ENDOCRINOLOGY RESEARCH AND PRACTICE

SOCIETY OF ENDOCRINOLOGY AND METABOLISM OF TURKEY

ORIGINAL ARTICLE
Endocrinol Res Pract. 2025;29(1):36-42

Nesfatin-1, Kisspeptin, 5-Alpha Reductase-1, and Aromatase in Men with Metabolic Syndrome and Hypogonadism

ABSTRACT

Objective: Metabolic syndrome (MS)-associated hypogonadism in men is a prominent issue and occurs through complex pathogenetic pathways. We investigated levels of kisspeptin, nesfatin-1, aromatase, 5-alpha reductase-1 (5AR-1), and gene expressions of aromatase and 5AR-1 that affect these pathways.

Methods: Twenty six hypogonadal males with MS (group 1), 26 eugonadal males with MS (group 2), and 26 healthy males (group 3) in Pamukkale University Hospital were included in this study. Serum kisspeptin, nesfatin-1, aromatase, and 5AR-1 levels were determined by ELISA, and gene expressions were determined by real-time polymerase chain reaction. Data were analyzed in the SPSS 25.0 program.

Results: Kisspeptin and nesfatin-1 levels in groups 1 and 2 were similar but lower than in group 3 ($P = .001$). Aromatase and 5AR-1 levels were significantly lower in group 1 than in group 3 ($P = .006$, $P < .001$). Aromatase levels of groups 1 and 2 were also similar. 5AR-1 and aromatase expressions were not statistically significant.

Conclusions: Nesfatin-1 can be a mediator in the pathogenesis of MS but not directly for hypogonadism. As a regulator of the hypothalamic–pituitary–gonadal axis, kisspeptin may affect this pathogenesis just by MS. 5-Alpha reductase-1 may also act on hypogonadism. Hypogonadism may be a case affected by the process rather than its main result of aromatase.

Keywords: Hypogonadism, metabolic syndrome, kisspeptin, 5-alpha reductase, nesfatin-1, aromatase, testosterone

Introduction

The combination of dyslipidemia, hypertension, hyperglycemia, and abdominal obesity that raises the risk of cardiovascular disease is known as metabolic syndrome (MS).[1] Male hypogonadism is a clinical disorder caused by low testosterone levels that is characterized by reduced muscle mass and bone mineralization, reduced fertility, sexual dysfunction, and impaired fat metabolism.[2] Metabolic syndrome and testosterone deficiency have a complex relationship. Testosterone deficiency leads to insulin resistance, while metabolic disorders and related clinical factors also reduce testosterone levels.[3,4]

Increased adipose tissue may suppress the hypothalamic–pituitary–gonadal (HPG) axis in men by increasing the conversion of testosterone to estradiol, leading to secondary hypogonadism. Testosterone is mainly metabolized to estradiol in adipose tissue by aromatase, whose activity is increased in visceral fat. Antiestrogens such as aromatase inhibitors have been demonstrated to elevate testosterone levels in men with secondary hypogonadism.[5]

Kisspeptin is a neuropeptide hormone that potently stimulates the HPG axis through gonadotropin-releasing hormone (GnRH).[6] Kisspeptin affects the pulsatile secretion of GnRH by providing information about energy stores, thus establishing the link between nutrition, metabolic status, and reproduction.[6]

Nesfatin-1 acts to regulate reproductive function and ensure energy balance. Plasma nesfatin-1 levels are correlated with body weight, fat mass, and body mass index (BMI). It plays a regulatory role in metabolism through antihyperglycemic effects and is related to MS and its components.[7] Studies have shown that nesfatin-1 administration promotes both GnRHs, luteinizing hormone (LH), and follicle-stimulating hormone (FSH) secretions in male

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Received: August 2, 2024
Revision requested: October 1, 2024
Last revision received: October 25, 2024
Accepted: November 25, 2024
Publication Date: January 2, 2025

Cite this article as: Güner H, Fidan Yaylalı G, Seçme M, Açıkbaş İ, Şenol H. Nesfatin-1, kisspeptin, 5-alpha reductase-1 and aromatase in men with metabolic syndrome and hypogonadism. *Endocrinol Res Pract.* 2025;29(1):36-42.

