Open Access

Case Study

PRNANO

Precis. Nanomed. 2025, 8(2): 1501-1510

ANDOVER HOUSE

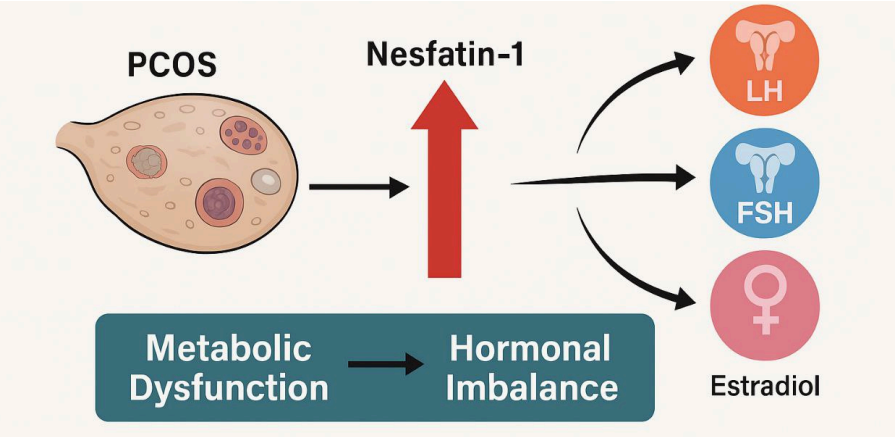
Elevated Nesfatin-1 and Altered Reproductive Hormones in Polycystic Ovary Syndrome: A Comparative Case-Control Study.

Zainab Ghazi Mohammed[1*], Hassan Ali Farman[2]

[1,2]*Department of Medical Laboratory Techniques, College of Health and Medical Techniques/ Kufa, Al-Furat Al-Awsat Technical University, 31003 Al-Kufa, Iraq*

Submitted: March 12, 2025 Accepted: April 11, 2025 Published: April 30, 2025

Graphical Abstract



Abstract

A case-control study was conducted on 60 women with polycystic ovary syndrome (PCOS) and 30 healthy controls. Blood samples were collected to measure reproductive hormones and nesfatin-1 levels. Women with PCOS had significantly higher nesfatin-1 levels compared to the controls. It was also found that nesfatin-1 levels positively correlated with testosterone and estradiol concentrations. Further longitudinal and mechanistic studies are needed to clarify Nesfatin-1's role in PCOS pathophysiology and its therapeutic implications

Keywords: Polycystic Ovary Syndrome; Nesfatin-1; Hyperandrogenism; Metabolic Dysfunction; Reproductive Hormones; Biomarker.

Purpose, Rationale, and Limitations

Polycystic ovary syndrome (PCOS) is a common hormonal disorder in women that is often associated with metabolic problems and fertility issues. Nesfatin-1 is a hormone known for regulating appetite and metabolism, but its role in reproductive health remains unclear. This study aimed to examine whether nesfatin-1 is associated with hormonal imbalances in women with PCOS. Existing studies are contradictory; clarifying nesfatin-1's role could aid in future biomarker development. However, a case–control design and lack of mechanistic insight limit causal interpretation.

Introduction

Polycystic ovary syndrome (PCOS) is a prevalent endocrine disorder in women of reproductive age, marked by hyperandrogenism, ovarian dysfunction, and polycystic ovarian morphology [1].

Its clinical manifestations, including menstrual irregularities, hirsutism, and infertility, often coexist with metabolic abnormalities such as insulin resistance, obesity, and dyslipidemia

* Corresponding Author: Zainab Ghazi Mohammed E-mail: zainabgazhi7@gmail.com

[2]. The etiology of PCOS involves dysregulated signaling within the hypothalamic–pituitary–ovarian (HPO) axis and disturbances in numerous metabolic pathways [3]. Reproductive biomarkers, including luteinizing hormone (LH), follicle-stimulating hormone (FSH), and sex steroids (testosterone and estradiol), are pivotal for understanding the pathophysiology of PCOS and are commonly dysregulated in affected individuals [4].

Recent attention has focused on the roles of appetite-regulating peptides in the pathogenesis of PCOS [5]. Nesfatin-1, a peptide derived from the nucleobindin-2 (NUCB2) gene, has been identified as an anorexigenic factor that modulates energy intake and may influence hypothalamic-pituitary-gonadal (HPG) axis activity [6]. The mechanistic underpinnings by which nesfatin-1 may contribute to the pathophysiology of PCOS extend beyond its well-recognized anorexigenic and energy-homeostatic roles [7]. In addition to its central effects on feeding behavior, nesfatin-1 is increasingly recognized as an active modulator of the hypothalamic–pituitary–ovarian (HPO) axis [8]. Several studies indicate that nesfatin-1 is expressed in key brain regions such as the arcuate and paraventricular nuclei, which co-localize with gonadotropin-releasing hormone (GnRH) neurons [9-11]. Through this colocalization, nesfatin-1 is thought to influence GnRH synthesis and secretion, thereby indirectly modulating pituitary release of LH and FSH, which are essential for ovarian follicular development and steroidogenesis. [12, 13]

