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Serum Phoenixin-14 levels of women with polycystic ovary syndrome increase proportionally with BMI

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Abstract. – OBJECTIVE: This study aimed to compare serum PNX-14 values of women with PCOS classified as lean or overweight according to the BMI values.

PATIENTS AND METHODS: Fifty lean or overweight women diagnosed with PCOS according to the revised Rotterdam criteria were included in the study. They were divided into two groups according to their BMI values. Thirty patients with BMI values of 18.5-24.9 kg/m^2 constituted the normal-weight PCOS group. Twenty patients with BMI values of 25-29.9 kg/m^2 formed the overweight PCOS group. Thirty patients with regular menstrual cycles who did not exhibit clinical and laboratory findings of PCOS were selected as the control group. The patients in the control group were also divided into two different groups as normal weight (n=17) and overweight (n=13). In anovulatory PCOS group, blood samples were collected on the third day of progesterone withdrawal bleeding. Both in ovulatory PCOS and control groups, blood samples were collected on the third day of spontaneous menstrual cycle. In addition to basal hormonal parameters, serum Phoenixin-14 concentrations were measured by enzyme-linked immunosorbent assay.

RESULTS: LH values of the overweight or lean PCOS group were significantly higher than the overweight or lean non-PCOS group (p<0.01). The LH/FSH ratios of the lean and obese PCOS groups were significantly higher than the non-PCOS control group (p<0.01). Testosterone levels of both lean and obese PCOS groups were significantly higher than non-PCOS groups (p<0.02). The HOMA-IR value of the obese PCOS group was significantly higher than the lean PCOS group (p<0.03). The HOMA-IR values of the patients in the PCOS group were significantly higher than the non-PCOS controls. Phoenixin-14 levels of the obese PCOS group were approximately three times higher than the lean PCOS group (p<0.01). Phoenixin-14 levels of the obese non-PCOS group were also three times higher than the lean non-PCOS group (p<0.01). Serum Phoenixin-14 levels of patients in the lean PCOS group were significantly higher than in the lean non-PCOS group (9.11±2.09 pg/mL *vs.* 2.04±0.11 pg/mL, p<0.01). Serum Phoenixin-14 levels of the patients in the obese PCOS group were significantly higher than in the obese non-PCOS group (27.4±3.04 pg/mL *vs.* 6.44±1.09 pg/mL, p<0.01). A positive and significant correlation was found between serum PNX-14 levels and BMI, HOMA-IR, LH, and testosterone levels in both lean and obese PCOS patients.

CONCLUSIONS: This study showed for the first time that serum PNX-14 levels are significantly increased in lean and obese PCOS patients. The increase in PNX-14 showed a proportional trend with BMI levels. Serum PNX-14 levels were correlated positively with serum LH, testosterone, and HOMA-IR.

Key Words:
PCOS, Lean, Overweight, Phoenixin-14, Testosterone, LH, Insulin.

Introduction

Phoenixin (PNX) is a new neuropeptide formed by cleavage from small integral membrane protein 20 known as C4orf52 and it is involved in the biogenesis of mitochondrial cytochrome c oxidase. PNX isoforms (PNX 14 and PNX 20) containing 14 and 20 amino acids are the most common amide peptides[1,2]. Amidation makes the peptide active, while nonamidated forms are inactive. PNX-14 and 20 are two separate forms with similar biological mechanisms of action and receptors[2]. Although the amino acid sequences are very similar in animals and humans, the amino acid sequence of PNX-20 may differ in humans and rodents[1]. Although PNX is primarily a central neuropeptide, adipose cells and pancreatic cells can also secrete PNX[3]. The hypothalamus is the region where PNX release is most intense in the central nervous system[3]. Hypothalamus se-

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cretes PNX into small capillaries, allowing it to be mixed into the systemic circulation[4].

