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Research Article / Araştırma Makalesi

The relationship of menstrual irregularity with AMH, ghrelin and leptin levels in athletes

Sporcularda adet düzensizliğinin AMH, ghrelin ve leptin düzeyleri ile ilişkisi

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ABSTRACT

Objective: To explore menstrual irregularity rates in female professional basketball and volleyball players and investigate the relationship with Anti-Müllerian Hormone (AMH), ghrelin, leptin levels and biochemical parameters.

Material and Methods: Forty-one professional female athletes and forty-one non-athlete controls aged 18-35 years participated in the study. Questioning of menstrual function has been performed and whole blood counts, AMH, leptin, ghrelin and other endocrine and metabolic parameters were evaluated.

Results: 41% of female athletes and in 24% of non-athlete controls revealed menstrual irregularities. AMH, ghrelin, leptin levels were similar in athletes and control group (p>0.05). While athletes with menstrual irregularities tend to have lower ghrelin and leptin levels and higher AMH than athletes without irregularities, the difference was not statistically significant (p>0.05). No significant difference was found between the groups in terms of endocrine and other metabolic parameters (p>0.05). There was a significant negative correlation between ghrelin and menstrual irregularity (r=-0.240, p=0.031) and a statistically significant positive correlation between AMH and testosterone levels (r=-0.247, p=0.025). There was no significant relationship between other parameters (p>0.05).

Conclusions: Although it was not significant, athletes were found to have more menstrual irregularities. The leading factors and preventive measures should be investigated in detail.

Keywords: *Anti-Mullerian Hormone (AMH), ghrelin, leptin, menstrual irregularity, sport*

ÖZ

Amaç: Profesyonel basketbol ve voleybol sporcularında adet düzensizliği oranlarının, bunların Anti-Müllerian Hormon (AMH), ghrelin, leptin düzeyleri ve biyokimyasal parametrelerle ilişkisini araştırmaktır.

Gereç ve Yöntemler: Çalışmaya 18-35 yaşları arasında 41 kadın sporcu ve 41 sporcu olmayan kadın kontrol katıldı. Katılımcıların menstruel işlev sorgulaması yapıldı ve tam kan sayımları, AMH, ghrelin, leptin düzeyleri ile diğer endokrin ve metabolik parametreleri değerlendirildi.

Bulgular: Sporcuların %41'inde (n=17), sporcu olmayan kontrollerin %24'ünde (n=10) menstruasyon düzensizliği bulundu. AMH, ghrelin, leptin düzeyleri sporcularda ve kontrol grubunda benzerdi (p>0.05). Menstruasyon düzensizliği olan sporcularda düzensizliği olmayan sporculara göre ghrelin ve leptin düzeyleri daha düşük, AMH düzeyi ise daha yüksek olmakla birlikte istatiksel olarak anlamlı fark yoktu (p>0.05). Ayrıca endokrin ve metabolik parametreler olarak bilinen insülin, glukoz, TSH, T3, kortizol gibi diğer kan değerleri açısından da gruplar arasında anlamlı bir fark saptanmadı (p>0.05). Adet düzensizliği ve ghrelin arasında anlamlı negatif korelasyon (r=-0.240, p=0.031) ve AMH ile testosteron düzeyleri arasında anlamlı pozitif korelasyon (r=0.247, p=0.025) saptandı. Diğer parametreler arasında anlamlı ilişki saptanmadı (p> 0.05).

Sonuçlar: Sporcularda adet düzensizliği sıklığının anlamlı düzeyde olmasa da daha fazla olduğu görülmektedir. Bunun nedenlerinin araştırılması ve önlenmesi için izlenecek yollar detaylı biçimde araştırılmalıdır.