At the cellular level, nesfatin-1 is proposed to act via a G protein-coupled receptor (with GPCR12 emerging as a potential candidate) that, once activated, triggers intracellular cascades such as the extracellular signal-regulated kinase (ERK1/2) pathway and modulates intracellular calcium levels [14]. These signaling events regulate GnRH neuronal activity and have direct effects on peripheral tissues. For example, in pancreatic beta cells, nesfatin-1 has been shown to enhance glucose-stimulated insulin secretion by promoting calcium influx through voltage-dependent potassium (Kv) channels. This mechanism may contribute to insulin resistance, a common metabolic abnormality in PCOS [10, 15].

Furthermore, emerging evidence suggests that nesfatin-1 directly influences ovarian function by increasing the expression of anti-apoptotic gene bcl-2, stimulating proliferation/differentiation markers, enhancing ovarian steroidogenesis, and promoting glucose uptake and metabolism in the ovary [16]. It appears that through autocrine and paracrine signaling, nesfatin-1 can alter the ovary's local microenvironment, potentially impacting both folliculogenesis and the synthesis of sex steroids such as estradiol and testosterone [16]. Such actions may provide a mechanistic link between the metabolic and reproductive dysfunction [17]. Moreover, regulating nesfatin-1 expression by sex steroids such as estradiol and progesterone further supports its role as a local modulator within reproductive tissues [18].

Several investigations propose that alterations in Nesfatin-1 expression may serve as a link between disordered metabolic status and reproductive dysfunction. Studies examining Nesfatin-1 levels in PCOS have produced variable findings [19]. While Wang et al. [20] reported significantly higher Nesfatin-1 concentrations in women with PCOS, Wang, et al. [21] found no pronounced disparity when compared to healthy controls. These discrepancies have been attributed to differences in study design, ethnic variation, methodological approaches, and the heterogeneity inherent in PCOS phenotypes. For example, a study by Psilopanagioti, et al. [22] indicated that nesfatin-1 levels showed a gradual increase from low-weight individuals to those who were overweight, but decreased significantly in obese (Group IV) and morbidly obese (Group V) individuals, while the study found no clear relationship between nesfatin-1 and body-mass index (BMI), it highlights the complexity of peptide hormone interactions in obesity [22]. The relationship between Nesfatin-1 and PCOS remains contentious, with studies reporting both elevated and comparable levels in affected individuals. For instance, the meta-analysis by Bahreiny, et al. [23] found a significant relationship between PCOS and nesfatin-1 levels, with a pooled standardized mean difference (SMD) of 0.54 (95% CI: 0.00-1.07; $p = 0.04$). Women with PCOS and higher insulin resistance showed even greater nesfatin-1 levels (SMD = 1.46; 95% CI: 0.92-2.00; $p < 0.001$). This suggests that elevated nesfatin-1 concentrations may play a compensatory role in metabolic dysregu-

lation associated with obesity and insulin resistance in women with PCOS. Conversely, Faeza, et al. [7] reported no significant differences after adjusting for BMI, highlighting adiposity as a critical confounder. A recent meta-analysis by Bahreiny, et al. [23] attributed these discrepancies to methodological variability (e.g., enzyme-linked immunosorbent assay [ELISA] kits, fasting status) and phenotypic heterogeneity, underscoring the need for standardized protocols and phenotype stratification in future studies.

Given these gaps, the current study aimed to measure serum nesfatin-1 levels in a well-characterized cohort of women with PCOS and age-/BMI-matched healthy controls while simultaneously examining correlations with reproductive hormones (LH, FSH, testosterone) and metabolic parameters. By adopting strict diagnostic criteria (Rotterdam consensus) and controlling for confounding factors such as BMI and insulin resistance, this work seeks to clarify the conflicting findings reported in prior studies and elucidate the potential dual role of nesfatin-1 as a biomarker linking metabolic and reproductive dysfunction in PCOS.

Materials and Methods

Study Design and Ethical Considerations

This case-control study was conducted at Babel Teaching Hospital for Pediatrics and Gynecology, Imam Sadiq Teaching Hospital, and Iskandaria General Hospital from January 1, 2024, to June 30, 2024. Ethical approval was granted by the Institutional Review Board (Approval Number: 4162), and all participants provided written informed consent before their inclusion in the study. The research protocol was designed and conducted in accordance with the ethical principles outlined in the Declaration of Helsinki [24].