The ovaries are reproductive tissues in which PNX is intensely secreted[5]. Ovarian tissue and follicles are reproductive structures in which PNX is intensely secreted[5]. PNX acts by attaching to the G protein-coupled receptor 173 in the brain and peripheral tissues. These receptors are intensely expressed in gonads and granulosa cells[3-5]. PNX-20 is more active in the brain and PNX-14 is more active in peripheral tissues. PNX-20 increases both GnRh and GnRH stimulated LH secretion. FSH and testosterone secretion is also stimulated by PNX[6]. In summary, PNX is a noropeptide that has a role in hypothalamic pituitary gonadal axis regulation and follicular development[5-7]. Since polycystic ovary syndrome is an endocrine disease characterized by hypothalamo-pituitary ovarian axis defect[8-10], serum PNX-14 levels may be affected by the presence of this syndrome.

Really peripheral and central peptide synthesis and secretion are significantly impaired in women with polycystic ovary syndrome (PCOS). The synthesis and release of many adipokines, central and peripheral peptides are impaired in PCOS[8-10]. Combined deficiency or overexpression of more than one peptide is one of the etiological causes of PCOS. The interaction between natriuretic peptide precursor type C natriuretic peptide receptor 2 is critical for follicle development[8-10]. The relationship between PNX levels and PCOS has not been evaluated except in a few isolated studies[7,11]. It has been reported that serum PNX levels of PCOS patients are higher than healthy controls. Serum PNX levels also correlated positively with serum LH, testosterone, and progesterone levels[11]. There is only one clinical study reporting a correlation between BMI and PNX values in women[11]. However, there is no study on how BMI values affect serum PNX values in PCOS patients. This study was designed to compare serum PNX-14 values of PCOS patients classified as lean or overweight according to their BMI values. Lean or overweight non-PCOS healthy patients were taken as the control group. Thus, it will be revealed whether circulating PNX levels are affected by BMI or PCOS.

Patients and Methods

Fifty lean and overweight patients diagnosed with PCOS according to the revised Rotterdam criteria were included in the study. Presence of at least two of the following criteria was accepted as PCOS: (i) ovulatory dysfunction, (ii) clinically or laboratory-confirmed hyperandrogenemia, (iii) detection of 12 or more follicles between 2 and 9 mm on ultrasound examination or an ovarian volume greater than 10 mL. All participants with the diagnosis of PCOS were divided into two groups according to their BMI values. BMI was determined according to the World Health Organization (WHO) guidelines as the ratio of patient weight to the square of patient height (kg/m^2). Thirty patients with BMI values of 18.5-24.9 kg/m2 constituted the normal weight PCOS group. Tweenty patients with BMI values of 25-29.9 kg/m2 formed the overweight PCOS group. Thirty patients with regular menstrual cycles who did not exhibit clinical and laboratory findings of PCOS were selected as the control group. The patients in the control group were in turn divided into two different groups as normal weight (n=17) and overweight (n=13). Thus, each patient in the PCOS group was matched with the control group in terms of BMI and age. Local ethics committee approval and patient consent were obtained before starting the study (Kayseri City Hospital 2023/805).

Patients in both the control and PCOS groups were invited to collect blood samples on the third day of the follicular phase. Blood samples were collected on the third day of the cycle from women who underwent progesterone withdrawal bleeding due to ovulatory dysfunction. In ovulatory PCOS patients, blood samples were collected on the third day of spontaneous menstrual cycle. Blood samples were collected the morning following an overnight fast. Because the patients presented at different times, the blood sample from each patient was centrifuged and serum aliquots were aliquots stored at -20°C until analysis. When the target number of patients was reached, frozen samples were thawed and luteinizing hormone (LH), follicle stimulating hormone (FSH), testosterone, estrogen, glucose, and insulin levels were measured, and insulin resistance values were calculated (HOMA-IR). Patients with endocrine diseases such as diabetes, thyroid, and adrenal glands, and those using insulin, cholesterol lowering, or other hormonal drugs for the last 6 months were not included in the study. Those with chronic systemic inflammatory diseases other than PCOS, those who had ovarian drilling, those who were found to have adrenal or pituitary pathology were not included in the study.

