

SERUM NESFATIN-1 LEVELS IN EXPERIMENTALLY-INDUCED POLYCYSTIC OVARY IN LEAN AND OBESE ALBINO RATS

By

Nawal Khalil Gerges and Abeer Albiomy Khalefa

Department of Physiology, Faculty of Medicine, Zagazig University

ABSTRACT

Background: Polycystic ovary syndrome (PCOS) is a very common problem in reproductive age; Obesity and insulin resistance (IR) play critical roles in its etiology. Limited studies have investigated the link between nesfatin-1 levels and PCOS. However, these studies are controversial.

Objectives: This study aimed to evaluate possible changes of serum nesfatin-1 Levels in letrozole-induced PCOS in lean and obese rats, and its association with some hormonal and metabolic parameters.

Material and methods: Forty two young virgin healthy female albino rats were used. Rats were divided into three equal groups: Group I (control), group II (lean PCOS) rats fed ordinary rodent diet for 9 weeks, and then received a daily single dose of letrozole orally (0.5 mg/kg/BW) for 21 days. Group III (obese PCOS) rats fed high fat diet for 9 weeks, and then received a daily single dose of letrozole (0.5 mg/kg/BW) orally for 21 consecutive days. At the end of experiment, serum levels of Nesfatin-1, free testosterone, LH, FSH, estradiol, progesterone, glucose and insulin were detected. BMI and HOMA-IR were calculated. Ovarian histopathology was done.

Results: In obese polycystic ovary group, serum nesfatin-1 level was significantly lower and accompanied by significant hyperinsulinemia, hyperglycemia, insulin resistance, and high BMI when compared to both of lean polycystic and control groups. Moreover, serum nesfatin-1 level significantly negative correlated with serum insulin levels, serum glucose levels, HOMA-IR, and BMI, but correlated positively with LH levels. In lean polycystic group, serum nesfatin-1 level did not significantly change, when compared to control group, In addition, there was an absence of any significant change in serum insulin levels, serum glucose levels, HOMA-IR, or BMI.

Conclusion: Serum nesfatin-1 level seemed to be related to several metabolic syndrome parameters rather than to polycystic ovary syndrome. Our findings raised a possibility that nesfatin-1 level played some role in PCOS. Therefore, larger scale and more detailed molecular studies in vivo and vitro on ovarian function are needed.

Key words: Nesfatin-1, poly cystic ovary, lean, obese, rats.

INTRODUCTION

Nesfatin-1 is an 82 amino acid polypeptide derived from nucleobindin 2 (NUCB2) (Oh et al., 2006 and Stengel et al., 2012).

Nesfatin-1 is expressed in the brain and peripheral tissues of both human and rodents. It is expressed in appetite-controlling hypothalamic nuclei, i.e. the arcuate nucleus, paraventricular nucleus, supraoptic nucleus, the lateral hypothalamic area (Oh et al., 2006), the

solitary tract and dorsal nucleus of vagus (**Brailoiu et al., 2007 and Kohno et al., 2008**), pancreatic beta cells (**Gonzalez et al., 2009 and Stengel and Tache, 2011**), adipose tissues (**Ramanjaneya et al., 2010**), and gastric mucosa (**Stengel et al., 2009a**). Such a wide pattern of distribution of nesfatin-1 was already taken as an indirect sign of its potential function as an integral regulator of energy homeostasis.

Identification of nesfatin-1 as a hypothalamic neuropeptide was immediately followed by the characterization of its pivotal role in regulating feeding by reducing food intake (**Brailoiu et al., 2007 and Mejima et al., 2009**).

Nesfatin/NUCB2 co-localizes with pancreatic beta cells producing insulin in both mice and rats, suggesting the possible role in the regulation of insulin secretion (**Gonzalez et al., 2009**). In addition, nesfatin-1 promotes Ca²⁺ influx through L-type channels and enhances glucose-induced insulin secretion in mouse islet b-cells (**Stengel et al., 2009b and Nakata et al., 2011**). Continuous peripheral infusion of nesfatin-1 improved insulin sensitivity, increase spontaneous physical activity, and whole-body fat oxidation in rats (**Gonzalez et al., 2011**). Collectively, these data suggested that nesfatin-1 participated in energy homeostasis.

Polycystic ovary syndrome (PCOS) is the most common endocrine disorder in reproductive age women, and it is characterized by chronic anovulation and hyperandrogenism (**Ehrman 2005; Bremer, 2010 and Shannon & Wang, 2012**). Although PCOS is a common

disease, the pathophysiology of PCOS is still not clear.

PCOS is a multi-factorial disease resulting from the dysfunction of different systems (**Ehrman, 2005**). Insulin resistance (IR) is a common feature of PCOS in both obese and non-obese women and contributes to development of hyperinsulinemia, which drives hyperandrogenism in these patients (**Palomba et al., 2010 and Hu & Qia, 2011**). Obesity, present in approximately one-half of patients with PCOS, often is the initial complaint of PCOS most of them having central obesity (**Gambineri et al., 2002, Stankiewicz & Norman, 2006 and Wilkes & Murdoch, 2009**). Even normal-weighted PCOS women have fat accumulation in central abdominal region (**Fica et al., 2008 and Shannon & Wang, 2012**).

