Disease and Diagnosis

Dis Diagn. 2023; 12(1):21-28

doi 10.34172/ddj.2023.426

Original Article

The Effect of Aerobic Exercise and Ginkgo biloba Herbal Supplementation on Lipocalin 2 Levels and Insulin Resistance in Obese Men

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Abstract

Background: Performing sports activities along with nutritional interventions is recommended as an effective way to reduce inflammatory cytokines and improve insulin resistance in obese people. This study aimed to evaluate the effect of aerobic exercise and Ginkgo biloba herbal supplementation on lipocalin 2 (Lcn2) levels and insulin resistance in obese men.

Materials and Methods: For this purpose, 40 obese men in Kashan were selected in an accessible and purposeful manner. Subjects were randomly divided into 4 groups, including aerobic exercise (n=10), aerobic exercise and ginkgo biloba supplementation (n=10), ginkgo biloba supplementation (n=10), and control group (n=10). Subjects performed aerobic exercise and supplementation for 6 weeks. Accordingly, the subjects started aerobic exercise for 25 minutes with an intensity of 65% of the maximum heart rate in the first and second weeks. In the third and fourth weeks, they practiced for 35 minutes with an intensity of 65%-75% of the maximum heart rate, and finally for 40 minutes with 75%-85% of maximum heart rate in the fifth and sixth weeks. Ginkgo biloba extract was prepared in the form of gelatin capsules, two of which were daily consumed by subjects after breakfast for 6 weeks. The subjects performed their usual daily activities in the control group. The obtained data were analyzed by analysis of covariance (ANCOVA) in SPSS software (version 22) at the level of 0.05.

Results: The results of the study showed that 6 weeks of aerobic exercise and Ginkgo biloba herbal supplementation significantly reduced Lcn2 levels ($P<0.001$ and $\eta^2=0.61$) and insulin resistance ($P<0.001$ and $\eta^2=0.74$) in obese men.

Conclusion: According to the findings of the present study, it seems that aerobic exercise and *Ginkgo biloba* herbal supplementation can significantly contribute to regulating body weight in obese men through a reduction in insulin resistance and Lcn2.

Keywords: Aerobic exercise, *Ginkgo biloba* supplement, Lipocalin 2, Insulin resistance

Received: June 5, 2022, Accepted: August 20, 2022, ePublished: November 10, 2022

Introduction

Some biochemical factors, secreted by adipocytes, are considered inflammatory markers that also affect the occurrence of some metabolic disorders. These factors are involved in safety functions, energy cost regulation, and insulin action (1). Some of these factors (e.g., alpha necrosis factor, interleukin 6, leptin, adiponectin, apelin, and visfatin) can directly affect glucose intolerance and insulin resistance by disrupting the process of glucose and insulin metabolism in peripheral tissues, especially stimulating the liver and skeletal muscles (2).

Lipocalin 2 (Lcn2) is an adipocytokine that is considered an important marker in obesity and glucose metabolism (3). It has also been reported in mice and humans that the Lcn2 concentration is associated with obesity and insulin resistance (4). The expression of Lcn2 increases by factors that increase insulin resistance (hyperinsulinemia and hyperglycemia) and decreases by thiazolidinediones (4). Lcn2 levels are higher in obese compared to lean people (3). In addition, a significant positive relationship has been reported between Lcn2 levels and variables associated with metabolic syndrome in humans, including a lipid profile, hyperinsulinemia, hyperglycemia, and insulin resistance (4).

Low levels of physical activity in obese people reduce the anti-inflammatory effects of cytokines and lead to obesity, insulin resistance, and diabetes (5). One of the best strategies to prevent obesity and its associated inflammation is to engage in regular physical activity and exercise (6). Regular exercise has been shown to

positively affect the inflammatory profile and immune system of inactive obese individuals (6). Although changes in adipokine levels may be among the main ways to understand the effect of exercise, the data on Lcn2 changes resulting from exercise are inconsistent and unclear (7-11).

