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The effect of exercise on GDF-15 levels in individuals with prediabetes: A randomized controlled trial

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ABSTRACT

Aims: Growth differentiation factor-15 (GDF-15) is an inflammatory cytokine that increases in prediabetes and is known for its anorexigenic effects. This study aims to evaluate the effects of a 12-week exercise program on GDF-15 in individuals with prediabetes.

Materials and Methods: In this multicenter, parallel-group, randomized-controlled trial, 64 patients aged 18–60 diagnosed with prediabetes were randomized in a 1:1 ratio into the exercise group (E) and the control group (C). Additionally, 32 patients who were planned to start metformin were included in the metformin group (M). Participants in the exercise group engaged in aerobic exercise at 50–70% of their maximum heart rate for 60 min, 3 days a week. Serum GDF-15 levels were evaluated at the beginning and the end of the 12th week.

Results: The mean age of the 91 participants who completed the study was 46.13 ± 8.52 years, and 23.1% were male. Basal GDF-15 levels were similar among the groups (E = 668.6 ± 415.1, C = 651.8 ± 352.5, M = 603.6 ± 387.2, $P = 0.47$). At the 12th week, GDF-15 levels were lower in the E compared to the C, while higher in the M compared to the C (E = 383.1 ± 215.6, C = 556.4 ± 285.6, M = 810.8 ± 498.0, $P < 0.001$). In inter-group comparisons, no significant change was observed in the C between the 0th and 12th weeks, while GDF-15 decreased in the E ($P < 0.001$) and increased in the M ($P < 0.001$).

Conclusions: It was determined that in individuals with prediabetes, GDF-15, which serves both as a biomarker of metabolic disorder and has a negative regulatory effect on appetite, decreased with 12 weeks of aerobic exercise and increased with metformin administration.

INTRODUCTION

The global prevalence of prediabetes has increased along with changing lifestyle factors, significantly elevating the risk of progression to type 2 diabetes and other metabolic diseases, including cardiovascular disease[1]. Based on data from the Diabetes Prevention Program study, exercise and dietary changes are recommended for all patients with prediabetes, while metformin therapy should be considered for individuals with prediabetes under 60 years of age[2, 3].

Various cytokines continue to be investigated regarding the relationship between the positive effects of exercise on metabolic health and changes at the cellular level. Growth differentiation factor-15 (GDF-15) is a dimeric cytokine from the transforming growth factor-β (TGF-β) superfamily involved in the regulation of macrophage activation[4]. Apoptosis is involved in signal transduction, cell repair, and cell growth and is lowly

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expressed in organs under physiological conditions[5, 6]. When damage, inflammation, or other stress conditions occur in the body, GDF-15 expression increases, and it assumes critical roles in processes such as repair of damaged tissues, modulation of inflammatory processes, and regulation of cell death[7].

Several studies have reported that GDF-15 can be used as a biomarker in cardiovascular diseases[8–10]. GDF-15 has been found to be increased in patients with impaired glucose tolerance and positively correlated with insulin resistance[11].

Growth differentiation factor-15 is also an essential adipokine released in thermogenic activation and increases energy consumption[12]. Metformin decreases body weight by increasing the secretion of GDF-15, an important cytokine in the gut-brain axis independent of the insulin pathway[13]. Studies in the literature have implicated GDF-15 as a therapeutic target in the treatment of obesity[14]. It has been shown that exercise, especially when it involves intense and continuous physical activity, can acutely increase the expression of GDF-15[15].

In light of the information available in the literature, it is expected that GDF-15 levels are already increased in individuals with prediabetes. However, considering the increase in GDF-15 with metformin and acute exercise, the effect of guideline-based aerobic exercise programs on GDF-15 in individuals with prediabetes emerges as an important matter to be examined scientifically. No comparative study examining the effect of exercise and metformin on GDF-15 in individuals with prediabetes was identified in the literature. The present study aimed to determine the effect of a 12-week aerobic exercise program on GDF-15 in individuals with prediabetes and to compare the effects of exercise and metformin on GDF-15 levels.

