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Effects of 60%-70%F.C. Exercise Intensity on Plasma Vaspin and Related Indexes of Overweight and Obese College Students

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Abstract:

Objective: To investigate the effect of 60%-70%F.C. exercise intensity on plasma Vaspin and related indexes of overweight and obese college students. Methods: The subjects were all overweight and obese college students, including 9 males and 13 females. Body composition and exercise ability tests were measured, weight loss goals and exercise programs were determined for each individual, and the intensity of exercise was set as F.C. 60%-70%, RPE between 13 and 15, Exercise for 60 minutes, five times a week, measured 8 weeks before and after the relevant indicators. Results: After 8 weeks of exercise intervention, the body weight of male and female groups decreased from 73.02kg and 66.68kg to 68.05kg and 61.84kg, WHR decreased from 0.87 to 0.83, and subcutaneous adipose layer thickness was significantly improved (P<0.01). TC in female group decreased from 3.76mmol/L to 3.38mmol/L (P<0.05), HDL increased from 1.31 mmol/L to 1.58 mmol/L (P<0.05), and HDL/LDL increased from 0.67 mmol/L to 0.85 mmol/L (P<0.01). The level of Vaspin in male and female groups increased from 4.88 ng/ml and 5.48 ng/ml to 5.42ng/ml and 6.01ng/ml, but the difference was not statistically significant (P>0.05). There was no significant correlation between Vaspin level and obesity-related indexes in male and female groups (P>0.05). Conclusion: In the absence of diet control, the exercise prescription of 60%-70%F.C. exercise intensity for 8 weeks can significantly improve the weight, WHR and subcutaneous fat layer thickness of overweight/obese male and female college students, and the effect of exercise weight loss is obvious. The intervention had no significant effect on blood lipid and plasma Vaspin levels.

Keywords*: Exercise intensity, Overweight and obesity, Students, Subcutaneous fat, Plasma Vaspin.*

I. INTRODUCTION

In recent years, the obesity problem has spread rapidly around the world. According to the research by Wang Xiaohong et al. [1], the prevalence of abdominal obesity among adolescent

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boys and girls in China increased from 4.1% and 6.3% to 20.6% and 21.0% respectively from 1993 to 2015. Adolescent obesity has been regarded as a public health problem to be solved urgently, because it may cause the health burden in adulthood and may increase the level of carotid intima-media thickness and the detection rate of carotid intima-media thickness thickening in adolescents[2]. Moreover, effective measures should be taken to prevent and control adolescent obesity, because it will not only cause metabolic diseases and psychological problems among adolescents, but also cause future complications[3].

Adipose tissue is considered as an endocrine organ that regulates energy balance by releasing bioactive factors. These substances secreted by adipose tissues are called adipokines[4], which may be involved in the regulation of glycolipid metabolism, inflammation, blood pressure and atherosclerosis, and also serve as the intermediate substance connecting obesity with metabolic syndrome[5]. Visceral adipose tissue-derived serineprotease inhibitor (Vaspin), one of the recently identified adipokines, plays a bridging role in the improvement of metabolic diseases such as obesity, and better controls the plasma Vaspin level of mice, thereby further improving glucose tolerance and insulin sensitivity and reducing calorie intake[6-7], which has been the focus of research in recent years. Weight loss by exercise is a kind of sports prescription that has been known by the public[8] and formulated in accordance with the personal physical condition as a scientific, quantitative and periodic weight loss exercise plan. The mechanism of action of exercise on Vaspin in obese people is still uncertain, and whether to fight obesity by regulating/inducing or inhibiting the concentration of Vaspin needs further verification. In this study, the exercise prescription with specific exercise intensity was used to intervene overweight and obese college students, and the effect of exercise intensity on obesity-related indicators and plasma Vaspin was explored, which can provide certain reference for effectively improving body shape, blood lipid changes and good healthy weight loss.