Anahtar Sözcükler: *Anti-Müllerian Hormon (AMH), ghrelin, leptin, adet düzensizliği, spor*

INTRODUCTION

Female athletes are at risk for developing menstrual period abnormalities. Depending on the increase in energy expenditure, exercise can cause chronic metabolic stress and psychogenic stress, and disrupt the stimulation of gonadotropin-releasing hormone (GnRH) and the endocrine balance (1). As a result of this, women with athletic amenorrhea

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have less release of follicle-stimulating hormone (FSH) and luteinizing hormone (LH), leading to impaired ovulation (2). The prevalence of secondary amenorrhea and oligomenorrhea in the general non-athletic population is between 2% and 5% (3). The incidence of menstrual disorders varies in female athletes and can be as high as 44% (4).

As a result of strenuous and repetitive exercise, partially negative energy balance occurs. If energy expenditure exceeds energy intake, homeostatic neuroendocrine mechanisms such as leptin and ghrelin hormone responses are activated to conserve energy. In addition to appetite regulation, leptin and ghrelin have an effect on the mechanisms which regulate the reproductive function. Leptin concentrations in circulation are directly proportional to body fat storages. Leptin stimulates GnRH release and gonadotropin production. It has been shown that critical leptin level is required for the continuation of menstruation and there is a deterioration in menstrual function in women with low leptin levels (5). Leptin levels in circulation generally decrease with exercise (6). It has been reported that serum leptin levels decrease in athletes who are highly trained, and daily leptin release pattern disappears in athletes with amenorrhea (7). Ghrelin in circulation is inversely proportional to body weight (8). Ghrelin decreases GnRH release and gonadotropin production by acting in reverse of leptin (9). It has been shown that increased ghrelin levels cause disruption in menstrual function. It has also been shown that ghrelin levels increase in people who exercise (10,11). Ghrelin levels were found to be higher in people with amenorrhea who are exercising (12).

Anti-Mullerian Hormone (AMH) is a peptide produced by granulosa cells of growing ovarian pre-antral and small antral follicles (13). Serum AMH level is related to the total number of antral follicles in the ovaries. Therefore, the increase in serum AMH level can be used as an ovarian reserve biomarker for the diagnosis of ovulatory dysfunction and polycystic ovary syndrome (PCOS) (13.14). The continued growth of the follicles leads to increased AMH levels and causes the menstrual cycle to be disrupted. Therefore, AMH levels are high in women who do not menstruate or have irregular menstruation (15). The purpose of this cross-sectional study is to compare the frequency of menstrual dysfunction in women who do team sports, such as basketball and volleyball, with non-athlete female control group, and to investigate whether it shows a correlation with leptin, ghrelin and AMH hormones if there is a disorder.

MATERIAL and METHODS

Our study was conducted between August 2018 and January 2019 together with four sports clubs in the Turkish Basketball First League and Turkish Volleyball First League. Forty-one female athletes between the ages of 18-35, who were professionally engaged in sports without any previous pregnancy history, and forty-one women of similar ages who did not exercise regularly with similar body mass indexes participated in the study. A cross-sectional study involving one-time blood analysis was performed.

"Personal Information Form" was used to obtain research data. Relevant forms were distributed to the participants of the study and they were asked to read and answer by themselves. The Personal Information Form consisted three parts. In the first part, there were questions to determine the socio demographic information (age, use of medication, smoking, chronic diseases, and eating disorders) of the participants. In the second part, training schedules of the athletes were questioned. The activity levels of the controls were noted, as well. The second part of the form contains the query of daily diet regimens. Third part includes questions to determine the menstrual characteristics of the participants (age of menarche, menstruation period, number of pads used, oligomenorrhea, polymenorrhea, secondary amenorrhea). Menstruation intervals longer than 30-35 days were accepted as oligomenorrhea (1,2), and shorter than 21 days, as polymenorrhea (1,3). Secondary amenorrhea was considered to be the absence of three or more consecutive menstrual bleeding after at least one normal period (3).

Functional hypogonadism is a reversible disorder characterized by GnRH deficiency without an anatomical cause. Since this disorder is associated with variable patterns of abnormal LH pulsatility and low leptin levels, these blood parameters were investigated to evaluate functional hypogonadism in participants.