Participant Recruitment and Selection

This study enrolled a total of 60 women diagnosed with PCOS and 30 age-matched healthy controls. The diagnosis of PCOS was established according to the 2003 Rotterdam criteria, requiring the presence of at least two of the following: oligo- or anovulation, clinical or biochemical hyperandrogenism, or polycystic ovarian morphology on ultrasound [25]. Individuals with thyroid dysfunction, hyperprolactinemia, congenital adrenal hyperplasia, Cushing's syndrome, or other endocrine disorders were excluded. Free from endocrine or metabolic conditions, healthy controls were selected from the same community or outpatient clinic. Participants were women aged 18–40 years with a BMI ranging from 18 to 40 kg/m², who had not used hormonal therapy for at least three months before study enrollment. Exclusion criteria encompassed pregnancy, lactation, severe systemic illnesses (e.g., cardiovascular disease, malignancy), autoimmune disorders, and recent use of insulin-sensitizing agents or corticosteroids.

Clinical Assessment and Anthropometry

Height was measured to the nearest 0.1 cm using a wall-mounted stadiometer (Seca 213, Hamburg, Germany), and body weight was recorded to the nearest 0.1 kg with a calibrated digital scale (Seca 799, Hamburg, Germany). BMI was calculated as weight in kilograms divided by the square of height in meters (kg/m²). Blood pressure was measured in the seated position using an automatic sphygmomanometer (Omron M6 Comfort, Omron Healthcare Co., Ltd., Kyoto, Japan), with three readings obtained at five-minute intervals; the mean of the final two measurements was recorded.

Sample Collection and Processing

After an overnight fast of at least 12 hours, venous blood samples (10 mL) were collected from all participants between 08:00 and 09:00 a.m. in the early follicular phase (days 2–5) of their menstrual cycle (or on a random day in those with oligomenorrhea/amenorrhea). Samples were drawn into serum separator tubes (BD Vacutainer SST II, BD Biosciences, Franklin Lakes, NJ, USA) and allowed to clot at room temperature for 30 minutes. The tubes were centrifuged at 3000 × g for 10 minutes, and serum aliquots were stored at −80 °C until further analysis.

Biochemical Measurements

Serum levels of LH, FSH, estradiol (E2), progesterone (P4), and total testosterone (T) were quantified using a fully automated electrochemiluminescence immunoassay (ECLIA) on the Cobas e 601 analyzer (Roche Diagnostics, Mannheim, Germany). The intra-assay and inter-assay coefficients of variation (CV) for these assays ranged between 2% and 6%, and

manufacturer-provided quality controls were run daily to ensure assay performance.

Nesfatin-1 Measurement

Serum Nesfatin-1 concentrations were determined via ELISA using a commercially available kit (Elabscience, USA; Cat. no. EL-H2373) with a lower detection limit of 15.0 pg/mL. Briefly, 50 µL of each standard or sample was added to the appropriate microplate well precoated with specific anti-Nesfatin-1 antibodies. After incubation at 37°C for 90 minutes, plates were washed five times using the manufacturer-supplied buffer. An HRP-conjugated secondary antibody (50 µL) was added, and incubation proceeded for 30 minutes at 37°C. Following another series of washes, 90 µL of 3,3′,5,5′-Tetramethylbenzidine (TMB) was applied for color development for 15 minutes in the dark. The reaction was stopped by adding 50 µL of stop solution, and optical densities were measured at 450 nm using a microplate reader (BioTek ELx808, BioTek Instruments, Winooski, VT, USA). Sample concentrations were calculated from the standard curve generated by four-parameter logistic (4-PL) regression, and duplicate measurements were performed for all specimens to ensure reproducibility. Per the manufacturer's specifications, the intra-assay and inter-assay CVs were <8% and <10%, respectively.

Experiments

This study followed a comparative case-control design aimed at exploring the relationship between nesfatin-1 levels and reproductive hormones in women with PCOS. The hypothesis was that nesfatin-1 levels would be significantly elevated in PCOS and correlate with specific hormonal patterns.

Subjects were stratified into PCOS and control groups based on strict diagnostic criteria. All procedures, including anthropometric measurements, fasting sample collection, and hormone assays, were standardized. Comparisons between groups were performed using appropriate statistical methods, and correlation analyses were used to assess the relationships between Nesfatin-1 and hormonal variables.

Statistical Analysis

Statistical analyses were conducted using SPSS version 28 (IBM Corp., Armonk, NY, USA) or an equivalent statistical software package. Continuous variables were summarized as mean ± standard deviation (SD), while categorical variables were reported as frequencies and percentages. The normality of data distribution was assessed using the Shapiro–Wilk test. The unpaired t-test was applied to normally distributed variables for comparisons between groups, whereas the Mann–Whitney U test was used for non-normally distributed data. Categorical variables were analyzed using the chi-square test (χ^2). Correlations between epiregulin levels and various hormonal or metabolic parameters were examined using Pearson's correlation coefficient for normally distributed data and Spearman's correlation coefficient for non-normally distributed data. A p-value of less than 0.05 was considered indicative of statistical significance [26].

