Available online at www.medicinescience.org

ORIGINAL ARTICLE

Medicine Science International Medical Journal

Medicine Science 2024;13(3):661-6

Serum relaxin-3 and galanin levels in individuals with erectile dysfunction with and without type 2 diabetes mellitus

Umit Yilmaz[1], Salih Burlukkara[2], Demirhan Orsan Demir[2]

[1]Karabük University, Faculty of Medicine, Department of Physiology, Karabük, Türkiye
[2]Karabük University, Faculty of Medicine, Department of Urology, Karabük, Türkiye

Received 18 July 2024; Accepted 14 August 2024
Available online 23.08.2024 with doi: 10.5455/medscience.2024.07.077

Abstract

This study was designed to determine the serum relaxin-3 and galanin levels in individuals with erectile dysfunction with and without type 2 diabetes mellitus (T2DM). A total of 154 individuals aged 30-60 were included in the study and the participants were divided into three groups: D-ED group (individuals with T2DM and ED) (n=49), ND-ED group (individuals with ED without T2DM) (n=55), and control group (individuals without T2DM and ED) (n=50). The age, BMI, and IIEF-5 score were recorded for all participants. The HDL, LDL, triglycerides, cholesterol, HbA1c and testosterone were determined from the blood taken from the participants, and galanin and relaxin-3 levels were measured in serum samples by ELISA method. The IIEF-5 score of the D-ED group was statistically lower than that of the ND-ED and control groups, and the IIEF-5 score of the ND-ED group was also lower than that of the control group. Serum testosterone levels in the control group were statistically higher than in the D-ED and ND-ED groups. The galanin level of the D-ED group was elevated than the control and ND-ED groups, but the galanin level of the ND-ED group was lower than the control group. The relaxin-3 levels were decreased in the D-ED and ND-ED groups compared to the control group. Galanin levels increased in individuals with T2DM and ED, however decreased in individuals with ED without T2DM. In addition, reduced relaxin-3 level may play a role in the development of ED.

Keywords: Erectile dysfunction, type 2 diabetes mellitus, testosterone, IIEF-5, galanin, relaxin-3

Introduction

The ED is the inability to have an erection sufficient for initiation or maintenance penile erection at the level required for satisfactory sexual performance due to organic and/or psychological reasons [1]. ED is a major health problem affecting men's quality of life and the number of people affected is increasing. It is estimated that there will be around three hundred million men in the world suffering from ED by the year 2025 [2]. The diabetes, a major cause of corpus cavernosum dysfunction, is one of the leading pathological conditions in the etiology of ED, and diabetic ED is emerging as a much more common complication in these patients than diabetic nephropathy and retinopathy [1].

Testosterone is the most important androgenic hormone in both men and women. Testosterone hormone is expressed by Leydig cells in the testicles under the control of luteinizing hormone (LH) in men. Testosterone plays a critical role in the male sexuality and penile erection, and there is a positive relationship between decreased testosterone and decreased erectile function. ED caused by treatment for hypogonadism or low testosterone may improve with testosterone replacement therapy [3].

Relaxin-3 is also expressed in the pre-optic region, which is important for the neurons that produce gonadotropin-releasing hormone (GnRH) and for reproductive function [4]. Intracerebroventricular administration of relaxin-3 increased GnRH release [5], and LH levels and follicle-stimulating hormone (FSH) levels in rats [6]. Relaxin-3 plays a role in the process of puberty and there is a positive relationship between the level of relaxin-3 and the levels of testosterone, FSH and LH [7].

CITATION
Yilmaz U, Burlukkara S, Demir DO. Serum relaxin-3 and galanin levels in individuals with erectile dysfunction with and without type 2 diabetes mellitusitus. Med Science. 2024;13(3):661-6.

Corresponding Author: Umit Yilmaz, Karabük University, Faculty of Medicine, Department of Physiology, Karabük, Türkiye
Email: umityilmaz@karabuk.edu.tr

DOI: 10.5455/medscience.2024.07.077

Galanin is a neuropeptide expressed by GnRH in hypothalamic neurons and plays a role in sex differentiation [8]. Both male and female rats have high concentrations of galanin in the hypothalamic-hypophyseal portal circulation and galanin receptors in their gonadotrophs [9]. Galanin mRNA levels increase in GnRH neurons during the transition from juvenile to adult rats, and galanin is required for maturation of the reproductive axis [10]. Galanin potentiates the steroidogenic effect of acute gonadotropin or LH in rat Leydig cells [11] and acts a role as a regulator of LH and testosterone release in male rats [9].