DOI: 10.5152/erp.2025.24360

rats. In addition, serum nesfatin-1 levels in obese, young adult men were found to be significantly lower than those of normal weight. Therefore, obesity-related decreased serum nesfatin-1 levels may eventually lead to reduced GnRH levels and impaired reproductive function in obese men.[8]

To have an androgenic effect on tissues, testosterone must be converted to its active metabolite, dihydrotestosterone, by the 5-alpha reductase (5AR) enzyme.[9] In a study evaluating the effects of 5AR enzymes on insulin sensitivity, it was observed that the co-inhibition of 5AR-1 and 5AR-2 increases body weight and insulin resistance compared to the inhibition of 5AR-2 alone.[10] These data highlight a previously unknown role for 5AR-1 in regulating metabolic signaling in humans. It may explain the relationship between hypogonadism and MS.

This study investigated whether nesfatin-1, kisspeptin, 5AR, and aromatase play a role in pathogenic processes in hypogonadal or eugonadal men with MS.

Material and Methods

Participants and Study Design

This cross-sectional study was conducted in Pamukkale -- University Hospital Endocrinology and Metabolism and Internal Medicine clinics between 2019 and 2020. Seventy-eight men aged between 25 and 65 years were included in this study. Twenty-six men diagnosed with MS according to the US National Cholesterol Education Programme Adult Treatment Panel III criteria and hypogonadotropic hypogonadism (group 1), 26 eugonadal men with MS (group 2), and 26 healthy men (group 3) were included in the research.

Patients with acute or chronic infections, cancer, chronic renal failure, cirrhosis, chronic liver failure, heart failure, and hypogonadal men under treatment or using drugs that could alter testosterone levels were excluded from the research. Patients with MS did not have any other comorbidities except MS components.

Patients with HbA1c values of 9 and above were excluded from the study to ensure that glucotoxicity did not affect the results and to preserve the homogeneity of the study group. The control group was selected from healthy volunteers who were hospital employees, did not have any diseases, had no history of drug use, and did not meet the diagnostic criteria for NCEP ATP III MS.

MAIN POINTS

- Nesfatin-1 may be a mediator in the development of metabolic syndrome (MS) and may indirectly affect MS-associated hypogonadism in men in this way.
- Studies point out that kisspeptin is one of the hypothalamic regulators of the hypothalamic–pituitary–gonadal axis. Considering its relationship with MS, it may be an essential peptide in the pathogenesis of hypogonadism associated with MS in men.
- Serum 5-alpha reductase-1 (5AR-1) levels may have potential in the pathogenesis of MS-associated hypogonadism in men.
- It may be more appropriate to consider aromatase not as the leading cause of hypogonadism but as a parameter affected by the process and to reconsider the hypotheses on this subject.

Statement of Ethics

This research was conducted ethically in accordance with the World Medical Association Declaration of Helsinki. It was approved by the Pamukkale University Clinical Research Ethics Committee at its meeting dated August 07, 2018, and numbered 16 (60116787-020/53189). Informed consent was obtained from all participants to participate in the study.

Hormone Analysis

Blood samples were taken between 08:00 and 09.00 in the morning after at least 8 hours of fasting. Vacuum tubes with gel were used for biochemical and hormonal analyses, and EDTA tubes were used for hemogram and ACTH. Blood samples were sent to the laboratory immediately after collection. Testosterone and other hormones were analyzed using the electrochemiluminescence immunoassay method on the Roche Cobas 8000 device. According to the reference range of this device, the lower limit for total testosterone was determined as 2.49 ng/mL for the age group of 20-49 years and 1.93 ng/mL for those aged 50 and over. Again, the lower limit for the free testosterone level calculated according to the reference ranges of the device was determined as 0.19 nmol/L for the age range of 20-49 years and 0.16 nmol/L for those aged 50 and over. Serum-free testosterone levels were calculated using the Vermeulen formula.[11]