On the other hand, various natural phytochemicals have recently been derived from fruits and vegetables and used to suppress obesity and its associated metabolic syndrome (12). Ginkgoaceae is a Chinese native tree, also widely cultivated in Europe, Australia, Korea, Japan, and the United States due to its health characteristics (13). It was also used in Chinese medical sciences about 5000 years ago because of its beneficial properties. The leaves of this tree, called Maidenhair, are the source of this medicinal effect. According to previous research (13), *Ginkgo biloba* extract (GbE) is obtained from the green leaves of this tree, and its compounds contain 24% of flavonoids (ginkgo-flavon glycosides) and 6% of terpenoids (ginkgolides A, B, C, J, and bilobalide). GbE is one of the most widely used herbal remedies in the world for the treatment of neurological diseases such as Alzheimer's, memory loss, schizophrenia, mild cognitive impairment, dementia, or dementia (14). This herbal extract has also been applied in cancer and cardiovascular diseases (15). The beneficial effects of GbE on obesity and diabetes have recently been demonstrated as well (15). GbE has been studied as a factor in hypoglycemia and insulin sensitivity due to its antioxidant and anti-inflammatory properties (13). Moreover, it has recently been introduced as an anti-obesity agent with inhibitory effects on energy intake, body weight, and visceral fat in obese rats. In addition, GbE significantly improves insulin sensitivity and increases the adiponectin receptor gene, along with decreasing tumor necrosis factor-alpha levels (15).

Due to the prevalence of obesity in the country, limited and contradictory results of the effect of exercise on Lcn2 levels, and the potential of GbE in improving energy and glucose homeostasis, this study sought to investigate the effect of 6 weeks of aerobic exercise and *Ginkgo biloba* supplementation on the serum levels of Lcn2 and insulin resistance in obese men.

Materials and Methods

The present research is of semi-experimental and laboratory type and according to the length of time, it is cross-sectional type; in terms of using the results, it is an applied study, conducted as a pre-test and post-test with the control group (in 2020). The statistical sample of the study consisted of 40 obese men in Kashan (using software G*Power – G), who were randomly selected using a table of random numbers. The inclusion criteria were 20-30 years of age, inactivity (lack of physical activity and regular exercise), and a body mass index of > 30 kg/m^2. On the other hand, the exclusion criteria were a history of a specific disease, a history of smoking, consumption of alcohol, and a history of diseases such as diabetes, hypertension, or cardiovascular disease. After screening for people with certain diseases and those who were taking medication, 40 people were selected and randomly (simple randomization) divided into 4 groups of aerobic exercise (n = 10), aerobic exercise and *Ginkgo biloba* supplementation (n = 10), *Ginkgo biloba* supplement (n = 10), and control group (n = 10). The sample size was determined using the fleece sample volume estimation equation and considering the test power of 0.8 and alpha equivalent of 0.05 for each group.

Ginkgo biloba

Ginkgo is one of the oldest Asian plants. The leaves of this tree are highly delicate, beautiful, and fan-shaped. *Ginkgo biloba* is a tree known as a living fossil that is native to China, and the used part is its leaves. In the present study, GbE was taken in the form of two capsules once a day. GbE capsules contained 80 mg of standardized GbE, including 19.2 mg of flavonoid glycosides (24%), 4.8 mg of terpene lactones (6%), and other substrates such as maltodextrin, microcrystalline cellulose, and magnesium stearate. The placebo capsule also contained microcrystalline cellulose, magnesium stearate, and maltodextrin (16).

The study consisted of pre-test and post-test stages, between which the subjects performed aerobic exercises and supplementary for 6 weeks. First, the subjects were asked to come to the gym for initial measurements such as height and weight and to explain the test steps. Then, four days later, blood sampling was performed in the pre-test stage (before aerobic exercises and supplementation). Biochemical indices were measured by a specialized laboratory at 8 AM after 12 hours of fasting. The primary blood sample of 10 ccs was taken from the anterior vein of the subjects' arms and frozen at -30°C. All measurements were performed by a laboratory expert who was unaware of the subjects' condition. Glucose, insulin, and Lcn2 levels were measured using special kits.

Serum Lcn2 levels were determined by the ELISA method with a special kit (R&D system, Minneapolis, MN, USA). Fasting glucose was measured enzymatically (Hitachi T, Tokyo, Japan), while radioimmunoassay (Monobind, Inc, USA) was used to measure fasting insulin. The coefficient of change inside and outside the test for insulin was less than 4%. Homeostatic model assessment for insulin resistance (HOMA-IR) evaluation was used to determine fasting insulin resistance by fasting blood glucose and insulin levels.