The hypothalamic satiety peptides which are known to be effective on energy balance having both physiologic and pathologic regulatory roles on the ovaries (**Gambineri et al., 2002**). Delayed puberty in female rats was associated with low nesfatin-1 levels. Moreover, nesfatin-1 injection influenced dramatically the levels of luteinizing hormone (LH) (**García-Galiano et al., 2010a and Gonzalez et al., 2012**).

Ademoglu et al. (2014) found that serum nesfatin-1 levels are significantly higher in women with PCOS independently of IR and BMI. On the other hand, **Deniz et al. (2012)** and **Alp et al. (2015)** demonstrated that Nesfatin-1 levels were lower in PCOS patients. However, **Binnetoğlu et al. (2014)** reported that Nesfatin-1 levels were not significantly different in patients with PCOS.

In the face of this controversy, this research was designed to evaluate possible changes of serum nesfatin-1 levels in letrozole-induced PCOS in lean and obese rats, and assess its correlation with some hormonal levels, and metabolic parameters (serum glucose levels, serum insulin, and insulin sensitivity).

MATERIALS AND METHODS

Forty two young virgin healthy female albino rats of a local strain, 6 weeks old with body weight 90-105 gm, obtained from the animal house of Faculty of Veterinary Medicine, Zagazig University. Animals were kept in nine steel wire cages (40 x 28 x18 Cm. 4-5 rats /cage) under hygienic conditions in animal house of Faculty of Medicine, Zagazig University. All animals received care in accordance with the guide to the care and use of experimental animals of **Institute of Laboratory Animal Resources (1996)**. The experimental protocol was approved by the Institutional Review Board and research ethics committee of Faculty of Medicine Zagazig University (IRB).

Rats were divided into three equal groups: Group I (control), rats fed standard rodent diet for 9 weeks, and then rats received 1 ml water orally by gavage daily for 21 days. Group II (lean PCOS) rats fed standard rodent diet for 9 weeks, and then rats received a daily single dose of letrozole (non-steroidal aromatase inhibitor, ACDIMA international) orally (0.5 mg/kg dissolved in water) by gavage for 21 consecutive days (**Kafali et al., 2004**). Group III (obese PCOS) rats fed high fat diet formulated in Faculty of Veterinary Medicine, Zagazig University (protein 20%, carbohydrates 35% and fat

45%, mainly in form of lard and soy bean) for 9 weeks (**He et al., 2012**), and then rats continued on HFD and received a daily single dose of letrozole (0.5 mg/kg dissolved in water) orally by gavage for 21 consecutive days (**Kafali et al., 2004**).

Smears were obtained daily by vaginal washing with saline. The fresh unstained samples were evaluated microscopically during the treatment period. Estrus phases were determined according to **Marcondes et al. (2002)** and **Goldman et al. (2007)**. The microscopic examination of vaginal smear revealed persistent estrus phase in all rats of group II and group III. So, blood and tissue samples were obtained from group I in the estrus phase of sexual cycle.

Twenty four hours after the end of the study (after the last dose of letrozole) and after overnight fasting, rats weighed and BMI were calculated according to the equation: body weight (gm)/length² (cm²) (**Novelli et al., 2008**).

Rats were anesthetized by ether inhalation. Blood samples were collected from orbital sinus (sampling of controls taken in the estrus phase) and ovaries were dissected and immediately fixed in 4% paraformaldehyde. Blood was centrifuged at 3000 rpm for 15 minutes. The supernatant serum was stored at -80°C.

Serum was examined for Nesfatin-1 levels, according to **Oh et al. (2006)** using nesfatin-1 Enzyme-linked immunosorbent assay (ELISA) rat kits (Sun Red Biotechnology Company, shanghai). Glucose level was determined according to **Tietz (1995)** using glucose enzymatic-liquizyme rat kits (Biotechnology, Egypt). Insulin levels were estimated according to **Temple et al. (1992)** using KAP1251-

INS-EASIA rat kits (BioSource Europe S.A., Belgium). Homeostatic model assessment of insulin resistance index (HOMA-IR) based on serum insulin level (μ IU/ml) and serum glucose level (mg/dl) was calculated according to the formula described by **Matthews et al. (1985)** as $HOMA-IR = \text{fasting serum glucose (mg/dl)} \times \text{fasting serum insulin } (\mu\text{IU/ml}) / 405$. LH, FSH, estradiol, progesterone and free testosterone levels were detected according to **Tietz (1995)** using ELISA rat kits: BC-1031, BC-1029, BC-1111, BC-1113 and BC-1115, respectively, Bio Check Inc 323 Vintage Park Dr. Foster City.

Histopathological examination: The abdominal cavities of the rats were opened. Ovaries were dissected and fixed in 10% buffered formalin for 6 hours at room temperature and washed in a phosphate buffer saline solution. For light microscopy, fixed tissues were dehydrated in an ascending series of ethanol, cleared in xylene and embedded in paraffin. 5 μ m thick sections were mounted in slides previously treated with 3-aminopropyl triethoxysilane and stained with hematoxylin-eosin preliminary observation (**Baravalle et al., 2007**). The pathologist was blinded to the treatment.