Results

In Table 1, the control group (n=30) had a mean age of 29.13 ± 5.26 years, while the PCOS group (n=60) had a mean age of 30.63 ± 4.61 years (p=0.23, NS). BMI was significantly higher in the PCOS group (32.64 ± 6.73 kg/m²) compared to controls (26.76 ± 4.50 kg/m²) (p<0.001). The PCOS group also had a markedly longer cycle length (42.3 ± 4.42 vs. 27.76 ± 2.32 days; p<0.001).

In Table 2, women with PCOS (n=60) showed significantly elevated LH (0.871 ± 0.116 vs. 0.244 ± 0.160 µIU/mL) and testosterone levels (0.728 ± 0.091 vs. 0.424 ± 0.108 ng/mL). Still, markedly lower FSH (3.176 ± 0.643 vs. 5.428 ± 1.148 µIU/mL), estradiol (15.254 ± 2.185 vs. 88.452 ± 14.164 pg/mL), and progesterone (0.065 ± 0.082 vs. 0.317 ± 0.055 ng/mL) compared to controls (n=30), with all differences reaching p<0.001.

In Table 3, the PCOS group (n=60) showed a markedly higher mean serum Nesfatin-1 level (580.182 ± 133.691 pg/mL) compared to the control group (266.232 ± 69.0825 pg/mL), and this difference was highly significant (p<0.001).

Figure 1 demonstrates a positive, although modest correlation (R=0.264, p=0.048) between Nesfatin-1 and testosterone levels, indicating that higher testosterone levels are associated with increased Nesfatin-1 in women with PCOS.

Table 1. Comparison of Baseline Demographic and Clinical Characteristics Between Control and PCOS Groups[a]

Characteristics	**Control** ***n* = 30**	**PCOS** ***n* = 60**	***P.value***
Age (years)	29.13 ± 5.26	30.63 ± 4.61	0.23 I
BMI (kg/m²)	26.76±4.502	32.64±6.73	<0.001 I***
Cycle length	27.76 ± 2.32	42.3 ± 4.42	<0.001 I***

n = number of cases; SD = standard deviation.

[a]Statistical significance was indicated by ***p<0.001. I: independent samples t-test.

Table 2. Comparison of Key Reproductive Hormone Levels Between Control and PCOS Groups[a]

Characteristic	**Control** ***n* =30**	**PCOS** ***n* = 60**	***p***
LH (mIU/mL)	2. 44 ±1.60	8.71±1.16	<0.001 I***
FSH (mIU/mL)	5.43 ±1.148	3.176 ±0.643	<0.001 I***
Testosterone (ng/mL)	0.424±0.108	0.728±0.091	<0.001 I***
Estradiol(pg/mL)	88.452± 14.164	15.254±2.185	<0.001 I***
Progestrone(ng/mL)	0.317±0.055	0.065±0.082	<0.001 I***

n = number of cases; SD = standard deviation.

[a]Statistical significance was indicated by ***p<0.001. I: independent samples t-test.

Table 3. Serum Nesfatin-1 Levels in Control vs. PCOS Groups[a]

Characteristic	**Control** ***n* =30**	**PCOS** ***n* = 60**	**p**
Nesfatin-1(pg/ml)	266.23 ± 69.08	580.18 ± 133.69	<0.001 I***

n = number of cases; SD = standard deviation.

[a]Statistical significance was indicated by ***p<0.001. I: independent samples t-test.

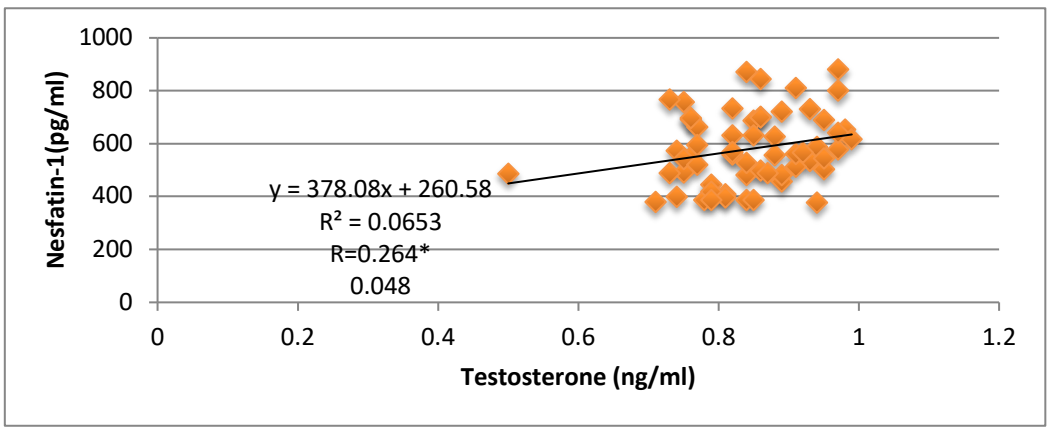


Figure 1. Correlation relationship between nesfatin-1(pg/mL) and testosterone(ng/mL).

