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The Effect of 8 Weeks of TRX and Pilates Training on Serum Afamin Levels in Overweight Young Women

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A B S T R A C T

Afamin is a vitamin E-binding glycoprotein in plasma that is primarily expressed in the liver. It facilitates the transport of vitamin E across the blood-brain barrier and is strongly associated with all components of the metabolic syndrome (Dieplinger & Dieplinger, 2015). The primary aim of this study was to examine the effect of 8 weeks of TRX and Pilates training on serum afamin levels in overweight young women. In this study, 30 inactive overweight women with a BMI over 25, aged between 25 and 35 years, were randomly selected and assigned to three groups: TRX (N=10), Pilates (N=10), and control (N=10). The Pilates training group performed exercises three sessions per week for 60 minutes per session, including postural exercises, posture identification, relaxation, stretching and balance movements, respiratory endurance, and strength exercises. The TRX group performed TRX workouts three times per week, each session lasting 60 minutes. Blood samples were taken 24 hours before the first training session and 48 hours after the last session. Paired t-tests and mixed analysis of variance (Mixed ANOVA) were used. Data were analyzed using SPSS version 26. Eight weeks of TRX and Pilates training resulted in significant changes in the BMI of overweight young women (P = 0.006). Additionally, eight weeks of Pilates training led to a significant decrease in afamin levels (P = 0.008). However, neither intervention produced significant changes in blood glucose levels (P = 0.280), insulin (P = 0.140), or insulin resistance (P = 0.162). Based on the findings of this study, it can be cautiously stated that Pilates training, in comparison to TRX, may reduce serum afamin levels in inactive obese women. This reduction could potentially serve as a predictive factor for weight loss, fat reduction, and a decrease in BMI in this population.

Keywords: *Pilates, TRX, Afamin, Overweight.*

1. Introduction

Obesity and overweight are chronic disorders that occur due to an increase in fat mass beyond normal limits, leading to a disruption in body composition. It is projected that by the

year 2030, three billion people worldwide will be affected by overweight and obesity (1). In addition to the high prevalence of various diseases (e.g., cardiovascular disease, diabetes) among individuals with overweight and obesity, research indicates that mortality rates in obese individuals are 75% higher compared to those with normal weight (2).

Body composition is defined as the ratio of fat mass to lean mass in the body. Various anthropometric indices have been introduced to assess body composition and diagnose overweight and obesity. Among these, body fat percentage measurement is considered the ideal method (3). However, Body Mass Index (BMI) is more widely used due to its simplicity, safety, and accuracy (4). Additionally, waist circumference and waist-to-hip ratio are suggested as predictors of central and abdominal obesity (5).

Another emerging biomarker for predicting obesity and diabetes is afamin. Afamin was first described in 1994 as the fourth member of the human albumin gene family, which includes albumin, α-fetoprotein, and vitamin D-binding protein. It is primarily expressed in the liver, as well as in tissues such as the brain, testes, ovaries, and kidneys. Knowledge regarding the (patho)physiological functions of this protein remains limited. Transgenic mice overexpressing the human afamin gene exhibited increased body weight and elevated blood lipid and glucose levels (6).

In a study conducted in 2019, Borzoi, Khodamradi, and Ranjbar Nejat Kheiripour examined the effect of nano-curcumin on insulin resistance and serum afamin levels in patients with metabolic syndrome. They described metabolic syndrome as a metabolic disorder consisting of clinical symptoms such as central obesity, insulin resistance, hyperglycemia, dyslipidemia, and hypertension. With the rising prevalence of obesity, the incidence of this syndrome is also expected to increase. This metabolic disorder significantly elevates the risk of cardiovascular disease (CVD) and type 2 diabetes (7).

In another study, Polkowska et al. (2019) investigated serum concentrations of adropin, afamin, and neudesin in children with type 1 diabetes. Their findings suggested that these biomarkers might play significant roles in glucose metabolism regulation and possess substantial potential as novel biomarkers for predicting future metabolic disorders. However, further studies involving larger patient cohorts are needed to determine the role of these proteins in the progression and treatment of type 1 diabetes (8).

Similarly, Kollerits et al. (2017), in a study titled "Plasma Concentrations of Afamin Are Associated With Prevalent and Incident Type 2 Diabetes: A Pooled Analysis in More Than 20,000 Individuals," reported that afamin is strongly associated with insulin resistance, and with both the prevalence and incidence of type 2 diabetes, independent of key metabolic risk factors. Afamin may serve as a promising novel biomarker for identifying individuals at risk of developing type 2 diabetes (9).