Statistical analysis: Results were presented as mean \pm standard deviation (SD). Statistical analysis was performed using the Statistical Package for the Social Sciences (SPSS), version 22 (SPSS Inc., Chicago, IL, United States). Repeated measures of analysis of variance (ANOVA) were applied followed by the least significant difference (LSD) post hoc test to compare means of each two different groups. Pearson's correlation

analysis was performed to screen potential relations between serum nesfatin-1 and all parameters. For all statistical tests done, P value < 0.05 was considered to be statistically significant.

RESULTS

Regarding lean PCO (group II), while the mean value of serum free testosterone level (235.60 ± 12.27 pg/ml) significantly increased ($P < 0.001$). The serum levels of LH (1.17 ± 0.40 IU/ml), estradiol (15.56 ± 2.52 pg/ml) and progesterone (4.16 ± 0.81 ng/ml) significantly decreased when compared with that of control (group I) (77.92 ± 8.64 , 1.94 ± 0.47 , 37.24 ± 4.22 , 7.79 ± 0.91 respectively) ($P < 0.001$). The remaining parameters measured in lean PCO group failed to achieve any significant change when compared with that of control group, as there were non-significant changes in the mean values of serum nesfatin-1 (2.28 ± 0.12 ng/ml), calculated BMI (0.49 ± 0.04 gm/cm²), serum FSH (6.64 ± 0.66 IU/ml), serum glucose (76.21 ± 9.62 mg/dl), serum insulin (8.08 ± 0.82 μ IU/ml) and calculated HOMA-IR (1.53 ± 0.29) in comparison to that of control group (2.31 ± 0.08 , 0.49 ± 0.02 , 7.01 ± 0.39 , 78.78 ± 10.76 , 7.92 ± 0.79 and 1.48 ± 0.46 respectively) ($P > 0.05$ - Table 1).

Concerning obese PCO (group III), the mean value of serum levels of nesfatin-1 (0.85 ± 0.06 ng/ml) significantly decreased when compared to that of both control group ($P < 0.001$), and lean PCO group ($P < 0.001$). The obese PCO group showed significant higher levels of the mean values of BMI (0.96 ± 0.14 gm/cm²), free testosterone (244.93 ± 9.11 pg/ml) Glucose (198.24 ± 9.41 mg/dl), Insulin (28.07 ± 2.70 μ IU/ml) and calculated HOMA-IR

(13.74± 1.50) when compared to that of control group (P< 0.001) and that of lean PCO group. In addition, the mean values of serum levels of estradiol (14.179 ± 2.37 pg/ml) and progesterone (4.71 ± 0.88 ng/ml) were significantly lower than that of control group, but no significant difference detected when compared to that of lean PCO group (P> 0.05). Moreover, FSH level showed non significant difference among the three studied groups (P > 0.05-Table 1).

Serum levels of nesfatin-1 in obese PCO group revealed significant negative correlation with BMI (r= -0.605, P< 0.05), serum glucose (r= -0.836, P< 0.001), serum insulin (r= -0.597, P< 0.05) and calculated HOMA-IR (r= -0.695, P < 0.01). However, serum levels of nesfatin-1

in obese PCO group revealed significant positive correlation with serum LH (r= 0.952, P< 0.001- Table 2 and figures 1,2,3,4,5).

Histopathological examination: Ovaries from the control group (group I) had follicles in various stages of development including secondary follicles, graafian follicles, and recently formed corpus luteum (Figure 6). In ovaries from PCO rats (group II -Figures 7, and group III- Figures 8), follicular cysts were visible as fluid-filled sacs on the ovarian surface. Histologically, it showed increased numbers of atretic and large cystic follicles, and the cystic wall was thickened, characterized by a thickened theca cell layer and a diminished granulosa cell layer.

Table (1): Comparison between the three studied groups (control, lean PCOS and obese PCOS)as regard measured variables.

Parameters	Group I (Control)	Group II (Lean PCOS)		Group III (Obese PCOS)		
	Mean ± SD	Mean ± SD	P VS group I	Mean ± SD	P VS group I	P VS group II
Nesfatin-1 (ng/ml)	2.31± 0 .08	2.28 ± 0.12	P = 0.31	0.85 ±.06	P <0.001	P <0.001
BMI (gm/cm ²)	0.49± 0.02	0.49 ±0.04	P = 0.930	0.96± 0.14	P <0.001	P= 0.000
LH (IU/ml)	1.94 ± 0.47	1.17 ± 0.40	P = 0.000	0.84 ± 0.24	P <0.001	P= 0.034
FSH (IU/ml)	7.01 ± 0.39	6.64 ± 0.66	P = 0.063	6.65 ± 0.69	P= 0.066	P= 0.985
Estradiol (pg/ml)	37.24 ± 4.22	15.56 ± 2.52	P <0.001	14.18 ±2.37	P <0.001	P = 0.253
Progesterone (ng/ml)	7.79±0 .91	4.16 ± 0.81	P <0.001	4.71 ± 0.88	P <0.001	P= 0.098
Testosterone (pg/ml)	77.92 ± 8.64	235.60 ±12.27	P <0.001	244.93 ±9.11	P <0.001	P= 0.020
Glucose (mg/ dl)	78.78±10.76	76.21 ±9.62	P=0 .498	198.24± 9.41	P <0.001	P <0.001
Insulin (?IU/ml)	7.92± 0.79	8.08 ± 0.82	P = 0.811	28.07 ±2.70	P <0.001	P <0.001
HOMA-IR	1.48 ± 0.46	1.53 ± 0.29	P= 0.907	13.74±1.50	P <0.001	P <0.001

Table (2): Correlation between serum nesfatin-1 levels and the levels of the other measured parameters.