Exclusion criteria were; having diagnosis of Polycystic Ovary Syndrome (PCOS), showing clinical signs of PCOS detected by an obstetrician, having premature ovarian failure, being pregnant, using oral contraceptive drugs or smoking. On the day of blood sampling, body weight, height, body mass index (BMI), and body fat levels of participants were measured. Height (cm) and weight (kg) measurements were taken using a wall-mounted height meter and digital scale. BMI were calculated by using the formula: Weight/Height^2 (kg/m^2). Body fat levels were measured using Tanita model TBF-300 A.

Written informed consent was obtained from all participants and the study was approved by the Ege University Faculty of Medicine Clinical Research Ethics Committee, (No: 17-9 / 7, 29/09/2017).

Collection and Preparation of Serum Samples

Blood samples were taken from the antecubital vein between 08.00 - 09.00 in the morning after one day of rest and 12 hours of fasting. Whole blood counts, lipid profiles, glucose, hemoglobin A1c (HbA1c), insulin, FSH, LH, estradiol, thyroid-stimulating hormone (TSH), free thyroxine (fT4), free tri-iodothyronine (fT3), prolactin, progesterone, testosterone, dehydroepiandrosterone sulfate (DHEAS), cortisol, leptin, ghrelin, AMH, vitamin B12, folate, iron, ferritin, aspartate aminotransferase (AST), alanin aminotransferase (ALT), creatine kinase (CK), alkaline phosphatase (ALP) and lactate dehydrogenase (LDH) levels were obtained. Separate samples were taken into gel vacuum tubes without anticoagulants for AMH, leptin and ghrelin measurement. Samples taken were allowed to clot for 30 minutes at room temperature and were centrifuged at 2000xg for 10 minutes. They were stored in capped storage tubes at -20˚C with the condition of keeping for a maximum of 6 months. Each sample was frozen and thawed only once.

Test Procedures

Routine biochemistry tests, hormone tests and whole blood counts were studied on the day which samples were taken.

Routine biochemistry tests such as glucose, AST, ALT, total cholesterol, triglyceride and HDL cholesterol tests were studied on Beckman Coulter AU 5800 (Beckman Coulter Inc., CA, USA) autoanalyzer by spectrophotometric method.

Hormone tests such as FSH, LH, estradiol, TSH, prolactin, progesterone, total testosterone and DHEAS tests were studied with chemiluminescence method on Beckman Coulter DXI 800 analyzer (Beckman Coulter Inc., CA, USA).

Complete blood counts were studied on the Coulter LH 750 hematology analyzer (Beckman Coulter Inc., Miami, FL, USA) with impedance method.

After all frozen serum samples were thawed on another day, the AMH test was run using the Human Mullerian Inhibiting Substance / Anti-Mullerian Hormone ELISA kit (Bioassay Technology Laboratory, China) using the chemiluminescence method on the Beckman Coulter DXI 800 device.

Ghrelin and leptin levels were also tested on a separate day by ELISA method according to appropriate protocols using the Human Ghrelin (GHRL) ELISA Kit and the Human Leptin (Lep) ELISA Kit (Bioassay Technology Laboratory, China) after being thawed. Standard graphics were created.

Statistical Analysis

IBM SPSS Statistics for Windows, Version 25.0 (Released 2017, Armonk, NY: IBM Corp.) software was used for statistical analysis. Data were evaluated as mean and standard deviation and $p < 0.05$ was accepted as statistically significant.

The Chi-Square Test was used to compare categorical variables between the athlete group and the control group. To examine whether numerical variables show normal distribution or not, Shapiro - Wilk Test was applied and parametric methods were preferred in comparisons since there was fit ($p > 0.05$). In order to examine whether there is a difference between the values of the athlete group and the control group, Student's t test was performed.

Both athlete and non-athlete control groups were separated in itself according to their menstrual conditions (regular/irregular). Inter-group differences were accessed by nonparametric Mann-Whitney U test, since the number of participants of each group was below 30.

