RESEARCH
O&G Forum 2024; 34-3s: 1541-1544

ESTIMATION OF NESFATIN-1 AND FETUIN-A WITH SAME RELATED PARAMETERS IN OBESE AND NON OBESE MENOPAUSE WOMAN

Khawla Jassim Mohammed Qassim[1], Abeer Ataallah Ayyed Al-Hadidy[2]

[1,2]University of Mosul, Iraq, College of Science, Department of Biology.
Khawla.23scp59@student.uomosul.edu.iq, abesbio53@uomosul.edu.iq

Abstract
Background: Menopause is an aging in woman, it begins in most of them at 45 years old, the main reason of menopause is the decline of the Estrogen hormone result from decline of ovaries function, it's include many of symptoms such as nocturnal sweats, hot flashes, depression, elevated of anxiety, insomnia, sleep disturbance, genitalia include inflammation, dryness, urinary tract infections, painful urination, gaining weight, usually in the abdomen, hips and joint pain. Menopause leads to many disease from them insulin resistance and obesity. The adipose tissue it's secretes active proteins such as nesfatin-1 which is adipocytokines regulates food intake. Fetuin-A is adipokines and hepatokiens that generated mainly by liver it serves to act as carrier of free fatty acid in circulation.
Objectives: The aim of this study is to estimate nesfatin-1, fetuin-A hormones and other related parameters in obese and non-obese menopause woman.
Material and Methods: This study involved 90 woman. For comparison, included (45) non-obese menopause woman weight with BMI (22.08±3.39) Kg/ m2 as a control group (group1), another (45) obese menopause woman with BMI (35.38±2.52) Kg/ m2 group2.
Results: There is significant decrease at (P≤ 0.001) in the level of nesfatin-1, E2 and significant increase at (P≤ 0.001) in the level of fetuin-A, FSH, LH and TNF-α in group2 compered to control group, also there is a significant elevation at (P≤ 0.001) in homeostatic model assessment- insulin resistance
(HOMA-IR) compared to control group.
Conclusion: nesfatin-1, E2 hormone decrease significantly, but fetuin-A, FSH, LH and TNF-α significantly increase and elevated with insulin resistance in obese menopause woman compered to control group.
Key words: Nesfatin-1, Fetuin-A, menopause, insulin resistance, hepatokines and adipokines.

Introduction

Menopause is the cessation of menstruation caused by the decline in ovarian follicular activity, resulting in its permanent cessation. Menopause naturally occurs between the ages of 45 and 55 as a normal mean of biological ageing. It is identified by 12 months of amenorrhea (1). The menstrual cycle modifications known as "perimenopause" which can endure for several years and impact one's physical, emotional, mental, and social well-being, are the first step in the slow shift to menopause (2).

Ovulatory dysfunction may theoretically raise the risk of obesity since it prevents woman from experiencing the customary "reduced appetite" time. The female sex steroid hormones especially estradiol (E_2) play a major role in mediating this relationship between body weight regulation and reproductive function. E_2 generally controls woman's homeostatic nutrition by reducing food intake and raising energy expenditure (3), in other word (with the loss of estradiol activity) lead to an increase in food consumption (4).

Nesfatin-1and its precursors described for the first time by Oh *et al* (2006) (5). Nesfatin-1, a potent anorexigenic peptide, encoded a fat-influencing and satiety-influencing protein that controlled homeostatic eating (6), the blood-brain barrier can be crossed by nesfatin-1, according to a study by Unniappan that discover the inhibition of anorexigenic neurons and the stimulation of pancreatic beta cell secretion may be the mechanisms behind the nesfatin-1-induced suppression of hunger (7).

The glycoprotein fetuin-A is a heterodimeric form found in the liver. Adult hepatocytes and embryonic cells express it the most frequently, whereas adipocytes and monocytes express it less frequently (8). Fetuin A is a member of the inhibitory class of cysteine proteinases, it is released into the blood, where it binds to and inhibits the tyrosine kinase of the insulin receptor in muscle and hepatocytes causing insulin resistance in these target tissues (9) so, increased fetuin A levels in humans are linked to insulin resistance and obesity (10).