II. RESEARCH OBJECTS AND METHODS

2.1 Research Objects

A total of 74 subjects (aged 17–22) with a body mass index (BMI) calculated based on height and weight [weight (kg)/ height ^{2} (m)]>24 and according to the overweight registration form filled out by the subjects were selected from the freshmen and sophomores in Anhui Normal University (except students majoring in music, sports and fine arts). Their height, weight and body composition were re-measured in the laboratory. Experimental subjects were simple overweight and obese students, without any weight loss behavior or medication related to weight loss. Besides, their answers on the Physical Activity Readiness Questionnaire

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(PAR-Q) were all "No", with no contraindications to exercise and low risk of exercise risk assessment. After the researchers explained the nature, purpose, possible benefits and risks of the experiment to the subjects according to the ethical requirements, 30 students, including 13 males and 17 females, were finally willing to accept the experiment and signed the informed consent form. In this experiment, the related indexes before and after exercise were compared, with no control group. In the 8-week exercise intervention, 22 subjects (9 males and 13 females) completed the experiment. Specific conditions are shown in Table I:

TABLE I. Baseline measures in the males and females two group (X±SD)

Gender	Number of people	Age (years old)	Height (cm)	Weight (kg)	BMI （kg/m^2）	FAT%
Male	9	19.97±1.27	168.7±0.06	73.02±6.35	25.64±1.42	23.89±1.46
Female	13	20.71±1.12	160.7±0.05	66.68±6.92	25.95±2.55	34.54±4.10

2.2 Research methods

2.2.1 Questionnaire

A questionnaire about obesity was designed before exercise, covering (1) Basic information: The basic information of the students (age, grade, height, and weight). (2) Dietary and exercise habits (whether or not you like a high-fat and high-sugar diet; whether to do physical exercise; if so, how often do physical exercise, etc.). Twenty-five students were randomly selected from the primary candidates to fill in the questionnaire, and the questionnaires were filled out repeatedly as required after one month. Six experts in exercise prescription and physical activity evaluated the validity of the two questionnaires, and the average score was 95 points. The reliability coefficient of the two questionnaires was 0.45, indicating that the reliability of the questionnaire was good.

2.2.2 Experimental design

A questionnaire about obesity was designed before exercise →"PAR-Q" questionnaire was used for investigation to assess exercise risk →Experimental informed consent form was signed →Body shape and body composition were measured→Blood lipid and plasma Vaspin were

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measured → Exercise function and exercise ability were tested→Functional Capacity (F.C.) was tested→Exercise prescription of 60%-70% F.C. exercise intensity was formulated→Eight weeks of this exercise prescription was implemented→The corresponding indexes after implementation were determined.

2.2.3 Main test indexes and test methods

2.2.3.1 Measurement of body composition

The body composition was measured using a Biospace Inbody3.0/ Korea. Test method: The subject stood barefoot on the foot electrode appropriately and grasped the specified position with both hands for measurement. In order to ensure the accuracy and effectiveness of the experimental data, the relevant operators were trained before the experimental detection, and the experimental test instruments were subjected to multiple correction pre-tests. The experimental objects were tested by the same tester, instrument and time node during the whole process. The main indicators collected in the body composition test were FAT%, waist-to-hip ratio, outer diameter of upper arm, inner diameter of upper arm, outer diameter of chest, inner diameter of chest, outer diameter of abdomen, inner diameter of abdomen, outer diameter of thigh and inner diameter of thigh.

2.2.3.2 Measurement of blood biochemical indexes

Before blood collection, subjects were deprived of food for 12 hours and water for 6 hours. Professional blood collectors from Yijishan Hospital of Wuhu City were invited to collect the blood of the subjects using disposable sterile blood collection equipment and the data were measured using Hitachi 7600 automatic biochemical analyzer from Japan. The main collected indicators were total cholesterol (TC), triglyceride (TG), high density lipoprotein cholesterol (HDL-C) and low density lipoprotein cholesterol (LDL-C).

