that patients with obesity (especially abdominal) have higher risk of insulin resistance or T2DM development. At the same time, adipose tissue that is increased in obesity can play a role of nesfatin-1 producing organ [4]. Furthermore, a negative correlation was found between levels of nesfatin-1 and postprandial glucose ($r = -0.430$, $p < 0.05$) in Group 2. As was already stated earlier, according to some studies, nesfatin-1 is also produced by pancreatic β-cells in addition to other organs, increasing insulin secretion and the sensitivity of skeletal muscle, liver and adipose tissues to insulin [6]. As a result of these mechanisms, rising of nesfatin-1 concentration may lead to reduction of blood glucose levels.

Patients of Group 3 were characterized by similar anthropometric data with patients of Group 1. There were negative correlations of nesfatin-1 level with body weight ($r = -0.318$, $p < 0.05$), BMI ($r = -0.285$, $p < 0.05$) and WC ($r = -0.271$, $p < 0.05$) in this group. No significant correlations between nesfatin-1 level and parameters of the carbohydrate metabolism have been identified that may be due to the influence of hypoglycemic drugs.

Thus, hypertensive patients had significantly increased nesfatin-1 levels. It can be assumed possible antihyperglycemic and insulinotropic effect of this adipocytokine in hypertensive patients with normoglycemia or prediabetes. These data may indicate a role of nesfatin-1 in the mechanism of metabolic disorders formation in hypertensive patients.

CONCLUSIONS

1. Nesfatin-1 levels in hypertensive patients (7.63 ng/ml) were significantly higher than in the control group (4.53 ng/ml). No significant differences were found between nesfatin-1 levels in hypertensive patients with normoglycemia, prediabetes or T2DM (7.62, 7.21 and 7.76 ng/ml respectively). However patients of Group 2 had significantly higher BMI.
2. Nesfatin-1 levels positively correlate with the fasting glucose levels in patients with hypertension and without disturbances of carbohydrate profile. Increasing of nesfatin-1 level in blood is accompanied by increased secretion of insulin. These links are not observed among parameters of hypertensive patients with dysglycemia.
3. Determination of nesfatin-1 level in hypertensive patients with normoglycemia in combination with the carbohydrate metabolism parameters may have important diagnostic value in the assessment of glucose tolerance.

PROSPECTS FOR FUTURE STUDIES

It seems appropriate to investigate the role of nesfatin-1 on the carbohydrate metabolism in isolated pathology (in patients without EH and with normal weight), given the diversity of its effects in polymorbidity.

REFERENCES

1. Kovalenko V. M. Sertsevo-sudinni hvorobi: medichno-sotsialne znachennya ta strategiya rozvitku kardiologiyi v Ukrayini / V. M. Kovalenko, A. P. Dorogoy // Ukrayinskiy kardiologichniy zhurnal. – 2016. – Dod. 3. – S. 5–14.
2. Bratus V. V. Ozhirenie, insulinorezistetnost, metabolicheskiy sindrom: fundamentalnyie i klinicheskie aspektyi / V. V. Bratus, T. V. Talaeva, V. A.Shumakov; pod red. V. N. Kovalenko. – K.: Chetverta hvilya, 2009. – 413 s.
3. Oh-I S. Identification of nesfatin-1 as a satiety molecule in the hypothalamus / S. Oh-I, H. Shimizu // Nature. – 2006. – № 443. – P. 709–712.

4. Goebel-Stengel M. Central and peripheral expression and distribution of NUCB2/nesfatin-1 / M. Goebel-Stengel, L. Wang // Current Pharmaceutical Design. – 2013. – № 19. – P. 6935–6940.
5. Nakata M. Nesfatin-1 enhances glucose-induced insulin secretion by promoting Ca(2+) influx through L-type channels in mouse islet beta-cells / M. Nakata, K. Manaka, S. Yamamoto [et al.] // Endocrine Journal. – 2011. – № 58. – P. 305–313.
6. Li Z. Peripheral effects of nesfatin-1 on glucose homeostasis / Z. Li, L. Gao, H. Tang [et al.] // PLoS ONE. – 2013. – № 8(8). – e71513.
7. Shimizu H. Peripheral administration of nesfatin-1 reduces food intake in mice: the leptin-independent mechanism / H. Shimizu, S. Oh-I // Endocrinology. – 2009. – № 150. – P. 662–671.
8. Tanida M. Nesfatin-1 stimulates renal sympathetic nerve activity in rats / M. Tanida, M. Mori // Neuroreport. – 2011. – № 22. – P. 309–312.
9. Liu F. Decreased plasma nesfatin-1 level is related to the thyroid dysfunction in patients with type 2 diabetes mellitus / F. Liu, Q. Yang, N. Gao [et al.] // Journal of Diabetes Research. – 2014. – № 2014. – 128014.
10. Li J. B. Effects of aging on the plasma levels of nesfatin-1 and adiponectin / J. B. Li, M. Nishida, K. Kaimoto [et al.] // Biomedical Reports. – 2014. – № 2. – P. 15–156.
11. Demidenko G. V. DisfunktsIya endoteliyu yak faktor rozvitku insulinorezistentnosti u hvorih na gipertonichnu hvorobu / G. V. Demidenko // Meditsina sogodni i zavtra. – 2012. – № 3–4. – S. 75–81.
12. Lastra G. Type 2 diabetes mellitus and hypertension: An update / G. Lastra, S. Syed, L. R. Kurukulasuriya [et al.] // Endocrinology and metabolism clinics of North America. – 2014. – № 43(1). – S. 103–122.
13. Yosten G. L. Nesfatin-1 exerts cardiovascular actions in brain^ possible interaction with the central melanocortin system / G. L. Yosten, W. K. Samson // American journal of physiology. Regulatory, integrative and comparative physiology. – 2009. – № 297(2). – P. 330–336.
14. Yamawaki H. A novel adipocytokine? Nesfatin-1 modulates peripheral arterial contractility and blood pressure in rats / H. Yamawaki, M. Takahashi, M. Mukohda [et al.] // Biochemical and Biophysical Research Communications. – 2012. – № 418(4). – P. 676–681.
15. Ramanjaneya M. Identification of nesfatin-1 in human and murine adipose tissue: a novel depot-specific adipokine with increased levels in obesity / M. Ramanjaneya, J Chen, J. E. Brown [et al.] // Endocrinology. – 2010. – № 151. – 3169–3180.