Spearman correlation test was applied to examine the relations between blood parameters with each other and with menstrual irregularity.

Power Analysis

Power analysis were performed considering the Mann-Whitney U Test for two independent groups, with a type 1 error rate of 0.05 and statistical power of $p(1-\beta) = 0.90$. Under these conditions it was determined that at least 36 participants should be recruited to each group, to find a difference in LARGE-CLASS ($d = 0.8$) effects size between the two groups. For each group five extra participants were also recruited to prevent missing values.

RESULTS

All of the participants were in a normal regular diet and had no history or presence of eating disorder. None of the athletes were doing individual training additional to their team training program, the participants of control group were not doing any regular physical activities.

Compared to the control group, athletes had more menstrual irregularities. However, the difference was not statistically significant ($p=0.10$). While 24 of the female athletes who participated in the study had no menstrual irregularities, 17 had irregularities (41%; 10 oligomenorrhea, 7 amenorrhea). In the control group, while 31 of the women had no irregularities, 10 had (24%; 8 oligomenorrhea, 2 amenorrhea).

In terms of anthropometric features, female athletes were younger, taller and heavier than the non-athlete control group, and their body fat levels were lower. These differences were statistically significant ($p < 0.05$) (**Table 1**). Body structure features were similar in women with and without menstrual irregularities ($p > 0.05$).

Table 1. Comparison of athlete and non-athlete women according to their general characteristics (**=p<0.01)

	Athletes (n=41) (mean ± SD)	Non-athletes (n=41) (mean ± SD)	Student's t test
Age (year)	23.66 ± 5.55	28.83 ± 4.41	p<0.001**
Height (cm)	175.95 ± 8.25	164.37 ± 5.89	p<0.001**
Weight (kg)	67.76 ± 9.01	59.77 ± 8.18	p<0.001**
Body mass index (kg/m²)	21.82 ± 2.00	22.12 ± 2.48	p=0.553
Body Fat Percentage (%)	20.91 ± 4.42	24.67 ± 6.99	p=0.005**

When both groups were compared in terms of blood tests, AST, LDH, CK and HbA1c values were significantly higher, while triglyceride levels were lower in athletes (p <0.05). Other blood tests revealed no statistically significant difference (p> 0.05) (Table 2).

Table 2. Comparison of groups in terms of blood test results (**=p<0.01)