Phoenixin-14 Measurement With ELISA

Phoenixin-14 concentrations in serum was measured by enzyme-linked immunosorbent assay (ELISA) using Human PNX-14 (Phoenixin-14) ELISA Kit (Wuhan Fine Biotech Co., Ltd., Wuhan, Hubei, China). This kit is used to measure the Phoenixin-14 concentration in serum, plasma, tissue homogenates and other biological fluids. The detection range of the Phoenixin-14 kit (assay range) was 1.563-100 pg/ml and the minimum measurable level (sensitivity) was < 0.938 pg/ml. The intra- and inter-assay coefficients of variation were <8% and <10%, respectively.

The primary objectives of our study can be listed as follows:

(i) To compare serum Phoenixin-14 levels of lean and overweight PCOS patients.
(ii) To compare serum Phoenixin-14 levels of patients in the lean and overweight PCOS and the lean and overweight non-PCOS control group.
(iii) To reveal the possible correlation between serum Phoenixin-14 levels, demographic, and metabolic parameters.

Statistical Analysis

Data were analyzed with the use of the Statistical Package for Social Sciences software 21.0 for Windows package software (IBM, Armonk. NY, USA). Normality of data was examined by the use of the Shapiro-Wilk test. Continuous variables were analysed using the Mann-Whitney U-test. Pearson's correlation analysis was performed to correlate serum PNX-14 and other demographic and hormonal parameteres. Data were presented as mean ± SD. Differences were considered statistically significant at $p<0.05$.

Results

Table I shows the distribution of demographic and hormonal parameters of all groups. Age and BMI values of the patients in the PCOS and non-PCOS control groups were found to be similar ($p>0.05$). The BMI values of PCOS or non-PCOS patients in the lean or overweight groups were consistent with the BMI of the group in which they were present. The distribution of fertility status of the patients in each group was similar. LH values of the overweight PCOS group were higher than the lean PCOS group ($p<0.02$). The LH values of the patients in the lean PCOS group were significantly higher than the lean non-PCOS group ($p<0.01$). LH values of the overweight PCOS group were significantly higher than the overweight non-PCOS group ($p<0.01$). There was no significant difference between and within the groups in terms of FSH values. LH/FSH ratios were similar in lean and overweight PCOS groups. The LH/FSH ratios of the lean and obese PCOS groups were significantly higher than the non-PCOS control group ($p<0.01$). The testosterone levels of the lean and obese PCOS groups were similar. Testosterone levels of both lean and obese PCOS groups

Table I. Comparison of demographic and hormonal findings of lean and obese patients in PCOS and non-PCOS groups.

	Lean PCOS	Overweight PCOS	Lean non-PCOS	Overweight non-PCOS
N	30	20	17	13
Age (y)	25.7±3.20	26.8±4.01	26.0±2.40	27.7±5.11
BMI (Kg/m^2)	22.4±3.04*	27.6±4.02	23.1±3.06$^{\$}$	28.0±6.10
Fertility status n (fertil/infertile)	14/16	12/8	9/8	7/6
Phoenixin-14 (pg/mL)	9.11±2.09*	27.4±3.04	2.04±0.11$^{\$}$	6.44±1.09
LH (mIU/mL)	10.2±3.08*	12.9±1.01	6.03±2.06	7.13±3.04
FSH (mIU/mL)	5.20±1.20	5.42±2.04	5.29±2.09	5.12±3.05
LH/FSH ratio	1.92	2.2	1.15	1.56
Testosterone (ng/dL)	47.5±8.01*	51.1±9.22	33.2±6.09$^{\$}$	40.6±8.30
Estradiol (pg/mL)	49.7±6.04	51.3±7.01	44.5±5.09	48.1±6.44
Insulin resistance (HOMA index)	7.11±1.20*	11.2±2.09	4.22±1.08$^{\$}$	7.92±2.04
Glucose (mg/dL)	89.3±9.22*	113.4±11.4	83.7±6.7$^{\$}$	110.2±10.3