MATERIALS AND METHODS

Study design

The study was a 12-week, multicenter, statistical analysis-blinded, parallel-group, randomized controlled trial. A total of 96 participants admitted to the Sultan Abdülhamid Han II Training and Research Hospital, Fatih Sultan Mehmet Training and Research Hospital, and Prof. Dr. Suleyman Yalcin City Hospital between 08.04.2021 and 07.12.2022 were included in the study. Participants were informed about the study objectives, procedures, and data confidentiality before participating in the study, and they were assured that participation was voluntary and that they could withdraw from the study at any time. Participants were fully informed about the risks and benefits of exercise. This cohort study was reported in accordance with the Consolidated Standards of Reporting Trials (CONSORT) reporting guidelines.

Study patients

In the study, patients aged 18–60 years who had been newly diagnosed with prediabetes, evaluated as physically inadeaquate active according to the International Physical Activity Questionnaire, had sufficient motivation to participate in the exercise program (assessed through individual interviews conducted by researchers), were deemed suitable for participation in the exercise program following a cardiac examination performed by a cardiologist (including resting electrocardiogram and questioning of cardiac symptoms, advanced tests such as exercise electrocardiogram if necessary), and had maintained the same weight for the last 6 months before the study (±2.5 kg) were included. The exclusion criteria for the study were as follows: having a serious medical condition that would prevent the person from participating in the exercise study (e.g., advanced malignancy or major neurological, psychiatric, or endocrine disease, respiratory failure, etc.), using metformin for the past 6 months prior to the study, having cardiovascular disease, heart failure, cancer, BMI <19 kg/m^2, undergoing hormone replacement therapy, having a life expectancy of less than 1 year, being HIV positive, substance use, functional dependence, orthopedic diseases that prevent physical activity, cognitive impairment, and other diseases that would affect the outcome of the study (e.g., respiratory diseases, muscle diseases, non-alcoholic fatty liver disease, etc.), and the use of medications or dietary supplements that would affect the outcome of the study. In addition to all these inclusion and exclusion criteria, individuals who were planned to start metformin by their physician were recruited and assigned to the metformin group before starting metformin.

Randomization and masking

A total of 32 patients who were diagnosed with prediabetes and planned to start metformin by their physicians were included in the metformin group. Block randomization in a 1:1 ratio was applied to 64 patients who would not be started on metformin, using computer-generated randomization by "www.randomizer.org" in blocks of two. All participants were provided guideline-based standardized lifestyle recommendations. Participants and practitioners were not blinded to allocation. Blinding was applied for the study of blood samples and statistical analyses. The flow diagram of the participants is presented in Figure 1.

Follow-up visits

The socio-demographic characteristics and habits of the participants who met the inclusion criteria were recorded. All participants were then invited to the first visit. Weight and body mass index (BMI) measurements were performed. The International Physical Activity Questionnaire and Mediterranean Diet Adherence Screener were applied. Blood samples were taken, and body mass indices were measured. Participants in the exercise group started the exercise program 24–48 h after the first visit. Participants in the metformin group had their first visit the day after they were prescribed metformin, before taking the first medication. Intermediate visits were carried out in the event of any complication or condition that the patient wished to report upon the patient's request. At the end of 12 weeks, second visits were performed. It was performed 24–48 h after the last exercise of the participants in the exercise group. At the