Exercises and supplementation protocols started 24 hours after the pretest. In the aerobic training group, performed for 6 weeks and 3 sessions per week, the subjects followed the training protocol. The workout began with 15 minutes of warm-up and ended with 15

minutes of cooling. In the first and second weeks, the subjects started aerobic exercise for 25 minutes with an intensity of 65% of the maximum heart rate. In the third to fourth weeks, they practiced for 35 minutes with an intensity of 65%-75% of the maximum heart rate, and finally for 40 minutes with 75%-85% of the maximum heart rate in weeks 5-6 (17). GbE was prepared in the form of gelatin capsules, and the subjects were asked to consume two of which daily after breakfast for 6 weeks (18).

The subjects of the control group performed their usual daily activities. Post-test blood sampling was performed 48 hours after the end of the training period and supplementation. Further, a 100-item food questionnaire was employed on three even and odd days to control the diet and calorie intake of the subjects. Then, the subjects' diet was simulated according to their reports to reduce the effect of the daily calorie intake.

Descriptive statistical methods were applied to analyze the data, calculate the central indicators, and scatter and draw graphs. Shapiro-Wilk test was used to check the normality of the data, while the Levene's test was utilized to evaluate the equality of variance of the variables. In the inferential statistics section, the effects of aerobic exercise and *Ginkgo biloba* supplementation on dependent variables were determined through the analysis of covariance (ANCOVA), and the training groups in each of the dependent variables were compared using the Bonferroni post hoc test. Within-group changes were analyzed using the paired *t* test. Data were analyzed using SPSS software, version 22.

Results

Table 1 presents the descriptive information of contextual variables (i.e., age, height, weight, and body mass index).

Table 2 lists the statistical indicators related to the main variables of the study, including Lcn2 and insulin resistance in different training groups.

As shown in Table 2, serum Lcn2 and insulin resistance had a significant decrease ($P<0.05$) in the post-test of training and complementary groups compared to the pre-test. Furthermore, the level of serum insulin was significantly lower in the post-test of training and supplement groups than in the pre-test ($P<0.05$), but there was no significant difference in the post-test of the serum glucose supplement group in comparison to the pre-test ($P\geq0.05$).

Tables 3 and 4 summarize the results of the ANCOVA to evaluate the effect of exercise interventions and compare the two groups in terms of lipocalin levels in obese men.

According to the results of the ANCOVA, 6 weeks of aerobic exercise together with *Ginkgo biloba* consumption led to a significant difference in serum Lcn2 levels with a significance of 0.00 and effect size of 0.61. Table 4 lists the results of the Bonferroni post hoc test to compare the two groups.

The results of the Bonferroni post hoc test (Table 4) demonstrated a significant difference in the serum Lcn2 levels of obese men between the two groups "aerobic exercise and *Ginkgo biloba* consumption" and "aerobic exercise" ($P=0.03$), "aerobic exercise and *Ginkgo biloba* consumption" and "*Ginkgo biloba* consumption" ($P<0.001$), "Aerobic exercise" and "control" ($P<0.001$), "Aerobic exercise and *Ginkgo biloba* consumption" and "control" ($P<0.001$), and "*Ginkgo biloba* consumption" and "control" ($P=0.01$). However, the test results represented no significant difference between the two groups "aerobic exercise" and "*Ginkgo biloba* consumption" ($P=0.99$). Tables 5 and 6 provide the results of the ANCOVA to evaluate the effect of exercise interventions and compare the two groups in terms of insulin resistance levels in obese men.

Considering that the assumptions of the covariance analysis test are valid, the result of the covariance analysis test shows a significant difference in insulin resistance levels after 6 weeks of aerobic exercise with *Ginkgo biloba* consumption with a significant difference of 0.00 and an effect size of 0.74. Table 6 presents the results of the Bonferroni post hoc test to compare the two groups

Based on the results of the Bonferroni post hoc test (Table 6), a significant difference was found between the two groups "aerobic training and consumption of *Ginkgo biloba*" and "aerobic training" ($P<0.001$), "aerobic training and consumption of *Ginkgo biloba*" and "*Ginkgo biloba* consumption" ($P<0.001$), "aerobic exercise" and "control" ($P<0.001$), "Aerobic Exercise and consumption of *Ginkgo biloba*" and "control" ($P<0.001$), and "Consumption of *Ginkgo biloba*" and "control" ($P<0.001$) in terms of serum levels of insulin resistance in obese men. However, the test results revealed no

Table 1. Individual Characteristics of the Subjects at the Basic Level

Variable	Groups			
	Aerobic Exercise and *Ginkgo biloba* Consumption	Aerobic Exercise	*Ginkgo biloba* Consumption	Control
Age (y)	25.60±3.40	24±3.12	27.10±3.28	25.30±3.77
Height (cm)	167.60±6.48	165.50±6.15	163.20±4.07	167.40±5.75
Weight (kg)	88±6.73	85.30±7.91	83.20±3.91	88±6.44
BMI ($kg.m^{-2}$)	31.31±1.30	31.07±0.88	31.24±1.14	31.38±0.94

Abbreviation: BMI: Body mass index.
Data are expressed as mean and standard deviations. *Significant sign of the difference between the two groups. Significance level ($P<0.05$).