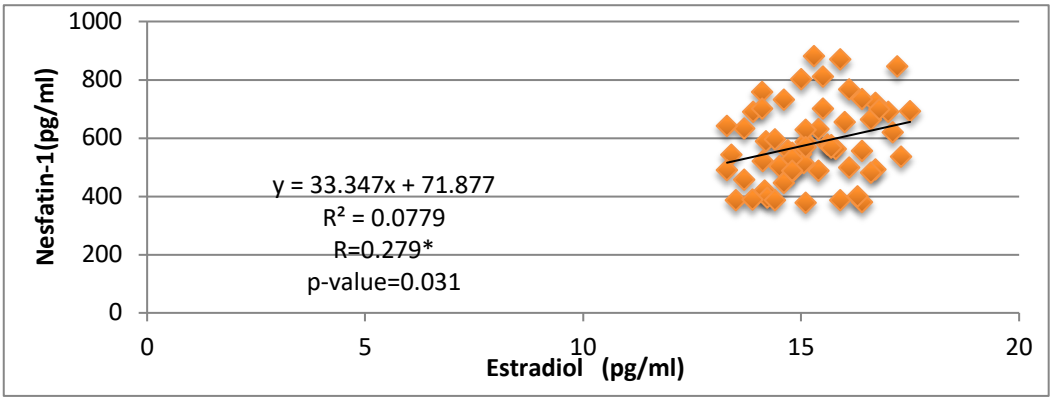


Figure 2. Correlation relationship between nesfatin-1(pg/ml) and estradiol(pg/mL).

Discussion

This study thoroughly compared anthropometric indices, reproductive hormone levels, and circulating Nesfatin-1 concentrations between women with PCOS and healthy controls. Our findings revealed that individuals with PCOS had notably higher BMIs, longer menstrual cycles, and altered gonadotropin levels characterized by elevated LH and diminished FSH. In line with the classical hyperandrogenic signature, the PCOS patients also exhibited increased testosterone. Of particular importance, our investigation demonstrated significantly elevated nesfatin-1 levels in PCOS, with positive correlations observed between Nesfatin-1 and both testosterone and estradiol. These results highlight the potential involvement of Nesfatin-1 in both the metabolic and reproductive disturbances characteristic of PCOS.

Our demonstration of higher nesfatin-1 concentrations in PCOS aligns with the findings of Ademoglu, et al. [27], who reported mean nesfatin-1 levels of approximately 371.43 ± 2.50 pg/mL and also with Faeza, et al. [7] that reported significant difference in serum nesfatin-1 levels between PCOS subjects (8.6 ng/ml) and controls (0.75 ng/mL, $p<0.01$), in PCOS groups. By contrast, Wang, et al. [21] did not observe a statistically significant difference in nesfatin-1 between PCOS and control populations, suggesting that discrepancies may stem from variations in diagnostic criteria, sample size, ethnic background, menstrual phase during sample collection, or the diverse phenotypic presentations of PCOS.

Nesfatin-1 is a cleavage product of the nucleobindin-2 (NUCB2) gene, widely recognized for its appetite-regulating functions in the hypothalamus [6]. Beyond central energy homeostasis, emerging evidence indicates that nesfatin-1 can modulate the HPG axis by influencing the release of gonadotropin-releasing hormone (GnRH) [8]. This modulation may be especially pertinent in PCOS, as GnRH pulsatility is frequently altered, resulting in preferential LH secretion and subsequent hyperandrogenemia.

Furthermore, nesfatin-1 may be involved in peripheral metabolism, where chronic hyperinsulinemia, a hallmark of insulin resistance in PCOS, may upregulate NUCB2/nesfatin-1 gene expression centrally and in peripheral tissues [28]. Additionally, low-grade inflammation, commonly observed in PCOS, can stimulate inflammatory mediators that enhance nesfatin-1 release [6, 29]. Hyperandrogenemia itself might create a feedback loop: elevated androgens can disrupt normal metabolic signaling in adipose tissue and the liver, leading to compensatory increases in appetite-regulating and insulin-sensitizing peptides, including nesfatin-1[30, 31]