The mechanisms underlying the relationship between afamin and weight loss or type 2 diabetes remain unclear. Initial findings indicating a hyperglycemic phenotype in transgenic mice for the human afamin gene support the important role of afamin in the development of type 2 diabetes (10). Recently, Shen et al. (2016) demonstrated a direct role of afamin in glucose metabolism (11).

A comprehensive analysis of population-based studies has revealed that afamin is significantly associated with obesity, prediabetes, insulin resistance, and both the prevalence and development of type 2 diabetes, independent of major metabolic parameters. Therefore, elevated plasma afamin concentrations may indicate the early stages of obesity and type 2 diabetes. A review of previous literature shows a lack of studies examining the effect of exercise and physical activity on afamin levels. Given the known effects of exercise on body fat and the potential of serum afamin as a predictor in obesity, diabetes, cardiovascular disease, and metabolic syndrome, the present study seeks to answer the question: Do exercise programs such as Pilates and TRX affect serum afamin levels, body composition, blood glucose, and insulin levels, and can afamin levels be used as a predictive factor for body fat and obesity?

2. Methods and Materials

This study was quasi-experimental and applied in nature, conducted using a pretest-posttest design. The statistical population consisted of inactive women with overweight who attended the Elm va Niroo Sports Club in Varamin city. The participants had a BMI above 25 and were between the ages of 25 and 35. None of the participants had engaged in any regular exercise program for at least six months prior to the study and only performed routine daily activities.

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Participants were excluded if they smoked, had a history of cardiovascular disease, diabetes, hereditary blood disorders, respiratory problems, pregnancy, or were taking any medication for weight loss or other medical conditions.

Two types of exercise interventions were used in this study: TRX and Pilates. The research employed a pretest-posttest design with two experimental groups and one control group. All selected participants were first assessed via pretest, then participated in their respective training protocols, and the dependent variables were measured again at the end of the intervention. In total, 30 overweight inactive women with a BMI above 25 and aged between 25 and 35 years were randomly selected from among those attending the Elm va Niroo Sports Club and assigned into three groups: TRX (N=10), Pilates (N=10), and control (N=10).

Participants were given a health questionnaire to ensure that they met the inclusion criteria. After evaluation, 30 participants were selected and distributed into the control, Pilates, and TRX groups. Efforts were made to precisely control all influencing variables during various stages of the research, including nutrition, temperature, BMI, environment, age, sex, absence of disease, health status and history, and sleep timing prior to testing. Participants followed a scheduled plan to participate in the tests. They were fully informed of the study's procedures, risks, and potential consequences, and gave written consent.

A few days before the start of testing, participants received detailed instructions on pre-test sleep and nutrition schedules. Two weeks prior to the start of training, baseline assessments were conducted, including height, weight, BMI, waist circumference, and hip circumference. Insulin resistance was calculated using the homeostatic model assessment (HOMA-IR) as follows (12):

HOMA-IR = (Fasting Glucose [mg/dL] × Fasting Insulin [µU/mL]) / 405

Blood samples (10 mL) were drawn from the antecubital vein of each participant in all three groups 24 hours before the first training session and 48 hours after the last session, at 8:30 a.m. in a fasting state. All participants had fasted for approximately 12–14 hours under the same conditions. The blood samples were centrifuged at 3,000 rpm for 10 minutes, and the desired variables were analyzed using commercially available kits in a laboratory.

This study was conducted during the summer of 2020 at the Elm va Niroo Sports Club in Varamin. Data collection instruments included: an afamin kit (manufactured by ZellBio GmbH, Germany, measured using the ELISA method with a Hiperion device), a glucose kit (manufactured by Pars Azmun, Iran, measured by photometric method using Hitachi 917), an insulin kit (manufactured by Siemens, Germany, measured by chemiluminescence using Immulite 2000xpi), a demographic and consent questionnaire, a SECA medical scale and stadiometer (Germany; precision: 0.5 kg and 0.1 cm respectively), a Polar heart rate monitor, and a 2019 model TRX Pro System suspension trainer.

The Pilates intervention lasted for 8 weeks, with 3 sessions per week, each lasting 60 minutes. Each session included 10 minutes of specialized Pilates warm-up, 40 minutes of basic Pilates exercises including postural training, posture recognition, relaxation, stretching and balance movements, respiratory endurance, and strength training, followed by 10 minutes of Pilates-specific cool-down.