Parameters \ Nesfatin-1	Group I (Control)		Group II (Lean PCOS)		Group III (Obese PCOS)	
	r	P	r	P	r	P
BMI (gm/cm ²)	-0.618	0.019	-0.403	0.06	-0.605	0.022
LH (IU/ml)	0.498	0.07	0.160	0.586	0.952	0.000
FSH (IU/ml)	0.315	0.273	0.077	0.794	0.292	0.311
Estradiol (pg/ml)	0.147	0.616	-0.115	0.695	-0.324	0.259
Progesterone (ng/ml)	-0.001	0.997	0.084	0.776	-0.101	0.731
Testosterone (pg/ml)	0.374	0.187	0.114	0.699	0.012	0.967
Glucose (mg/ dl)	0.246	0.396	-0.552	0.041	-0.836	0.000
Insulin (?IU/ml)	-0.311	-0.279	-0.538	0.047	-0.597	0.024
HOMA-IR	-0.361	-0.205	-0.658	0.010	-0.695	0.006

r= correlation with Nesfatin-1 levels.

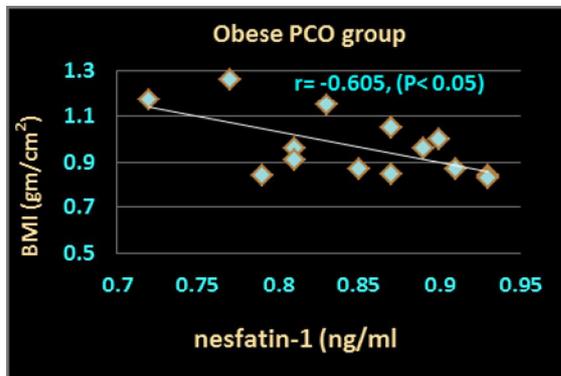


Figure (1): Correlation between serum nesfatin-1 levels (ng/ml) and calculated BMI values (gm/Cm²) in obese PCO rats (group III).

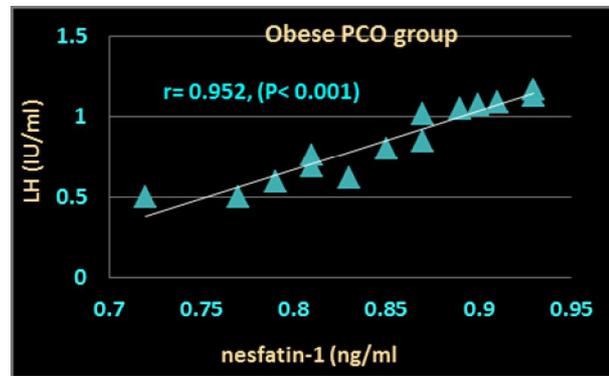


Figure (2): Correlation between serum nesfatin-1 levels (ng/ml) and serum LH levels (IU/ml) in obese PCO rats (group III).

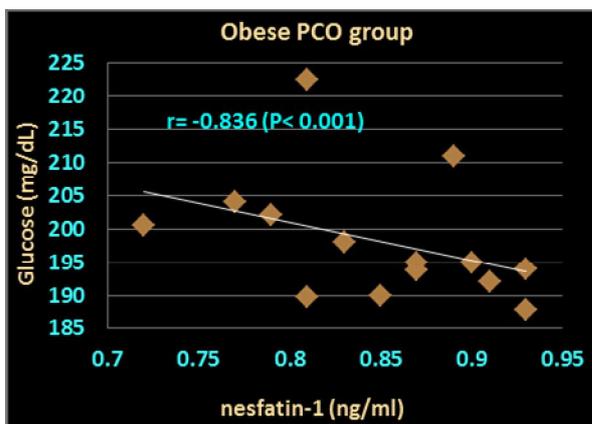


Figure (3): Correlation between serum nesfatin-1 levels (ng/ml) and serum glucose levels (mg/ dl) in obese PCO rats (group III).

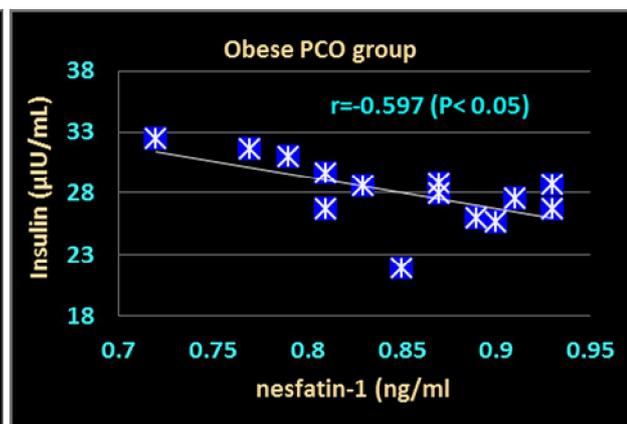


Figure (4): Correlation between serum nesfatin-1 levels (ng/ml) and serum insulin levels (?IU/ml) in obese PCO rats (group III).

