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Effect of high-fat diet and aerobic exercise on total body weight of male C57BL/6 mice

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Abstract

The aim of this research was the effect of high-fat diet and moderate aerobic exercise on total body weight in C57BL/6 mice. Based on this, 15 male C57BL/6 mice were randomly divided into two groups: mice fed with a normal diet (ND, 5) and high-fat diet (HFD, 10). Mice were fed for 12 weeks. After detecting the induction of prediabetes by diagnostic tests in the HFD group, this group was divided into two subgroups: pre diabetic (PD) mice and pre diabetic mice with endurance exercise (PD-Ex) which was running on a treadmill 5 days a week and 45 minutes each day and with an increasing speed from 15 to 23 meters per minute. Independent t-test and one-way analysis of variance at $p \leq 0.05$ level were used for statistical analysis of data. The findings showed a significant increase in the total body weight of the HFD group mice compared to the ND group. While endurance training caused a significant decrease in body weight ($p<0.01$). According to the results of the research, we conclude that a high-fat diet causes weight gain, which then causes the negative effects of pre-diabetic conditions. It also seems that aerobic exercise improves the effects of prediabetes with weight loss.

Keywords: High-fat diet, aerobic exercise, prediabetes, C57BL/6 mice

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1. Introduction

Lifestyle changes, lack of physical activity and high-fat diets have made overweight and obesity one of the major health problems in developing countries. Obesity is the result of the lack of balance between energy consumption and energy intake, which mainly high consumption of high-fat food levels and lack of physical activity will disrupt this balance. Skeletal muscle is one of the endocrine organs that plays a vital and necessary role in maintaining this balance and body metabolism [1]. Therefore, in each stage of the life cycle, it is necessary to maintain the balance between received and consumed energy. Modifying eating habits is not only the problem of individual members of society, but also the problem of the whole society. Therefore, it requires a multi-sectoral, population-based and culturally adapted approach. It turns out that eating is about much more than just satisfying hunger. Eating is a social factor that acts as a means of communication between people in a culture, and behaviors related to eating in the family have a socially acquired context [2]. Among the non-modifiable risk factors, we can mention family history, which is a very useful variable for identifying people at risk. Because diabetes occurs in the relatives of people who have been diagnosed with this disease, more often than in non-afflicted people. Additionally, relatives of people with diabetes have been found to follow lifestyle patterns similar to those of patients with diabetes, which also increases the risk[3]. In other words, the family becomes a mechanism that can benefit or harm health! The family is an essential element in the development of health and caregiving behaviors that provide emotional, adaptive, informational, economic, and functional support. Consuming high-calorie foods, followed by obesity and overweight, can lead to other diseases, including type 2 diabetes, cardiovascular diseases, musculoskeletal disorders, and some cancers. One of the