Table 2. Statistical Indicators Related to Research Variables

Variable	Group	Measurement Step	Mean ± Standard Deviation	*P* Value
Lipocalin 2 (ng/mL)	Aerobic exercise and *Ginkgo biloba* consumption	Pre-test	76.45 ± 5.99	<0.001*
		Post-test	60.50 ± 3.52	
	Aerobic exercise	Pre-test	74.20 ± 5.70	0.01*
		Post-test	66.45 ± 4.73	
	Ginkgo biloba consumption	Pre-test	74.29 ± 7.08	0.04*
		Post-test	68.84 ± 5.09	
	Control	Pre-test	74.81 ± 6.82	0.12
		Post-test	75.21 ± 4.07	
Insulin resistance	Aerobic exercise and *Ginkgo biloba* consumption	Pre-test	2.08 ± 0.28	<0.001*
		Post-test	1.85 ± 0.27	
	Aerobic exercise	Pre-test	2.005 ± 0.21	0.02*
		Post-test	1.92 ± 0.22	
	Ginkgo biloba consumption	Pre-test	2.20 ± 0.29	0.04*
		Post-test	2.07 ± 0.27	
	Control	Pre-test	2.07 ± 0.32	0.18
		Post-test	2.10 ± 0.30	
Glucose (mg/dL)	Aerobic exercise and *Ginkgo biloba* consumption	Pre-test	117.10 ± 7.69	0.03*
		Post-test	106.40 ± 8.77	
	Aerobic exercise	Pre-test	112.80 ± 7.36	0.04*
		Post-test	109.20 ± 8.40	
	Ginkgo biloba consumption	Pre-test	120.70 ± 10.22	0.09
		Post-test	115.20 ± 10.05	
	Control	Pre-test	117.70 ± 11.005	0.19
		Post-test	119.20 ± 10.45	
Insulin (U/mL)	Aerobic exercise and *Ginkgo biloba* consumption	Pre-test	7.18 ± 0.71	0.02*
		Post-test	7.03 ± 0.74	
	Aerobic exercise	Pre-test	7.19 ± 0.53	0.04*
		Post-test	7.12 ± 0.52	
	Ginkgo biloba consumption	Pre-test	7.37 ± 0.58	0.04*
		Post-test	7.29 ± 0.57	
	Control	Pre-test	7.13 ± 0.81	0.14
		Post-test	7.15 ± 0.80	

*Sign of significant difference in level $P<0.05$

Table 3. Results of Analysis of Covariance Test for Evaluating the Effect of Exercise Interventions on Serum Lipocalin 2 Levels

Source of Effect	Sum of Squares	Degrees of Freedom	Average of Squares	Statistical Value F	*P* Value	Effect Size
Corrective model	111.07	4	277.76	13.94	0.01	0.61
Width of origin	1236.13	1	1236.13	62.04	<0.001	0.63
Pre-test lipocalin 2	0.15	1	0.155	0.008	0.93	0
Group	1099.65	3	366.55	18.39	<0.001	0.61

Table 4. Findings of Bonferroni Test Concerning Lipocalin 2

Group (I)	Group (J)	Difference Between the Means	Standard Error	*P* Value
Aerobic exercise and *Ginkgo biloba* consumption	Aerobic exercise	-5.92	2.01	.03
	Ginkgo biloba consumption	-8.31	2.01	<0.001
	Control	-14.69	2.00	0.00
Aerobic exercise	*Ginkgo biloba* consumption	-2.39	1.99	<0.001
	Control	-8.76	1.99	<0.001
Ginkgo biloba consumption	Control	-6.37	1.99	0.01

Table 5. Results of the Analysis of Covariance to Evaluate the Effect of Exercise Interventions on Insulin Resistance Levels