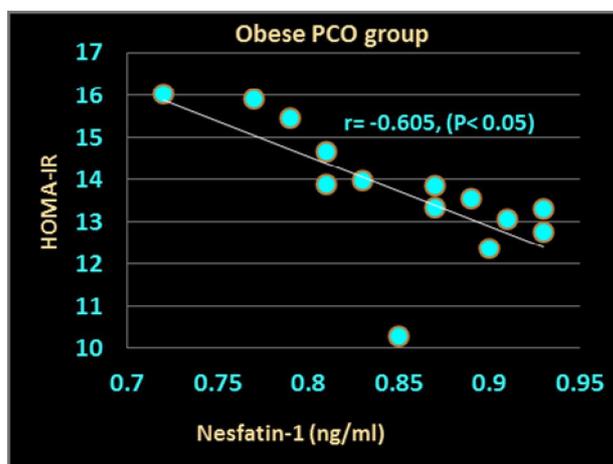


Figure (5): Correlation between serum nesfatin-1 levels (ng/ml) and calculated HOMA-IR values in obese PCO rats (group III).

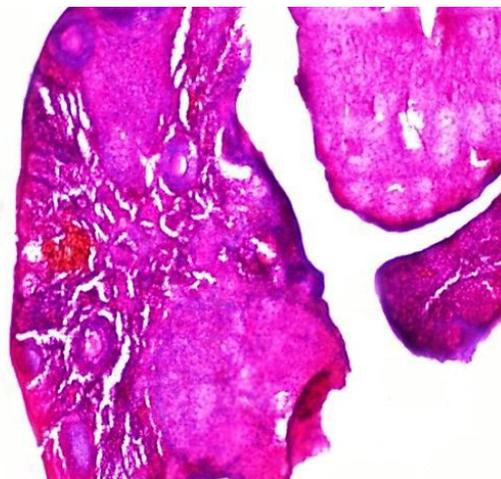


Figure (6): Rat ovary in group 1 (control) showing variable sized graffian follicles surrounded by normal ovarian stroma (LM, H& E, X 200).

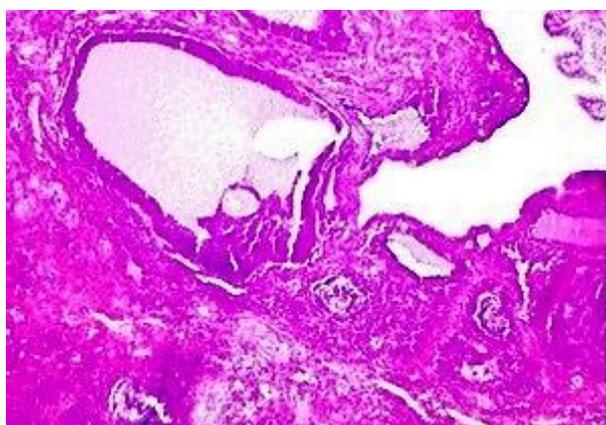


Figure (7): Rat ovary in group II (Lean PCO) showing some atretic and large cystic follicles. The cystic wall was thickened, characterized by thickened theca cell layer with diminished granulosa cell layer (LM, H& E, X 200).

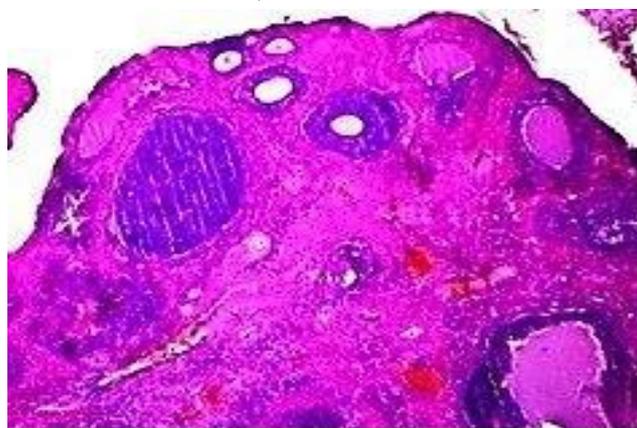


Figure (8): Rat ovary in group III (obese PCO) showing atretic follicles and large cystic follicles. The cystic walls were thickened, (thickened theca cell layer with diminished granulosa cell layer) (LM, H& E, X 200).

DISCUSSION

Nesfatin-1 is a hormone, involved in the regulation of nutritional status, food intake (Tan et al., 2011) and insulin secretion (Zhang et al., 2012). In addition, it is thought to be related to ovarian functions (Gonzalez et al., 2009).

PCOS is a very common syndrome in reproductive age, but the exact etiology is still unknown. The results of the previous studies considered that obesity and IR

play an important role in the etiology (Shimizu et al., 2009, Garcia-Galiano et al., 2010, Li et al., 2010 and Garcia-Galiano et al., 2012).