2.2.3.3 F.C.

The German professional exercise treadmill (h/p/cosmos) was used to measure F.C., and the Bruce-Protocol exercise treadmill design was adopted in the plan, with a total of seven grades, 3min/ grade. The slope and speed in the experiment were continuously increased, until F.C. was obtained [9] after the exercise was terminated. See Table II for details:

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TABLE II. The F.C. test results and 60%-70%F.C. of corresponding to the target heart rate ($\bar{X}$±SD)

Gender	Corresponding maximum heart rate of F.C.	F.C.（METs）	Corresponding heart rate of 60% F.C.	Corresponding heart rate of 60% F.C.
Male (n=9)	191.11±6.51	12.11±0.03	114.67±3.74	133.78±4.47
Female (n=13)	185.38±5.75	11.89±0.66	111.08±3.35	129.55±4.00

2.2.3.4 Plasma Vaspin test

After fasting for 12 hours and water deprivation for 6 hours, 5mL of fasting venous blood was collected for rapid separation to obtain plasma, which was then aliquoted into 1.5ml EP tube and stored in a -80℃ refrigerator for subsequent use. The microplate reader (Tecan Sunrise/ Switzerland) and plate washer (Tecan Columbus Washer/ Switzerland) produced in Switzerland, and the human Vaspin ELISA kit (Shanghai Yanyu Biotechnology Co., Ltd.) produced by R&D Company in the United States were used. Plasma Vaspin concentrations were determined using the double-antibody one-step sandwich method in an ELISA assay (Enzyme-Linked Immunosorbnent Assay). Experimental procedures: (1) Forty-four EP tubes (1.5ml) of frozen plasma were taken before and after the experiment. The samples were uniformly and fully thawed at room temperature. The human Vaspin kit was taken out from the refrigerator at 4℃ and balanced at room temperature for 20min. The pre-coated Vaspin plates were taken out from the aluminum foil bag. (2) The wells of standard substance were set as two, with 50μl of standard substance of different concentrations added to each. (3) The sample well was set as a single well to add 10μl of the sample to be tested, and then 40μl of the sample diluent was added, without adding the blank well. (4) 100μl horseradish peroxidase (HRP)-labeled detection antibody (except for the blank well) was added into each of the standard well and sample well, the reaction well was sealed with a sealing plate membrane, and then incubated in a 37℃ incubator for 1 hour. (5) Five times of washing with an automatic plate washer was performed, which was set as 350μl of washing solution was injected into each well for 1min, and the washing was circulated five times. (6) 50μl substrates α and β were added into each well for incubation in the dark at 37℃ for 15min. (7) 50μl stop solution was added into each well, and the OD value of each well was measured at the wavelength of 450nm (620nm reference) within 15min. In the Excel worksheet, the linear regression curve of the standard was drawn with the concentration of the standard as abscissa and the corresponding OD value as ordinate, and the Vaspin regression equation y=0.159x-0.008, and the correlation

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coefficient between the measured value of the standard reagent and the actual measured value r=0.999(P<0.001) were obtained. The equation was substituted into the Excel worksheet to get the corresponding concentration value, which was multiplied by 5 to get the actual concentration of the sample.See Table III for details:

2.2.4 Formulation and implementation of exercise prescription

The values of each element of this exercise prescription are as follows: Sports methods mainly include running and rope skipping (for easy control of THR), with intensity of the heart rate corresponding to 60%–70% F.C., namely, Target Heart Rate (THR) for 20 to 30 minutes each time, with an accumulated duration of 1 hour. The frequency was five times per week, and activities should be prepared and organized for 5–10 min before and after each exercise. After one week on the treadmill and in the outdoor playground, the subjects were taught to test the THR and the rating of percent fatigue (RPE) and their interchangeability during exercise, and the radial artery rate (10 seconds ×6) immediately after exercise was measured so that the subjects could feel the RPE subjectively (Grades 13–15) and master the consistency of 60%–70% F.C. The testers were present for guidance and supervision every time to ensure that each subject maintained the cumulative time of 60%-70% F.C. for 20-30 minutes during each exercise, and required the subjects to record their body and exercise conditions every day, and timely made slight adjustments to the exercise prescription according to the body changes of subjects during the implementation of the exercise prescription.

TABLE III. Vaspin standard OD and the corresponding concentration

Items	Blank	S1	S2	S3	S4	S5
OD value	0	0.154	0.253	0.404	0.92	1.934
Concentration of standard preparation (ng/ml)	0	0.75	1.5	3	6	12
Measured concentration of standard preparation (ng/ml)	0	1.019	1.642	2.591	5.836	12.214

2.3 Mathematical Statistics

SPSS13.0 statistical software package was used to process and analyze the relevant data, and the mean standard deviation ($\overline{X}$±SD) was used. Paired sample t-test was used to compare

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the indexes before and after exercise intervention, and two-variable correlation analysis was used for some data. $P<0.05$ indicated the significant difference, and $P<0.01$ indicated the very significant difference.

