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Relationship Between Orexin-A and Insulin Resistance in Patients with Type 2 Diabetes Mellitus

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Abstract
Background: Orexin-A is an orexigenic hormone that plays an important role in the metabolism of blood glucose, insulin, and insulin resistance (IR). The pathogenesis of type 2 diabetes mellitus (T2DM) is related to the abnormality in insulin and IR. However, no sufficient studies to date have clearly shown the association of orexin-A with biochemical parameters related to T2DM.
Objectives: The aim of this study was to determine the relation of orexin-A with IR and how they associate with physiological changes in T2DM patients. Understanding this relation will offer some pharmacological tools to reduce some complications in diabetes.
Materials and Methods: A total of 41 T2DM and 43 non-DM subjects, aged between 40-60 years with body mass index (BMI) ≤25 kg/m^2, participated in the present study.
Fasting serum orexin-A, IR, fasting blood glucose (FBG), glycated hemoglobin (HbA1C), lipid profile, liver enzymes (alanine aminotransferase (ALT) and aspartate transaminase (AST)), nitric oxide (NO), and malondialdehyde (MDA) parameters were evaluated. Orexin-A was evaluated by using enzyme-linked immunosorbent assay (ELISA). For statistical analysis, GraphPad Prism 7.0 and SPSS version 24.0 programs were used.
Results: Orexin-A was positively correlated with blood pressure, FBG, HbA1c, insulin, and IR but inversely related to insulin sensitivity (IS), leptin, and gender. Stepwise multiple regression presented HOMA-IR, diastolic blood pressure, and very-low-density lipoprotein-cholesterol as predictors for orexin-A. The area under control value showed orexin-A, FBG, HbA1c, HOMA-IR, IS, ALT, AST, NO and MDA as biomarkers for T2DM disease.
Conclusion: Orexin-A has a predictive ability to diagnose T2DM, as it is significantly associated with hyperglycemia, IR, and IS.

Keywords: orexin-A; leptin; HOMA-IR; insulin sensitivity; oxidative stress; free radicals

العلاقة بين هورمون الاوركسين ومقاومة الانسولين في المرضى المصابين بداء السكري من النوع الثاني

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الخلاصة

إن عمليات ألأيض في جسم الانسان خصوصًا أيض السكرو الأنسولين ومقاومة الأنسولين تتأثر بهرمون أوركسين– أ. والهدف من الدراسة هي تحديد علاقة أوركسين– أ مع الأنسولين وكيفية ارتباطها بالتغيرات الكيميائية والفسيولوجية في مرضى داء السكري من النوع الثاني في مدينة السليمانية. إن فهم هذه العلاقة قد يقدم بعض الطرق العلاجية لتقليل نسبة من المضاعفات في مرضى السكري .

تم تقدير أوركسين– أ ، مقاومة الأنسولين، حساسية الأنسولين ، جلوكوز الدم، الهيموجلوبين الجلوكوزيلاتي، الدهون الكيميائية ، إنزيمات الكبد ، أكسيد النيتريك ، المالونديالديهايد، هرمون الجريلين واللبتين في مصل 41 مريضا يعانون من داء السكري من النوع الثاني والذين يراجعون مركز السكري والغدد الصماء و43 شخصا تم اختيارهم عشوائيا من سكان مدينة السليمانية. أعمارهم تتراوح ما بين (40–60) سنة، ومؤشر كتلة الجسم أقل من 25. وقد تم قياس هرمون أوركسين– أ باستخدام تقنية الفحص المناعي المرتبط بالإنزيم (إلايزا).