The TRX group trained three times a week for 60 minutes per session, which included 10 minutes of general and TRX-specific warm-up, 40 minutes of TRX-specific exercises, and 10 minutes of stretching and cool-down. Initially, the exercises for both Pilates and TRX were simple and aimed at familiarizing participants with the principles of the methods. As participants progressed, the exercises increased in intensity and complexity.

Exercise intensity in the Pilates group was monitored using heart rate, starting at 55% of maximum heart rate. To apply the principle of progressive overload, intensity increased by 5% every two weeks—60% in week 2, 65% in week 4, and 70% in week 6. A 30-second rest interval was given between each exercise (13). In the TRX group, exercise intensity was set at 55% of one-repetition maximum (1RM), with 5% increments every two weeks—reaching 60% in week 2, 65% in week 4, and 70% in week 6 (14).

Data were analyzed using SPSS version 26. The Shapiro-Wilk test was used to assess the normality of the data. After confirming normal distribution, paired t-tests and mixed analysis of variance (Mixed ANOVA) were used to analyze the data. In cases of significant differences between groups, Bonferroni post-hoc tests were applied. Statistical analysis was conducted at a significance level of $P \leq 0.05$.

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3. Findings and Results

The means and standard deviations of participants' height, body weight, and BMI in the three groups are presented. The mean and standard deviation of height across all three groups are relatively close, suggesting that the groups are approximately homogeneous. There was no significant difference in participants' weight prior to the intervention; however, after the training period, the mean weight of the Pilates and TRX groups decreased, though the decrease was not statistically significant (Table 1).

Table 1

Descriptive statistics for height, body weight, and BMI by group (Mean ± SD)

Variable	Control Group	Pilates Training Group	TRX Training Group
Height (cm)	162.00 ± 4.09 (pre) / 160.55 ± 4.27 (post)	—	161.66 ± 6.67 (pre)
Weight (kg)	73.70 ± 6.32 (pre) / 74.16 ± 6.36 (post)	74.44 ± 12.77 (pre) / 71.93 ± 13.02 (post)	70.08 ± 8.89 (pre) / 68.40 ± 8.75 (post)

The means and standard deviations of body composition, afamin levels, blood glucose, insulin, and insulin resistance in the three groups are presented below (Table 2).

Table 2

Descriptive statistics of key variables by group (Mean ± SD)

Variable	Control Group	Pilates Training Group	TRX Training Group
BMI (kg/m²)	28.05 ± 2.24 (pre) / 28.25 ± 2.20 (post)	28.84 ± 4.67 (pre) / 27.87 ± 4.81 (post)	26.78 ± 2.60 (pre) / 26.12 ± 2.43 (post)
Afamin (ng/mL)	6.78 ± 5.38 (pre) / 8.10 ± 4.17 (post)	33.65 ± 21.58 (pre) / 27.00 ± 17.28 (post)	25.60 ± 13.68 (pre) / 22.23 ± 10.91 (post)
Glucose (mg/dL)	93.66 ± 5.31 (pre) / 93.83 ± 6.21 (post)	90.66 ± 7.08 (pre) / 87.22 ± 6.01 (post)	96.11 ± 5.46 (pre) / 93.44 ± 6.94 (post)
Insulin (µIU/mL)	5.50 ± 3.74 (pre) / 5.65 ± 3.14 (post)	8.04 ± 4.24 (pre) / 6.15 ± 2.23 (post)	11.82 ± 10.33 (pre) / 5.78 ± 2.28 (post)
HOMA-IR	1.283 ± 0.890 (pre) / 1.306 ± 0.718 (post)	1.853 ± 1.147 (pre) / 1.339 ± 0.523 (post)	2.882 ± 2.67 (pre) / 1.343 ± 0.562 (post)

As shown in Table 2, the mean and standard deviation of body composition and afamin in the TRX group were low in both pretest and posttest. Although posttest body composition values in the Pilates and TRX groups decreased compared to the pretest, the reduction was not statistically significant. Mean blood glucose values in the pretest were similar across the groups and did not differ significantly. Although blood glucose levels in the intervention groups decreased after training compared to the control group, the decrease was not statistically significant. Similarly, there were no significant differences in insulin levels between groups in the pretest and posttest, though a reduction in insulin was noted in both intervention groups after eight weeks of training. No significant differences were observed in HOMA-IR between groups before and after training, although values decreased slightly in both intervention groups.

