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The Effect of Resistance Training in Water and Land with Vitamin D Supplementation on Anti-Mullerian Hormone in Women with Polycystic Ovary Syndrome

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Abstract

Objectives: This study aimed to investigate the effect of resistance training in water and land with vitamin D on anti-Mullerian hormone in women with polycystic ovary syndrome (PCOS).

Methods: Sixty women with PCOS (20 - 35 years old) referred to the Hafez Hospital in Shiraz in 2018 were selected and randomly divided into six groups of (1) control, (2) water training, (3) land training, (4) vitamin D, (5) water training with vitamin D, and (6) land training with vitamin D. Groups 2, 3, 5, and 6 performed resistance training in water and land for eight weeks, three sessions per week, while groups 4, 5 and 6 consumed vitamin D for eight weeks. For statistical analysis of data independent samples *t*-test, one-way ANOVA and Tukey's post hoc tests were used ($P \leq 0.05$).

Results: Training in water and land have no significant effect on anti-Mullerian hormone ($P \geq 0.05$); vitamin D, training in water with vitamin D, and training in land with vitamin D have significant effect on reduction of anti-Mullerian hormone ($P = 0.001$); training in water with vitamin D and training in land with vitamin D have more effect on reduction of anti-Mullerian hormone rather than vitamin D ($P \leq 0.05$) and after training in water with vitamin D ($P = 0.01$) and training in land with vitamin D ($P = 0.001$) there is a significant relationship between anti-Mullerian hormone changes and weight.

Conclusions: It appears that resistance training in water and land combined with vitamin D consumption may decrease the anti-Mullerian hormone by reduction in weight and as a result improve ovarian and reproductive function in women with PCOS.

Keywords: Fertility Disorder, Anti-Mullerian, Training, Vitamin D

1. Background

Polycystic ovarian syndrome (PCOS) is the most important cause of lack or absence of ovulation in infertile women, and about 6% - 20% of women in reproductive age develop it (1). This complication was initially recognized by increased androgen adrenal and ovarian secretion, irregular menstruation, fibrous and enlarged ovaries, increased primary and pre-antral follicles, disturbances in the formation of dominant follicles and hirsutism (2). However, today it is characterized as a disorder with multiple causes and metabolic consequences that increase the incidence of metabolic syndrome (3). Increase in the levels of androgens and intrinsic increase in the number of follicles in women with PCOS escalate the production of anti-Mullerian hormone. The amount of anti-Mullerian hormone indicates the size of the growing follicles and the function of the ovarian reserves in women. Studies have also shown that vitamin D in the form of 2D (OH) 1,25 is a steroid hormone that plays a moderating role in the reproductive stages of men and women, and its deficiency is an important factor in the pathogenesis of metabolic syndrome in women with PCOS (4). Since PCOS treatment involves both pharmacological and non-pharmacological therapies including lifestyle changes, weight loss, proper diet and dietary supplements, it seems that using proper diet and supplementation can help improve this condition. Some studies on the effects of lifestyle interventions including diet, exercise and behavioral changes in overweight infertile women with or without PCOS, showed significant improvement in the rate of ovulation, regular menstruation and decreased risk of abortion compared with pre-intervention (5). However, the effect of different types of exercise activity on the level of anti-Mullerian hormone in PCOS women is controversial. In this regard, a significant decrease (6, 7) and no change (8, 9) in the amount

of anti-Mullerian hormone in PCOS women after various sports activities have been reported. Exercise in water is one of the methods of motion therapy that has recently been taken up by sports science experts and is becoming increasingly widespread. Research has shown that various types of exercise activities (aerobic exercise activity versus resistance, land-based activity, and water-based activities in the present research and some previously-conducted research) have different benefits for the body (10). It seems that the anti-Mullerian hormone changes depend on the type, intensity, and duration of the exercise. The use of new therapeutic approaches in the field of exercise for health and well-being in PCOS women is necessary.