تم استخدام تقنية جراف بادبريزم 7.0 و الحزمة الإحصائية للعلوم الاجتماعية رقم الرخصة 24 للتحليل الإحصائي ووفقًا لاختبارات التشخيص بأستعمال منحنى الخاصية العملياتية للمستقبل تم تشخيص أوركسين– أ، مقاومة الأنسولين، حساسية الأنسولين، مستوى جلوكوزالدم، قيمة الهيموجلوبين الجلوكوزيلاتي، الدهون الكيميائية، إنزيمات الكبد، أكسيد النيتريك، المالونديالديهايد و إنزيمات الكبد كعلامات بايوكيميائية لداء السكري من النوع الثاني في مجتمعنا الدراسي . وأحصائيا أظهرت هرمون أوركسين–أ ارتباطا إيجابيا مع ضغط الدم ، جلوكوزالدم، الهيموجلوبين الجلوكوزيلاتي، الأنسولين و مقاومة الأنسولين ولكن علاقته مع حساسية الأنسولين، هرمون اللبتين ونوع الجنس هي علاقة عكسية. وجدت نتائج تحليل الأنحدار المتدرج مقاومة الأنسولين ، وضغط الدم الانبساطي و البروتين الدهني منخفض الكثافة جدا كمتغيرات متنبأة لهرمون ألأوركسين–أ في المجموعات الدراسية بأكملها. نستنتج من هذه الدراسة إن الأوركسين–أ وجميع الدلائل المرتبطة بالجلوكوز والأنسولين هي علامات حيوية لداء السكري من النوع الثاني ، وإن أوركسين –أ يحفز مقاومة الأنسولين، فرط السكر و ارتفاع ضغط الدم في مجموعتنا الدراسية.

Introduction

Diabetes mellitus is a complex noncontagious chronic disease.T2DM is classified as the most common form of diabetes, which is characterized by hyperglycemia [1] due to insufficient insulin production, improper responses of the peripheral cells to insulin, or both [2]. The untreated hyperglycemia causes several serious complications [1]. The hormonal disturbance is one of the consequences of T2DM.

Orexin-A (hypocretin) is an orexigenic hormone which has an important role in appetite, arousal, and energy homeostasis regulation [3]. Orexin-A hormone has an effectual role on the metabolic processes of the body, especially glucose metabolism [4], insulin, IR [5] and IS [6]. Orexin-A also affects other hormones, such as leptin and the anorexigenic hormone [5]. Furthermore, orexin-A is involved in increasing blood pressure, as Li and Nattie recorded the hypertensive effect of orexin on normal rats [7].

According to our knowledge, the mechanisms of the modulation of orexin-A and T2DM are not fully understood and more studies are needed to determine these mechanisms in T2DM. Thus, we have designed the current study to determine orexin-A association with insulin, IR, and some clinical and physiological changes in T2DM patients. Understanding of this relation will offer certain pharmacological tools to reduce certain complications in diabetes.

Subjects and Methods

Subjects

The study was performed by enrolling eighty-four Kurdish subjects from Al-Sulaymaniyah city. Forty-one subjects (16 men and 25 women) with T2DM disease attending the Diabetic and Endocrine Clinical Center at Al-Sulaymaniyah city were included. Forty-three healthy subjects (14 men and 29 women) were randomly selected as a control group. The age of the participants ranged between 40-60

years, with BMI ≤ 25. They were free from any chronic diseases (hypertension, cancer, and cardiovascular, digestive, hepatic, renal, autoimmune, and thyroid diseases). Furthermore, none of them were pregnant, smokers, or alcoholic consumer subjects. The blood samples were collected from April to October 2018.

Clinical parameters

The clinical history of each participant was recorded, while their BMI and mean arterial pressure (MAP) values were calculated [8].

HOMA-IR was used as an insulin resistance index and calculated by applying the following homeostasis model assessment formula.

HOMA-IR= (Fasting Serum Insulin (μIU/ml)×FBG (mg/dl)) / 405

In addition, the quantitative insulin sensitivity check index (QUICKI) was used for assessing IS [9].

Laboratory measurements

About 10ml of fasting blood was withdrawn from the antecubital vein of all participants. For estimation of all biochemical parameters, Diagnosticum Zrt (TOKYO BOEKI MEDISYS) Biolis kits were used. The amount of insulin was determined by utilizing MAGLUMI Insulin (CLIN) Snib kit. Ref. no. 130205002M (Shenzhen New Industries, Shenzhen, China). Friedewald's equation was applied for the calculation of very-low-density lipoprotein-cholesterol (VLDL-C) [10]. Serum NO was estimated by colorimetric method based on Griess reaction [11]. For the determination of serum MDA, a simple and sensitive spectrophotometric method and a 2-Thiobarbituric acid (TBA) reagent (Merck KGaA, Darmstadt, Germany) were used [12].