*Shows statistical signicance between normal weight PCOS *vs.* obese PCOS. $^{\$}$Shows statistical significant between normal weight non-PCOS *vs.* obese non-PCOS. $p<0.05$ shows significant difference. Data are presented as Mean±SD.

were significantly higher than non-PCOS groups (p<0.02). Serum estradiol levels were similar within and between groups. The HOMA-IR value of the obese PCOS group was significantly higher than the lean PCOS group (p<0.03). The HOMA-IR values of the patients in the PCOS group were significantly higher than the non-PCOS controls. The blood glucose values of the obese PCOS group were found to be significantly higher than the lean PCOS group (p<0.02). Blood sugar values were similar between the groups.

Phoenixin-14 levels of the obese PCOS group were approximately three times higher than the lean PCOS group (p<0.01). Phoenixin-14 levels of the obese non-PCOS group were also three times higher than the lean non-PCOS group (p<0.01). Serum Phoenixin-14 levels of patients in the lean PCOS group were significantly higher than in the lean non-PCOS group (9.11±2.09 pg/mL *vs.* 2.04±0.11 pg/mL, p<0.01). Serum Phoenixin-14 levels of the patients in the obese PCOS group were significantly higher than in the obes non-PCOS group (27.4±3.04 pg/mL *vs.* 6.44±1.09 pg/mL, p<0.01). A positive and significant correlation was found between serum PNX-14 levels and BMI, HOMA-IR, LH, and testosterone levels in both lean and obese PCOS patients (Table II). In the non-PCOS control group, no correlation was found between PNX-14 levels and other parameters.

Discussion

PCOS is one of the most common endocrinopathies detected in women of reproductive age. It is a spectrum of diseases that give clinical manifestations with different phenotypic features rather than a single clinical picture[12]. Promising new drugs have come into use in the medical treatment of PCOS. The short-term data of myoinositol treatment in PCOS are encouraging[13]. It has also been reported that the combination of myoinositol and melatonin provides improvement in glucose and thyroid functions[14]. Similarly, in anovulatory patients treated with d-chiro-inositol, ovulation was restored in two patients[15]. Low-dose vitamin D administration has also been recommended for improvement in metabolic parameters and luteal support in PCOS patients[16]. In the last decade, the view that PCOS-related changes occur as a result of defects in the synthesis and release of central or peripheral peptides is gaining weight[8-12]. PCOS, characterized by varying degrees of disruption in the function of dozens of peptides rather than a single peptide, leads to disruption of the pulsatile pattern of central LH release[9]. Since the defect in LH secretion impairs the endocrine functions of the ovaries, ovulatory dysfunction or androgen hypersecretion may occur. Since the chronic inflammatory background and hyperandrogenemia impair insulin hemostasis, it causes an increase in lipogenesis and a decrease in lipolysis[10-12]. Our study is important in terms of presenting the first clinical data investigating serum PNX-14 levels in lean and obese PCOS patients. Serum PNX-14 levels of both lean and obese PCOS patients were found to be significantly higher than healthy controls without PCOS. We also found a positive and significant correlation between PNX-14 levels and serum LH. PNX is known to increase GnRH-mediated LH secretion[1]. The fact that PNX injection into the rat brain ventricles resulted in an increase in LH release is an important proof that PNX has a positive modulatory effect on LH release[17].

PNX exerts its hypothalamic and pituitary effects mostly *via* GPR 173 receptors and cAMP/PKA pathway[18]. However, the mechanism by which PNX increases serum FSH and testosterone levels is not fully known. In the current study, no significant correlation was found be-

Table II. Correlation analysis of serum PNX-14 levels and demographic and hormonal parameters of lean and obese PCOS patients.