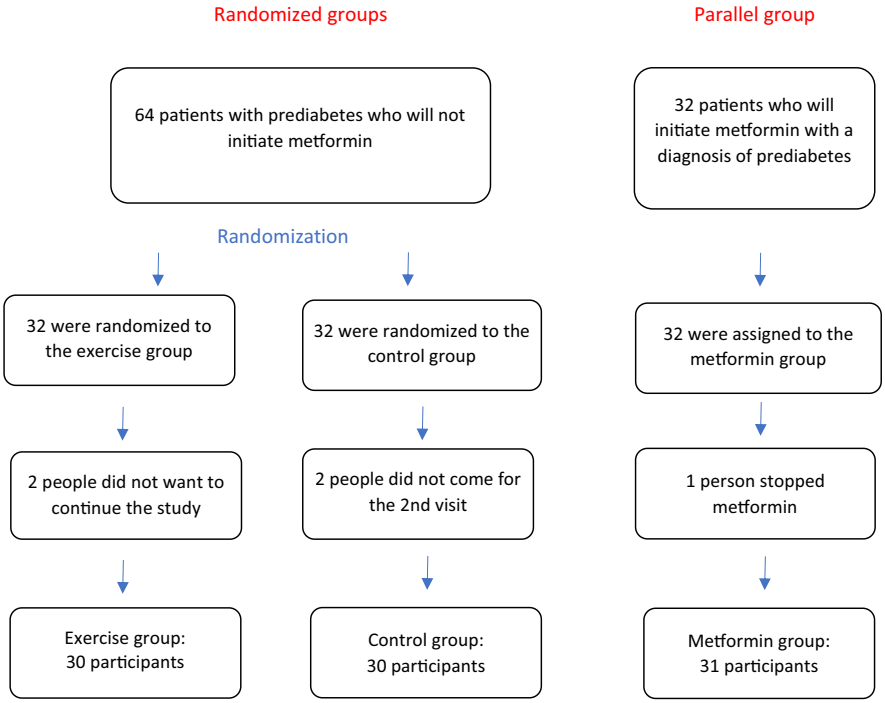


Figure 1 | The figure shows the flow diagram of participants during the research process.

second visit at the 12th week, all measurements performed at the first visit were repeated.

Blood samples

At the beginning and end of the study, 6 mL peripheral blood samples were collected from all prediabetic subjects following an 8-h overnight fast. GDF-15 level was measured in the serum sample obtained using a human ELISA kit (BT Lab, Korea, Shangai). Glucose, insulin, low-density lipoprotein (LDL), high-density lipoprotein (HDL), triglyceride, c-peptide, and HbA1C values were also measured in the serum sample. HOMA-IR (Homeostatic Model Assessment of Insulin Resistance) value was calculated with the formula glucose (mg/dL) × and insulin (mU/mL)/405.

Exercise intervention

Participants in the intervention group underwent aerobic exercise for 12 weeks at the Fatih Sultan Mehmet Training and Research Hospital, Diabetes and Obesity Sports Center. Participants were instructed to perform aerobic exercise at 50–70% of their maximum heart rate (moderate weight) for 60 min each 3 days a week under the supervision of specialist physicians, accompanied by a sports trainer and a sports physiotherapist. Maximum heart rate was calculated with the Karvonen formula (220-age), pulse was monitored with a fingertip pulse oximeter during exercise, and the exercise rhythm corresponding to moderate-weight exercise was determined. Aerobic exercises were performed at 50% of the maximum heart rate for the first 4 weeks, 60% of the maximum heart rate between weeks 4–8, and 70% of the maximum heart rate between weeks 8 and 12. The exercise performed for 30 min on a treadmill and 30 min on a bicycle ergometer was monitored by physiotherapists, and the working speeds of the exercise equipment were adjusted. Heart rate, blood pressure, and saturation measurements were recorded before and after the exercise sessions. During the recovery period, participants were monitored until their vital signs returned to resting values. One day of rest was given between exercise days, and a 2-day rest was provided after the third exercise day. Exercise adherence was controlled and monitored by the researchers. The adherence of the metformin group to the medication was monitored by the number of pills consumed. To ensure that the physician supervision did not differ between the intervention, control, and metformin groups, all participants were offered continuous one-to-one contact with the attending physician. All patients on metformin were followed up 1 week, 1, 2, and 3 months after drug initiation by questioning symptoms (nausea, vomiting, dyspepsia, constipation, diarrhea, etc.).

The control group received only standard lifestyle recommendations. These recommendations included dietary and exercise advice typically provided to all individuals with prediabetes in outpatient clinic settings. Based on the surveys assessing dietary and exercise habits recorded at baseline and at the 12th week, no behavioral changes were observed in the participants,

except for the expected increase in physical activity levels in the exercise group.