main problems of obesity is type 2 diabetes, which is characterized by defects in insulin secretion and activity, hyperglycemia, and disturbances in carbohydrate, fat, and protein metabolism[4]. The global epidemic of T2D is one of the biggest challenges of the World Health Organization and it entails very high human and economic costs. The American Diabetes Association has introduced the following four criteria for diagnosing diabetes: 1- Glycosylated hemoglobin (A1c) value of 6.6% or higher, 2- Fasting glucose higher than 126 mg/dL, 3- 2-hour plasma glucose higher than 200 mg/dL during an oral glucose tolerance test using 75 grams of glucose and 4- symptoms of increased blood glucose, including increased urine volume, excessive thirst and weight loss for no reason, on the one hand, diagnosis of prediabetes through plasma glucose A1c of about 5.7 to 6.4%, fasting glucose of 100 to 125 mg/dL and impaired glucose tolerance, i.e. 2-hour plasma glucose of 140 to 199 mg/dL after glucose loading, are identified[5]. Statistics show that the number of diabetic patients worldwide in 2014 was about 422 million and it is estimated that by 2030, there will be at least 592 million cases of diabetes worldwide[6]. T2D is a growing global health problem that is closely related to the obesity epidemic. Of the three main types of diabetes, T2D is far more common than type 1 diabetes (T1D) or Gestational diabetes (GDM) is more than 90% of diabetic patients. People with T2D are at high risk of developing microvascular complications (including retinopathy, nephropathy, and nephropathy) and macrovascular complications (such as cardiovascular disease) due to hyperglycemia and insulin resistance (metabolic) syndrome. Environmental factors (obesity, unhealthy diet, and physical inactivity) and genetics play a role in the occurrence of T2D in several pathophysiological disorders that are responsible for impaired glucose homeostasis. T2D is characterized by impaired carbohydrate, lipid, and protein metabolism and is caused by impaired insulin secretion, insulin resistance, or a combination of both (as the main factors in this disease) [7]. Over the past several decades, our understanding of the development and progression of T2D has evolved rapidly. Its main cause is the gradual impairment of insulin secretion by the beta cells of the pancreas, which is usually on a background of insulin resistance already established in skeletal muscles, liver and adipose tissue. Pre-diabetes, which was introduced by the American Diabetes Association in 2002, is a pre-diabetes condition in which blood sugar levels are higher than normal and lower than the diagnostic criteria for T2D. In pre-diabetes, there is a disorder in insulin secretion and an increase in insulin resistance, and it appears before the development of type 2 diabetes. The symptoms of pre-diabetes are hunger at all times, unexplained weight loss or gain, extreme thirst, nausea and fatigue, changes in visual perception, blurred vision, and leg pain. On the other hand, factors such as genetics, sedentary lifestyle, obesity, high blood pressure and sleeping less than 5.5 hours a day have been identified as the most important causes of prediabetes[8]. In addition to increasing the risk of diabetes, pre-diabetes damages the kidneys and nerves. Considering that pre-diabetes is considered a high-risk condition for diabetes, therefore, timely diagnosis and treatment of pre-diabetes is very important and with lifestyle modification through diet control and physical activity at this time. It can lead a person to a healthy life [9]. Obesity and physical inactivity lead to insulin resistance, which, together with a genetic predisposition, puts stress on beta-cells and leads to the failure of beta-cells and a gradual decrease in insulin secretion. Insulin resistance manifests years before prediabetes and T2D. Insulin resistance occurs in most organs, especially in muscles and liver [10]. (Impaired cell function and increased insulin resistance are two pathologies that lead to prediabetes and T2D The onset of increased insulin resistance begins years before diabetes and even in prediabetes [9]. The primary and main cause of prediabetes is insulin resistance in muscle, liver and fat tissues, which is followed by increased insulin production by the pancreas. This situation ultimately leads to the destruction of pancreatic beta cells and the complete failure of insulin production and T2D (Sherrington et al., 1998). Insulin resistance in skeletal muscle is the main cause of Impaired glucose tolerance (IGT) [11,12]. Change in lifestyle, in the form of improving nutrition, obesity and doing physical activity in order to lose weight, has an important role in the prevention and treatment of T2D. One of the most effective prevention and treatment methods is physical activity, which is used due to increased insulin sensitivity [13]. On the other hand, physical activity due to weight control, improving body composition, increasing cardiovascular fitness, controlling blood glucose and increasing insulin sensitivity; It is one of the most effective ways to prevent and treat diabetes. Aerobic exercise by using large muscle groups increases insulin sensitivity, decreases insulin resistance and improves beta cell function in diabetics. The recommended duration of aerobic exercise for people is at least 150 minutes of moderate-intensity activity or 75 minutes of high-intensity activity during a week [14,15]. Therefore, in this research, the researchers decided to create pre-diabetic model mice by using a high-fat diet and its effect on total body weight, and then to investigate the effect of aerobic exercise separately and together, to examine the negative effects of pre-diabetes on the amount of total weight and its nature.