	Reference Ranges	Athletes (n=41) (mean ± SD)	Non-athletes (n=41) (mean ± SD)	Student's t test
Ghrelin (ng/mL)	a*	2.82 ± 1.57	3.52 ± 1.61	p=0.050
Leptin (ng/mL)	a*	15.26 ± 7.28	18.40 ± 7.84	p=0.066
Antimüllerian Hormone (AMH) (ng/mL)	18-25 years: 0.96-13.34 26-30 years : 0.17-7.37 31-35 years: 0.07-7.35	3.83 ± 2.67	3.52 ± 1.98	p=0.547
Leukocyte (10^3/µL)	3.53-9.57	6.30 ± 1.43	22.27 ± 98.25	p=0.301
Erythrocyte (10^6/µL)	3.96-5.50	4.34 ± 0.49	4.53 ± 0.37	p=0.056
Hemoglobin (g/dL)	12.14-16.27	12.60 ± 1.17	12.73 ± 1.26	p=0.843
Hematocrit (%)	35.39-47.19	38.63 ± 3.20	38.53 ± 3.66	p=0.896
Glukoz (mg/dL)	70-105	84.24 ± 9.37	85.46 ± 12.35	p=0.616
Aspartate Aminotransferase (AST) (U/L)	13-38	25.85 ± 10.51	18.63 ± 4.89	p=0.0001**
Alanine Aminotransferase (ALT) (U/L)	7-52	19.70 ± 10.33	15.90 ± 10.38	p=0.100
Cholesterol (mg/dL)	136-190	185.80 ± 30.23	179.70 ± 32.09	p=0.379
Triglycerides (mg/dL)	<150	66.07 ± 20.45	92.68 ± 42.33	p=0.001**
HDL-Cholesterol (mg/dL)	30-85	63.46 ± 12.91	59.34 ± 11.15	p=0.126
FSH (mIU/mL)	Mid follicular phase: 3.85-8.78 Mid luteal phase: 1.79-5.12 Ovulatory peak: 4.54-22.51	6.68 ± 10.12	5.90 ± 2.87	p=0.634
LH (mIU/mL)	Mid follicular phase: 2.12-10.89 Mid luteal phase: 1.20-12.86 Ovulatory peak: 19.18-103.03	6.70 ± 4.66	6.97 ± 5.40	p=0.805
Estradiol (E2) (pg/mL)	Mid follicular phase: 27-122 Mid luteal phase: 49-291 Pre-ovulatory phase: 95-433	124.61 ± 96.21	112.20 ± 79.91	p=0.530
TSH (mIU/mL)	0.38-5.33	2.08 ± 1.06	1.74 ± 0.97	p=0.133
Prolactin (mIU/mL)	3.34-26.72	12.16 ± 4.68	11.67 ± 6.59	p=0.700
Progesterone (ng/mL)	Mid follicular phase: 0.31-1.52 Mid luteal phase: 5.16-18.56	3.98 ± 4.11	2.47 ± 3.78	p=0.087
Testosterone (ng/dL)	10-75	52.14 ± 26.42	53.98 ± 15.85	p=0.703
DHEAS (µg/dL)	18-391	236.49 ± 104.74	254.73 ± 92.42	p=0.406
LDH (U/L)	140-271	189.46 ± 33.33	156.31 ± 22.72	p=0.0001**
ALP (IU/L)	34-104	61.53 ± 17.61	56.97 ± 14.62	p=0.206
Iron (µg/dL)	50-212	88.24 ± 45.23	76.80 ± 36.20	p=0.210
Ferritin (µg/L)	10-158	29.78 ± 25.71	33.23 ± 82.23	p=0.798
CK (U/L)	30-223	253.68 ± 277.74	91.85 ± 60.56	p=0.001**
Cortisol (µg/dL)	6.7-22.6	10.14 ± 3.88	9.05 ± 3.59	p=0.192
Folate (ng/mL)	3.1-19.9	9.04 ± 4.23	8.16 ± 3.39	p=0.298
FT4 (ng/dL)	0.61-1.12	0.85 ± 0.14	0.80 ± 0.10	p=0.095
FT3 (pg/mL)	2.5-3.9	3.05 ± 0.40	3.02 ± 0.42	p=0.704
Insulin (µIU/mL)	1.9-23	8.61 ± 7.15	11.41 ± 8.85	p=0.119
Vitamin B12 (pg/mL)	127-525	268.75 ± 104.81	245.80 ± 104.59	p=0.324
HbA1c (%)	4-6	5.08 ± 0.38	4.78 ± 0.23	p=0.0001**

a* : Reference range is not available.

Ghrelin and leptin levels were lower and AMH values were higher in athletes with menstrual irregularities. However, these differences were not statistically significant (p>0.05). CK levels were higher in athletes with menstrual irregularities (p=0.019). There were no statistically significant differences between athletes with and without menstrual irregularities in terms of other blood parameters (p>0.05) (Table 3).

Table 3. Comparison of athletes with and without menstrual irregularities in terms of blood tests (*= p<0.05).