Insulin resistance was determined by the mathematical formula, Homeostasis model assessment of insulin resistance (HOMA-IR: fasting plasma insulin (mU/L) × fasting glucose (mg/dL)/405) and those with HOMA-IR ≥2.5 were considered as insulin resistance.[12]

Leukocyte Isolation From Blood Samples

A total of 4 mL of blood sample in the hemogram tube was taken into a 15 mL centrifuge tube, and the final volume was completed by adding RBC lysis buffer (89.9 g NH_4Cl; 10 g $KHCO_3$; 2 mL 0.5 M EDTA) to 10 mL. The samples were centrifuged at 2500 rpm for 10 minutes. Afterward, the supernatant was removed, and 10 mL of RBC lysis buffer was added to the remaining pellet. The tubes were placed in the centrifuge again and centrifuged at 2500 rpm for 10 minutes. After the second centrifugation, the supernatant in the tubes was removed, and 10 mL of RBC lysis buffer was added to the pellet. The tubes were subjected to centrifugation again. These processes were repeated 3 or 4 times until a clean pellet was obtained. In the last stage, 500 µL of triazole chemical was added to the clean pellet remaining in the tubes, and the samples were transferred to 1.7 mL microcentrifuge tubes with the help of a micropipette. The samples were stored in a deep freezer at −20°C until the RNA isolation process.

RNA isolation and Real-Time PCR

Total RNA isolation was performed from lymphocyte cells using Trizol Reagent (Invitrogen, USA). cDNA synthesis from the RNA template was performed by reverse transcription using the commercial kit "VitaScript FirstStrand cDNA Synthesis Kit" (PCCSKU1301). Aromatase and 5-alpha reductase 1 gene expression analyses were performed with Step One Plus Real-Time RT-PCR (Applied Biosystems, USA) according to the protocol of SYBR Green qPCR Master Mix (Thermo Scientific, USA). The RT-PCR assay was performed using gene-specific primers. Expression results were normalized to beta-actin gene (housekeeping gene) expression levels to calculate relative expression ratios.

Determination of Kisspeptin, Nesfatin-1, Aromatase, and 5AR1 Concentrations by Enzyme-Linked Immunosorbent Assay Method

Blood samples were transferred to a yellow-capped biochemistry tube containing gel and centrifuged at 2500 rpm for 10 minutes. The serum formed after centrifugation was transferred to a 1.7 mL microcentrifuge tube with the help of a micropipette and stored in a deep freezer at −20°C until the Enzyme-Linked Immunosorbent Assay (ELISA) experiment was performed. To determine the amount of Aromatase, 5AR1, Kisspeptin, and Nesfatin-1 from serum samples, the necessary ELISA kits were obtained commercially, and analysis procedures were carried out by applying the protocols of the relevant kits.

Statistical Analysis of Data

The required sample size was calculated as 26 people for each group. The data were analyzed using the SPSS 25.0 (IBM SPSS Corp.; Armonk, NY, USA) program. Continuous variables are given as mean ± standard deviation, and categorical variables are presented as numbers and percentages. The data's compliance with normal distribution was examined using the Shapiro–Wilk test. One-way analysis of variance (post hoc: Tukey test) was used to compare independent group differences when parametric test assumptions were met. When parametric test assumptions were not met, Kruskal–Wallis variance analysis (post hoc: Mann–Whitney *U* test with Bonferroni correction) was used to compare independent group differences. Chi-square analysis was used to compare categorical variables. In addition, the relationships between continuous variables were examined using Spearman or Pearson correlation analysis. $P < .050$ was considered statistically significant.

Results

A total of 78 men participated in the study. Three groups were formed: 26 individuals with MS-associated hypogonadism (group 1), 26 individuals with MS (group 2), and 26 healthy individuals (group 3). In total, 15 (19.2%) patients had type 2 diabetes mellitus (DM), 15 (19.2%) hypertension, and 50 (64.1%) dyslipidemia.