2. Objectives

Regarding the above-mentioned, the present study aimed to investigate the effect of resistance training in water and land with vitamin D supplementation on anti-Mullerian hormone levels in women with PCOS.

3. Methods

In this quasi-experimental study, was performed among women aged between 20 - 35 years referring to the Infertility Department of Hafez Hospital. At first, 83 women with PCOS volunteered to participate in the study. Among them, 70 women with PCOS, after their diagnosis and examination, were selected by a gynecologist at Motahari Hospital in Shiraz based on inclusion criteria. Then, according to Morgan's sampling table, 60 women were selected as statistical sample. Inclusion criteria included having at least two criteria of the three criteria of PCOS (these three criteria were: (1) low ovulation or non-ovulation (usually expressed as oligomenorrhoea, amenorrhea, polyamorphic), (2) increased blood circulation of androgens, hirsutism with ratio of FSH/LH > 2, and (3) polycystic ovaries (seen in ultrasonography). In addition, other criteria included not having metabolic diseases, malignancy, endocrine disorders and not using drugs that interfere with the level of anti-Mullerian hormone, as well as not having heart disease, kidney and liver disease or diabetes and not consuming alcohol, cigarettes, and medications such as contraceptives, progesterone conjugate, metformin, clomiphene citrate, gonadotropin and troglitazone during the past three months, and not exercising during the last three months. Exclusion criteria included the presence of adrenal enzyme disorders, suspected ovarian tumors or very large cysts in the ovary. To evaluate the morphology of the ovaries, subjects were observed at the early follicular phase (2nd to 4th day of menstruation) under ultrasound of the womb and appendages by a radiologist. Subjects were taken to the lab 48 hours before the intervention and pre-rest blood samples were collected. After measuring the demographic characteristics, subjects were divided into six groups of 10 including (1) control, (2) water training, (3) land training, (4) vitamin D, (5) water training with vitamin D, and (6) land training with vitamin D. Groups 2, 3, 5, and 6 performed resistance training for eight weeks, three sessions per week, also groups 4, 5 and 6 consumed vitamin D3 supplement for eight weeks, which was dosed by the physician and was used based on the nature and severity of vitamin D deficiency in subjects. Each session of land resistance training included 15 minutes of warming up in the form of static stretching movements, then dynamic movements, resistance trainings with weights (first sessions began with 30 minutes and 40% of a maximal repetition, and the intensity of the trainings per week was increased to 70% of a maximal repetition in the final sessions) and 5 minutes of cooling (tensile movements) at the end of each training session. Resistance training sessions in water were divided into three parts: (1) warming, which lasted about 5 to 15 minutes, and generally included warming the joints and focusing on muscles that were under dynamic working power, including walking and running in the water; (2) the main part, involving trunk strength training, upper trunk and lower trunk with dumbbell (3 sets, 12 repetitions) for 60 minutes, and (3) the final part consisting of 15 minutes of cooling (walking and Jacuzzi) (11). Blood samples were collected 48 hours before the intervention and 48 hours after the last training session. The serum level of anti-Mullerian hormone was then measured by ELISA using the French Beckman kit (ng/mL). It is worth mentioning that regarding the subjects at the end of the study, two drops in control group, two drops in training in land conditions, one drop in vitamin D consumption, and one drop in training in land with vitamin D suplementation took place. To analyze the findings, Kolmogorov-Smirnov test, Pearson correlation coefficient, paired sample *t*-test and one-way ANOVA test along with Tukey's test were used ($P \leq 0.05$).