The relaxin-3 plays a role in glucose metabolism [12], while galanin has a role in the control of hyperinsulinemia, sensitivity and secretion of insulin in type 2 diabetes mellitus (T2DM) [13]. In addition, the relaxin-3 and galanin neuropeptides are released from GnRH-secreting neurons and have an effect on testosterone levels through LH [7,9]. This study was designed to determine the serum relaxin-3 and galanin levels in individuals with erectile dysfunction with and without T2DM.

Material and Methods

Ethical Statement and Study Groups

This study was conducted in accordance with the necessary approvals obtained from the Ethics Committee of Karabük University, Faculty of Medicine (#2023/1212). A total of 154 individuals aged 30-60 were included in the study. The participants in the study were divided into three groups: D-ED group (individuals with T2DM and ED), ND-ED group (individuals with ED and without T2DM) and control group (individuals without T2DM and ED). Demographic characteristics, age, medical history (comorbidity), physical examination findings, BMI, smoking, and IIEF-5 score were recorded for all participants. The HDL, LDL, triglycerides, cholesterol, HbA1c, testosterone, galanin, and relaxin-3 levels were determined from serum samples obtained from the blood taken from the participants.

Inclusion Criteria of the Patients

According to the Turkish version of the short form of the IIEF-5, patients with an IIEF-5 score ≤21 were considered to have ED. In addition, T2DM was diagnosed according to the ESC (European Society of Cardiology) and EASD (European Association for the Study of Diabetes) guidelines. The IIEF-5 survey was administered to individuals who agreed to participate in the study, in a way that would not disturb them, and the data was recorded. These recorded data were stored in a way to protect the privacy of individuals. A score of 21 or less on the 5-question test in the IIEF-5 survey is considered to be sexual dysfunction [14].

Inclusion Criteria of the Controls

The control group consisted of individuals (individuals without T2DM and without ED) matched for age and BMI with an IIEF-5 score of ≥22 [14].

Exclusion Criteria of the Patients

The patients with acute or chronic urinary tract disease, end-stage renal disease, and hepatic insufficiency in the past 6 months, prostate cancer, individuals with a history of history of pelvic trauma, psychogenic ED or severe psychiatric disorders, chronic infectious diseases and immunomodulatory/anti-inflammatory drug treatment were excluded. The individuals taking medications that affect erectile function or sex hormones were also excluded.

Laboratory Measurements

The levels of HDL, LDL, triglycerides, cholesterol, HbA1c, and testosterone were determined in the biochemistry laboratory of Karabük Training and Research Hospital from venous blood collected for routine blood tests from patients attending the urology outpatient clinic.

Determination of serum relaxin-3 and galanin levels

The fresh venous blood samples collected from all participants in the morning (08:00-10:00) were centrifuged at 3000 rpm for 10 minutes on the same day to separate the serum. Then, the serum samples were frozen and stored at -20°C until ELISA analysis. The relaxin-3 and galanin levels were determined from the serum samples using commercially available specific relaxin-3 (cat. no: 201-12-4772, SunRed Biotechnology, China) and galanin (cat. no: 201-12-1333, SunRed Biotechnology, China) ELISA kits. The absorbance was measured at 450 nm with a spectrophotometer (Multiskan GO, Thermo Scientific, USA).

Statistical Analysis

The IBM statistics 22.0 package was used for statistical analyses. The Shapiro-Wilk test was used to assess the suitability of the data for normal distribution. The one-way analysis of variance test was used for multiple comparisons of normally distributed data. Tukey's test was used for homogeneous variances and Tamhane's test was used for non-homogeneous variances. Data are presented as mean ± standard deviation. The Mann-Whitney U test with Bonferroni correction was used to compare groups of data that did not show a normal distribution. Data are presented as median (minimum-maximum). The categorical variables were evaluated using the chi-squared test.