Age, BMI, and waist circumference measurements are presented in Table 1.

Analysis of fasting glucose, fasting insulin, HOMA-IR, HbA1c, hemoglobin, creatinine, and alanine aminotransferase (ALT) by groups is presented in Table 2. Fasting glucose, fasting insulin, HOMA-IR, TG, ALT, and sex hormone-binding globulin (SHBG) were found to be significantly higher in the first and second groups compared to the third group, while high-density lipoprotein (HDL) was significantly lower ($P < .001$). However, these parameters were not significant between groups 1 and 2. Total cholesterol was highest in group 2

Table 1. Intergroup Analysis of Clinical Features

	Hypogonadal Men with Metabolic Syndrome, Mean ± SD	Eugonadal Men with Metabolic Syndrome, Mean ± SD	Healthy Men, Mean ± SD	*P*
Age (year)	36.80 ± 7.90	36.60 ± 9.70	33.50 ± 6.30	.300
Body mass index (kg/m^2)	36.00 ± 6.30	34.20 ± 4.40	27.70 ± 1.50	<.001[1]
Waist circumference (cm)	123.50 ± 18.70	114.10 ± 9.80	92.60 ± 60	<.001[1]

Values below $P = .05$ are statistically significant.
Mean, arithmetic mean; SD, standard deviation.
[1]Statistically significant difference is between hypogonadal men with metabolic syndrome and healty men, between eugonadal men with metabolic syndrome and healty men.

Table 2. Analysis of Laboratory Results by Groups

	Hypogonadal Men with Metabolic Syndrome, Mean ± SD	Eugonadal Men with Metabolic Syndrome, Mean ± SD	Healthy Men, Mean ± SD	*P*
Fasting glucose (mg/dL)	112.40 ± 25.80	105.00 ± 23.30	91.30 ± 6.10	**<.001[1]**
Fasting insulin (mU/L)	21.50 ± 9.90	16.30 ± 9.10	8.40 ± 2.40	**<.001[1]**
HOMA-IR	5.90 ± 3.50	4.10 ± 2.40	1.80 ± 0.60	**<.001[1]**
HbA1c (%)	6.70 ± 1.10	6.80 ± 1.20	–	.800
Total cholesterol (mg/dL)	192.90 ± 51.00	208.30 ± 53.60	166.40 ± 23.30	**.005[2]**
HDL (mg/dL)	38.10 ± 13.20	38.20 ± 6.50	48.80 ± 6.00	**<.001[1]**
LDL (mg/dL)	112.50 ± 42.60	125.80 ± 44.60	98.20 ± 21.60	.050
Triglyceride (mg/dL)	244.50 ± 174.20	223.60 ± 96.90	95.80 ± 33.10	**<.001[1]**
Creatinine (mg/dL)	0.80 ± 0.20	0.90 ± 0.10	0.80 ± 0.10	.100
ALT (IU/L)	37.60 ± 19.60	40.90 ± 19.50	22.10 ± 0.60	**<.001[1]**
Hemoglobin (g/dL)	15.20 ± 1.10	20.90 ± 30.00	15.40 ± 0.50	.500
Leukocyte (×10^9/L)	8.00 ± 1.60	8.20 ± 1.60	7.10 ± 1.50	**.030[2]**
Albumin (g/L)	47.60 ± 2.08	48.00 ± 3.20	48.00 ± 2.00	.800
SHBG (nmol/L)	18.40 ± 10.00	23.10 ± 9.10	31.30 ± 10.30	**<.001[1]**

Values below $P = .05$ are statistically significant.
ALT, alanine aminotransferase; HDL, high-density lipoprotein; LD,: low-density lipoprotein; mean, arithmetic mean; SD, standard deviation; SHBG, sex hormone-binding globulin.
[1]Statistically significant difference is between hypogonadal men with metabolic syndrome and healty men, between eugonadal men with metabolic syndrome and healty men.
[2]Statistically significant difference is between eugonadal men with metabolic syndrome and healty men.