2. Materials and methods

In this research, 4-week-old C57BL/6 male mice with an average weight of 12 to 14 grams were used. Mice were kept under standard conditions of light-dark cycle (12 hours of light and 12 hours of darkness) with 50-60% humidity and 21±2°C temperature, and all mice had free access to food and water. The present research was carried out in two stages. The first stage aimed at inducing pre-diabetes using a high-fat diet, matched rats were randomly divided into two groups: the first group (number = five: N), normal diet (ND) containing 10% fat, 70% carbohydrate, received 20% protein and the second group (number = ten: N), which received high-fat diet (HFD) containing 60% fat, 25% carbohydrate and 15% protein for 12 weeks. After 12 weeks and comparing the weight of two groups and using diagnostic tests, the induction of prediabetes was proven in the high-fat diet group compared to the normal group. Considering that the range of pre-diabetes levels in C57BL/6 mice is 100 to 150 mg/dL of blood glucose. After ensuring the induction of prediabetes in the high-fat diet group, this group was divided into two subgroups: the pre diabetic (PD) group only received the high-fat diet until the end of the experiment, and the pre diabetic rats that underwent a period of aerobic exercise (PD-Ex) to They exercised on a treadmill for 5 days a week (45 minutes a day) for 10 weeks[1]. The exercise intensity protocol was designed in an incremental

manner. In this way, the training intensity was increased once every two weeks (with an average intensity of 50-60% of oxygen consumption) [16]. Initially, before starting the main exercise intervention, all rats started running on a treadmill (10 minutes, using different speeds) for one week to familiarize the rats with running. The training intervention weeks were: the first and second weeks of rats with an intensity of 15 meters per minute, the third and fourth weeks with a speed of 17 meters per minute, the fifth and sixth weeks with a speed of 19 meters per minute, the seventh and eighth weeks with a speed of 21 meters per minute. minutes and in the 9th and 10th weeks, they did their training at a speed of 23 meters per minute. It is noteworthy that each training session included 3 minutes of warm-up at a speed equivalent to 15% less than the training intensity every week, 40 minutes of training and 2 minutes of cooling down, equivalent to 20% less than the training intensity, along with receiving a high-fat diet[17,18]. At the end of 12 weeks, in order to determine the effect of the high-fat diet and to confirm the induction of prediabetes, compared to its corresponding group (the group receiving a normal diet) and at the end of 22 weeks to check the effectiveness of the interventions, and to compare the normal, prediabetes and prediabetes groups to Along with exercise, weight and biochemical factors were evaluated. The weight of mice was measured by a digital scale. Fasting blood glucose (FBS) and glucose tolerance test (GTT) were measured using a blood glucose meter (Sannuo GA-3 Blood Glucose Meter, Changsha Sinocare Inc) from the tail end of the mice. In order to measure glucose tolerance, rats were fasted for 6 hours and then gavage with 200 microliters of glucose solution. Blood was taken for the glucose tolerance test with an animal glucometer in the form of drops and measured at time intervals of 0, 30, 60, 90 and 120 minutes later. Plasma insulin levels were determined with a mouse insulin ultrasensitive ELISA kit (ALPCO 80-INSMS-E01, Keewaydin Drive, USA) according to the manufacturer's instructions. At the end of the training protocol, the normal, pre-diabetic and pre-diabetic groups with exercise were compared and the effectiveness of endurance exercise was reported.

3. Results and Discussions

The results showed that after consuming high-fat food for 12 weeks, the weight of the rats that received the high-fat diet increased significantly (31.0±2.92 mg/dL) compared to the rats that had received a normal diet (28.39 ± 0.3 mg/dL) ($p<0.0001$) (Chart 1) The obtained results showed that during fasting, the blood glucose of rats that received a high-fat diet had a noticeable and significant increase (136.6 ± 9.92 mg/dL) compared to rats that received a normal diet (39 ± 3). ($p<0.0001$) (Table 1). This means that the induction of prediabetes has been done correctly. Regarding the level of plasma insulin, the high-fat diet group had a significant increase compared to the normal diet group ($p<0.0001$). This again can be proof of insulin resistance (Table 1). The results showed that after consuming high-fat food and induction of prediabetes, which lasted for 12 weeks, the weight of PD rats increased significantly (31 ± 2.92 g) compared to rats that received ND (3 30.39 ± 0.0 g) ($p < 0.01$). While the exercise group (PD-Ex) showed a significant reduction (31±0.982 grams) compared to the PD group ($p<0.01$) (Chart 2). At the end of the training period, which lasted for 10 weeks, the results showed that the blood glucose in the PD group rats had a considerable and significant increase (136.6 ± 9.92 mg/dL) compared to the ND rats (95.39 ± 3 mg per deciliter) showed ($p<0.0001$), while the PD-Ex group had a significant decrease in fasting blood glucose (94±14.60 mg/dL) compared to the PD group (144±11.09 mg/dL) liter) showed $p>0.0001$) (Chart 3 A). Regarding the level of plasma insulin at the end of the second intervention, the level of plasma insulin in the PD group showed a significant increase compared to the ND group ($p<0.0001$). Also, the PD-Ex group showed a significant decrease in insulin levels compared to the PD group ($p<0.0001$) (Chart 3b). (Table 2).