Source of Effect	Sum of Squares	Degrees of Freedom	Average of Squares	Statistical Value F	*P* Value	Effect Size
Corrective model	3.02	4	0.75	220.09	<0.001	0.96
Width of origin	3.78	1	3.78	0.00	0.97	0.00
Pre-test resistance insulin	2.57	1	2.57	748.59	<0.001	0.95
Group	0.34	3	.01	33.58	<0.001	0.74

Table 6. Findings of Bonferroni Test Regarding Insulin Resistance

Group (I)	Group (J)	Difference Between the Means	Standard Error	*P* Value
Aerobic exercise and *Ginkgo biloba* consumption	Aerobic exercise	-0.14	0.02	<0.001
	Ginkgo biloba consumption	-0.11	0.02	<0.001
	Control	-0.26	0.02	<0.001
Aerobic exercise	*Ginkgo biloba* consumption	0.03	0.02	0.99
	Control	-0.11	0.02	<0.001
Ginkgo biloba consumption	Control	-0.15	0.02	<0.001

significant difference between the two groups "aerobic exercise" and "*Ginkgo biloba* consumption" ($P=0.99$).

Discussion

The results of the present study demonstrated that aerobic exercise, *Ginkgo biloba* herbal supplementation, and a combination of both had a significant effect on Lcn2 levels in obese men. There was also a significant difference between the groups compared to the control group.

Although more than a decade has passed since the recognition of Lcn2, the physiological function of this protein is still incomprehensible, and changes in the blood levels of this protein, especially due to adaptation to physical activity, have been studied in very few human specimens. Although this cytokine was previously known as a secretory protein from kidney tissue (18, 19), it was first introduced as an inflammatory marker associated with obesity. Subsequently, this adipocytokine has been cited in various studies as a mediator for obesity and insulin resistance, and other obesity-related metabolic disorders (20, 21). The findings of the present study are consistent with the results of the studies conducted by Mehrabani et al (9) and Ceylan et al (22), while contradicting those of the research conducted by Moghadasi et al (23) and Choi et al (8). These differences include the physiological characteristics of the subjects, as well as the dose and intensity of the exercise.

Lcn2 is reported to be involved in iron accumulation within heart cells, inflammation, and apoptosis, whereas adiponectin, unlike Lcn2, modulates iron through an unknown mechanism of entry into heart cells. On the other hand, iron accumulation in cardiomyocytes leads to the production of free radicals, and since GbE increases the activity of the released antioxidant enzymes in the heart, this process is expected to be controlled to some extent by taking antioxidant supplements and exercising. Motamedi et al stated that changes in Lcn2 and plasma glucose levels were not significant after eight weeks of intermittent aerobic exercise with moderate intensity. They also suggested complex links between Lcn2 and metabolic disorders caused by obesity and inflammation, and other factors, including hormonal changes and substrate metabolism, which alter plasma levels of Lcn2, independent of the effects of exercise (24). Hosseini et al reported that among the plasma levels of Lcn2 in the five groups, exercise and consumption of the coriander extract did not represent a significant difference, which was probably due to the short duration of exercise and the dose of coriander extract (25). Ghorbanian et al demonstrated the absence of a significant effect of exercise on Lcn2 by examining the effect of an increasing period of resistance training on Lcn2 levels and lipid profile in inactive men. Although resistance training significantly reduced some of the lipid markers, they stated that since Lcn2 is secreted from sources other than adipose tissues (i.e., epithelial cells, liver, kidney, and lung), it is likely that exercise would stimulate these cells and other inflammatory factors to secrete more Lcn2, although this increase was not significant (10). In a study on obese Korean women performing aerobic and resistance training 5 sessions per week, Choi et al reported the lack of any significant effect on Lcn2, while anthropometric indices decreased significantly. They attributed the absence of changes to other factors such as adipocyte fatty acid-binding protein (8).

The results of the present study showed that aerobic exercise, *Ginkgo biloba* herbal supplementation, and a combination of both significantly reduce insulin resistance levels in obese men. There was also a significant difference between the intervention group compared to the control group. The present findings are consistent with those of Atashak et al (26) and Zhu and Xie (27). On the other hand, they contradict the results of Hasanvand

and Farhadi (16), Barbalho et al (28), and Eisvand et al (15). Perhaps this discrepancy in the training program could be attributed to the intensity of the exercise, the type of subjects, the type of exercise, and the gender and age of the subjects. Likewise, Guelfi et al measured the effect of 12 weeks of aerobic and resistance training with a controlled diet on 30 overweight men and concluded that insulin resistance in both aerobic and resistance groups was significantly lower than in the diet group (29).