Results

Demographic Characteristics of the Participants

Demographic characteristics of individuals including age, BMI, smoking, medical history (comorbidity), HDL, LDL, triglyceride, cholesterol, HbA1C measurements are presented in Table 1. The mean age of the D-ED group was statistically elevated than that of the ND-ED and control groups ($p<0.05$), and the difference between them was not statistically significant in age between the ND-ED and control groups. The groups were also compared for BMI and statistically the D-ED group had the highest BMI ($p<0.05$). The D-ED, ND-ED and control groups were compared for HDL and LDL levels and there was no statistically significant difference between the groups for these parameters. There was no difference in cholesterol levels between the D-ED and ND-ED

DOI: 10.5455/medscience.2024.07.077

groups and the ND-ED and control groups, but the cholesterol level in the D-ED group was statistically elevated than in the control ($p<0.05$). There was no statistically significant difference in triglyceride levels between the D-ED and ND-ED groups, but the triglyceride level in the control was statistically lower than in the D-ED and ND-ED groups ($p<0.05$). In addition, the HbA1C level of the D-ED group was statistically higher than that of the ND-ED and control groups ($p<0.05$), and the difference between them was not statistically significant in HbA1c level between the ND-ED and control groups.

Table 1. Demographic characteristics of the participants

	D-ED (n=49)	ND-ED (n=55)	Control (n=50)	p value
Age	51.55±8.57[a]	46.44±12.57[b]	45.84±14.20[b]	<0.05
BMI (kg/m²)	28.34±2.66[a]	24.14±2.8[b]	26.26±3.68[c]	<0.05
Smoking (%)	57.14	50.9	60	-
HDL (mg/dL)	41.39±10.58	44±11.77	44.30±9.7	>0.05
LDL (mg/dL)	106.9±27.68	105.81±32.9	94.6±35.54	>0.05
Triglyceride (mg/dL)	174.04 (92-622)[a]	150 (37-777)[a]	125.5 (40-333)[b]	<0.05
Cholesterol (mg/dL)	186±26.19[a]	179.45±45[a,b]	163±40.04[b]	<0.05
HbA1c	7.29±1[a]	4.67±1.17[b]	4.83±0.63[b]	<0.05

Groups marked with different letters were statistically different from each other; ($p<0.05$)

Serum Testosterone, Galanin, and Relaxin-3 Levels and IIEF-5 Score of the Participants

Serum testosterone, galanin and relaxin-3 levels, and IIEF-5 scores of the participants are given in Table 2. The IIEF-5 score of the D-ED group was statistically lower than that of the ND-ED and control groups ($p<0.05$), and the IIEF-5 score of the ND-ED group was also decreased than that of the control group ($p<0.05$). Serum testosterone levels in the control group were statistically higher than in the D-ED and ND-ED groups ($p<0.05$), but the difference between them was not statistically significant in serum testosterone levels between the D-ED and ND-ED groups. It was determined that the galanin level of individuals in the D-ED group was higher than that of individuals in the control and ND-ED groups ($p<0.05$). However, it was found that the galanin level of individuals in the ND-ED group decreased compared to healthy individuals ($p<0.05$). The serum relaxin-3 levels decreased in the D-ED and ND-ED groups compared to the healthy individuals ($p<0.05$). However, there was no statistical difference in serum relaxin-3 levels between the D-ED and ND-ED groups.

Table 2. Serum testosterone, galanin, and relaxin-3 levels and IIEF-5 score of the participants

	D-ED (n=49)	ND-ED (n=55)	Control (n=50)	p value
Testosterone (ng/dL)	350.57±175.21[a]	402.13±169.96[a]	483.32±94.26[b]	<0.05
Galanin level (ng/L)	35.38±11.71[a]	11.77±2.48[b]	23.38±3.24[c]	<0.05
Relaxin-3 level (ng/L)	228.6±45.87[a]	239.1±44.53[b]	402.31±125.08[c]	<0.05
IIEF-5 score	11 (5-20)[a]	15 (5-21)[b]	23 (22-25)[c]	<0.05

Groups marked with different letters were statistically different from each other; ($p<0.05$)

Correlations Between IIEF-5 Score, Serum Testosterone, Galanin, and Relaxin-3 Levels in the Current Study

The correlation analysis results between IIEF-5 score, serum testosterone, galanin, and relaxin-3 levels are given in Table 3. Correlation analysis demonstrated a negative correlation between serum galanin level and IIEF-5 score ($r=-0.175$, $p=0.030$). There was also a negative correlation between serum galanin level and serum testosterone level ($r=-0.134$, $p=0.097$). There was a significant positive correlation between serum relaxin-3 level and IIEF-5 score ($r=0.694$, $p=0.0001$). Again, there was a positive correlation between serum relaxin-3 and serum testosterone levels ($r=0.370$, $p=0.0001$). ROC curve was used to predict ED in diabetic patients using serum relaxin-3 levels. The cut-off value of serum relaxin 3 was 228.79 with a sensitivity of 71% and a specificity of 78% (AUC, 0.242; 95% CI, 0.168–0.316; $p<0.0001$) (Figure 1).