	Lean PCOS		Overweight PCOS	
	Serum Phoenixin-14			
	r	p	r	p
BMI	0.677	0.04	0.54	0.02
HOMA-IR	0.65	0.03	0.69	0.02
Glucose	0.45	0.33	0.31	0.32
LH	0.66	0.01	0.73	0.01
FSH	0.12	0.40	0.36	0.60
Testosterone	0.59	0.03	0.81	0.01

tween PNX-14 and serum FSH levels in neither lean nor obese PCOS group. However, a positive and significant correlation was found between serum testosterone levels and PNX-14. In addition, the increase in PNX-14 levels showed a positive and significant correlation with serum LH levels. The PNX-testosterone relationship is more pronounced in obese women with PCOS than in lean women with PCOS. In the light of these findings, we can state that serum PNX-14 levels increase proportionally with BMI in the presence of PCOS. There was a 3-fold increase in PNX levels in the obese PCOS group compared to the lean group (9.11±2.09 pg/mL *vs.* 27.4±3.04 pg/mL). In obese non-PCOS patients, PNX-14 levels increased nearly three times compared to the lean non-PCOS group (2.04±0.11 pg/mL *vs.* 6.44±1.09 pg/m/L). However, PNX increases in non-PCOS groups were significantly lower than in PCOS patients. In the light of these findings, we can make the following comments; (i) PCOS significantly increases serum PNX-14 levels compared to healthy controls, (ii) serum PNX increase is potentiated in obese PCOS patients, (iii) obesity increases serum PNX-14 levels nearly three times in the presence of PCOS, (iv) serum PNX values of non-PCOS lean patients are within normal limits, (v) obese women have an increase in serum PNX-14 levels even without a diagnosis of PCOS, (vi) however, PNX-14 increase in non-PCOS obese individuals is approximately one-fifth of obese women with PCOS (6.44±1.09 pg/m/L *vs.* 27.4±3.04 pg/mL).

Since there are not enough clinical studies, it is not possible to make a clear comment about the relationship between BMI and PNX-14. Synthesis and secretion of many peripheral and central peptides are impaired in obese PCOS patients[9,10]. Adipokines are involved in lipogenesis as well as in the regulation of communication between adipose tissue and endocrine and reproductive organs[12]. Since PNX is secreted in both central and adipose tissue, its synthesis and release may change in the presence of PCOS. Ullah et al[11] showed that BMI and serum PNX levels were correlated in women with PCOS. It has also been shown that both mature adipocytes and preadipocytes contain a large amount of the PNX receptor GPR173[2,3]. By binding to these receptors, PNX-14 transforms preadipocytes into mature adipocytes and increases the formation of white adipose tissue[2,3]. Consistent with the above, we found a positive correlation between serum PNX-14 levels and BMI in both the lean and obese PCOS groups. However, the positive correlation between PNX and BMI was more pronounced in the lean group than in the obese group. The increase in the presence of mature adipocytes due to obesity may cause desensitization of PNX-14 secreting cells and PNX receptors. Obese and lean PCOS patients may have extensive expression of the PNX receptor *GPR173* mRNA in their white preadipocytes. PNX may contribute to obesity due to PCOS through the hypertrophy and hyperplasia it creates in adipose tissue[2,19]. The fact that the PNX-BMI correlation strengths of obese cases and lean PCOS cases are different from each other is a topic worth investigating.

Since PNX is expressed in both alpha and beta cells of the pancreas, it may have a role in insulin and glucose hemostasis[20,21]. PNX stimulates glucose-mediated insulin release *via* the cAMP/Epac pathway in pancreatic cells[20]. We found a positive correlation between serum PNX-14 levels and HOMA-IR in both lean and obese PCOS groups. The possible reason why the increase in PNX-14 potentiates insulin resistance may be that PNX transforms preadipocytes into mature adipocytes[2,3]. Increasing adipocytes may stimulate insulin resistance by stimulating adipokine secretion[12]. In summary, although PNX positively regulates insulin secretion, insulin resistance may occur due to stimulation of lipogenesis. The emergence of insulin resistance in both lean and obese PCOS patients suggests that PNX induces insulin resistance independent of adiposity. Increasing androgen levels may also have induced insulin resistance independently of PNX. Impaired PNX synthesis in the presence of obesity may also be responsible for insulin resistance[22].