Statistical analysis

The data obtained in the study were analyzed using the SPSS (Statistical Package for Social Sciences) for Windows 25.0 software. Descriptive statistical methods were used to evaluate the data. Continuous variables used in the study were tested for conformity to normal distribution. The normal distribution of the data used in this study was ensured by checking the Shapiro–Wilk values.

Parametric and non-parametric tests were used in statistical evaluations for normally distributed variables. In the comparison of quantitative data in normally distributed variables, ANOVA was used for three-group comparisons, and the Kruskal-Wallis test was used for non-normally distributed variables. A multiple comparison test with Bonferroni correction was performed to identify groups showing differences. The variation within each group was analyzed using the dependent sample test. The dependent sample *t*-test was employed for groups with a normal distribution, while the Wilcoxon Signed-Rank test was utilized for groups without a normal distribution. Moreover, the homogeneity of the groups was analyzed using the chi-square test. The correlation between the change in GDF-15 levels and the change in weight was separately analyzed for the exercise and control groups using Pearson correlation analysis. Cohen's *d* value and 95% confidence interval (CI) were used to determine the statistical effect size, presented as a forest plot.

The statistical power analysis of this study was based on GDF-15, HbA1C, and HOMA-IR parameters. The G-POWER analysis indicated that with 30 participants in each group, a statistical power of 86% and a significance level of 5% ($\alpha = 0.05$) were achieved. These results demonstrate that the sample size determined for the study has sufficient statistical power to detect significant differences in the targeted parameters. In case of data loss, the sample size was increased, and 32 people were planned to be included in each group.

RESULTS

The mean age of the 91 participants who completed the study was 46.13 ± 8.52 years, with 23.1% being male. Table 1 presents the distribution of demographic characteristics, weight, BMI, and participant habits across the study groups.

Baseline GDF-15 levels were comparable between groups (exercise [E] = 668.6 ± 415.1 ng/L, control [C] = 651.8 ± 352.5 ng/L, metformin [M] = 603.6 ± 387.2 ng/L, $P = 0.47$). At week 12, GDF-15 levels were significantly lower

Table 1 | Distribution of socio-demographic characteristics, weight, BMI, and habits

		Groups						Test value	*P* value
		Control		Metformin		Exercise			
		n	%	*n*	%	*n*	%		
Gender	Male	7	23.3	8	25.8	6	20.0	0.29^{P}	0.86
	Female	23	76.7	23	74.2	24	80.0		
Smoking status	No	23	76.7	23	74.2	19	63.3	1.48^{P}	0.48
	Yes	7	23.3	8	25.8	11	36.7		
Alcohol use	No	30	100.0	30	96.8	27	90.0	3.05^{FE}	0.22
	Yes	0	0.0	1	3.2	3	10.0		
Marital status	Single	2	6.7	3	9.7	3	10.0	2.97^{FE}	0.58
	Married	26	86.6	23	74.2	21	70.0		
	Widowed	2	6.7	5	16.1	6	20.0		
Educational status (years)	Grades 1–5	18	60.0	18	58.1	15	50.0	6.79^{FE}	0.34
	Grades 6–8	5	16.7	6	19.3	2	6.7		
	Grades 9–12	2	6.7	2	6.5	9	30.0		
	Grades13+	5	16.7	5	16.1	4	13.3		
Age (years)	Mean ± SD	45.3 ± 9.1		47.7 ± 7.6		45.2 ± 8.9		0.85^{F}	0.43
BMI (kg/m^2)	Mean ± SD	30.5 ± 5.6		30.7 ± 5.2		29.6 ± 4.6		0.40^{F}	0.67
Weight (kg)	Mean ± SD	77.7 ± 15.6		78.6 ± 14.0		79.9 ± 13.1		0.19^{F}	0.83
IPAQ score (METs-min/week)	Mean ± SD	893.9 ± 356.8		832.7 ± 310.3		904.4 ± 342.2		0.41^{F}	0.67
MEDAS score	Mean ± SD	7.7 ± 1.8		7.7 ± 1.6		7.6 ± 1.5		0.06^{F}	0.95
	Single	2	6.7	3	10.0	3	10.0	2.65^{FE}	0.58
	Married	26	86.7	22	73.3	21	70.0		
	Widowed	2	6.7	5	16.7	6	20.0		