III. RESULTS

3.1 Changes of Obesity-Related Indexes before and after the Experiment

Table IV shows commonly used indexes related to obesity. After eight weeks of implementation of the exercise prescription, the body weight and waist-to-hip ratio of the male and female groups were significantly lower than those before the experiment ($P<0.01$). The thickness of subcutaneous adipose layer in upper arm, chest, waist and thigh representing the relevant indexes of subcutaneous fat was tested by paired t-test. After eight weeks of exercise prescription intervention, the subcutaneous fat layer thicknesses of upper arm, chest, waist and thigh in boys and girls were significantly reduced before and after the experiment ($P<0.01$), especially the average reduction in waist circumference of girls was 0.61cm, and the average reduction in thigh circumference was 0.24cm, suggesting that the exercise prescription had good weight loss effect.

TABLE IV. Changes before and after the experiment of obesity related indexes ($\overline{X}$±SD)

Items	Male (n=9)		t	P	Female (n=13)		t	P
	Before experiment	After experiment			Before experiment	After experiment		
Weight (kg)	73.02±6.35	68.05±6.89	10.853	0.000	66.68±6.92	61.84±6.99	12.861	0.000
waist-to-hip ratio	0.87±0.02	0.83±0.02	10.193	0.000	0.87±0.05	0.83±0.04	7.852	0.000
Upper arm (cm)	0.93±0.11	0.76±0.07	5.689	0.000	1.23±0.18	1.14±0.17	7.300	0.000
Chest (cm)	0.80±0.13	0.61±0.11	6.541	0.000	1.20±0.22	1.10±0.19	4.291	0.001
Waist (cm)	1.39±0.17	1.10±0.16	6.107	0.000	1.72±0.39	1.11±0.18	10.175	0.000
Thigh (cm)	1.00±0.12	0.81±0.10	5.713	0.000	1.52±0.19	1.28±0.25	6.028	0.000

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TABLE V. Changes before and after the experiment of plasma lipids and Vaspin ($\bar{X}$±SD)

Items	Male (n=9)		t	P	Female (n=13)		t	P
	Before experiment	After experiment			Before experiment	After experiment		
TG（mmol/L）	2.06±2.93	1.28±0.73	1.131	0.284	0.97±0.57	1.09±0.53	-1.249	0.232
TC（mmol/L）	3.59±0.56	3.36±0.40	2.146	0.057	3.76±0.64	3.38±0.32	2.520	0.025
HDL-C（mmol/L）	1.10±0.28	1.13±0.22	-1.018	0.333	1.31±0.24	1.58±0.21	-2.856	0.013
LDL-C（mmol/L）	1.56±1.00	1.65±0.43	-0.377	0.714	2.00±0.37	1.94±0.41	1.051	0.311
HDL/LDL	0.71±0.35	0.73±0.24	-0.494	0.632	0.67±0.14	0.85±0.20	-3.243	0.006
Vaspin（ng/ml）	4.88±2.05	5.42±2.23	-1.753	0.118	5.48±1.22	6.01±1.69	-1.840	0.091

3.2 Changes of Blood Lipids and Plasma Vaspin before and after Experiment

Table V shows that through the implementation of the eight-week exercise prescription, TG, HDL-C, LDL-C and HDL-C /LDL-C in the male group were changed without significant difference (P>0.05), while TC, HDL-C and HDL-C /LDL-C in the female group all showed significant differences (P<0.05, P<0.01), with no difference in TG and LDL-C (P>0.05). The data before and after the experiment showed that the plasma Vaspin of both groups were increased but without significant difference (P>0.05).

3.3 Gender Differences of Blood Lipids and Plasma Vaspin before and after Experiment

Table VI shows the difference between the male and female groups in blood lipid and plasma Vaspin before and after the experiment. Independent sample T test was performed for the data. There was no significant difference in the series of blood lipid indexes between the male and female groups before and after the experiment (P>0.05), indicating that there was no gender difference in blood lipid. Plasma Vaspin concentration in female group was higher than that in male group but without significant difference (P>0.05).