Today, consumption of high-fat foods in the form of receiving extra calories on the one hand, and inactivity on the other hand, has caused obesity and overweight, and as a result, many metabolic disorders, including diabetes. Since diabetes is one of the most important health problems in the world today, it is associated with an increase in the death rate[19]and the prediabetes state is considered as the boundary of entering diabetes in which the blood glucose is abnormally high but The threshold considered for the diagnosis of diabetes is not reached, so the aim of the present study is to investigate the effect of high-fat food consumption and aerobic exercise on the total body weight of male C57BL/6 mice as a possible factor involved in prediabetes and diabetes. The importance of prediabetes and its harmful effects and possible solutions to improve its complications should be clarified. The researchers stated that consuming a high-fat diet not only leads to obesity and diabetes, but also leads to metabolic syndrome, including elevated levels of fasting blood glucose (FBS), insulin, and more. which can have undesirable effects on the whole body systemically [20]. Overweight and obesity are one of the important factors and the background of various diseases, including diabetes and cardiovascular diseases, which cause a lot of problems for a person. Also, observations of body weight increase can be a warning sign for metabolic disorders and pre-diabetes. The results of the present research showed that the high-fat diet led to a significant increase in the total body weight of mice compared to the normal group. These findings were consistent with the results of Xiaopu's studies [21]. Accumulation of triacylglycerols in skeletal muscle due to excess weight leads to competition between fat and glucose for harvesting and oxidation, which will reduce glucose oxidation. Some free-fatty acids are decomposed into diacylglycerol, fatty acyl-CoA and ceramides [9].

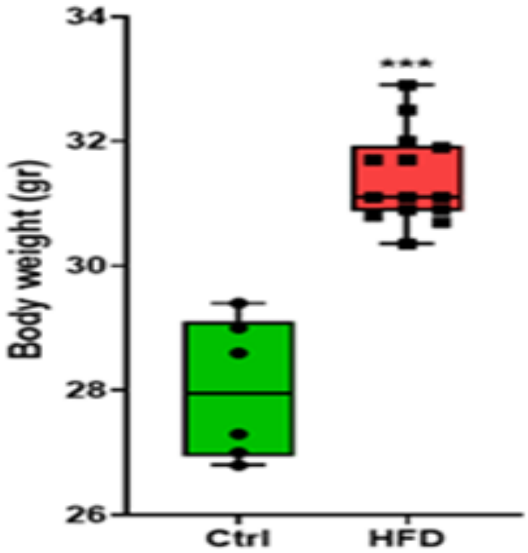

Chart 1: Effect of pre-diabetes on body weight. Comparison of body weight between HFD group and ND group. All graphs are presented as (mean ± standard error) as well as significance levels, ($p<0.001$) ** ($p<0.0001$).***

Table 1: Effect of prediabetes on blood glucose and insulin. Display of the descriptive level of significant ameri values comparing fasting blood glucose (FBS) and plasma insulin levels between the HFD group and the ND group.