Hormonal tests (orexin-A, ghrelin, and leptin) were estimated by ELISA. Serum orexin-A and ghrelin levels were estimated by using Cloud-clone corp. USA kits, Cat. No.: CEA607Hu and CEA991, respectively. For leptin assessment, human leptin ELISA kit (Biovender research and diagnostic products, Cat no.: RD191001100, Czech Republic) was used.

Statistical analysis

GraphPad Prism 7 software (San Diego, CA, USA) was used. To compare the sensitivity and specificity of variables in both control and patient groups, the receiver operating characteristic (ROC) curve was created. To analyze the correlation coefficient of orexin-A and all other biochemical parameters, Pearson correlation coefficient (r) was used. Stepwise multiple regression was performed to predict the relationship of orexin-A with all other variables. Statistical package for social science (SPSS) version 24.0 (IBM, Armonk, NY, USA) was used for all the analyses. A p-value <0.05 was considered statistically significant.

Ethical approval

The study was approved ethically by the ethical committee of the College of Medicine at University of Sulaimani-Kurdistan Region of Iraq.

Results

Fasting serum orexin-A and several biochemical parameters are biomarkers of T2DM

A total of 84 participants (41 diabetic and 43 non-diabetic) were involved in the study. The results of the ROC curve analysis of all study subjects are demonstrated in Figure-1, which demonstrates FBG, HbA1c, HOMA-IR, and QUICKI as excellent markers for T2DM disease, as their area under control (AUC) values are nearly equal to 1. While orexin-A, AST, ALT, MDA and NO are good biochemical markers for T2DM in all study subjects.

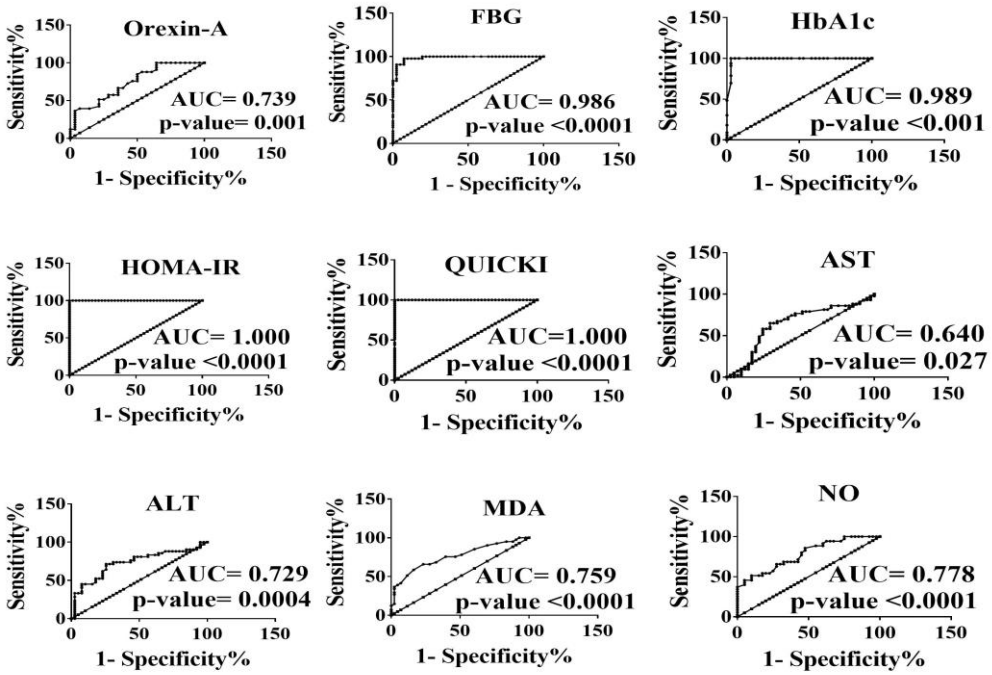

Figure 1-The area under the control (AUC) value presents Orexin-A, FBG, HbA1c, HOMA-IR, QUICKI, AST, ALT, MDA, and NO as biomarkers of T2DM in all studied groups. FBG: fasting blood glucose; HbA1c; glycated hemoglobin; HOMA-IR: Homeostatic Model Assessment of Insulin Resistance; QUICKI: Quantitative Insulin Sensitivity Check Index; AST: Aspartate transaminase; ALT: Alanine transaminase; MDA: Malondialdehyde and NO: Nitric oxide.