TABLE VI. Changes before and after the experiment Sex differences of plasma lipids and Vaspin ($\bar{X}$±SD)

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Items	Before experiment		F	P	After experiment		F	P
	Male (n=9)	Female (n=13)			Male (n=9)	Female (n=13)		
TG (mmol/L)	2.06±2.93	0.97±0.57	3.701	0.066	1.28±0.73	1.09±0.53	0.318	0.578
TC (mmol/L)	3.59±0.56	3.76±0.64	0.053	0.819	3.36±0.40	3.38±0.32	0.963	0.336
HDL-C (mmol/L)	1.10±0.28	1.31±0.24	1.395	0.249	1.13±0.22	1.58±0.21	0.001	0.977
LDL-C (mmol/L)	1.56±1.00	2.00±0.37	3.485	0.074	1.65±0.43	1.94±0.41	0.029	0.866
HDL/LDL	0.71±0.35	0.67±0.14	3.991	0.057	0.73±0.24	0.85±0.20	0.268	0.609
Vaspin (ng/ml)	4.88±2.05	5.48±1.22	1.412	0.249	5.42±2.23	6.01±1.69	1.202	0.286

Note: In independent sample T test; P > 0.05 indicated no significant difference. P<0.05 indicated significant difference; P<0.01 indicated very significant difference.

3.4 Correlation Analysis of Plasma Vaspin Level and Obesity Index and Blood Lipid in Overweight and Obesity Patients

As shown in Table VII, the results of correlation analysis on the experiment data of boys and girls before experiment by SPSS13.0 showed that there was no significant difference between the plasma Vaspin levels of boys and girls and the indexes related to blood lipids and obesity (P>0.05).

TABLE VII. Correlation analysis between Vaspin and indexes of overweight and obesity

Vaspin concentration		Body fat	Body mass	FAT%	WHR	BMI	TC	TG	HDL-C	LDL-C	HDL/LDL
Male (n=9)	Pearson Correlation	0.30	0.19	0.37	0.20	0.03	0.10	0.39	0.01	0.39	0.59
	Sig.(2-tailed)	0.42	0.63	0.32	0.62	0.95	0.79	0.30	0.99	0.30	0.10
Female (n=13)	Pearson Correlation	0.29	0.21	0.25	0.34	0.42	0.12	0.10	0.22	0.40	0.45

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Sig.(2-tailed)	0.33	0.49	0.41	0.25	0.16	0.69	0.74	0.67	0.18	0.13

IV. DISCUSSION

4.1 Analysis on the Influence of 60%-70% F.C. Exercise Intensity on Obesity and Lipid Indexes of Overweight and Obese College Students

Relevant research evidence shows that the negative body shape resulted from the gradual decrease of physical activity participation of adolescent population[10] will bring many adverse effects to adolescent men and women[11]. Waist-to-hip ratio has gradually become a new measurement indicator, which can more accurately predict the risk of cardiovascular disease related to obesity. A smaller value indicates a lower risk of cardiovascular disease[12]. After the experiment, the waist-to-hip ratio of both male and female students showed a downward trend, and their body weight and the thickness of subcutaneous adipose layers in all parts were significantly improved, indicating that this exercise prescription could effectively improve the body composition and body fat distribution of obese people, reduce the accumulation of adipose layers, and play a good role in reducing weight and preventing obesity-related metabolic syndrome.

Jia Lei[13] proved through experiments that swimming could effectively improve the concentrations of serum TC and LDL-C in nutritional obese mice and increase the serum HDL-C level. According to the research by Guo Yin et al.[14], four weeks of aerobic exercise intervention could well improve the LDL-C and TC levels in children, but HDL-C was not significantly improved. In this experiment, it was found that there was no significant change in blood lipid of male students, but TC of female students was significantly decreased and HDL-C and HDL-C /LDL-C were significantly increased, which might be related to appropriate intensity and duration of exercise, because exercise can promote the activity or content of enzymes related to lipid metabolism and the expression of receptors related to lipid metabolism, and increase the speed of lipid movement, decomposition and excretion, so as to improve the blood lipid level of obese patients.