4. Results

The demographic characteristics and levels of anti-Mullerian hormone along with the body mass index (BMI) of the subjects are presented in Tables 1 and 2, respectively. The results of paired sample *t*-test in Table 2 show that there is no significant difference in the pre-test (12.65 ± 1.23) and post-test (12.41 ± 0.94) levels of anti-Mullerian hormone in the control group ($P = 0.16$). However, the levels of anti-Mullerian hormone in water training group ($P = 0.001$) (pre-test: 12.32 ± 1.15 and post-test: 11.97 ± 1.15), land training group ($P = 0.001$) (pre-test: 10.67 ± 0.75 and

10.40 ± 0.77), vitamin D (P = 0.001) (pre-test: 11.85 ± 1.52 and 9.71 ± 1.12), water training with vitamin D (P = 0.001) (pre-test: 12.02 ± 1.51 and post-test: 7.44 ± 0.62) and land training with vitamin D (P = 0.001) (pre-test: 11.91 ± 1.45 and post-test: 7.47 ± 0.65) in the post-test were significantly decreased compared to the pre-test. Also, the results of paired sample *t*-test showed that in the control group BMI increased significantly in the post-test (26.90 ± 0.97) compared to the pre-test (P = 0.001) (26.70 ± 0.99), however, the BMI of water training (P = 0.004) (pre-test: 27.11 ± 0.74 and post-test: 26.82 ± 0.81), land training (P = 0.001) (pre-test: 27.01 ± 1.15 and post-test: 26.54 ± 1.03), vitamin D (P = 0.001) (pre-test: 26.71 ± 0.91 and post-test: 26.49 ± 0.90), water training with vitamin D (P = 0.001) (pre-test: 27.72 ± 1.47 and post-test: 26.69 ± 1.22) and land training with vitamin D (P = 0.001) (pre-test: 27.86 ± 1.54 and post-test: 27.41 ± 1.57) groups in the post-test was significantly decreased compared to the pre-test. The results of one-way ANOVA test (to review the changes of the pre-test to the post-test) in Table 2 showed that there was a significant difference in the level of anti-Mullerian hormone changes and BMI in the six groups of the study (P = 0.001). Results of Tukey's post hoc test in Table 3 showed that anti-Mullerian hormone levels in vitamin D, water training with vitamin D, and land training with vitamin D groups were significantly reduced compared to the control group (P = 0.001); in the vitamin D, water training with vitamin D and land training with vitamin D decreased significantly compared to the water training (P = 0.001); in the vitamin D, water training with vitamin D and land training with vitamin D decreased significantly compared to the land training (P = 0.001); in water training with vitamin D and land training with vitamin D was significantly lower than vitamin D (P = 0.001). However, there was no significant difference in anti-Mullerian hormone levels between the control group and water training (P = 0.99) and land training (P = 0.99), between water training and land training (P = 0.99), and between water training with vitamin D and land training with vitamin D (P = 0.99). Also, the results of Tukey's post hoc test showed that BMI levels in land training (P = 0.001), water training (P = 0.001), vitamin D (P = 0.004), water training with vitamin D (P = 0.001) and land training with vitamin D (P = 0.001) decreased significantly compared to control group. Furthermore, in water trainig with vitamin D compared to water training (P = 0.001), land training (P = 0.001), vitamin D (P = 0.001) and land training with vitamin D (P = 0.001) significantly decreased. However, there was no significant difference in BMI changes between water training and land training (P = 0.56) and vitamin D (P = 0.97), between land training with vitamin D (P = 0.20) and water training with vitamin D (P = 0.99) and between vitamin D and land training with vitamin D (P = 0.23). The results of Pearson correlation coefficient test in Table 4 show that there is a significant correlation between anti-Mullerian hormone changes and weight in water training with vitamin D (P = 0.01) and land training with vitamin D (P = 0.001). However, there was no significant correlation between anti-Mullerian hormone changes and weight in control (P = 0.43), water training (P = 0.69), land training (P = 0.74) and vitamin D (P = 0.98) (Table 4).