Another possible mechanism relates to the interaction between nesfatin-1 and other metabolic hormones such as leptin and ghrelin. Weibert, et al. [32] reported that nesfatin-1 may share downstream signaling pathways with leptin, another crucial regulator of appetite and energy expenditure. Since leptin levels are often dysregulated in obesity (frequently comorbid with PCOS) [33], elevated nesfatin-1 may represent a compensatory response to counteract hyperphagia or metabolic imbalance. Additionally, nesfatin-1 can influence glucose homeo-

stasis by enhancing insulin secretion from pancreatic beta cells [10, 15]; thus, its elevation in PCOS could be linked to a compensatory drive to improve glycemic control in an insulin-resistant state. Overall, these interconnected pathways underscore a dual role for Nesfatin-1 in modulating both metabolic and reproductive physiology, potentially amplifying the pathophysiologic loops inherent in PCOS.

From a clinical perspective, our findings suggest that Nesfatin-1 measurement may offer added insight into the degree of metabolic and hormonal dysregulation in PCOS. In particular, the positive relationships between nesfatin-1 and testosterone/estradiol imply that elevated nesfatin-1 could be a surrogate marker for heightened androgenic and estrogenic activity, providing a more nuanced view of disease severity. Future research substantiates that nesfatin-1 levels improve or normalize with interventions such as weight reduction, insulin-sensitizing medications (e.g., metformin), or anti-androgen therapy. In that case, clinicians might consider nesfatin-1 as a complementary biomarker to track treatment efficacy or disease progression. On a therapeutic front, targeted modulation of nesfatin-1 or its upstream regulators might represent a novel intervention strategy to correct both metabolic and reproductive abnormalities in PCOS.

A key strength of this study is the well-defined diagnostic criteria for PCOS, ensuring the enrollment of a relatively homogenous patient group. Additionally, we used standardized, validated assays to measure reproductive hormones and nesfatin-1, enhancing the reliability and reproducibility of our results. The inclusion of correlation analyses between nesfatin-1 and sex steroids further augments the mechanistic relevance of our findings, positioning nesfatin-1 at the nexus of metabolic and endocrine disturbances in PCOS.

Nonetheless, the present investigation has several limitations. First, the cross-sectional design restricts our capacity to infer causal relationships, whether elevated nesfatin-1 triggers or simply reflects the metabolic and hormonal imbalances in PCOS remains unclear. Second, while adequately powered for detecting group differences, our sample size may not capture the full phenotypic diversity of PCOS. Third, unmeasured confounders such as dietary habits, physical activity levels, and psychological stress could influence nesfatin-1 and hormone levels. Finally, although we attempted to standardize the timing of blood sampling, fluctuations during different phases of the menstrual cycle may still affect hormone and Nesfatin-1 measurements.

Conclusion

The present findings highlight the heightened nesfatin-1 concentrations in women with PCOS and their significant associations with androgenic and estrogenic imbalances. This interplay underscores nesfatin-1's potential role as a biomarker bridging metabolic and reproductive dysfunctions. By delineating these neuroendocrine interactions, our results contribute valuable insight into the multifactorial nature of PCOS. Nonetheless, longitudinal and mechanistic investigations are warranted to establish causal links and potential therapeutic implications. Such work may ultimately refine diagnostic precision and enhance management strategies for PCOS.

Acknowledgements: We sincerely thank the medical and administrative staff at Babel Teaching Hospital for Pediatrics and Gynecology, Imam Sadiq Teaching Hospital, and Iskandaria General Hospital for their invaluable support throughout the study. We also thank the Institutional Review Board for granting ethical approval, and all participants for generously contributing their time and effort.

Conflicts of Interest: The authors declare no conflict of interest. The funders had no role in the study's design, data collection, analysis, interpretation, manuscript writing, or decision to publish the results. For a written confirmation, please write to the editorial office.

Ethics Approval: This study was approved by the Institutional Review Board (Approval No: 4162) and conducted in accordance with the Declaration of Helsinki. Informed consent was obtained from all participants.

Funding: This research did not receive a specific grant from any funding agency in the public, commercial, or not-for-profit sectors.

Quote this article as Zainab Ghazi Mohammed and Hassan Ali Farman, Elevated Nesfatin-1 and Altered Reproductive Hormones in Polycystic Ovary Syndrome: A Comparative Case-Control Study, Precis. Nanomed. 2025, 8(2):1501-1510, https://doi.org/10.33218/001c.xxxxxx.

References

[1] R. Shukla et al., "Ongoing Clinical Trials for Polycystic Ovarian Syndrome (PCOS) Afflicted Infertility in Women: A Narrative Review," Reviews on recent clinical trials, 2024.