Material and Methods:

In this study (90) woman between (50-65) years old precipitate from Iraqi hospitals and privet laboratories in Mosul governorate from the period 15/8/2023 to 15/11/2023. The woman divided into two groups:

$Group_1$: involved (45) non obese menopause woman with BMI (22.08±3.39) Kg/ m^2 as control group.

$Group_2$: involved (45) obese menopause woman with BMI (35.38±2.52) Kg/ m^2.

Collecting Samples: The woman were asked to fast for 12 hours, and then 5 ml of their venous blood was drawn and put into gel tubes. After the serum was centrifuged and separated, it was put in sterile Eppendorf plastic tubes and refrigerated at -20 °C until it was tested.

Hormonal and Biochemical Tests: The laboratories tests conducted in this study comprised the measurement of Body Mass Index (BMI), nesfatin-1, fetuin-A, E_2, FSH, LH, HOMA-IR and TNF-α. BMI was estimated by using the formula Kg/m^2. The levels of nesfatin-1, fetuin-A, E_2, FSH, LH, insulin and TNF-α estimated using ELISA techniques, while glucose levels were tested using the Fujifilm dives. The HOMA-IR index, which measures insulin resistance, was computed using the Matthews equation:

$$\frac{Fasting\ insulin\ (\mu U/\mathrm{ml} \times Fasting\ glocose\ (\mathrm{mg}/dl}{405} \quad (11).$$

Statistical Analysis: Data were collected, revised, coded and entered to the Statistical Package for Social Science (IBM SPSS) version (16). The quantitative data were presented as mean, standard deviations and ranges when their distribution found parametric. The data were analyzed according to the system of simple experiments and a complete random design, as the different coefficients were significantly distinguished by different letters of the alphabet under the probability level of ($P \leq 0.001$) according to Duncun's multiple range test. The correlation was also conducted between the variables (12).

RESULTS

Table 1: The parameters value in the different groups of study.

Groups / Parameters	Group 1 (no.=45)	Group 2 (no.=45)
Nesfatin-1 (pg/ ml)	(23.83±3.73) a	(1.83±1.15) b
Fetuin-A (ng/ ml)	(44.6±4.99) b	(70.0±3.34) a
E2 (pg/ ml)	(2.41±0.82) a	(0.78±0.13) b
FSH (mIU/ ml)	(47.5±5.10) b	(85.7±20.3) a
LH (mIU/ ml)	(31.0±7.24) b	(83.2±14.6) a
HOMA-IR	(1.76±0.21)b	(3.83±1.11) a
TNF-α (pg/ ml)	(6.66±1.06)b	(20.8±1.79) a
BMI (kg/ m^2)	(22.08±3.39) b	(35.38±2.52) a

There is a significant difference, the no. is followed by different letters.

The values are means ± standard deviation SD.

Table (2): Correlations between Nesfatin-1 and Fetuin-A with other parameters

	E_2 (Pg/ml)	FSH (mIU/ ml)	LH (mIU/ ml)	HOMA-IR	TNF-α (Pg/ ml)	BMI (kg/ $m^{2)}$
Nesfatin-1 (pg/ ml)	.783**	-.771**	-.885**	-.764**	-.953**	-.898**
Fetuin-A (ng/ ml)	-.760**	.766**	.856**	.757**	.938**	.871**

The table (1 and 2) demonstrates a substantial reduction at ($P \leq 0.001$) in the levels of nesfatin-1, E_2 as (1.83±1.15) pg/ ml, (0.78±0.13) pg/ ml respectively in the group$_2$, compared to the control group (23.83±3.73) pg/ ml, (2.41±0.82) pg/ ml respectively. The results indicated a significant increase at ($P \leq 0.001$) in the level of fetuin-A (70.0±3.34) ng/ ml, FSH (85.7±20.3) mIU/ ml, LH (83.2±14.6) mIU/ ml, HOMA-IR (3.83±1.11), TNF-α (20.8±1.79) and BMI (35.38±2.52), in the group$_2$ compared to the control group (44.6±4.99) ng/ ml, (47.5±5.10) mIU/ ml, (31.0±7.24) mIU/ ml, (1.76±0.21), (6.66±1.06) (pg/ ml), (22.08±3.39) kg/ m^2 respectively.