Conclusions

In addition to the above metabolic and hormonal effects, PNX-14 positively regulates the synthesis of reactive oxygen derivatives and anti-inflammatory cytokines[15]. However, we did not study changes in serum levels of ROS or anti-inflammatory cytokines. Since PNX-14 works in coordination with other peptides such as kisspeptin and nesfatin for its central and peripheral effects, determining the levels of these peptides is important to reveal the actual functions of PNX. Despite all these limitations, it has been shown for the first time that serum PNX-14 levels are significantly increased in lean and obese PCOS patients. The increase in PNX-14 shows a proportional trend with BMI levels. The increase in

PNX-14 also correlates positively with serum LH, testosterone, and HOMA-IR.

Conflict of Interst
All authors have nothing to disclose.

Ethics Approval
The authors declare that all procedures contributing to this work comply with the ethical standards of the relevant national and institutional committees on human experimentation and with the Helsinki Declaration of 1975, as revised in 2008. The Ethical Committee of the Kayseri City Hospital approved the study protocol (2023/805).

Authors' Contributions
All authors contributed to the study conception and design. Material preparation was performed by Cevat Rifat Cundubey and Seyma Daglituncezdi Cam. Data were collected by Cevat Rifat Cundubey and Seyma Daglituncezdi Cam. All authors contribıted to statistical analysis. The first draft was written by Cevat Rifat Cundubey. All authors approved the final version of the manuscript.

Informed Consent
Informed consents were obtained from all participants.

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References

1) Yosten GL, Lyu RM, Hsueh AJ, Avsian-Kretchmer O, Chang JK, Tullock CW, Dun SL, Dun N, Samson WK. A novel reproductive peptide, phoenixin. J Neuroendocrinol 2013; 25: 206-215.
2) Billert M, Rak A, Nowak KW, Skrzypski M. Phoenixin: More than Reproductive Peptide. Int J Mol Sci 2020; 21: 8378.
3) Billert M, Wojciechowicz T, Jasaszwili M, Szczepankiewicz D, Waśko J, Kaźmierczak S, Strowski MZ, Nowak KW, Skrzypski M. Phoenixin-14 stimulates differentiation of 3T3-L1 preadipocytes via cAMP/Epac-dependent mechanism. Biochim Biophys Acta Mol Cell Biol Lipidss 2018; 1863: 1449-1457.
4) Pałasz A, Rojczyk E, Bogus K, Worthington JJ, Wiaderkiewicz R. The novel neuropeptide phoenixin is highly co-expressed with nesfatin-1 in the rat hypothalamus, an immunohistochemical study. Neurosci Lett 2015; 592: 17-21.
5) Nguyen XP, Nakamura T, Osuka S, Bayasula B, Nakanishi N, Kasahara Y, Muraoka A, Hayashi S, Nagai T, Murase T, Goto M, Iwase A, Kikkawa F. Effect of the neuropeptide phoenixin and its receptor GPR173 during folliculogenesis. Reproduction 2019; 158: 25-34.
6) Guvenc G, Altinbas B, Kasikci E, Ozyurt E, Bas A, Udum D, Niaz N, Yalcin M. Contingent role of phoenixin and nesfatin-1 on secretions of the male reproductive hormones. Andrologia 2019; 51: e13410.
7) Kalamon N, Błaszczyk K, Szlaga A, Billert M, Skrzypski M, Pawlicki P, Górowska-Wójtowicz E, Kotula-Balak M, Błasiak A, Rak A. Levels of the neuropeptide phoenixin-14 and its receptor GRP173 in the hypothalamus, ovary and perioovarian adipose tissue in rat model of polycystic ovary syndrome. Biochem Biophys Res Commun 2020; 528: 628-635.
8) Celik O, Celik N, Ugur K, Hatirnaz S, Celik S, Muderris II, Yavuzkir S, Sahin İ, Yardim M, Aydin S. Nppc/Npr2/cGMP signaling cascade maintains oocyte developmental capacity. Cell Mol Biol (Noisy-le-grand) 2019; 65: 83-89.
9) Celik O, Aydin S, Celik N, Yilmaz M. Peptides: Basic determinants of reproductive functions. Peptides 2015; 72: 34-43.
10) Celik O, Aydin S, Celik N, Ugur K, Yavuzkir S, Hatirnaz S, Yardim M, Celik S. Molecular role of peptides/proteins in subfertility of polycystic ovarian syndrome. Cell Mol Biol (Noisy-le-grand) 2019; 65: 32-40.
11) Ullah K, Ur Rahman T, Wu DD, Lin XH, Liu Y, Guo XY, Leung PCK, Zhang RJ, Huang HF, Sheng JZ. Phoenixin-14 concentrations are increased in association with luteinizing hormone and nesfatin-1 concentrations in women with polycystic ovary syndrome. Clin Chim Acta 2017; 471: 243-247.
12) Celik N, Aydin S, Ugur K, Yardim M, Acet M, Yavuzkir S, Sahin İ, Celik O. Patatin-like phospholipase domain containing 3-gene (adiponutrin), preptin, kisspeptin and amylin regulates oocyte developmental capacity in PCOS. Cell Mol Biol (Noisy-le-grand) 2018; 64: 7-12.
13) Gullo G, Carlomagno G, Unfer V, D'Anna R. Myo-inositol: from induction of ovulation to menopausal disorder management. Minerva Ginecol 2015; 67: 485-486.
14) D'Anna R, Santamaria A, Giorgianni G, Vaiarelli A, Gullo G, Di Bari F, Benvenga S. Myo-inositol and melatonin in the menopausal transition. Gynecol Endocrinol 2017; 33: 279-282.
15) Bezerra Espinola MS, Laganà AS, Bilotta G, Gullo G, Aragona C, Unfer V. D-chiro-inositol Induces Ovulation in Non-Polycystic Ovary Syndrome