Table 3. Spearman's analysis of the correlation between IIEF-5 score, serum testosterone, galanin, and relaxin-3 levels in the current study

	IIEF-5 score		Serum testosterone levels	
	Correlation coefficient	p-value	Correlation coefficient	p-value
Serum galanin levels	−0.175	0.030	−0.134	0.097
Serum relaxin-3 levels	0.694	0.0001	0.370	0.0001

DOI: 10.5455/medscience.2024.07.077

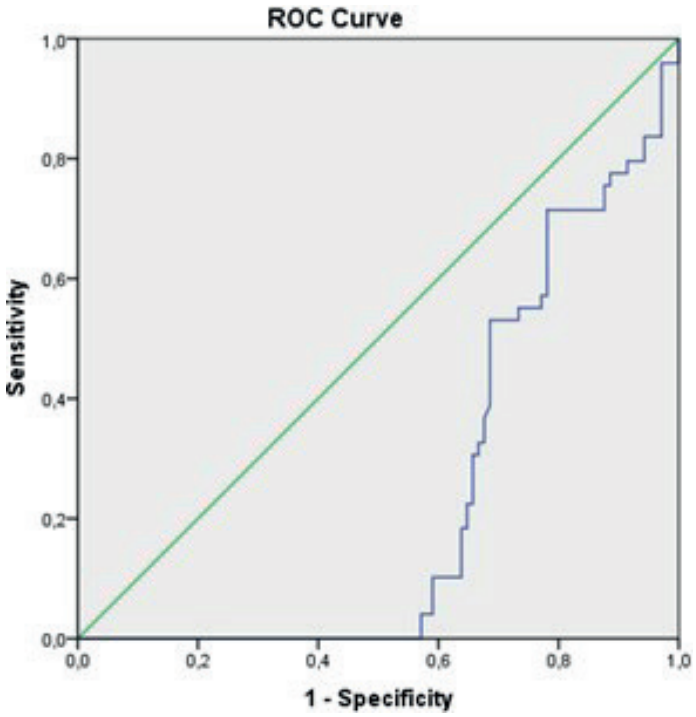

Figure 1. Sensitivity and specificity of serum relaxin-3 in predicting erectile dysfunction in diabetic patients

Discussion

The ED is the inability to have an erection sufficient for initiation or maintenance of sexual intercourse. The pathology of ED in diabetic men is known to be multifactorial and complex, involving vascular, neurogenic, endothelial, and hormonal mechanisms, although the pathophysiology is still not clearly defined [1,2].