	Reference Ranges	Athletes (n=41) (mean ± SD)	Athletes with menstrual irregularities (n=17) (mean ± SD)	Athletes without menstrual irregularities (n=24) (mean ± SD)	Mann-Whitney U test
Ghrelin (ng/ml)	a*	2.82 ± 1.57	2.31 ± 1.21	3.19 ± 1.72	p=0.092
Leptin (ng/ml)	a*	15.26 ± 7.28	13.65 ± 6.26	16.45 ± 7.87	p=0.203
Antimüllerian Hormone (AMH) (ng/ml)	18-25 years: 0.96-13.34 26-30 years : 0.17-7.37 31-35 years: 0.07-7.35	3.83 ± 2.67	4.12 ± 3.15	3.63 ± 2.32	p=0.781
Leukocyte (10^3/µl)	3.53-9.57	6.30 ± 1.43	6.65 ± 1.51	6.04 ± 1.35	p=0.204
Erythrocyte (10^6/µl)	3.96-5.50	4.34 ± 0.49	4.35 ± 0.23	4.34 ± 0.62	p=0.284
Hemoglobin (g/dl)	12.14-16.27	12.60 ± 1.17	12.50 ± 0.74	12.80 ± 1.40	p=0.109
Hematocrit (%)	35.39-47.19	38.63 ± 3.20	37.95 ± 1.83	39.12 ± 3.87	p=0.072
Glukoz (mg/dl)	70-105	84.24 ± 9.37	85.88 ± 9.07	83.08 ± 9.60	p=0.347
Aspartate Aminotransferase (AST) (U/L)	13-38	25.85 ± 10.51	25.29 ± 6.28	26.25 ± 12.82	p=0.449
Alanine Aminotransferase (ALT) (U/L)	7-52	19.70 ± 10.33	19.00 ± 6.43	20.20 ± 12.50	p=0.577
Cholesterol (mg/dl)	136-190	185.80 ± 30.23	194.00 ± 30.98	180.00 ± 28.93	p=0.173
Triglycerides (mg/dl)	<150	66.07 ± 20.45	64.70 ± 19.35	67.04 ± 21.56	p=0.682
HDL-Cholesterol (mg/dl)	30-85	63.46 ± 12.91	66.58 ± 14.43	61.25 ± 11.52	p=0.278
FSH (mIU/ml)	Mid follicular phase: 3.85-8.78 Mid luteal phase: 1.79-5.12 Ovulatory peak: 4.54-22.51	6.68 ± 10.12	8.50 ± 15.57	5.40 ± 2.31	p=0.682
LH (mIU/ml)	Mid follicular phase: 2.12-10.89 Mid luteal phase: 1.20-12.86 Ovulatory peak: 19.18-103.03	6.70 ± 4.66	7.03 ± 5.25	6.46 ± 4.29	p=1.00
Estradiol (E2) (pg/ml)	Mid follicular phase: 27-122 Mid luteal phase: 49-291 Pre-ovulatory phase: 95-433	124.61 ± 96.21	137.76 ± 128.09	115.29 ± 66.84	p=0.814
TSH (mIU/ml)	0.38-5.33	2.08 ± 1.06	1.85 ± 1.06	2.25 ± 1.06	p=0.234
Prolactin (mIU/ml)	3.34-26.72	12.16 ± 4.68	13.12 ± 4.93	11.48 ± 4.48	p=0.296
Progesterone (ng/ml)	Mid follicular phase: 0.31-1.52 Mid luteal phase: 5.16-18.56	3.98 ± 4.11	3.11 ± 3.36	4.60 ± 4.53	p=0.435
Testosterone (ng/ml)	10-75	52.14 ± 26.42	48.12 ± 25.92	54.98 ± 26.95	p=0.296
DHEAS (µg/dl)	18-391	236.49 ± 104.74	221.77 ± 90.90	246.92 ± 114.27	p=0.443
LDH (U/L)	140-271	189.46 ± 33.33	195.41 ± 27.88	185.25 ± 36.69	p=0.106
ALP (IU/L)	34-104	61.53 ± 17.61	66.70 ± 21.33	57.87 ± 13.75	p=0.208
Iron (µg/dl)	50-212	88.24 ± 45.23	91.82 ± 53.83	85.70 ± 39.08	p=0.874
Ferritin (µg/L)	10-158	29.78 ± 25.71	36.82 ± 33.35	24.79 ± 17.70	p=0.361
CK (U/L)	30-223	253.68 ± 277.74	279.70 ± 161.13	235.25 ± 339.46	p=0.019*
Cortisol (µg/dl)	6.7-22.6	10.14 ± 3.88	10.75 ± 3.96	9.70 ± 3.85	p=0.451
Folate (ng/ml)	3.1-19.9	9.04 ± 4.23	9.83 ± 4.32	8.49 ± 4.17	p=0.153