Discussion

Adipose tissue releases many adipokines including nesfatin-1 which have a role in regulating insulin resistance, glucose metabolism and lipid metabolism. The results of our investigation indicate that serum nesfatin-1 levels were significantly lower in the group of menopausal obese woman compared to the control group. These findings are consistent with the study conducted by Dokumacioglu *et al* 2020 (13).

The main reason for the low levels of nesfatin-1 in obese menopause is the decrease in E_2 hormone levels. This decrease in E_2 hormone leads to an increase in the mRNA expression of NUCB2 the gene responsible for encoding the nesfatin-1 protein in lacto-somatotroph cells. On the other hand, the mRNA expression of NUCB2 is increased by Progesterone in endometrial stromal cells (14). These findings are consistent with a previous study that showed an increase in NUCB2 mRNA expression in the pituitary gland due to E_2 hormone (15). Therefore, in normal physiology the decrease in E_2 and progesterone hormones during menopause results in a decrease in NUCB2 mRNA expression of nesfatin-1, this explained the correlation between nesfatin-1 and E_2 at (r= .783) as show in table (2), because of an increase in LH and FSH production to make up for the lowering E_2 brought on by ovarian function decline, which is a normal part of menopause, and because FSH is thought to be a notable indicator of E2 drop (16), this explain the correlation between nesfatin-1 with FSH and LH at (r= -.771), (r=-.88) respectively.

Multiple investigations have proven the correlation between nesfatin-1 levels and obesity. There have been reports indicating that the activity of the nesfatin-1 gene was increased in the adipose tissue of subjects on a high-fat diet. This indicates that nesfatin-1 may play a role in the process of lipid buildup and perhaps contribute to food-induced obesity (17). Previous study shown that continuous consumption of a high-fat obesogenic diet led to a decrease in stomach NUCB2 mRNA expression in mice which might caused the obesity (18).

Nesfatin-1 has been found to have appetite-suppressing effects, to evidence has shown that when nesfatin-1 was injected directly into the brain ventricles of mice it decreases their food intake during the dark phase, this decrease in food intake is due to a reduction in meal size, indicating increased satiation, as well as a decrease in meal frequency and longer intervals between meals indicating increased satiety (19). Therefore, the decrease in nesfatin-1 levels can lead to an increase in appetite and ultimately contribute to obesity. The anorexigenic effects of nesfatin-1 can also lead to a reduction in body fat deposition and weight. Nesfatin-1 acts as an inhibitory factor for food consumption by crossing the blood-brain barrier, once it reaches the hypothalamic regions nesfatin-1 suppresses hunger and reduces food intake (20).

Menopause-related chronic inflammation increased TNF-α from adipose tissue, which in turn caused inflammation and decreased adipose tissue's secretion of nesfatin-1. These hormones in the bloodstream caused hyperphegia, and women in the menopause who were obese due to increased food intake and a high BMI (21), this explained correlation between nesfatin-1 and TNF-α as (r=-.95).

Our findings indicate that serum fetuin-A levels were significantly elevated in obese woman menopause in comparison to the control group. A decline in E_2 levels in

postmenopausal woman may lead to a rise in overall body fat particularly in the central region with reduction in subcutaneous fat and an increase in visceral fat, this change in fat distribution is associated with elevated levels of fetuin-A in the bloodstream (22), this explained correlation between fetuin-A and E_2 at (r=-.76). So based on the negative association between E2 and FSH and LH, where a low level of E2 does not cause the pituitary to stop secreting FSH and LH (22), this lined with our result and explains the significant correlation between fetuin-A with FSH and LH as (r=.76), (r=.85) respectively.