Fasting serum orexin-A is correlated with blood pressure, glucose, and insulin-related parameters

The results also demonstrated that orexin-A correlated significantly with systolic blood pressure (SBP), diastolic blood pressure (DBP), MAP, FBG, insulin, HOMA-IR, and HbA1c. Whereas it was inversely correlated with sex, QUICKI, and leptin level (Table-1).

Table 1-The Pearson correlation coefficient of orexin-A with anthropometrics and metabolic parameters of the study population. (Coefficients and p-values)

Parameters	Orexin-A (n=84)	
	r	p-value
Age	0.15	0.080
Sex	**-0.23**	**0.019**
Weight	0.13	0.118
Height	0.15	0.093
BMI	0.03	0.390
SBP	**0.24**	**0.015**
DBP	**0.20**	**0.037**
MAP	**0.23**	**0.017**
FBG	**0.31**	**0.002**
Insulin	**0.29**	**0.005**
HOMA-IR	**0.39**	**0.0001**
QUICKI	**-0.38**	**0.0002**
HbA1c	**0.35**	**0.001**
Cholesterol	-0.11	0.155

Triglyceride	-0.01	0.475
HDL-C	0.08	0.226
LDL-C	0.14	0.101
VLDL-C	-0.01	0.474
AST	0.05	0.326
ALT	0.17	0.056
NO	-0.01	0.475
MDA	0.06	0.282
Leptin	**-0.24**	**0.014**
Ghrelin	0.01	0.444

Pearson correlation coefficient (r) was used for testing orexin-A correlation with other parameters. BMI: body mass index; SBP: systolic blood pressure; DBP: diastolic blood pressure; MAP: mean arterial pressure; FBG: fasting blood glucose; HOMA-IR: homeostasis model assessment of insulin resistance; QUICKI: quantitative insulin sensitivity check index; HbA1c: glycated hemoglobin A1c; HDL-C: high-density lipoprotein-cholesterol; LDL-C: low-density lipoprotein-cholesterol; VLDL-C: very low-density lipoprotein-cholesterol; AST: aspartate transaminase; ALT: alanine transaminase; NO: nitric oxide and MDA: Malondialdehyde.

IR, diastolic blood pressure (DBP), and VLDL-C are correlated with serum fasting orexin-A levels

Stepwise multiple regression was performed to test the relation of orexin-A with all the studied parameters, as shown in Table-2. The results demonstrated that the main independent predictor for orexin-A in all studied groups is HOMA-IR, followed by DBP, height, and VLDL-C.

Table 2-The independent predictors for serum orexin-A hormone in the whole study sample

Model	B	Beta	Partial correlation	R^2	Adjusted R^2	F	p-value
1			0.39	0.15	0.14	14.42	**0.000**
Constant	0.93						**0.000**
HOMA-IR	0.25	0.39					**0.000**
2			0.48	0.23	0.21	11.84	**0.000**
Constant	-1.82						0.064
HOMA-IR	0.28	0.44					**0.000**
DBP	1.46	0.28					**0.006**
3			0.52	0.27	0.24	9.78	**0.000**
Constant	-1.88						0.051
HOMA-IR	0.31	0.49					**0.000**
DBP	1.23	0.24					**0.019**
Height	2.19	0.22					**0.035**
4			0.55	0.31	0.27	8.75	**0.000**
Constant	-1.56						0.102
HOMA-IR	0.37	0.59					**0.000**
DBP	1.16	0.22					**0.025**
Height	2.42	0.24					**0.019**
VLDL-C	-0.19	-0.22					**0.039**

By applying stepwise multiple regression, the studied independent predictors were arranged simultaneously to different model. The excluded variables are age, sex, weight, BMI, SBP, MAP,

FBG, insulin, QUICKI, HbA1c, serum cholesterol, triglyceride, HDL-C, LDL-C, AST, ALT , NO, MDA, ghrelin and leptin. B: the unstandardized coefficient regression, Beta: the standardized coefficient regression, R^2: the coefficient of determination.