Table 3. Hormone Analyses of the Groups

	Hypogonadal Men with Metabolic Syndrome, Mean ± SD	Eugonadal Men with Metabolic Syndrome, Mean ± SD	Healthy Men, Mean ± SD	*P*
Prolactin (µg/L)	13.60 ± 6.10	12.00 ± 4.50	12.50 ± 4.50	.500
Estradiol (ng/L)	23.10 ± 10.70	37.50 ± 13.70	24.60 ± 11.40	**<.001**[1]
ACTH (ng/L)	33.20 ± 17.10	28.30 ± 15.30	25.70 ± 11.90	.200
Cortisol (µg/L)	13.70 ± 4.20	11.8 ± 4.70	13.90 ± 3.60	.200
FSH (U/L)	4.80 ± 2.80	4.10 ± 2.20	3.40 ± 1.50	.090
LH (U/L)	4.40 ± 1.90	5.40 ± 1.50	5.10 ± 1.50	.100
Total testosterone (ng/mL)	1.80 ± 0.30	3.60 ± 0.80	4.60 ± 1.00	**<.001**[2]
Free testosterone (nmol/L)	0.10 ± 0.04	0.20 ± 0.06	0.30 ± 0.07	**<001**[3]
DHEA-S (µg/dL)	267.20 ± 147.80	296.60 ± 125.80	344.00 ± 146.00	.100
GH (µg/L)	0.20 ± 0.50	0.10 ± 0.10	0.20 ± 0.400	.200
IGF-1 (µg/L)	144.00 ± 42.80	151.20 ± 43.10	184.50 ± 53.00	**.005**[4]
TSH (mU/L)	2.40 ± 1.00	2.10 ± 1.40	1.60 ± 0.50	**.020**[5]
Free T3 (ng/dL)	3.30 ± 0.50	3.40 ± 0.30	3.50 ± 0.30	.200
Free T4 (ng/dL)	1.20 ± 0.20	1.20 ± 0.20	1.30 ± 0.20	.100

Values below *P* = .05 are statistically significant.
ACTH, adrenocorticotropic hormone; DHEA-S, dehydroepiandrosterone; FSH, follicle-stimulating hormone; GH, growth hormone; IGF-1, insulin-like growth factor-1; LH, luteinizing hormone; mean, arithmetic mean; SD, standard deviation; TSH, thyroid-stimulating hormone; T3, triiodothyronine; T4, thyroxine.
[1]Statistically significant difference is between hypogonadal men with metabolic syndrome and eugonadal men with metabolic syndrome, hypogonadal men with metabolic syndrome, and healthy men.
[2]Statistically significant difference is between all groups.
[3]Statistically significant difference is between hypogonadal men with metabolic syndrome and eugonadal men with metabolic syndrome, between hypogonadal men with metabolic syndrome and healthy men.
[4]Statistically significant difference is between hypogonadal men with metabolic syndrome and healty men, between eugonadal men with metabolic syndrome and healthy men.
[5]Statistically significant difference is between hypogonadal men with metabolic syndrome and healthy men.

and lowest in group 3, and there was significance between groups 2 and 3 ($P = 0.005$).

Hormone analysis of the groups is presented in Table 3. Estradiol levels were the lowest in group 1 and the highest in group 2, and the difference between group 2 and other groups was statistically significant ($P < .001$). Total testosterone was the lowest in group 1 and the highest in group 3, and the difference between all 3 groups was statistically significant ($P < .001$). Free testosterone levels were also statistically significantly lower in group 1 compared to other groups ($P < .001$). Although the free testosterone level of the second group was lower than the third group, there was no statistical difference between them. Although FSH levels were the highest in group 1 and the lowest in group 3, there was no statistical difference between the groups ($P = .090$). Luteinizing hormone values were lowest in group 1 and highest in group 2, but no statistical difference was found between the groups ($P = .100$)

Nesfatin-1, kisspeptin, 5AR-1, and aromatase levels, as well as 5AR-1 and aromatase gene expressions, are shown in Table 4.