4.2 Analysis of the Influence of 60%-70% F.C. Exercise Intensity on Vaspin of Overweight and Obese College Students

Vaspin, first proposed by Japanese researcher Hida et al.[15] in 2005, was found in the white adipose tissue of OLETF rats. The animal model is characterized by abdominal obesity, insulin resistance, dyslipidemia and hypertension. The increased expression of Vaspin human

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adipose tissues was found to be related to insulin resistance, suggesting that the increased Vaspin serves as a compensation mechanism for insulin resistance, reducing and improving food intake and insulin resistance[16-17]. Animal experiment has shown that exercise can reduce the expression of Vaspin mRNA in the white adipose tissues of OLETF rats[18]. Mi Kyung Lee et al.[19] found through their research that exercise could inhibit the mRNA expression of Vaspin mRNA the visceral adipose tissues of OLETF rats, but the specific mechanism is still unclear and further research is needed. Youn et al.[20] reported that Vaspin level increased significantly after 4 weeks of exercise intervention, and found that the decrease of BMI and the increase of insulin sensitivity were predictors of Vaspin increase, and also observed that Vaspin level of women with normal glucose tolerance was 2.5 times higher than that of men. It has been reported in the literature[5] that gender difference is related to the secretion of Vaspin, and serum Vaspin level can be predicted by gender, but the specific mechanism is not clear. Whether it is affected by sex hormones or adrenal hormones like adiponectin and leptin[21] needs further investigation.

In this study, the serum Vaspin level of female students is higher than that of male students, with no statistical difference, which may be due to the small sample size in our study. After experimental intervention, the Vaspin concentrations in both male and female students were increased, probably because the increase in plasma Vaspin after exercise mediated the increase in insulin sensitivity caused by exercise. The increase of plasma Vaspin is closely related to insulin resistance, glycolipid metabolism disorder and atherosclerosis index increase. As an insulin sensitizing adipocyte factor, the increase of plasma Vaspin in may be a compensatory reaction of insulin resistance, and its increase may represent a temporary adaptation mechanism.

Aust et al.[22] found a correlation between serum Vaspin and BMI from the U-shaped curve. Klöting et al.[23] found a significant correlation between visceral Vaspin mRNA expression and BMI, and FAT%, and subjects without detected Vaspin expression all had the characteristics of lower FAT%. Based on these relationships, multivariate linear analysis showed that increases in body fat better predicted visceral fat, decreased insulin, and Vaspin expression. Through their research, Wada[24] and Seeger[5], et al. respectively found that the mRNA expression of Vaspin mRNA rat visceral adipose tissues had a significant correlation with FAT% and BMI. However, the correlation between Vaspin and BMI was still unclear in human body. In this study, it is found that Vaspin has no obvious correlation with obesity-related indexes and blood lipids, which indicates that Vaspin has certain complexity in the body, and Vaspin may play a role in the human body, which is an adaptive compensation mechanism by inducing/inhibiting the changes of some indexes. But this mechanism is still unclear and needs further exploration.

V. CONCLUSIONS

After 8 weeks of exercise with an exercise intensity of 60%-70% of FC and an RPE between grades 13-15, the weight of the male and female groups dropped from 73.02kg and 66.68kg to 68.05kg and 61.84kg, and WHR both decreased from 0.87 decreased to 0.83, and the thickness of the subcutaneous fat layer was significantly improved; the TC of the female group decreased from 3.76 mmol/L to 3.38 mmol/L, HDL increased from 1.31 mmol/L to 1.58 mmol/L, and HDL/LDL increased from 0.67 mmol /L increased to 0.85 mmol/L; Vaspin levels in the male and female groups increased from 4.88 ng/ml and 5.48 ng/ml to 5.42 ng/ml and 6.01 ng/ml. It can be seen that this weight loss exercise prescription can effectively improve the weight, WHR and thickness of the subcutaneous fat layer of overweight and obese college students, and can achieve a better weight loss effect; it can also reduce TC, increase HDL and HDL/LDL values to various degrees, and improve Blood lipid levels. Although the weight-loss exercise prescription has changed the plasma Vaspin level of college students, it has little effect. This provides some ideas for further research on the relationship between weight loss exercise prescription and Vaspin.

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