Previous research has shown that insulin secretion is inhibited by the increased levels of norepinephrine due to exercise. It is also possible that exercise-induced insulin depletion is associated with glucose savings, limiting muscle glucose uptake by muscle and making blood glucose more available to the brain. The possible causes of decreased insulin resistance because of activity include insulin-independent mechanisms such as increased GLUT-4 as a result of muscle contractions (10).

Zhu and Xie concluded that training duration increased insulin sensitivity, which could be related to an increase in insulin signaling receptors, an increase in glucose and mRNA transporter protein levels, activation of glycogen, and hexokinase synthesis or increased reversal muscle glucose, or changes in muscle composition. Exercise alone, without losing weight, can lead to changes in insulin sensitivity (27). Waters et al also showed that changes in insulin levels were associated with changes in the reduction of abdominal fat (30). Similarly, Xing et al found that both intensity and duration of exercise were effective so that improvements in insulin sensitivity occurred when applying the highest level of exercise (31). Therefore, probably 3 training sessions per week for 6 weeks were sufficient, according to the characteristics of the subjects in the present study, to achieve a significant change in insulin and the insulin resistance index. Insulin resistance and impaired glucose metabolism usually form a gradual process that begins with overweight and obesity.

Some studies demonstrated that exercise improves glucose homeostasis and increases insulin sensitivity. Waters et al introduced mechanisms to increase the action of insulin due to exercise, which increased the signaling of insulin receptors, increased glucose transporter protein, enhanced glycogen storage capacity due to higher enzyme activity glycogen synthetase and hexokinase, increased glucose release from blood to muscle due to increased muscle capillaries and changes in muscle composition to increase glucose uptake, and decreased release while increasing clearance of free fatty acids (30).

Regarding the effect of GbE on insulin resistance in people with metabolic syndrome and obesity, the finding of Zhu and Xie is in line with those of the present study. In a study on 17 patients with metabolic syndrome, they showed that 2 months of treatment with *Ginkgo biloba* reduced high-sensitivity C-reactive protein by 44.4% and HOMA-IR by 15.3% (27). Banin et al (32) also represented that GbE reduced insulin resistance in high-fat diet mice. They reported that 8 weeks of supplementation in mice with three different doses (100, 200, and 400 mg/kg) reduced insulin levels by 29%, 55%, and 70%, respectively, and caused resistance by 50%, 69%, and 80%, respectively. On the other hand, Kudolo et al indicated that the short-term use of GbE did not significantly improve or alter insulin sensitivity in diabetic and non-diabetic individuals. Possible differences may include the model of the subjects, the method of consumption, and its duration (33).

GbE is useful in the treatment of neurodegenerative diseases because of the clearance of free radicals (15). In addition, GbE effectively reduces lipid fat accumulation and inhibits the formation of atherosclerotic plaques (15). Further, it induces the release of adiponectin from adipocytes and insulin from beta cells in vitro (15). However, the association of GbE and insulin sensitivity has been rarely investigated previously.

Insulin resistance is a key pathogenic factor in type 2 diabetes. AMPK is an important node that links insulin signaling and lipid metabolism and is a positive regulator of insulin resistance. Downstream regulation of AMPK activity in HFD-fed mice, as associated with a decrease in AMPK phosphorylation, reflects the altered adverse effects of fat on insulin signaling. The activation of AMPK by GbE 761 and greater phosphorylation of AMPK after EGb761 treatment, not only improves hepatic lipid metabolism but also benefits from insulin signaling. However, more research is needed to identify the mechanisms of GbE 761 on AMPK activity and its effects on insulin signaling (15).

Conclusion

Performing aerobic exercise for six weeks with *Ginkgo biloba* supplementation could significantly reduce serum Lcn2 and insulin resistance index in obese men. It is recommended that obese men use a combination of aerobic exercise with the *Ginkgo biloba* supplement.

Acknowledgments

The authors acknowledge their deep gratitude toward all participants who helped us conduct this research project.

Author Contributions

Conceptualization: SAH; Study validation and supervision: BA; Data analysis and interpretation: SAH, BA; Writing and reviewing: MV.

Conflict of Interests

There is no conflict of interests to be declared.

Ethical Approval

Prior to the beginning of the study, ethical approval was obtained from the Scientific and Ethical Committee of Mahallat Branch, Islamic Azad University (Ethics No. 20021404972001).

Informed Consent

All participants gave their written informed consent after receiving explanations regarding the study objective and methodology.

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