Obesity causes an increase in fetuin-A levels and high levels of fetuin-A contribute to the development of obesity. This has been demonstrated in animal studies using a rat model of diet-induced obesity, where it was observed that there was a high expression of fetuin-A mRNA in the liver. Additionally, it has been discovered that fetuin-A plays a significant role as a carrier protein for Free fatty acids (FFAs) in the bloodstream (23). FFAs induce an increase in the expression of fetuin-A via the activation of Nuclear factor-kappa-B (NF-kB).

Our study discovered a rise in HOMA-IR in obese menopause. The results of Kim's research are consistent with our findings, suggesting that the menopausal condition affects insulin resistance levels (24). E_2 improves the body's response to insulin resulting in decreased absorption of glucose in insulin-sensitive tissues such as adipose tissue and skeletal muscle. The skeletal muscle is widely acknowledged as the primary component responsible for oxidative metabolism and glucose disposal (25).

The findings of this study established a significant correlation between nesfatin-1 and HOMA-IR. More precisely, an elevation in HOMA-IR was observed in obese postmenopausal woman following a decrease in nesfatin-1 levels, This described the anti-hyperglycemic properties of nesfatin-1 (26). Improve that firstly, by intravenous administration of nesfatin-1 decreases blood glucose levels (27). Secondly, by the role of nesfatin-1 in regulating insulin secretion is indicated by its production in pancreatic beta cells (28). The mechanism comprises three distinct stages: initially, nesfatin-1, which is secreted by the pancreas, stimulates an enhanced response to glucose (29). Subsequently, nesfatin-1 augments the secretion of insulin in response to glucose and finally, nesfatin-1 facilitates the release of glucagon-like peptide-1 (30).

This research demonstrates a favorable correlation between fetuin-A levels and HOMA-IR in obese individuals and/or those experiencing menopause, mechanism of insulin resistance proposed by fetuin- A is the reduction of insulin receptor tyrosine kinase activity which inhibits insulin receptor autophosphorylation (31). woman with elevated fetuin-A induces the production of proinflammatory cytokines such as IL-6 and TNF-α which in turn promote inflammation and damage to β-cells, this leads to persistent hyperglycemia and the development of type 2 diabetes mellitus (32), so a rise in fetuin-A levels in the bloodstream may lead to an increase in the production of TNF-α and a decrease in the synthesis of adiponectin from adipocytes (33), this explained the correlation fetuin-A and TNF-α as (r=0.93).

Conclusion:

We conclude from this research that there was a significant decrease in the nesfatin-1 hormone in obese menopause woman and the level of fetuin-A hormone increase in obese menopause woman the two hormone led to increase in HOMA-IR, TNF-α in menopause woman which led to obesity.

Acknowledgments

The author would like to express her gratitude to the College of Science's Department of Biology at the University of Mosul for their invaluable support and provision of resources throughout the course of this research. Additionally, the author wishes to salute the Nineveh Health Department.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

References

1. Jameson, J. (2013). "Harrison's Endocrinology". 3rd Edition, McGraw Hill Education. Philadelphia, USA.

2. Soares, C. and Taylor, V. (2007). Effects and management of the menopausal transition in woman with depression and bipolar disorder. Journal of Clinical Psychiatry, 68(9): 16.

3. Leeners, B.; Geary, N.; Tobler, P. and Asarian, L. (2017). Ovarian hormones and obesity. Human reproduction update, 23(3): 300-321.

4. Tumminia, A.; Vinciguerra, F.; Parisi, M. and Frittitta, L. (2018). Type 2 diabetes mellitus and Alzheimer's disease: role of insulin signalling and therapeutic implications. International journal of molecular sciences, 19(11): 3306.43

5. Oh-I, S.; Shimizu, H.; Satoh, T.; Okada, S.; Adachi, S.; Inoue, K. and Mori, M. (2006). Identification of nesfatin-1 as a satiety molecule in the hypothalamus. Nature, 443(7112): 709-712.