Studies have found a link between nesfatin-1 levels and PCOS. However, these studies are limited and controversial. Therefore, this study was designed to estimate serum nesfatin-1 levels in letrozole-induced PCOS in lean and HFD obese rats, and correlate these levels with some hormonal levels, BMI, and several

metabolic parameters to clarify the possible role of nesfatin-1 in the pathogenesis of PCOS.

The signs of PCOS induced by letrozole in lean and obese rats was proved by the significant hyperandrogenism (higher serum testosterone levels) accompanied by significant reduction in both estradiol and progesterone levels in comparison to control group, in addition to persistent estrus and histopathological features of cystogenesis. These signs occurred because letrozole blocked cytochrome P450 aromatase which is responsible for aromatization of testosterone to estradiol (**Van Voorhis et al., 1994**). Anovulation was expected because there was a decrease in serum progesterone concentrations (**Meenakumari et al., 2004**), increase in the number of atretic and cystic follicles due to disturbed folliculogenesis, and persistent estrus (**Rezvanfara et al., 2012**).

In the present study, it was found that serum nesfatin-1 significantly decreased in obese polycystic ovary group, when compared to control, and lean polycystic ovary groups, while there were insignificant changes in serum nesfatin-1 level between lean polycystic ovary and healthy control groups.

These results were in agreement with **Deniz et al. (2012)** and **Alp et al. (2015)** who reported low levels of nesfatin-1 levels in obese PCOS patients compared to the healthy control groups. However, **Binnetoglu et al. (2014)** detected non-significant difference in plasma nesfatin-1 levels in non-obese patients with PCOS as compared to healthy subjects. **Ademoglu et al. (2014)** found that the nesfatin-1 level is higher in the PCOS than the

control group that was not differing in BMI, and there is an association of nesfatin-1 with the existence of PCOS independently of IR and high BMI. This discrepancy may be attributed to the differences in species and the study design, including patient selection, small sample and experimental conditions (**Binnetoglu et al., 2014**).

The present study tried to explore the precise mechanism that leading to decrease serum nesfatin-1 level in obese PCOS. The first possible explanation was that decrease serum nesfatin-1 level may be a consequence of hyperinsulinemia, and the higher IR in obese polycystic ovary than both lean polycystic ovary and healthy control groups. It was found that there were a significant increase in serum insulin level and the high insulin resistance in obese polycystic ovary, when compared to lean polycystic ovary and healthy control groups. In addition, there was also a negative correlation between serum nesfatin-1 levels and serum insulin level, and HOMA-IR resistance in obese PCOS group.

These results were in agreement with **Deniz et al. (2012)** who explained the reduction in serum nesfatin-1 level in the patients with PCOS by hyperinsulinemia, and increased insulin resistance, because their patients with PCOS had higher insulin resistance than the control subjects, while the study of **Binnetoglu et al. (2014)** reported that plasma nesfatin-1 levels were not significantly different in non obese patients with PCOS as compared to healthy subjects as BMI and insulin resistance levels were similar in both groups.

This hypothesis is supported by previous studies that found that nesfatin-1 levels were lower in type 2 DM patients than those in type 1 DM patients and healthy individual (**Li et al., 2010**), and also lower in patients with gestational DM compared with control pregnant women (**Foo et al., 2010**). Moreover, pancreatic islet NUCB2 mRNA decreased in T2DM patients (**Riva et al., 2011**) and protein levels of NUCB2 also decreased in a type 2 diabetic rats model (**Xue and Kahn, 2006**). Furthermore, **Dong et al. (2013)** demonstrated that a high-fat diet also significantly decreased plasma nesfatin-1 level compared to that in the control group.

However, the mechanism by which hyperinsulinemia and increased insulin resistance decreased nesfatin-1 level is still unclear.

Pancreatic beta cells producing insulin colocalize with nesfatin/NUCB2 in the islets of both mice and rats (**Iguchi et al., 2007 and Wilkes & Murdoch, 2009**), suggesting the possible role of nesfatin-1 in the regulation of insulin secretion (**Gonzalez et al., 2009**). On the light of the fact that some of the brain-gut peptides, including glucagon-like peptide-1 (GLP-1), and ghrelin, regulate both the brain and pancreatic islet functions, it seems that nesfatin-1 may have a role in the regulation of insulin release (**Meier et al., 2002 and Yada et al., 2008**). Moreover, **Stengel et al. (2009b)** and **Nakata et al. (2011)** proved that nesfatin-1 enhances glucose induced insulin secretion by promoting Ca influx through L-type channels in mouse islets beta-cells.

Therefore, it could be concluded that there was a negative relationship between insulin and nesfatin-1 levels.

The second possible explanation for lowering nesfatin-1 in obese PCO was the increase in blood glucose level. The present study found that there was a significant increase in blood glucose level in obese POC group when compared to lean POC group and healthy control group, and there was also a negative correlation between nesfatin-1 and fasting blood glucose in obese PCO group.

These results were in agreement with other correlation between nesfatin-1 concentrations and fasting blood glucose level in the study of **Tsuchiya et al. (2010)** and **Deniz et al. (2012)**.