Table 3. Comparison of athletes with and without menstrual irregularities in terms of blood tests (*= p<0.05).

	Reference Ranges	Athletes (n=41) (mean ± SD)	Athletes with menstrual irregularities (n=17) (mean ± SD)	Athletes without menstrual irregularities (n=24) (mean ± SD)	Mann-Whitney U test
FT4 (ng/dl)	0.61-1.12	0.85 ± 0.14	0.85 ± 0.13	0.85 ± 0.15	p=0.731
FT3 (pg/ml)	2.5-3.9	3.05 ± 0.40	3.14 ± 0.44	2.99 ± 0.36	p=0.283
Insulin (IU/ml)	1.9-23	8.61 ± 7.15	9.87 ± 8.22	7.72 ± 6.32	p=0.427
Vitamin B12 (pg/ml)	127-525	268.75 ± 104.81	256.88 ± 101.13	277.16 ± 108.69	p=0.375
HbA1c (%)	4-6	5.08 ± 0.38	5.11 ± 0.44	5.07 ± 0.34	p=0.622

Similar to the athlete group, the blood values were not statistically significantly different between women with and without menstrual irregularities in the control group (p> 0.05).

There was only a statistically significant negative correlation between ghrelin and menstrual irregularity (r=-0.240, p=0.031). Additionally, a statistically significant positive correlation was found between AMH and testosterone levels (r=0.247, p=0.025). There was no significant relationship between other parameters (p> 0.05).

DISCUSSION

Menstrual dysfunctions were higher in athletes compared to the non-athlete controls (41%, 24%, respectively). Body fat levels were lower in athletes. In both groups, leptin, ghrelin and AMH levels were found to be similar in women with menstrual dysfunction.

Athletes had similar body composition and age characteristics who had menstrual dysfunctions and regular periods. This shows that the features such as body fat level and age have no effect on menstrual functions. Athletes who were trying to improve their physical performance through strict dietary restrictions or strenuous exercises that cause weight loss have been found to have higher levels of amenorrhea or oligomenorrhea (16,17). It was reported that menstrual dysfunction in runners and ballet dancers occurred between 65 to 69% (18,19), and approximately 45% in female athletes playing volleyball and basketball (20). In this study, the rate of menstrual dysfunction has been depicted as 41%. Unlike long distance running, basketball and volleyball are intermittent sports, and athletes have enough time to rest between periods of intense activities. In addition, basketball and volleyball players are more muscular and heavier than long distance runners and ballet dancers (21). Unlike runners and ballet dancers, team sports athletes do not have strict nutritional restrictions, so usually there is no significant negative energy balance. This may explain the lower frequency of menstrual dysfunction in basketball and volleyball players. In these athletes, menstrual disorders may result from very intense, strenuous training programs and long-term psychological stress.

Intense physical stress and energy imbalance cause activation of the hypothalamic - pituitary - adrenal axis and high cortisol levels. It is suggested that cortisol causes amenorrhea by disrupting GnRH release and LH's normal pulsatile release (22). Although low FSH and LH levels are seen in cases of hypogonadothropic hypogonadism, no statistically significant difference was found between the groups in terms of FSH, LH and estrogen levels in our study. In insufficient energy intake, metabolic and endocrine adaptations occur to conserve energy. These adaptations include lower T3 (23, insulin (24), IGF-1 (25) and leptin (7) levels, increased cortisol (2) and ghrelin levels (12). In our study, the levels of metabolic and endocrine parameters such as insulin, glucose, leptin, ghrelin, T3 and cortisol were similar among athletes with and without menstrual dysfunction. Also considering that none of the participants were in a calorie restriction diet, these findings indicated that the energy balance was not impaired in basketball and volleyball players in this study. If training does not cause a deterioration in energy balance, it may not have harmful effects on female reproductive functions by itself.