Discussion

According to our knowledge, this is the first study that evaluates the prevalence of orexin-A hormone in T2DM patients and non-DM subjects in Kurdistan Region/Iraq.

In this study, Pearson correlation analysis determined a negative significant correlation of orexin-A with sex , as was also previously shown [13], but a positive significant positive relation with blood pressure, FBG, HbA1c, insulin, and HOMA-IR. The positive correlation of orexin-A with FBG and insulin may be related to the roles of orexin-A in the protection against pancreatic cell dysfunction and the stimulation of insulin secretion from the pancreatic beta cells in T2DM patients [14]. The high level of orexin and the disorder in its secretion were shown to be associated with insulin resistance [15]. The significant correlation of orexin-A with SBP, DBP, and MAP may be related to the effects of orexin-A on the sympathetic nervous activities, which causes an increased blood pressure [7]. It also may be due to an increase in adrenalin and noradrenalin hormones [16], through its role on the hypothalamic-pituitary-adrenal pathway [17].

A significant inverse correlation of serum orexin-A with leptin was recorded in our analysis, which support that both have an inverse regulatory effect on energy and metabolic homoeostasis maintenances [5]. This correlation might be due to the inhibitory effect of leptin on orexigenic neuron [15] or due to the effect of orexin on the neuroendocrine system, including leptin level's setting [18]. On the other hand, an inverse correlation of orexin-A with QUICKI was obtained. It can be suggested that the possible hypertension conditions and chronic psychological stress on our population may cause chronic over activity of the sympathetic nervous system, which may conduce to a decline in IS [19]. Lipid profile has no significant correlation with orexin-A, which may be due to the normal body weight of our study participants, as indicated by BMI values. This result is confirms that of Rani *et al*., who showed a negative correlation of orexin with triglyceride in obese diabetic patients [5].

ALT and AST are markers for liver injury that showed no significant relation with orexin-A in our group of T2DM patients, although Lin *et al*. found the involvement of orexin-A in hepatic reperfusion-induced liver injury in rats [20]. Ghrelin has stimulatory effects on orexin neuron, leading to the stimulation of orexin mRNA expression in the hypothalamus [21]. Nevertheless, we obtained no significant association of ghrelin with orexin-A in our patients with T2DM disease. NO is a free radical that has an inhibitory effect on orexin neurons, as recorded by Yamakawa *et al*., who found that the level of circulating NO regulates the activation of orexin neurons [22]. As related to serum MDA, Zwirska-Korczala *et al*. found that orexin-A caused an increase in MDA level [23]. However, we did not record any significant relation of orexin-A with serum NO and MDA.

When the effects of all other parameters were excluded by stepwise multiple regression analysis, HOMA-IR was shown to be the first independent predictor of orexin-A (Table-2). This confirms the role of orexin-A in IR and T2DM disease [5, 24] and that HOMA-IR is a strong predictor of T2DM development [25].

As presented in Figure-1, and in accordance with our results established by the AUC value, orexin-A [26], HOMA-IR, QUICKI, FBG and HbA1c were considered as hallmarks for T2DM disease [27, 28]. Orexin-A is considered as a biomarker for T2DM disease due to its regulatory effect on glucose and insulin homeostasis. Orexin-A has a dual effect on blood glucose regulation through the autonomic nervous system, i.e. the sympathetic and parasympathetic nervous system. When the cytosolic energy is reduced, the orexin neuron is stimulated and causes an increase in blood glucose level through the sympathetic nervous system. This explanation applies to diabetes patients as they have a low level of cytosolic energy which leads to hepatic glucose production and, thus, hyperglycemia [4]. HOMA-IR is presented as a marker for T2DM disease in our study. This confirmed the critical role of insulin resistance in the development of T2DM disease [2]. Meanwhile, we found that MDA and NO are biomarkers for T2DM disease. MDA is a lipid peroxidation product which is evaluated as an oxidative stress marker and predicts T2DM [29]. NO has a potential role in the pathogenesis of type 2 DM [30].