6. Dore, R.; Levata, L.; Lehnert, H. and Schulz, C. (2017). Nesfatin-1: functions and physiology of a novel regulatory peptide. Journal of Endocrinology, 232(1): R45-R65.

7. Gonzalez, R.; Perry, R.; Gao, X.; Gaidhu, M.; Tsushima, R.; Ceddia, R. and Unniappan, S. (2011). Nutrient responsive nesfatin-1 regulates energy balance and induces glucose-stimulated insulin secretion in rats. Endocrinology, 152(10): 3628-3637.

8. Chekol, E.; Tilahun, Z.; Mengie, T.; Mekonnen, M.; Teshome, M., Abebe, E. and Asmamaw, M. (2022). The structure, biosynthesis, and biological roles of fetuin-A: A review. Frontiers in cell and developmental biology, 10: 945287.

9. Ismail, N.; Ragab, S.; El-Baky, A.; Hamed, M. and Ibrahim, A. (2014). Effect of oral curcumin administration on insulin resistance, serum resistin and fetuin-A in obese children: randomized placebo-controlled study. Research Journal of Pharmaceutical, Biological and Chemical Sciences, 5(1): 887-896.

10. Wojtysiak, B.; Malecha, A.; Burska, A.; Duma, D. and Donica, H. (2010). Serum fetuin-A levels in patients with type 2 diabetes mellitus. Annales Universitatis Mariae Curie-Sklodowska, Sectio DDD: Pharmacia, 23(2): 93-99.

11. Matthews, D.; Hosker, J.; Rudenski, A.; Naylor, B.; Treacher, D. and Turner, R. (1985). Homeostasis model assessment: insulin resistance and beta-cell function from