[2] P. Kabakchieva, "POLYCYSTIC OVARY SYNDROME: DIVERSE CLINICAL PRESENTATIONS ACROSS ADOLESCENCE, REPRODUCTIVE AGE, AND MENOPAUSE," Anti-Aging Eastern Europe, vol. 3, no. 2, pp. 78-86, 2024, doi: 10.56543/aaeeu.2024.3.2.04.

[3] M. Rahman, F. T. Rahman, M. U. Mallik, J. Saha, M. M. Rahman, and K. A. K. Azad, "Metabolic dysfunctions in polycystic ovary syndrome," Journal of Medicine, vol. 25, no. 1, pp. 68-77, 2024, doi: https://doi.org/10.3329/jom.v25i1.70703.

[4] A. M. Huffman, S. Rezq, J. Basnet, and D. G. Romero, "Biomarkers in polycystic ovary syndrome," Current Opinion in Physiology, vol. 36, p. 100717, 2023, doi: https://doi.org/10.1016/j.cophys.2023.100717.

[5] D. Jayamurali, N. Ravishankar, N. Manoharan, R. Parasuraman, S. K. Jayashankar, and S. N. Govindarajulu, "Neuropeptide Network of Polycystic Ovary Syndrome–A Review," Protein & Peptide Letters, 2024, doi: https://doi.org/10.2174/0109298665309949240822105900.

[6] T. L. Steffen, J. D. Stafford, W. K. Samson, and G. L. Yosten, "Nesfatin-1 is a regulator of inflammation with implications during obesity and metabolic syndrome," Appetite, vol. 203, p. 107669, 2024, doi: https://doi.org/10.1016/j.appet.2024.107669.

[7] F. Faeza, S. Pikee, and J. Anju, "Correlation of serum nesfatin 1 level with metabolic and clinical parameters in Indian women with and without polycystic ovarian syndrome," 2022.

[8] J. Ha, J. Shin, E. Seok, S. Kim, S. Sun, and H. Yang, "Estradiol and progesterone regulate NUCB2/nesfatin-1 expression and function in GH3 pituitary cells and THESC endometrial cells," Animal Cells and Systems, vol. 27, no. 1, pp. 129-137, 2023, doi: https://doi.org/10.1080/19768354.2023.2226735.

[9] A. Pałasz et al., "Spexin and nesfatin-1-expressing neurons in the male human claustrum," Journal of Chemical Neuroanatomy, vol. 136, p. 102400, 2024, doi: https://doi.org/10.1016/j.jchemneu.2024.102400.

[10] S. K. Rupp, E. Wölk, and A. Stengel, "Nesfatin-1 receptor: distribution, signaling and increasing evidence for a G protein-coupled receptor–a systematic review," Frontiers in Endocrinology, vol. 12, p. 740174, 2021, doi: https://doi.org/10.3389/FENDO.2021.740174.

[11] E. T. Uyar and E. P. Aydın, "Role of nesfatin-1 in major depression," in The Neuroscience of Depression: Elsevier, 2021, pp. 331-340.

[12] D. García-Galiano and M. Tena-Sempere, "Emerging roles of NUCB2/nesfatin-1 in the metabolic control of reproduction," Current pharmaceutical design, vol. 19, no. 39, pp. 6966-6972, 2013, doi: https://doi.org/10.2174/13816128193931127142531.

[13] S. Feijoo-Bandin, D. Rodriguez-Penas, V. Garcia-Rua, A. Mosquera-Leal, J. R. González-Juanatey, and F. Lago, "Nesfatin-1: a new energy-regulating peptide with pleiotropic functions. Implications at cardiovascular level," Endocrine, vol. 52, pp. 11-29, 2016, doi: https://doi.org/10.1007/S12020-015-0819-Z.

[14] E. Ishida et al., "Nesfatin-1 induces the phosphorylation levels of cAMP response element-binding protein for intracellular signaling in a neural cell line," PloS one, vol. 7, no. 12, p. e50918, 2012, doi: https://doi.org/10.1371/JOURNAL.PONE.0050918.

[15] H. Shimizu and A. Osaki, "Nesfatin/nucleobindin-2 (NUCB2) and glucose homeostasis," Current Hypertension Reviews, vol. 9, no. 4, pp. 270-273, 2013, doi: https://doi.org/10.2174/15734021106661407020926 57.