The Shapiro-Wilk test was used to assess the normal distribution of the data. Levene's test was used to evaluate homogeneity of variances, which was confirmed.

Table 3

Paired t-test results for each variable in the three groups

Variable	Group	Mean Change	df	t	p-value
BMI (kg/m²)	TRX	0.66	9	2.95	.018*
	Pilates	0.97	9	4.335	.002*
	Control	0.174	9	1.382	.226
Afamin (ng/mL)	TRX	3.37	9	1.718	.124
	Pilates	6.65	9	2.917	.019*
	Control	1.32	9	1.433	.211
Glucose (mg/dL)	TRX	2.66	9	1.455	.184
	Pilates	3.44	9	2.784	.024*

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	Control	0.166	9	0.164	.876
Insulin (µIU/mL)	TRX	6.03	9	2.08	.071
	Pilates	1.88	9	1.357	.212
	Control	0.15	9	0.443	.676
HOMA-IR	TRX	1.538	9	2.033	.077
	Pilates	0.513	9	1.401	.199
	Control	0.022	9	0.269	.798

*Significant difference between pretest and posttest ($p \leq .05$)

Table 3 indicates that the intervention significantly affected body composition in both training groups, and serum afamin and glucose levels in the Pilates group. However, paired t-tests revealed no significant differences in the control group after 8 weeks. There were also no significant effects on insulin or insulin resistance in any group.

Table 4

Mixed ANOVA results for between-group differences

Variable	Source of Variation	SS	df	MS	F	p	η^2
BMI (kg/m²)	Time	2.729	1	2.729	14.921	.001*	.415
	Time × Group	2.421	2	1.210	6.617	.006*	.387
Afamin (ng/mL)	Time	97.353	1	97.353	9.729	.023*	.223
	Time × Group	114.665	2	57.041	3.332	.047*	.253
Glucose (mg/dL)	Time	45.433	1	45.433	4.981	.037*	.192
	Time × Group	24.722	2	12.361	1.355	.280	.114
Insulin (µIU/mL)	Time	77.667	1	77.667	4.372	.057	.172
	Time × Group	76.675	2	38.337	2.158	.140	.170
HOMA-IR	Time	5.298	1	5.298	4.349	.057	.172
	Time × Group	4.841	2	2.420	1.987	.162	.159

*Significant difference ($p \leq .05$)

According to Table 4, the interaction of time × group was significant for body composition and afamin. This suggests that the changes from pretest to posttest varied significantly between the groups. For glucose, insulin, and insulin resistance, changes were not significant.

Table 5

Bonferroni multiple comparisons between groups for body composition and afamin

Variable	Comparison	F	p
BMI (kg/m²)	Control vs. Pilates	F = 0.183	p = .008*
	Control vs. TRX	F = 1.720	p = .121
	Pilates vs. TRX	F = 1.904	p = .001*
Afamin (ng/mL)	Control vs. Pilates	F = 22.88	p = .018*
	Control vs. TRX	F = 16.47	p = .117
	Pilates vs. TRX	F = 6.41	p = .008*

*Significant difference between groups ($p \leq .05$)

The mixed ANOVA revealed significant effects for body composition and serum afamin ($p < .05$). Bonferroni post hoc tests confirmed significant differences between the control and Pilates groups, and between the Pilates and TRX groups.

4. Discussion and Conclusion

To the best of our knowledge, the present study is the first to investigate the effect of physical activity on serum afamin levels. A significant change in serum afamin levels was

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observed following a period of Pilates training, along with improvements in body composition and blood glucose levels in overweight individuals.

Our current understanding of the potential physiological or pathophysiological functions of afamin remains limited. Recent animal studies have shown that overexpression of the afamin gene leads to increased body weight and elevated blood glucose and lipid concentrations. Preliminary findings in transgenic mice with a hyperglycemic phenotype expressing high levels of the afamin gene support the potential role of afamin in the development of type 2 diabetes and indicate that elevated plasma afamin concentrations may predict the onset of metabolic syndrome (10).

Human studies have also demonstrated an association between afamin and the development of metabolic syndrome. In a study conducted by Seres et al. (2017), increased afamin levels were found to be associated with components of metabolic syndrome, including visceral obesity (15).