Conclusions

Orexin-A possesses a linear correlation with hyperglycemia and insulin resistance, which indicates the stimulatory effects of orexin-A on glucose and insulin secretion from the liver. Accordingly, orexin-A and HOMA-IR may be suggested as predictors for the metabolic disorder and diabetic complications in our subjects. Furthermore, orexin-A can be considered as a good biomarker for T2DM disease.

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

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References

1. Federation ID. **2017**. Idf diabetes atlas 8th edition.Brussels, belgium: International diabetes federation.
2. Abbas KM, Alaaraji SFT and Al–Shawk RS. **2020**. A study of the association between IL-17 and HOMA-IR in Iraqi type 2 diabetic patients. *Iraqi Journal of Science*. 491-8. doi: 10.24996/ijs.2020.61.3.4
3. Skrzypski M, Khajavi N, Mergler S, Billert M, Szczepankiewicz D, Wojciechowicz T, et al. **2016**. Orexin a modulates ins-1e cell proliferation and insulin secretion via extracellular signal-regulated kinase and transient receptor potential channels. *J Physiol Pharmacol*. **67**(5): 643-52.
4. Tsuneki H, Wada T and Sasaoka T. **2012**. Role of orexin in the central regulation of glucose and energy homeostasis. *Endocr J*. **59**: 365-74.
5. Rani M, Kumar R and Krishan P. **2019**. Serum level of orexin a and its correlation with metabolic risk factors in type 2 diabetes mellitus patients. *Int J Diabetes Dev Ctries*. **39**(4): 647-53. doi: 10.1007/s13410-019-00728-z
6. Kaczmarek P, Skrzypski M, Pruszynska-Oszmalek E, Sassek M, Kolodziejski P, Billert M, et al. **2017**. Chronic orexin-a (hypocretin-1) treatment of type 2 diabetic rats improves glucose control and beta-cell functions. *J Physiol Pharmacol*. **68**(5): 669-81.
7. Li A and Nattie E. **2014**. Orexin, cardio-respiratory function, and hypertension. *Front Neurosci*. **8**: 22. doi: 10.3389/fnins.2014.00022
8. Yu D, Zhao Z and Simmons D. **2016**. Interaction between mean arterial pressure and HbA1c in prediction of cardiovascular disease hospitalisation: A population-based case-control study. *J Diabetes Res*. 2016. doi: 10.1155/2016/8714745
9. Chen H, Sullivan G, Yue LQ, Katz A and Quon MJ. **2003**. Quicki is a useful index of insulin sensitivity in subjects with hypertension. *American Journal of Physiology-Endocrinology and Metabolism*. **284**: 804–12.
10. Friedewald WT, Levy R and Fredrickson DS. **1972**. Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. *Clin Chem*. **18**(6): 499-502.
11. Sun J, Zhang X, Broderick M and Fein H. **2003**. Measurement of nitric oxide production in biological systems by using griess reaction assay. *Sensors*. **3**(8): 276-84.
12. Patait MR and Mody R. **2011**. Estimation of serum malondialdehyde before and after radiotherapy in oral squamous cell carcinoma patients undergoing antioxidant therapy. *Journal of Indian Academy of Oral Medicine and Radiology*. **23**(4): 563-8.
13. Al-Ghreaty H and Al-Tu'ma F and Hatrosh S. **2017**. The role of orexin hormone in sera of patients with metabolic syndrome of kerbala province: Iraq. *Iraq Medical Journal*. **1**(3): 57-.
14. Skrzypski M, Billert M, Nowak K and Strowski M. **2018**. The role of orexin in controlling the activity of the adipo-pancreatic axis. *J Endocrinol*. **238**(2): R95-R108.
15. Al-Kuraishy H, Abdulhadi M, Hussien N, Al-Niemi M, Rasheed H and Al-Gareeb A. **2020**. Involvement of orexinergic system in psychiatric and neurodegenerative disorders: A scoping review. *Brain Circulation*. **6**(2): 70. doi: 10.4103/bc.bc_42_19