Table 1. Demographic Characteristics of Subjects in the Six Groups of Research at the Beginning of the Research Period

Group	Age, y	Height, cm	Weight, kg
Control	29.23 ± 2.11	157.78 ± 1.47	66.48 ± 1.76
Water training	31.12 ± 2.42	158.64 ± 1.23	68.25 ± 1.84
Land training	30.01 ± 1.70	160.39 ± 1.54	69.55 ± 1.05
Vitamin D	28.56 ± 1.55	159.04 ± 1.63	67.63 ± 1.97
Water training with vitamin D	29.43 ± 2.73	157.19 ± 1.07	68.37 ± 1.92
Land training with vitamin D	30.21 ± 1.65	158.31 ± 1.03	69.64 ± 1.35

5. Discussion

The results of this study showed that the levels of anti-Mullerian hormone in water training, land training, vitamin D, water trainig with vitamin D and land trainig with vitamin D were significantly reduced. However, there was no significant difference in the levels of anti-Mullerian hormone between water trainig and land training; between water training and vitamin D as well as between land training and vitamin D. In many studies, the beneficial effects of participating in sports programs on various aspects of PCOS women have been studied. However, information on the effects of physical activity on reproductive status and especially anti-Mullerian hormone in PCOS women is limited. The results of the present study showed that the anti-Mullerian hormone levels decreased following resistance training in water and land, which are consistent with the findings of other studies (6, 7). Researches have shown that the increase and reduction of anti-Mullerian hormone has a significant effect on fertility, because in people with polycystic syndrome, the level of this hormone is high, but in patients with repeated abortions, the level of this hormone is low. In this regard, Pellatt and other colleagues reported higher levels of anti-Mullerian hormone in PCOS women compared to women with healthy ovaries, due to an increase in the number of antral follicles and increased production of this hormone by each follicle (12). Nontheless, the cause of increasing the production of anti-Mullerien hormone in PCOS women is not clear, but it is likely that the concentration of intra-ovarian androgens is a determining factor, because studies have shown correlation be-

Table 2. Results of Paired *t*-Test and One Way ANOVA to Assess the Pre-Test and Post-Test Changes of Anti-Mullerian Hormone and Body Mass Index in the Subjects of the Six Study Groups

Variable	Measurement Time[a]		Paired Sample *t*-Test		F Test[b]	
	Pre-Test	Post-Test	P Value	*t*	F	P Value
Anti-Mullerian, ng/mL					112.65	0.001*
Control	1.23 ± 12.65	0.94 ± 12.41	0.16	-1.54		
Water training	1.15 ± 12.32	11.97 ± 1.15	0.001*	-8.30		
Land training	0.75 ± 10.67	0.77 ± 10.40	0.001*	-9.79		
Vitamin D	1.52 ± 11.85	1.12 ± 9.71	0.001*	-13.27		
Water training with vitamin D	1.51 ± 12.02	0.62 ± 7.44	0.001*	-15.65		
Land training with vitamin D	1.45 ± 11.91	0.65 ± 7.47	0.001*	-15.83		
BMI, kg/m^2					29.05	0.001*
Control	0.99 ± 26.70	0.97 ± 26.90	0.004*	20.02		
Water training	0.74 ± 27.11	0.81 ± 26.82	0.001*	-3.77		
Land training	1.15 ± 27.01	1.03 ± 26.54	0.001*	-6.64		
Vitamin D	0.91 ± 26.71	0.90 ± 26.49	0.001*	-9.35		
Water training with vitamin D	1.47 ± 27.72	1.22 ± 26.69	0.001*	-8.86		
Land training with vitamin D	1.54 ± 27.86	1.57 ± 27.41	0.001*	-6.45		

[a] Values are expressed as mean ± SD.
[b] F test for comparing the changes of post-test and pre-test.

tween the levels of anti-Mullerian hormone and androgen (13). The resulting mechanism is likely to include improvements in insulin resistance and hyperandrogenism, which by reducing the stimulation of follicular growth and consequently reducing their number, decreases the anti-Mullerian hormone (14). However, the results of some investigations are contrary to the results of this research (8, 9). The reason for lack of changes in serum levels of anti-Mullerian hormone in patients with polycystic ovarian syndrome in the study of Saremi and other colleagues (6) was reported to be high levels of anti-Mullerian hormone; also the researchers stated that it is possible that the intensity of training for making a change in the level of anti-Mullerian hormone has not be sufficient. Although based on the findings of the present study, the mechanism for reducing anti-Mullerian hormone following training in water and land is not clear, it seems that improvement in cardiovascular function as well as increase in muscular mass as a result of resistance training reduce the levels of sex steroids and may also reduce the secretion of the anti-Mullerian hormone.