In group 1, waist circumference was positively correlated with kisspeptin ($r = 0.3$, $P = .004$) and serum aromatase level ($r = 0.4$, $P = .010$). A negative correlation was found between fasting glucose and kisspeptin ($r = -0.4$, $P = .030$) and serum aromatase levels ($r = -0.4$, $P = .030$). Aromatase gene expression was positively correlated with estradiol level ($r = 0.4$, $P = .020$). While 5AR-1 gene expression was positively correlated with LH level ($r = 0.4$, $P = .02$), it was negatively correlated with free T4 level ($r = -0.4$, $P = .020$).

Table 4. Analysis of Nesfatin-1, Kisspeptin, 5-Alpha Reductase-1, and Aromatase levels, also 5-Alpha Reductase-1 and Aromatase Gene Expression

	Hypogonadal Men with Metabolic Syndrome, Mean ± SD	Eugonadal Men with Metabolic Syndrome, Mean ± SD	Healthy Men, Mean ± SD	*P*
Nesfatin-1 (ng/mL)	12.30 ± 10.00	14.10 ± 11.30	26.10 ± 13.80	**.001**[1]
Kisspeptin (ng/L)	292.60 ± 173.00	346.60 ± 189.60	550.90 ± 236.30	**<.001**[1]
5-Alpha reductase-1 (ng/mL)	6.00 ± 3.30	6.50 ± 3.80	9.60 ± 3.70	**.006**[2]
Aromatase (ng/mL)	12.30 ± 6.90	14.40 ± 7.90	23.00 ± 9.90	**<.001**[1]
5-Alpha reductase-1 gene expression	27.80 ± 3.10	27.40 ± 3.70	27.40 ± 5.90	.800
Aromatase gene expression	32.80 ± 2.90	32.50 ± 2.70	31.60 ± 4.70	.500

Mean, arithmetic mean; SD, standard deviation. Values below *P* = .05 are statistically significant.
[1]Statistically significant difference is between hypogonadal men with metabolic syndrome and healty men, between eugonadal men with metabolic syndrome and healthy men.
[2]Statistically significant difference is between hypogonadal men with metabolic syndrome and healthy men.

A negative correlation was found between aromatase gene expression and serum aromatase level ($r = -0.4$, $P = .010$).

Discussion

Metabolic syndrome has a complicated association with testosterone deficiency through multiple pathways and various mediators. In this study, serum nesfatin-1, kisspeptin, 5AR-1, and aromatase levels, as well as 5AR-1 and aromatase gene expressions, were simultaneously investigated based on studies examining different pathways. All nesfatin, kisspeptin, and aromatase levels were lower in patients with MS. Whether or not hypogonadism accompanied MS did not change their levels. On the other hand, only 5AR-1 levels were lower in patients with both MS and hypogonadism.

Nesfatin has been associated with reducing insulin resistance by lowering gluconeogenesis and increasing peripheral glucose uptake,[13] and its levels were found to be low in MS.[14,15]

No study has been found in the available literature that directly investigates the association of nesfatin-1 levels and MS and male hypogonadism in humans. However, Chen et al[8] revealed results regarding the relation between obesity, testosterone, and nesfatin-1 in mice. This research showed that a high-fat diet for 18 weeks caused lower serum and hypothalamic NUCB2/nesfatin-1 levels, increased hypothalamic pro-inflammatory factors, and reduced hypothalamic GnRH levels. Obesity and related hypogonadotropic hypogonadism were the clinical consequences; with diet and/or exercise, adiposity was reduced, hypogonadotropic hypogonadi sm-associated obesity improved, and also serum and hypothalamic NUCB2/nesfatin-1 levels, inflammatory factors, and hypothalamic GnRH levels were all reversed.[8] In our research, nesfatin levels were lower in MS groups but similar in patients with or without hypogonadism. So, it can be concluded that it is not the link between MS and hypogonadism in men. Also, there was no correlation between FSH, LH, total or free testosterone, and nesfatin-1 in any of the groups.