[16] K. Dotania, M. Tripathy, and U. Rai, "Ovarian nesfatin-1 in Hemidactylus flaviviridis: Reproductive phase-dependent expression, role and hormonal regulation," Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology, vol. 288, p. 111556, 2024, doi: https://doi.org/10.1016/j.cbpa.2023.111556.
[17] E. A. Hamed, H. G. Sayyed, A. M. Abbas, M. M. A. Gaber, and H. M. Abd El Aleem, "Nesfatin-1, dopamine, and NADPH levels in infertile women with polycystic ovary syndrome: is there a relationship between their levels and metabolic and hormonal variables," Journal of reproduction & infertility, vol. 23, no. 3, p. 160, 2022, doi: https://doi.org/10.18502/jri.v23i3.10006.
[18] J. Ha and H. Yang, "Nesfatin-1 expressed in human endometrial stromal cell line (THESC) stimulates decidualization through FAK/PI3K/AKT signaling pathway," Peptides, vol. 182, p. 171315, 2024, doi: https://doi.org/10.1016/j.peptides.2024.171315.
[19] M. Rybska et al., "Nesfatin-1 expression and blood plasma concentration in female dogs suffering from cystic endometrial hyperplasia and pyometra and its possible interaction with phoenixin-14," BMC Veterinary Research, vol. 20, no. 1, p. 486, 2024.
[20] Y. Wang, R. Li, B. Sun, W. Song, X. Zhao, and Y. Hu, "Relationship of Serum Nesfatin-1 and Insulin-Like Growth Factor-1 Levels with Adverse Pregnancy Outcomes in Patients with Polycystic Ovary Syndrome," Age (years), vol. 30, no. 53, p. 75.71, 2024.
[21] Y. Wang, X. Ma, J. Luo, X. Wang, and L. Han, "Expression of serum PSA, nesfatin-1, and AMH in patients with polycystic ovary syndrome," Cellular and Molecular Biology, vol. 67, no. 5, pp. 57-63, 2021, doi: https://doi.org/10.14715/cmb/2021.67.5.8.
[22] A. Psilopanagioti, S. Nikou, and H. Papadaki, "Nucleobindin-2/nesfatin-1 in the human hypothalamus is reduced in obese subjects and co-localizes with oxytocin, vasopressin, melanin-concentrating hormone, and cocaine-and amphetamine-regulated transcript," Neuroendocrinology, vol. 108, no. 3, pp. 190-200, 2019.
[23] S. S. Bahreiny et al., "Circulating nesfatin-1 levels in women with polycystic ovary syndrome: A systematic review and meta-analysis," International journal of reproductive biomedicine, vol. 21, no. 10, p. 777, 2023.
[24] B. Shrestha and L. Dunn, "The declaration of Helsinki on medical research involving human subjects: a review of seventh revision," 2019, doi: 10.33314/jnhrc.v17i4.1042.
[25] E. A. Rotterdam, "Rotterdam ESHRE/ASRM-Sponsored PCOS Consensus Workshop Group. Revised 2003 consensus on diagnostic criteria and long-term health risks related to polycystic ovary syndrome," Fertil Steril, vol. 81, no. 1, pp. 19-25, 2004, doi: 10.1016/j.fertnstert.2003.10.004.
[26] D. George and P. Mallery, IBM SPSS statistics 26 step by step: A simple guide and reference. Routledge, 2019.
[27] E. N. Ademoglu et al., "Plasma nesfatin-1 levels are increased in patients with polycystic ovary syndrome," Journal of endocrinological investigation, vol. 37, pp. 715-719, 2014, doi: https://doi.org/10.1007/S40618-014-0089-2.
[28] K. V. Nagaraju et al., "Nucleobindin-2 derived nesfatin-1 in polycystic ovary syndrome: a PRISMA and GRADE-compliant systematic review and meta-analysis with diagnostic test accuracy," vol. 49, ed, 2024, pp. 111-121.
[29] E. Rudnicka et al., "Chronic low grade inflammation in pathogenesis of PCOS," International journal of molecular sciences, vol. 22, no. 7, p. 3789, 2021, doi: https://doi.org/10.3390/IJMS22073789.
[30] S. Andrisse et al., "Hepatocyte androgen receptor in females mediates androgen-induced hepatocellular glucose mishandling and systemic insulin resistance," bioRxiv, p. 2021.06. 09.447759, 2021, doi: https://doi.org/10.1101/2021.06.09.447759.
[31] D. Pignatelli, S. S. Pereira, and E. Kandaraki, "The pathogenic role of androgen excess in PCOS," Polycystic Ovary Syndrome, pp. 55-71, 2022, doi: https://doi.org/10.1016/b978-0-12-823045-9.00017-1.
[32] E. Weibert, T. Hofmann, U. Elbelt, M. Rose, and A. Stengel, "NUCB2/nesfatin-1 is associated with severity of eating disorder symptoms in female patients with obesity," Psychoneuroendocrinology, vol. 143, p. 105842, 2022, doi: https://doi.org/10.1016/j.psyneuen.2022.105842.

[33] M. H. Daghestani et al., "A study of ghrelin and leptin levels and their relationship to metabolic profiles in obese and lean Saudi women with polycystic ovary syndrome (PCOS)," Lipids in health and disease, vol. 17, pp. 1-9, 2018, doi: https://doi.org/10.1186/s12944-018-0839-9.