Independent Samples Test								
Levene's test for equality of variances				t-test for equality of Means				
		F	Sig.	t	df	Sig. (2-tailed)	MD	Std. error difference
FBS	Equal variances assumed	11.670	0.006	-8.671	11	0.000	-38.55556	4.44634
	Equal variances not assumed			-12.090	10.805	0.000	-38.55556	3.18901
Insline Plasma	Equal variances assumed	1.202	0.293	-18.185	13	0.000	-1.58200	0.08700
	Equal variances not assumed			-20.894	11.593	0.000	-1.58200	0.07572

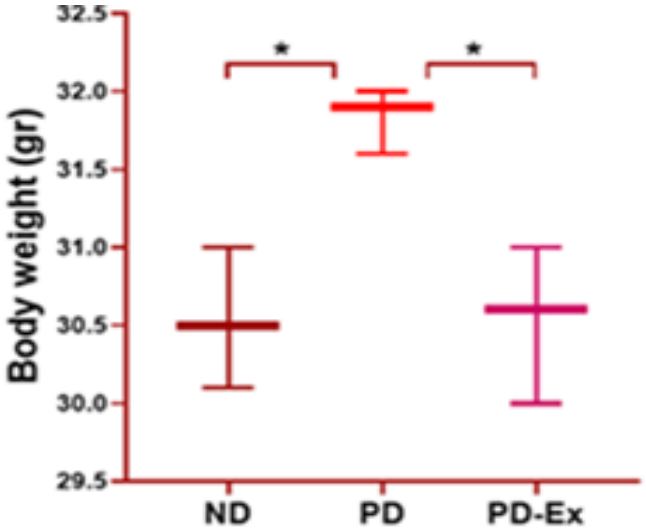

Chart 2: The effect of endurance training on body weight. Comparison of body weight between PD group, ND group and PD-Ex group. All graphs are presented as (mean ± standard error) as well as significance levels, (p<0.001) ** (p<0.0001).***

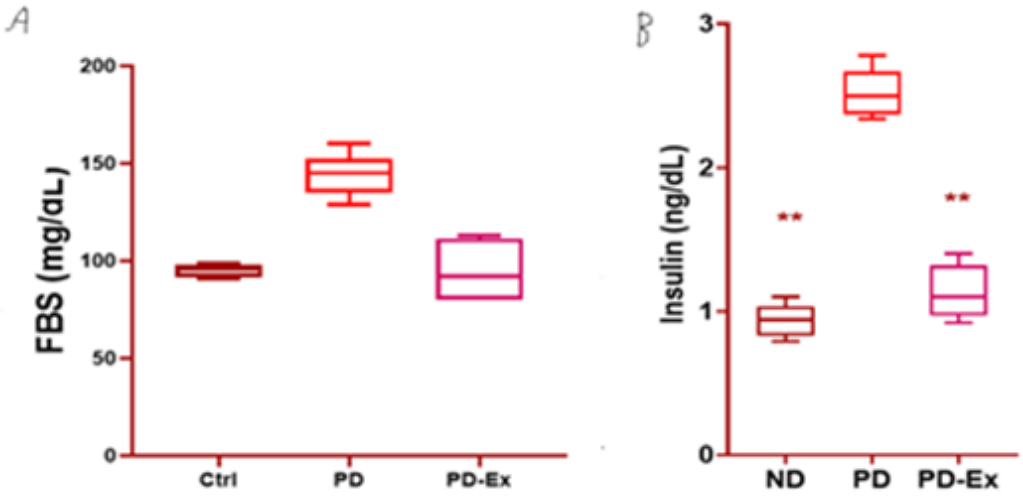

Chart 3: The effect of endurance training on blood glucose and insulin. A. Comparison of fasting blood sugar (FBS) and b. plasma insulin levels between the HFD group and the ND and PD-Ex groups. All graphs are presented as (mean ± standard error) as well as significance levels, (p<0.001) ** (p<0.0001).***

Table 2: The effect of endurance training on blood glucose and insulin. Showing the level of statistically significant values in comparison of fasting blood sugar level (FBS) and plasma insulin level between HFD group, ND group and PD-Ex group. Shown by SPSS software.