The regulators of homeostatic mechanism for energy intake; leptin and ghrelin can affect the mechanisms regulating the reproductive function. Low levels of leptin have been shown to cause impairment of menstrual function (5). Leptin levels in circulation generally decrease with exercise. The response of these hormones appears to be influenced by exercise intensity, type, and duration. Therefore, different results were reported in various studies (6,26). These results support the importance of the link between energy consumption and menstrual disorders. Athletes with menstrual dysfunctions showed no statistically significant difference compared to athletes with regular menstruations, although their leptin levels were lower. This result showed that although these athletes were training intensely, the energy balance has not been adversely affected.

In most studies investigating the effect of exercise on ghrelin level, it has been found that ghrelin level increases in response to weight loss (27). High ghrelin levels have been shown to suppress hypothalamic GnRH release and pulsatile LH release and cause amenorrhea in young athletes (28). De Souza et al. reported that ghrelin levels increase in pe-

ghrelin levels may help to explain that the impact of sports on menstrual function was less than expected in this study.

Serum Antimullerian hormone (AMH) is one of the most sensitive markers used to evaluate ovarian reserve. AMH levels are high in women who have menstrual dysfunction. The increase in serum AMH level can be used as an ovarian reserve marker of menstrual dysfunction (13,30). , Abbara A et al. found that serum AMH level was higher in women with menstrual disorder and the risk of menstrual disorder increased with increment in AMH level. They also showed that AMH level was more effective in distinguishing women with menstrual disorder than the number of antral follicles(30). In our study, the level of AMH in athletes with menstrual irregularities was higher, although not statistically significant compared to athletes with normal menstrual functions. Considering the findings from our study and previous studies, we can say that serum AMH level may provide valuable information in the diagnosis and follow-up of menstrual dysfunctions that may occur in athletes.

There are limitations for this study. First, the number of participants is relatively small compared to previous studies examining the relationship between AMH and lifestyle and menstrual cycle characteristics.

Second limitation is the lack of calculation of the daily energy expenditure. Athletes with BMI lower than 18.5kg/m^2 are shown to have higher risk for menstrual dysfunction (31). All of the participants of this study had BMI's higher than 18.5kg/m^2 and none were in a calorie restriction diet. Therefore, we assumed that none of the participants had negative energy balance.

Third limitation is that physical characteristics were not similar between the groups. In our study, the athlete group consisted of volleyball and basketball players. These individuals are known to be taller and heavier than the normal population. On the other hand, the non-athlete control group was representing the normal population.

CONCLUSION

Although there was no statistically significant difference, frequency of menstrual irregularities were higher in athletes playing basketball and volleyball than non-athlete group. AMH levels were higher in athletes with menstrual irregularities. However, it is necessary to conduct extensive studies in order to investigate whether AMH level can be used as a marker in determining athletic amenorrhea.

Ethics Committee Approval / Etik Komite Onayı

The approval for this study was obtained from Institutional Ethics Committee of Ege University, İzmir, Turkey (Decision no: E.248047 Date: 03.10.2017).

Conflict of Interest / Çıkar Çatışması

The authors declared no conflicts of interest with respect to authorship and/or publication of the article.

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Author Contributions / Yazar Katkıları

Concept All authors; Design All authors; Supervision MCT, BY; Materials AB, SS, OK; Data Collection and/or Processing SS, AB, OK, PB, OKG; Analysis and Interpretation PB, OKG; Literature Review SS, OK; Writing Manuscript SS, OK; Critical Reviews MCT, BY.

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