The ED is approximately three times more common in men with diabetes than in the normal population, and it occurs earlier age in people with diabetes than in people without diabetes - on average 10 to 15 years earlier [15]. Galanin and galanin receptors play a critical role in regulation of blood glucose levels and reproductive hormone levels [16]. Patients with impaired glucose tolerance have higher serum galanin levels, and galanin levels are negatively related to one-hour glucose levels [17]. Plasma glucose and galanin levels have been found to be high in patients with gestational diabetes (GDM), and it has been suggested that this high galanin level is a physiological adaptation to the high glucose levels associated with GDM [18]. In addition, galanin has been demonstrated to increase the concentration of GLUT4 in muscle tissue of rats with T2DM and, in addition to insulin, to promote glucose clearance and insulin sensitivity [19]. Galanin may increase insulin sensitivity to lower blood glucose levels. The incompatibility between circulating high galanin levels and low glucose utilization observed in diabetic individuals is called galanin resistance. In short, both galanin and insulin resistance are interrelated [20,21]. Moreover, Sandoval-Alzate et al. stated that serum galanin levels were increased in obese individuals than in lean individuals, supporting the concept of galanin resistance [22]. In T2DM, galanin plays a critical role in maintaining insulin sensitivity and inhibiting insulin secretion to reduce hyperinsulinemia. Therefore, plasma galanin levels may be used as a new marker of insulin resistance and as a clinical measure of diabetes mellitus risk [13]. Moreover, galanin has been suggested to modulate reproductive behavior via neurotransmitters, neuromodulators, or receptors [16]. In recent years, galanin has been demonstrated to affect the HPG axis. The galanin and LHRH are co-localized and secreted together into the hypophyseal portal system [23]. Also, galanin increases LHRH secretion, thereby enhancing LH release [24,25]. In addition, it has been shown that administration of galanin antiserum reduces LH secretion and surge in proestrus [26,27]. These effects may possibly be mediated by hypothalamic galanin, which has been suggested to trigger the preovulatory gonadotropin surge [28]. However, there is also evidence that galanin may have a minor role in the regulation of the HPG axis in women [29]. In addition, galanin has been shown that administration has no effect on the release of GnRH, LH and FSH hormones [30]. The galanin gene expression in GnRH neurons is triggered by a gonad-dependent process during the transition from juvenile to adult state in rats [10]. In addition, galanin and galanin receptors have been showed to decrease in the corpus cavernosum of castrated rats, and it has been suggested that increasing galanin and GalR1-3 may improve erectile function [31]. However, intracerebroventricular infusion of galanin-like peptide to rats has been demonstrated to stimulate non-contact erections but not touch [32]. According to our results, the galanin levels in individuals with T2DM and ED increased compared to healthy individuals, and individuals with ED without diabetes. However, it was determined that the galanin level in individuals with ED without diabetes was reduced compared to healthy individuals. Based on these results, the increased level of galanin in T2DM suggest that it may play a role in insulin/galanin resistance, as stated in previous studies [20, 21]. However, the fact that galanin levels of individuals with ED without T2DM are lower than healthy individuals suggests that galanin may regulate the hormones in the reproductive axis. However, the increased galanin levels in individuals with T2DM and ED seem a little complicated and difficult to explain. This is because, so far, attempts have been made to determine the effect of galanin peptide by giving galanin peptide to rodents, but the relationship between serum galanin levels and sexual function/hormones has not been studied. In this regard, our study is a first and provides new data to the literature. In our study, another parameter, testosterone hormone level, was decreased in individuals with ED with T2DM and ED without T2DM compared to healthy individuals. In addition, the IIEF-5 score was decreased in individuals with ED with T2DM and ED without T2DM compared to healthy individuals. However, galanin level and both IIEF-5 score, and testosterone hormone levels were not parallel between the groups. According to our results, galanin peptide may play a more dominant role in regulating blood glucose levels rather than its role in reproductive functions.

DOI: 10.5455/medscience.2024.07.077

Relaxin family peptides have a high structural similarity to insulin. This family belongs to the relaxin/insulin superfamily, including insulin-like growth factors [33]. The relaxin-3 have a role in mammalian reproductive functions [34], possibly involved in HPG axis stimulation affecting Leydig cell function or spermatogenesis [35]. The overexpression of relaxin family peptide receptor 1 (RXFP1) via lentiviruses may ameliorate ED caused by diabetes mellitus [36]. The relaxin-3 induce the HPG axis [5] and reproductive status in male rats [37]. Relaxin-3 stimulated the expression of GnRH, Kiss-1, and gonadotropin subunits in cell cultures consisting of mouse hypothalamus, pituitary and gonadal cells [38]. In addition, no differences in plasma relaxin-3 have been reported between patients with T2DM and controls [12]. In men with delayed puberty, it has been found that levels of LH, FSH, testosterone and relaxin-3 decrease. In addition, it has been shown that there is a positive relationship between serum relaxin-3 levels and testicular volume, serum testosterone, FSH and LH hormones, and a negative correlation with BMI [7]. According to our results, relaxin-3 levels decreased in individuals with T2DM, ED, and non-diabetic individuals with ED, compared to healthy individuals. The testosterone hormone and relaxin-3 hormone showed parallelism between the groups, but the IIEF-5 score, and relaxin-3 hormone did not show parallelism. In addition, according to our results, it can be said that the level of relaxin-3 hormone is not affected by the presence/absence of T2DM in individuals with ED, but the decrease in the level of relaxin-3 hormone may play a role in the emergence of ED.

Conclusion

According to our results, galanin levels were increased in individuals with diabetes and erectile dysfunction. In this regard, it can be suggested that galanin probably plays a more role in blood glucose homeostasis rather than ED. However, it can be said that the decrease in galanin may play a role in the ED due to the decrease in galanin levels in individuals with erectile dysfunction without diabetes compared to healthy individuals. In addition, since the level of relaxin-3 decreases in individuals with ED compared to healthy individuals, it can be suggested that relaxin-3 may play a role in erection formation.