(PCOS), Non-Insulin-Resistant Young Women, Likely by Modulating Aromatase Expression: A Report of 2 Cases. Am J Case Rep 2021; 22: e932722.
16) Menichini D, Forte G, Orrù B, Gullo G, Unfer V, Facchinetti F. The role of vitamin D in metabolic and reproductive disturbances of polycystic ovary syndrome: A narrative mini review. Int J Vitam Nutr Res 2022; 92: 126-133.
17) Stein LM, Tullock CW, Mathews SK, Garcia-Galiano D, Elias CF, Samson WK, Yosten GLC, Am J Physiol Regul Integr Comp Physiol 2016; 311: 489-496.
18) 18. Treen AK, Luo V, Belsham DD. Phoenixin Activates Immortalized GnRH and Kisspeptin Neurons Through the Novel Receptor GPR173. Mol Endocrinol 2016; 30: 872-888.
19) Muir LA, Neeley CK, Meyer KA, Baker NA, Brosius AM, Washabaugh AR, Varban OA, Finks JF, Zamarron BF, Flesher CG, Chang JS, DelProposto JB, Geletka L, Martinez-Santibanez G, Kaciroti N, Lumeng CN, O'Rourke RW. Adipose tissue fibrosis, hypertrophy, and hyperplasia: Correlations with diabetes in human obesity. Obesity (Silver Spring) 2016; 24: 597-605.
20) Billert M, Kołodziejski PA, Strowski MZ, Nowak KW, Skrzypski M. Phoenixin-14 stimulates proliferation and insulin secretion in insulin producing INS-1E cells. Biochim Biophys Acta Mol Cell Res 2019; 1866: 118533.
21) Prinz P, Scharner S, Friedrich T, Schalla M, Goebel-Stengel M, Rose M, Stengel A. Central and peripheral expression sites of phoenixin-14 immunoreactivity in rats. Biochem Biophys Res Commun 2017; 493: 195-201.
22) Rocca C, Scavello F, Granieri MC, Pasqua T, Amodio N, Imbrogno S, Gattuso A, Mazza R, Cerra MC, Angelone T. Phoenixin-14: detection and novel physiological implications in cardiac modulation and cardioprotection. Cell Mol Life Sci 2018; 75: 743-756.