16. Li A, Hindmarch CC, Nattie EE and Paton JF. **2013**. Antagonism of orexin receptors significantly lowers blood pressure in spontaneously hypertensive rats. *J Physiol*. **591**(17): 4237-48. doi: 10.1113/jphysiol.2013.256271

17. Litwack G. **2017**. *Human biochemistry*: Academic Press.

18. Kok S, Meinders A, Overeem S, Lammers G, Roelfsema F, Frolich M, et al. **2002**. Reduction of plasma leptin levels and loss of its circadian rhythmicity in hypocretin (orexin)-deficient narcoleptic humans. *The Journal of Clinical Endocrinology & Metabolism*. **87**(2): 805-9.

19. Messina G, Dalia C, Tafuri D, Monda V, Palmieri F, Dato A, et al. **2014**. Orexin-a controls sympathetic activity and eating behavior. *Front Psychol*. **5**: 997. doi: 10.3389/fpsyg.2014.00997

20. Lin J, Yan G, Xue H, Hao X, Zhang K and Wang L. **2009**. Orexin-a in hepatic reperfusion-induced liver injury in rats. *Zhong Nan Da Xue Xue Bao Yi Xue Ban*. **34**(11): 1078-85.

21. Yamanaka A, Beuckmann CT, Willie JT, Hara J, Tsujino N, Mieda M, et al. **2003**. Hypothalamic orexin neurons regulate arousal according to energy balance in mice. *Neuron*. **38**(5): 701-13.

22. Yamakawa T, Kurauchi Y, Hisatsune A, Seki T and Katsuki H. **2018**. Endogenous nitric oxide inhibits, whereas awakening stimuli increase, the activity of a subset of orexin neurons. *Biol Pharm Bull*. **41**(12): 1859-65. doi: 10.1248/bpb.b18-00633

23. Zwirska-Korczala K, Adamczyk-Sowa M, Sowa P, Pilc K, Suchanek R, Pierzchala K, et al. **2007**. Role of leptin, ghrelin, angiotensin ii and orexins in 3t3 l1 preadipocyte cells proliferation and oxidative metabolism. *J Physiol Pharmacol*. **58**(Suppl 1): 53-64.

24. Adeghate E, Lotfy M, D'Souza C, Alseiari SM, Alsaadi AA and Qahtan SA. **2020**. Hypocretin/orexin modulates body weight and the metabolism of glucose and insulin. *Diabetes Metab Res Rev*. **36**(3): e3229. doi: 10.1002/dmrr.3229

25. Purohit V and Tiwari V. **2015**. Study of insulin resistance in Type 2 Diabetes Mellitus by HOMA-IR score. *Int J Med Res Rev*. **3**(1): 3-9.

26. Rani M, Kumar R and Krishan P. 2017. Implicating the potential role of orexin in hypertension. *Naunyn Schmiedebergs Arch Pharmacol*. **390**(7): 667-76. doi: 10.1007/s00210-017-1378-z

27. Singh B and Saxena A. **2010**. Surrogate markers of insulin resistance: A review. *World J Diabetes*. **1**(2): 36.

28. Al-Attaby AKT and Al-Lami MQD. **2019**. Effects of duration and complications of type 2 diabetes mellitus on diabetic related parameters, adipocytokines and calcium regulating hormones. *Iraqi Journal of Science*.2335-61. doi: 10.24996/ijs.2019.60.11.5

29. Bouhajja H, Kacem FH, Abdelhedi R, Ncir M, Dimitrov JD, Marrakchi R, et al. **2018**. Potential predictive role of lipid peroxidation markers for type 2 diabetes in the adult tunisian population. *Can J Diabetes*. **42**(3): 263-71. doi: 10.1016/j.jcjd.2017.06.006

30. Mishra S and Mishra BB. **2017**. Study of lipid peroxidation, nitric oxide end product, and trace element status in type 2 diabetes mellitus with and without complications. *Int J Appl Basic Med Res*. **7**(2): 88-93. doi: 10.4103/2229-516X.205813