Conflict of Interests
The authors declare that there is no conflict of interest in the study.

Financial Disclosure
The present work was supported by the Research Fund of Karabük University [Project No: KBÜBAP-24-ABP-012]. This study was conducted with the approval of the Ethics Committee of the Faculty of Medicine of the Karabuk University (#2023/1212).

Ethical Approval
This study was conducted with the approval of the Ethics Committee of the Faculty of Medicine of the Karabük University (#2023/1212).

Acknowledgements
We would like to thank Dr. Mehmet Fatih Seyhan for allowing us to work in the laboratories of Istanbul Yeni Yüzyıl University.

References

1. Thorve VS, Kshirsagar AD, Vyawahare NS, et al. Diabetes-induced erectile dysfunction: epidemiology, pathophysiology and management. J Diabetes Complications. 2011;25:129-36.
2. Ragab A, Ahmed MH, Reda Sayed A, et al. Serum nesfatin-1 level in men with diabetes and erectile dysfunction correlates with generalized anxiety disorder-7: a prospective comparative study. Andrology. 2023;11:307-15.
3. Blute M, Hakimian P, Kashanian J, et al. Erectile dysfunction and testosterone deficiency. Front Horm Res. 2009;37:108-22.
4. Tanaka M, Iijima N, Miyamoto Y, et al. Neurons expressing relaxin 3/INSL 7 in the nucleus incertus respond to stress. Eur J Neurosci. 2005;21:1659-70.
5. McGowan BM, Stanley SA, Donovan J, et al. Relaxin-3 stimulates the hypothalamic-pituitary-gonadal axis. Am J Physiol Endocrinol Metab. 2008;295:E278-86.
6. Calvez J, de Avila C, Guevremont G, Timofeeva E. Sex-specific effects of chronic administration of relaxin-3 on food intake, body weight and the hypothalamic-pituitary-gonadal axis in rats. J Neuroendocrinol. 2016;28. doi: 10.1111/jne.12439.
7. Hanafy S, Sabry JH, Akl EM, et al. Serum relaxin-3 hormone relationship to male delayed puberty. Andrologia. 2018;50. doi: 10.1111/and.12882.
8. Finn PD, McFall TB, Clifton DK, Steiner RA. Sexual differentiation of galanin gene expression in gonadotropin-releasing hormone neurons. Endocrinology. 1996;137:4767-72.
9. Scheffen JR, Splett CL, Desotelle JA, Bauer-Dantoin AC. Testosterone-dependent effects of galanin on pituitary luteinizing hormone secretion in male rats. Biol Reprod. 2003;68:363-9.
10. Rossmanith WG, Marks DL, Clifton DK, Steiner RA. Induction of galanin gene expression in gonadotropin-releasing hormone neurons with puberty in the rat. Endocrinology. 1994;135:1401-8.
11. Romanelli F, Fillo S, Isidori A, et al. Galanin stimulates steroidogenesis in rat Leydig cells. Life Sci. 1998;63:255-63.
12. Zhang X, Zhu M, Zhao M, et al. The plasma levels of relaxin-2 and relaxin-3 in patients with diabetes. Clin Biochem. 2013;46:1713-6.
13. Fang P, Yu M, Shi M, et al. The neuropeptide galanin benefits insulin sensitivity in subjects with type 2 diabetes. Curr Protein Pept Sci. 2013;14:669-73.
14. Rosen RC, Cappelleri JC, Smith MD, et al. Development and evaluation of an abridged, 5-item version of the International Index of Erectile Function (IIEF-5) as a diagnostic tool for erectile dysfunction. Int J Impot Res. 1999;11:319-26.
15. Nisahan B, Kumanan T, Rajeshkannan N, et al. Erectile dysfunction and associated factors among men with diabetes mellitus from a tertiary diabetic center in Northern Sri Lanka. BMC Res Notes. 2019;12:210.
16. Mills EG, Izzi-Engbeaya C, Abbara A, et al. Functions of galanin, spexin and kisspeptin in metabolism, mood and behaviour. Nat Rev Endocrinol. 2021;17:97-113.
17. Zhang Z, Fang P, Yu M, et al. Serum galanin concentration is increased in subjects with impaired glucose tolerance. Can J Diabetes. 2017;41:563-6.