In another study, Ahmed et al. (2015) examined inflammatory markers and adipokines in obese children and their relationship with serum afamin levels—a novel vitamin E-binding protein. They reported that obesity is associated with chronic low-grade inflammation, which may play a role in the pathogenesis of obesity-related complications. A positive correlation was observed between increased inflammatory markers and weight gain. The study found that the mean serum levels of afamin and inflammatory markers such as CRP were higher in obese children compared to their normal-weight peers. A positive correlation between afamin and BMI was reported, along with a negative correlation between afamin and adiponectin. Weight loss may thus improve inflammatory markers and reduce serum afamin levels. However, the authors emphasized the need for further studies to clarify the role of afamin and its relationship with other inflammatory biomarkers, potentially leading to the identification of new therapeutic targets for obesity (16).

Seeber et al. (2014) also reached similar conclusions, noting that high afamin levels were associated with metabolic syndrome in young women and could serve as an independent predictive factor for the development of metabolic syndrome in women at risk for overweight and obesity (6). Their findings corroborated a previous population-based study showing that afamin concentrations were associated with several metabolic syndrome parameters in men and women aged 50 to 60. Among the metabolic syndrome laboratory parameters assessed, triglyceride level was the only independent predictor of afamin concentration (6). Similarly, Kronenberg et al. (2014) demonstrated that afamin was most strongly associated with triglyceride levels and waist circumference in older men and women (10).

Kollerits et al. (2017), in a prospective cohort study, also found that plasma afamin concentrations were associated with both the prevalence and incidence of type 2 diabetes. A 20% increase in type 2 diabetes incidence was observed with elevated plasma afamin levels. Most researchers described a strong correlation between afamin and prediabetes, as well as type 2 diabetes-related phenotypes such as insulin resistance. However, the mechanisms underlying this relationship and the causal pathways require further investigation (9). In another study by Köninger et al. (2018), afamin was proposed as a potential biomarker for glucose metabolism in individuals with gestational diabetes (17).

Physical activity, depending on its type, intensity, and duration, may influence factors related to metabolic syndrome. However, the mechanisms responsible for such changes remain unclear. Potential mechanisms include improvements in body composition and blood glucose regulation (18). The results of the present study are consistent with these findings. It can be stated that due to the reduction in serum afamin levels after eight weeks of aerobic Pilates training, there were observable reductions in body weight, BMI, and WHR—all of which are positively correlated with afamin levels—making these outcomes unsurprising. Improved glucose metabolism following the Pilates intervention also contributed to changes in afamin levels among overweight participants in the study. As noted, triglyceride levels are also a predictor of serum afamin levels (6). In the present study, triglyceride levels were not measured to assess their correlation with afamin, which represents a limitation of this research.

The present study found that TRX training did not have a significant effect on afamin levels. It is possible that the negative energy balance induced by this type of resistance training was insufficient to elicit changes in afamin concentrations. Furthermore, there were no significant

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improvements in some metabolic indicators (e.g., blood glucose) following TRX training, which could explain the lack of change in afamin levels.

It is recommended that future studies investigate the effects of endurance, resistance, and combined training on afamin across various health conditions such as diabetes, cancer, cardiovascular disease, and metabolic syndrome. Additionally, it is suggested that variables be measured at midpoints throughout the intervention, in addition to pre- and posttests, to determine the precise timing of observed changes.

In conclusion, eight weeks of Pilates training resulted in a significant reduction in serum afamin levels in the posttest and a significant improvement in body composition in the Pilates group compared to the control group. Based on the findings of this study, it can be cautiously stated that, in comparison to TRX training, Pilates may reduce serum afamin levels in inactive obese women, and this reduction could serve as a predictive factor for weight loss, fat reduction, and decreased BMI in this population.

Authors' Contributions

All authors equally contributed to this study.

Declaration

In order to correct and improve the academic writing of our paper, we have used the language model ChatGPT.

Transparency Statement

Data are available for research purposes upon reasonable request to the corresponding author.

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Declaration of Interest

The authors report no conflict of interest.

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Ethics Considerations

The study placed a high emphasis on ethical considerations. Informed consent obtained from all participants, ensuring they are fully aware of the nature of the study and their role in it. Confidentiality strictly maintained, with data anonymized to protect individual privacy. The study adhered to the ethical guidelines for research with human subjects as outlined in the Declaration of Helsinki. Ethical considerations included obtaining informed consent, ensuring confidentiality and anonymity, and avoiding any harm to participants.

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