The results of this study showed that the anti-Mullerian levels of groups with vitamin D significantly decreased. Regarding the insulin resistance effect on increasing levels of androgens and, as a result of the disappearance of growing follicles in PCOS patients, treatment with vitamin D is likely to improve metabolic rate, reduce androgen levels and even improve ovulation in people with PCOS (15). Vitamin D has inhibitory effects on the level of anti-Mullerian hormone in the ovary and by decreasing the rate of this factor, induces ovulation and improves the histological quality of the polycystic ovary and leads them to healthy and active ovaries. Studies have shown that following vitamin D deficiency, a decrease in the intracellular calcium serum concentration as well as abnormal oocyte function have been obseved (16). Regarding the above-mentioned, resistance training on land and water along with vitamin D supplementation by decreasing BMI increases ovulation. Therefore, it is hoped to treat polycystic ovary syndrome and thereby help to treat infertility with taking advantage of appropriate nutritional supplements. Finally, the results showed that body mass index levels of water training, land training, vitamin D consumption, water training with vitamin D supplementation and land training with vitamin D supplementation decreased significantly which was consistent with the results of different studies in this regard (17, 18). However, the results did not match the results of Randeva and other colleagues (19), which can be attributed to the difference in the rate of primary body mass index of the subjects and the population studied. The lack of access to subjects with the same vitamin D deficiency, small sample, lack of record of food intake by subjects and the cross-sectional nature of this study are among the limitations of this research; so as the small sample and food in-

Table 3. Results of Tukey's Post Hoc Test to Compare Anti-Mullerian Hormone and Body Mass Index Between Six Groups of Research

Group	BMI		Anti-Mullerian	
	Mean Differences	P Value	Mean Differences	P Value
Control				
Water training	0.50	0.001*	0.11	0.99
Land training	0.67	0.001*	0.02	0.99
Vitamin D	0.42	0.004*	1.91	0.001*
Water training with vitamin D	1.23	0.001*	4.34	0.001*
Land training with vitamin D	0.66	0.001*	4.20	0.001*
Water training				
Land training	0.17	0.56	-0.08	0.99
Vitamin D	0.07	0.97	1.80	0.001*
Water training with vitamin D	0.73	0.001*	4.23	0.001*
Land training with vitamin D	0.16	0.62	4.09	0.001*
Land training				
Vitamin D	0.25	0.20	1.88	0.001*
Water training with vitamin D	0.55	0.001*	4.31	0.001*
Land training with vitamin D	0.01	0.99	4.17	0.001*
Vitamin D				
Water training with vitamin D	0.80	0.001*	2.43	0.001*
Land training with vitamin D	0.23	0.23	2.29	0.001*
Water training with vitamin D				
Land training with vitamin D	-0.56	0.001*	-0.13	0.99

Table 4. Results of Pearson Correlation Coefficient to Determine the Relationship Between Anti-Mullerian and Weight Changes in the Six Groups of Study

Group	Correlation Coefficient	
	r	P Value
Control	0.32	0.43
Water training	-0.14	0.69
Land training	0.13	0.74
Vitamin D	-0.007	0.98
Water training with vitamin D	0.74	0.01*
Land training with vitamin D	0.89	0.001*

take can affect the results of research, it suggested that in future studies use high statistical sample and control the dietary intake among subjects.

5.1. Conclusions

Considering the findings of the research, it appears that resistance training in water and land combined with vitamin D consumption may decrease the anti-Mullerian hormone level and as a result, improve ovarian and reproductive function in women with fertility disorders. Therefore, it is recommended that these patients benefit from new therapeutic approaches in sport along with proper dietary supplements for their health and well-being.

Footnotes

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