DOI: 10.5455/medscience.2024.07.077

18. Fang P, Bo P, Shi M, et al. Circulating galanin levels are increased in patients with gestational diabetes mellitus. Clin Biochem. 2013;46:831-3.

19. He B, Shi M, Zhang L, et al. Beneficial effect of galanin on insulin sensitivity in muscle of type 2 diabetic rats. Physiol Behav. 2011;103:284-9.

20. Fang P, Shi M, Zhu Y, et al. Type 2 diabetes mellitus as a disorder of galanin resistance. Exp Gerontol. 2016;73:72-7.

21. Fang P, Yu M, Shi M, et al. Galanin peptide family regulation of glucose metabolism. Front Neuroendocrinol. 2020;56:100801.

22. Sandoval-Alzate HF, Agudelo-Zapata Y, Gonzalez-Clavijo AM, et al. Serum galanin levels in young healthy lean and obese non-diabetic men during an oral glucose tolerance test. Sci Rep. 2016;6:31661.

23. Merchenthaler I, Lopez FJ, Negro-Vilar A. Colocalization of galanin and luteinizing hormone-releasing hormone in a subset of preoptic hypothalamic neurons: anatomical and functional correlates. Proc Natl Acad Sci U S A. 1990;87:6326-30.

24. Lopez FJ, Merchenthaler I, Ching M, et al. Galanin: a hypothalamic-hypophysiotropic hormone modulating reproductive functions. Proc Natl Acad Sci U S A. 1991;88:4508-12.

25. Sahu A, Xu B, Kalra SP. Role of galanin in stimulation of pituitary luteinizing hormone secretion as revealed by a specific receptor antagonist, galantide. Endocrinology. 1994;134:529-36.

26. Xu B, Pu S, Kalra PS, et al. An interactive physiological role of neuropeptide Y and galanin in pulsatile pituitary luteinizing hormone secretion. Endocrinology. 1996;137:5297-302.

27. Lopez FJ, Meade EH Jr, Negro-Vilar A. Endogenous galanin modulates the gonadotropin and prolactin proestrous surges in the rat. Endocrinology. 1993;132:795-800.

28. Todd JF, Small CJ, Akinsanya KO, et al. Galanin is a paracrine inhibitor of gonadotroph function in the female rat. Endocrinology. 1998;139:4222-9.

29. Giustina A, Gastaldi C, Bugari G, et al. Role of galanin in the regulation of somatotrope and gonadotrope function in young ovulatory women. Metabolism. 1995;44:1028-32.

30. Arvat E, Gianotti L, Ramunni J, et al. Effect of galanin on basal and stimulated secretion of prolactin, gonadotropins, thyrotropin, adrenocorticotropin and cortisol in humans. Eur J Endocrinol. 1995;133:300-4.

31. Yuan P, Li X, Xiong WJ, er al. Downregulation of the expression of galanin impairs erectile function in hypoandrogenic rats. Sex Med. 2023;11:qfad029.

32. Fraley GS. ICV galanin-like peptide stimulates non-contact erections but not touch-based erections in adult, sexually experienced male rats. Neuropeptides. 2017;64:69-73.

33. Calvez J, de Avila C, Timofeeva E. Sex-specific effects of relaxin-3 on food intake and body weight gain. Br J Pharmacol. 2017;174:1049-60.

34. Xu Q, Ye M, Su Y, et al. Hypogonadotropic hypogonadism in male tilapia lacking a functional rln3b gene. Int J Biol Macromol. 2024;270:132165.

35. Silvertown JD, Neschadim A, Liu HN, et al. Relaxin-3 and receptors in the human and rhesus brain and reproductive tissues. Regul Pept. 2010;159:44-53.

36. Sun T, Xu W, Tu B, et al. Engineered adipose-derived stem cells overexpressing RXFP1 via CRISPR activation ameliorate erectile dysfunction in diabetic rats. Antioxidants (Basel). 2023;12:171.

37. McGowan BM, Stanley SA, Ghatei MA, Bloom SR. Relaxin-3 and its role in neuroendocrine function. Ann N Y Acad Sci. 2009;1160:250-5.

38. Kanasaki H, Tumurbaatar T, Tumurgan Z, et al. Effect of relaxin-3 on Kiss-1, gonadotropin-releasing hormone, and gonadotropin subunit gene expression. Reprod Med Biol. 2019;18:397-404.