fasting plasma glucose and insulin concentrations in man. Diabetologia, 28: 412–419.
12. Hommadi, S. (2017). ―Statistical Analysis for Agricultural Experiments. Using SAS program". 1st edition. Iraqi company.
13. Dokumacioglu, E.; Iskender, H.; Sahin, A.; Erturk, E. and Kaynar, O. (2020). Serum levels of nesfatin-1 and irisin in obese children. European Cytokine Network, 31: 39-43.
14. Ha, J.; Shin, J.; Seok, E.; Kim, S.; Sun, S. and Yang, H. (2023). Estradiol and progesterone regulate NUCB2/nesfatin-1 expression and function in GH3 pituitary cells and THESC endometrial cells. Animal Cells and Systems, 27(1): 129-137.
15. Chung, Y., Kim, J., Im, E., Kim, H., & Yang, H. (2015). Progesterone and 17β-estradiol regulate expression of nesfatin-1/NUCB2 in mouse pituitary gland. Peptides, 63, 4-9.
16. Kawakita, T.; Yasui, T.; Yoshida, K.; Matsui, S. and Iwasa, T. (2023). Associations of LH and FSH with reproductive hormones depending on each stage of the menopausal transition. BMC Woman's Health, 23 (1): 286.
17. Stengel, A., Mori, M., & Taché, Y. (2013). The role of nesfatin-1 in the regulation of food intake and body weight: recent developments and future endeavors. Obesity Reviews, 14(11), 859-870.
18. Mohan, H., Ramesh, N., Mortazavi, S., Le, A., Iwakura, H., & Unniappan, S. (2014). Nutrients differentially regulate nucleobindin-2/nesfatin-1 in vitro in cultured stomach ghrelinoma (MGN3-1) cells and in vivo in male mice. PLoS One, 9(12), e115102.
19. Goebel, M.; Stengel, A.; Wang, L. and Taché, Y. (2011). Central nesfatin-1 reduces the nocturnal food intake in mice by reducing meal size and increasing inter-meal intervals. Peptides, 32(1): 36-43.
20. Wu, D.; Yang, M.; Chen, Y.; Jia, Y.; Ma, Z.; Boden, G. and Yang, G. (2014). Hypothalamic nesfatin-1/NUCB2 knockdown augments hepatic gluconeogenesis that is correlated with inhibition of mTOR-STAT3 signaling pathway in rats. Diabetes, 63(4): 1234-1247.
21. Stubbins, R.; Najjar, K.; Holcomb, V.; Hong, J. and Núñez, N. (2012). Oestrogen alters adipocyte biology and protects female mice from adipocyte inflammation and insulin resistance. Diabetes, Obesity and Metabolism, 14 (1): 58-66.
22. Antonson, P.; Omoto, Y.; Humire, P. and Gustafsson, J. (2012). Generation of ERα-floxed and knockout mice using the Cre/LoxP system. Biochemical and biophysical research communications, 424(4): 710-716.
23. Pal, D.; Dasgupta, S.; Kundu, R.; Maitra, S.; Das, G.; Mukhopadhyay, S. and Bhattacharya, S. (2012). Fetuin-A acts as an endogenous ligand of TLR4 to promote lipid-induced insulin resistance. Nature medicine, 18(8): 1279-1285.
24. Dasgupta, S.; Bhattacharya, S.; Biswas, A.; Majumdar, S.; Mukhopadhyay, S.; Ray, S. and Bhattacharya, S. (2010). NF-κB mediates lipid-induced fetuin-A expression in hepatocytes that impairs adipocyte function effecting insulin resistance. Biochemical journal, 429(3): 451-462.
25. Kim, S.; Lee, J.; Im, J.; Kim, D.; Lee, H.; Kim, S. and Lee, J. (2013). Association between serum osteocalcin and insulin resistance in postmenopausal, but not premenopausal, woman in Korea. Menopause, 20(10): 1061-1066.
26. Saengsirisuwan, V.; Pongseeda, S.; Prasannarong, M.; Vichaiwong, K. and Toskulkao, C. (2009). Modulation of insulin resistance in ovariectomized rats by endurance exercise training and estrogen replacement. Metabolism, 58(1): 38-47.
27. Su, Y.; Zhang, J.; Tang, Y.; Bi, F. and Liu, J. (2010). The novel function of nesfatin-1: anti-hyperglycemia. Biochemical and biophysical research communications, 391(1): 1039-1042.
28. St-Pierre, D.; Martin, J.; Shimizu, H.; Tagaya, Y.; Tsuchiya, T.; Marceau, S. and Poirier, P. (2016). Association between nesfatin-1 levels and metabolic improvements in severely obese patients who underwent biliopancreatic derivation with duodenal switch. Peptides, 86: 6-12.
29. Khalili, S.; Khaniani, M.; Afkhami, F. and Derakhshan, S. (2017). NUCB2/Nesfatin-1: a potent meal regulatory hormone and its role in diabetes. Egyptian Journal of Medical Human Genetics, 18(2): 105-109.
30. Şafak, A.; Gulvicek, N.; Sksoy, D.; Karakaya, J. and Usman, A. (2017). Increased serum nesfatin-1 levels in patients with impaired glucose tolerance. Turkish Journal of Endocrinology and Metabolism, 21(3): 65.
31. Schalla, M. and Stengel, A. (2018). Current understanding of the role of nesfatin-1. Journal of the endocrine society, 2(10): 1188-1206.
32. Esser, N.; Legrand-Poels, S.; Piette, J.; Scheen, A. and Paquot, N. (2014). Inflammation as a link between obesity, metabolic syndrome and type 2 diabetes. Diabetes research and clinical practice, 105(2): 141-150.
33. Mathews, S.; Rakhade, S.; Zhou, X.; Parker, G.; Coscina, D. and Grunberger, G. (2006). Fetuin-null mice are protected against obesity and insulin resistance associated with aging. Biochemical and biophysical research communications, 350(2): 437-443.