Current information points to "kisspeptin" as a regulator of the HPG axis with its LH and testosterone-promoting effects. It was shown that plasma LH, FSH, and testosterone levels increased.[16] Kisspeptin-10 was found to increase LH secretion and testosterone levels in men.[17] It also increased LH pulse frequency, secretion, and testosterone in men with type 2 DM and testosterone deficiency.[18]

In another study, kisspeptin levels of 30 male patients with hypogonadotropic hypogonadism were higher compared to the control group ($P < .010$). Kisspeptin and LH/FSH levels were not correlated. Although kisspeptin and HOMA-IR were not correlated, this relationship was not statistically significant.[19] In our research, the lowest serum kisspeptin levels were detected in men with MS-associated hypogonadism, while the highest values were found in healthy men ($P < .001$). However, kisspeptin levels were similar between MS groups with or without hypogonadism. While there was no correlation between HOMA-IR and kisspeptin in any group, in the group with MS-associated hypogonadism, serum kisspeptin levels, and fasting glucose negatively correlated as predicted ($r = -0.4$, $P = .030$), while they positively correlated with waist circumference ($r = 0.3$, $P = .040$). In light of this information, we can say that kisspeptin is especially affected in MS, and its effect on metabolic parameters becomes more evident when MS and hypogonadism coexist.

Upreti et al[10] published research on 46 men hypothesizing that 5AR inhibition causes metabolic dysfunction. They performed a hyper insulinemic-euglycemic clamp test on subjects given oral dutasteride, finasteride, or tamsulosin. This study showed increased adipose tissue and insulin resistance due to the 5AR-1/5AR-2 inhibitor dutasteride. They concluded that dual inhibition of 5ARs, but not inhibition of 5AR2 alone, modulates insulin sensitivity in human peripheral tissues rather than the liver.[10]

In studies based on male hypogonadism, the expression of 5AR-1 was ignored because it was not expressed in human testicular tissue, and the focus was on 5AR-2. However, 5AR-1 is most expressed in many tissues and has simultaneous metabolic effects. So, in our study, we examined the effect of 5AR-1 on MS-associated hypogonadism. Serum 5AR-1 levels were found to be lowest in men with MS-associated hypogonadism and highest in healthy men ($P = .006$). However, the MS group was not significantly different from the others. Moreover, there was no statistical significance between 5AR-1 gene expressions. Although there was no change in the gene expression level, the significant difference in serum levels suggests that epigenetic mechanisms may be effective. 5AR enzymes were isolated from tissue samples such as the liver and testis, and their gene expressions were examined in some studies. However, since we isolated the 5AR-1 enzyme from blood samples in our study, gene expressions may result differently. Although there is no significant difference in 5AR-1 gene expressions, serum 5AR-1 levels, the end product of the gene, may be one of the main factors in the pathogenesis of MS-associated hypogonadism in men. Future studies, including larger populations, may clarify the possible role of 5AR-1 in mechanisms related to MS and hypogonadism.

Testosterone and obesity interact in a dual way. More fat tissue means more aromatase and more conversion of testosterone to estradiol, which reduces circulating testosterone. Low testosterone levels increase fat cell mass and fat accumulation, which gradually leads to a more depressing effect on testosterone. Also, the normal negative feedback effect of testosterone on the HPG axis is largely through the aromatization of testosterone to estradiol. Therefore, excessive aromatase activity in adipocytes, which increase in number in obesity, suppresses gonadotropin-mediated testosterone secretion and deepens hypogonadism.[20] de Boer et al presented a clinical study supporting this explanation.[21] In this study, after 6 weeks of treatment with letrozole to 10 obese men, estradiol levels ($P = .006$) decreased, LH ($P < .001$), and total testosterone levels ($P < .001$) decreased.[21] Similarly, Zumoff et al[22] observed that 6 obese men who were administered an aromatase inhibitor, testolact, for 6 weeks showed increased serum testosterone levels ($P < .001$), decreased estradiol levels ($P < .004$), and serum LH levels.[22]