However, other investigators did not find any correlation between those parameters (**Ademoglu et al., 2014 and Alp et al., 2015**). Those differences might be because of the variations in the study design and species difference.

Su et al. (2010) showed that iv injections of nesfatin-1 in rats significantly reduced the elevated blood glucose levels, producing an antihyperglycemic effect that arose through peripheral action, which was dose-time- and insulin-dependent. Therefore, it could be concluded that statistically higher glucose levels found in PCOS may also have an inhibitory action on serum nesfatin-1 level indirectly through hyperinsulinemia (**Deniz et al., 2012**).

Another reason for lowering nesfatin-1 level in obese PCOS may be the increase in the BMI in these cases. In this study, it was found that there was significant increase in BMI in obese PCO, when

compared to lean PCO, and control groups, while there was an insignificant change in BMI between lean PCO and control groups. In addition, there was significant negative correlation between nesfatin-1 level and BMI in obese PCO.

These results were potentiated by the previous study of **Tsuchiya et al. (2010)** who proved that fasting nesfatin-1 concentrations were significantly lower in subjects with high BMI. Similarly, the studies of **Deniz et al. (2012)** and **Ademoglu et al. (2014)** proved a negative correlation between nesfatin-1 level and BMI in obese PCO.

Administration of nesfatin-1 (intravenous, subcutaneous, intraperitoneal, intracerebrovascular and intranasal) inhibits short and long-term food intake in a dose - and time -dependent manner, resulting in a reduction in body weight (**Shimizu et al., 2009 and Shannon & Wang 2012**). Moreover, **Li et al. (2010)** showed that fasting serum nesfatin-1 was significantly lower in T2DM patients compared to healthy subjects. This reduction in nesfatin-1 may be due to one of the appetite related hormones involved in diabetic hyperphagia.

However, some studies revealed that nesfatin-1 levels showed positive correlation with BMI (**Ari et al., 2011 and Liu et al., 2014**), even though some reports found no correlation (**Aslan et al., 2012**). Recently, **Alp et al. (2015)** found that although BMI was not differing in between groups, waist hip ratio was higher in the patient group than in the control cases. This discrepancy may be attributed to the differences in species, and the study design.

PCOS patients suffer particularly from android type obesity (distribution of fat tissue is accompanied by glucose intolerance, hyperinsulinemia, DM and increase in androgen production) (**Guzick et al., 1994 and Norman et al., 2002**). In this study, it was found that there was a significant increase in serum testosterone level in both lean and obese polycystic ovary, when compared to control group. Moreover, there was a significant increase in serum testosterone level in obese polycystic ovary, when compared to lean polycystic ovary group.

This further hyperandrogenemia in obese POC group could be explained by hyperinsulinemia, as some investigators reported that insulin causes increasing androgen synthesis through its action on ovarian theca cells via an insulin-like growth factor (IGF-1). This is one of the critical mechanism that contributing to the development of PCOS (**Nahum et al., 1995 and Nelson et al., 2001**).

In spite of the peripheral IR in PCOS, insulin produces ovarian effects suggesting that insulin could acts through other receptors or via secondary precursors (**Deniz et al., 2012**).

Some investigators have shown that nesfatin-1 and its binding sites were detected in the theca cells and interstitial cells around follicle, suggesting that nesfatin-1 may play a role as a local regulator of energy homeostasis and steroidogenesis in the ovary via paracrine and autocrine signaling (**Kim et al., 2010 and Kim et al., 2011a &b**).

Gonzalez et al. (2012) demonstrated the expression of nesfatin-1 protein in the ovary and hypothalamus, suggesting the

regulatory effects of nesfatin-1 on the hypothalamo-pituitary-ovarian axis.

The present study found that there was a significant decrease in serum LH level in both lean and obese polycystic ovary, when compared to control group, while there was a significant decrease in serum LH level in obese polycystic ovary, when compared to lean polycystic ovary group. In addition, there was a positive correlation between serum LH level and nesfatin-1 level in obese POC group.

These results were in agreement with **García-Galiano et al. (2010a)** who showed that NUCB2 mRNA and protein levels increase in the hypothalamus during the pubertal transition of female rats. In addition, intracerebroventricular injections of nesfatin-1 induced significant elevation in circulating LH levels. Furthermore, central injections of anti-NUCB2 morpholino oligonucleotides reduced circulating LH levels in pubertal female rats. However, **Gonzalez et al. (2012)** found that nesfatin-1 has an opposite effect in goldfish LH secretion. This difference could be explained by that nesfatin-1 has species-specific effects on LH secretion. The mechanism by which nesfatin-1 alters LH synthesis and secretion remains unclear. However, it is possible to occur through the down regulation of GnRH and /or by a direct action in the pituitary.

Therefore, it could be concluded that Low nesfatin-1 levels in cases with PCOS may be involved in the pathogenesis of this syndrome through direct local effect on the ovary and/or affecting the hypothalamic-pituitary-ovarian axis. However, these hypotheses need further investigations to support them.