Multiple Comparisons							
Tukey HSD	MD					95% confidence interval	
Dependent variables	I Group	(J) Group	J	Std. error	Sig.	Lower Bound	Upper Bound
FBS	ND	PD	-48.00	7.70	.000	-68.55	-27.44
		PD-EX	1.33	7.41	.982	-18.44	21.11
	PD	ND	48.00	7.70	.000	27.44	68.55
		PD-EX	49.33	6.95	.000	30.77	67.88
	PD-EX	ND	-1.33	7.41	.982	-21.11	18.44
		PD	-49.33	6.95	.000	-67.88	-30.77
Insline Plasma	ND	PD	-1.582	.10197	.000	-1.85	-1.3099
		PD-EX	-.2020	.10197	.159	-.47	.0701
	PD	ND	1.582	.10197	.000	1.3099	1.8541
		PD-EX	1.38	.10197	.000	1.1079	1.6521
	PD-EX	ND	-.2020	.10197	.159	-.0701	.4741
		PD	-1.38	.10197	.000	-1.6521	-1.1070

Experimental studies have shown that ceramides rapidly activate protein phosphatase 2 A (PP2A) or protein kinase C (PKC) in the short term to inactivate protein kinase B (AKT). On the other hand, in the long term, ceramides activate C-Jun NH2-Terminal Kinase or Pbx regulating Protein 1axes. Therefore, by targeting insulin receptor substrate (Insulin receptor substrate-1/2) and AKT, ceramides inhibit insulin signaling and ultimately reduce glucose uptake caused by insulin stimulation, which increases the likelihood of developing diabetes[2]. On the other hand, obesity and high concentration of free fatty acids (FFA) causes endoplasmic reticulum stress, which further stimulates the response of tanned protein, which probably causes insulin resistance[22], Also, obesity and insulin resistance lead to the phosphorylation of serine amino acid at positions 307 (human) and 302 (mouse) instead of tyrosine amino acid in molecules, which results in a disturbance in the insulin signaling pathway [23]. According to the present findings, pre-diabetic mice fed with a high-fat diet showed metabolic disorders such as increased levels of fasting blood glucose, insulin and abnormal glucose tolerance compared to the control group that consumed a normal diet. While the pre-diabetic group showed a significant decrease in fasting blood glucose and insulin levels compared to the pre-diabetic group. In this regard, Putner et al. showed that feeding a high-fat diet can cause obesity and metabolic disorders in rodents, which is similar to human metabolic syndrome[24]. Winzel et al stated that the C57BL/6J mouse model fed a high-fat diet is a robust model for abnormal glucose tolerance and early T2D formation that may be used for pathophysiology studies and new treatment development[25]. Abdulahi et al (2022) as well as other researchers[26,27]. Sinitiskaya et al., (2007) showed that mice fed with a high-fat diet containing 60%, lead to the formation of prediabetes and early onset of chronic metabolic disorders[28]. such as increasing the level of fasting blood sugar and increasing the level of insulin in the blood in rats. In the present study, it was shown that aerobic exercises led to a significant reduction in weight and improved metabolic factors (fasting blood glucose and plasma insulin levels) in the PD-Ex group compared to the PD group as a control group. In this regard, the researchers stated that exercise is considered the cornerstone of T2D prevention and treatment, and various mechanisms may contribute to the benefits of exercise. Interestingly, exercise improves insulin sensitivity in both healthy and insulin-resistant individuals[29].The improvement in insulin sensitivity after a training session is short-lived. But repeated aerobic exercise improves insulin sensitivity beyond the acute effect of the last exercise session[30]. Importantly, the risk of developing T2D is reduced by regular activity over a year[31]. It has been emphasized that physical activity seems to have a significant relationship with increasing the effectiveness of insulin in skeletal muscles[32]. According to the results of the studies, it seems that the effect of exercise depends on various factors such as the type, intensity and frequency of exercise and the amount of glucose. Kodama et al. (2007) reported that the combination of aerobic and endurance training even with low intensity and volume can improve insulin resistance[33], and Church et al. stated that the combination of aerobic and resistance training is more effective than irregular training[34]. However, in relation to exercise, different factors such as the type, intensity and repetition of exercise and the level of glucose give rise to different theories.

4. Conclusions

The results of the present study show that a high-fat diet causes an increase in total weight, and that there is probably a reciprocal relationship between diabetes and body fat mass. Also, in the present study, it was observed that aerobic exercise as a prevention method can lead to the

improvement of metabolic factors involved in pre-diabetes, including fasting blood sugar and insulin levels, with weight loss.

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Conflict of interest

All the authors hereby declare that there is no conflict of interest regarding this research.

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