As expected in our study, aromatase gene expression was positively correlated with estradiol levels in the group with MS-associated hypogonadism. In this group, a positive correlation was found between serum aromatase levels and waist circumference, and a negative correlation was found with HDL. These findings support the hypothesis that elevated serum aromatase levels are associated with poor lipid profiles and poor body composition in hypogonadal men associated with MS. However, this association was not observed in men with MS with normal testosterone levels or in the control group.

Our analysis shows no significant difference between the groups regarding aromatase gene expressions. This may be because

aromatase gene expression, similar to 5AR-1 enzyme gene expression, was studied in serum samples, not at the tissue level. Interestingly, the negative correlation between serum aromatase levels and aromatase gene expression in the MS group also makes the suitability of serum samples for aromatase gene expression controversial.

There are also findings contrary to the classical thought about the effect of aromatase on male hypogonadism. In the study published by Ghanim et al, the mRNA expressions of androgen receptor, estrogen receptor and aromatase from adipose tissue of 32 hypogonadal and 32 eugonadal men with type 2 DM were examined. As a result, the expression of androgen receptor, estrogen receptor and aromatase in hypogonadal men was significantly lower than in eugonadal men.[23] In the continuation of the study, after testosterone or placebo (saline) injection in hypogonadal men for 22 weeks, the expression of all three genes significantly increased in the testosterone-treated group.[23] In our study, supporting this result, the serum aromatase level was highest in the healthy control group; it was found to be lower in the MS group and at the lowest level in the MS-associated hypogonadism group. These results support the hypothesis that when testosterone, the substrate of aromatase, decreases, aromatase and, therefore, estradiol levels will also decrease.

Another remarkable finding is that serum aromatase and serum 5AR-1 levels, which we expect to have opposite effects on testosterone, were positively correlated in all groups.

Although this study is important in analyzing possible parameters affecting both central and peripheral pathways in the pathogenesis of metabolic syndrome-related male hypogonadism, one of our limitations is that the number of cases could not be larger. For this reason, further studies with larger sample sizes are needed.

In conclusion, considering all these results, nesfatin-1 may be a mediator in the development of MS and may indirectly affect MS-associated hypogonadism in men in this way. Studies point out that kisspeptin is one of the hypothalamic regulators of the HPG axis. Considering its relationship with MS, it may be an important peptide in the pathogenesis of hypogonadism associated with MS in men. It is possible that serum 5AR-1 levels may have potential in the pathogenesis of MS-associated hypogonadism in men. It may be more appropriate to consider aromatase not as the main cause of hypogonadism but as a parameter affected by the process and to reconsider the hypotheses on this subject.

Availability of Data and Materials: The data that support the findings of this study are available on request from the corresponding author.

Ethics Committee Approval: This research was approved by the Pamukkale University Clinical Research Ethics Committee (approval number:16(60116 787-020/53189), date: 07.08.2018).

Informed Consent: Verbal informed consent was obtained from all participants to participate in the study.

Peer-review: Externally peer-reviewed.

Author Contributions: Concept – G.F.Y., H.G.; Design – G.F.Y., Halil Güner.; Supervision – H.G., G.F.Y.; Resources – M.S., İ.A.; Materials – M.S., İ.A.; Data Collection and/or Processing – H.Ş., M.S.; Analysis and/or Interpretation – H.Ş., M.S.; Literature Search – H.G., G.F.Y.; Writing – H.G., G.F.Y.; Critical Review – H.G., G.F.Y.

Declaration of Interests: The authors have no conflicts of interest to declare.

Funding: This study was supported by the Scientific Research Projects Coordination Unit of Pamukkale University (Grant number 2020TIPF002).

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