CONCLUSION

Serum nesfatin-1 level was lower in obese polycystic ovary group, which was having hyperinsulinemia, hyperglycemia, insulin resistance, and high BMI, when compared to lean polycystic and control groups. In lean polycystic group, serum nesfatin-1 level did not significantly change, when compared to control group due to absence of significant changes in serum insulin, glucose levels, insulin sensitivity, and body weight changes.

Serum Nesfatin 1 level seemed to be related to several metabolic syndrome parameters rather than to polycystic ovary syndrome. Accordingly, it can be hypothesized that the changes of serum nesfatin-1 level in polycystic ovary syndrome may be an additional mechanism by which hyperinsulinemia induces hyperandrogenism, which leads to polycystic ovary syndrome. However, nesfatin-1 level is not likely to be the only determinant factor in polycystic ovary syndrome.

Our findings raised a possibility that nesfatin-1 level plays some role in PCOS. Therefore, larger scale and more detailed molecular studies in vivo and in vitro on ovarian function are needed.

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مستويات النسفاتين -1 في مصل دم الجرذان البيضاء النحيلة والسمنية المحدث بها تجريبيا تكيس المبيض

نوال خليل جرجس - عبير البيومي خليفة

قسم الفسيولوجي- كلية الطب - جامعة الزقازيق

خلفية البحث: متلازمة المبيض المتعدد التكيسات مشكلة شائعة جدا في سن الإنجاب. وتلعب البدانة ومقاومة الإنسولين أدوارا حاسمة في المسببات لهذه المتلازمة، إلا أن الدراسات الخاصة بعلاقة مستويات هرمون النسفاتين -1 ومتلازمة تكيس المبايض محدودة و مثيرة للجدل لتضارب نتائجها.

الهدف من البحث: تقييم التغييرات المحتملة في مستويات النسفاتين-1 بمصل دم الجرذان المصابة بمتلازمة تكيس المبايض التي يسببها الليتروزول سواء العجاف منها أو السمنية، وإرتباطه مع بعض الدلالات الهرمونية والأيضية.

مواد وطرق البحث: أجريت الدراسة علي إثنين و أربعين من الجرذان السليمة البكر الشابات البيضاء. تم تقسيم الجرذان إلى ثلاث مجموعات متساوية: المجموعة الأولى (الضابطة)، والمجموعة الثانية (نحيلة محدث بها متلازمة المبيض المتعدد التكيسات) : الجرذان التي تغذت علي حمية القوارض المعتادة لمدة 9 أسابيع، وبعد ذلك تلقت جرعة واحدة يوميا من الليتروزول بالفم بإستخدام أنبوبة معديه (0.5 ملغم / كغم من وزن الجسم) لمدة 21 يوما، والمجموعة الثالثة (سمنية محدث بها متلازمة المبيض المتعدد التكيسات). وقد تغذت الجرذان بحمية غنية بالدهون لمدة 9 أسابيع، وبعد ذلك تلقي جرعة واحدة يوميا من الليتروزول (0.5 ملغ / كغم) عن طريق الفم باستخدام أنبوبة معديه لمدة 21 يوما متتالية. وفي نهاية التجربة تم قياس مستويات النسفاتين-1 و هرمون التستوستيرون الحر، والهرمون المحفز لجريبات المبيض، وهرمون اللتونة، وهرموني الاستراديول والبروجسترون، ومستويات السكر والإنسولين في مصل الدم. كما تم حساب مؤشر كتلة الجسم ومعامل المقاومة للإنسولين. وقد تم الفحص المجهرى لأنسجة المبيض.

النتائج: كان مستوى النسفاتين-1 بمصل الدم في مجموعة المبيض المتعدد التكيسات مع السمنة المفرطة منخفضاً وله دلالة إحصائية ويرافقه إرتفاع له دلالة إحصائية في الجلوكوز بمصل الدم وكذلك مقاومة الإنسولين، وإرتفاع بمؤشر كتلة الجسم بالمقارنة مع كل من المجموعة الضابطة و مجموعة الجرذان النحيلة المحدث بها تكيس المبايض. وعلاوة على ذلك، فقد كان لمستويات النسفاتين -1 إرتباطاً سلبياً له دلالة إحصائية مع مستويات الإنسولين، ومستويات الجلوكوز ودلالة المقاومة للإنسولين، ومؤشر كتلة الجسم، ولكن ثبت له إرتباطاً طردياً مع مستويات هرمون اللتونة.

ولم يحدث أي تغير له دلالة إحصائية في مستوى النسفاتين-1 في مجموعة الجرذان النحيلة المصابة بتكيس المبايض عندما قورنت بمثيلاتها في المجموعة الضابطة و المجموعة السمنية. وكذلك لم يحدث تغيير في أي من معدل كتلة الجسم أو مستوي كل من الجلوكوز أو الإنسولين أو دلالة المقاومة للإنسولين.

الإستنتاج: أن لمستوى النسفاتين-1 بمصل الدم صلة بدلالات متلازمة التمثيل الغذائي وليس بمتلازمة المبيض المتعدد التكيسات مباشرة. وهناك إحتمال أن يلعب هذا البيئد دوراً في متلازمة تكيس المبايض. لذلك، فهناك حاجة أكبر لإجراء العديد من الدراسات الجزيئية الأكثر تفصيلا داخل و خارج الجسم على مرضي تكيس المبايض.