F, One-way ANOVA Test; FE, Fisher Exact Chi-square Test; IPAQ, International Physical Activity Questionnaire; KW, Kruskal Wallis *H* test; MEDAS, Mediterranean Diet Adherence Screener; *P*, Pearson Chi-square Test.

Table 2 | Comparison of GDF-15 levels between groups at baseline and the 12th week

	Groups	Baseline		12 week		*z/t*	*P* value
		Mean ± SS	Med (Q25–Q75)	Mean ± SS	Med (Q25–Q75)		
GDF-15 (ng/L)	Control (1)	651.8 ± 352.5	536.0 (455.6–635.0)	556.4 ± 285.6	505.4 (372.6–648.5)	-1.82^{z}	0.07
	Metformin (2)	603.6 ± 387.2	505.8 (396.7–613.5)	810.8 ± 498.0	649.2 (514.5–825.8)	**-4.00^{z}**	**<0.001**
	Exercise (3)	668.6 ± 415.1	531.7 (409.2–731.3)	383.1 ± 215.6	327.5 (243.3–405.4)	**-4.78^{z}**	**<0.001**
F/KW test		1.53^{KW}		**28.27^{KW}**			
P value		0.47		**<0.001**			
				3 < 1 < 2			

F, One-way ANOVA Test; GDF-15, Growth-Differentiation Factor-15; KW, Kruskal Wallis *H* Test; *t*, Dependent Sample *t* Test; *z*, Wilcoxon Signed Rank Test.

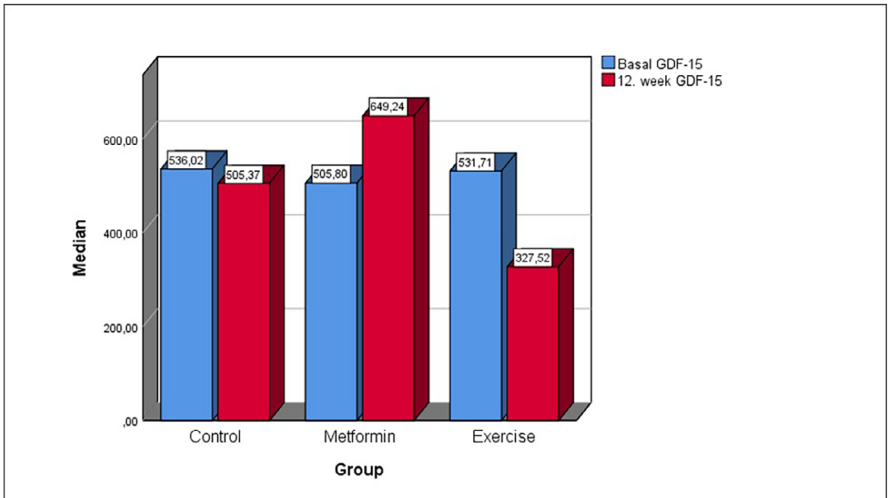
in the exercise group compared to the control group and significantly higher in the metformin group compared to the control group (E = 383.1 ± 215.6 ng/L, C = 556.4 ± 285.6 ng/L, M = 810.8 ± 498.0 ng/L, $P < 0.001$; Table 2).

In intra-group comparisons, no significant changes in GDF-15 levels were observed in the control group between baseline and week 12. However, GDF-15 levels decreased significantly in the exercise group ($P < 0.001$) and increased significantly in the metformin group ($P < 0.001$) from baseline to week 12 (Figure 2).

Table 3 displays the baseline and week 12 blood glucose and insulin parameters. At baseline, fasting glucose and HbA1c levels were similar across groups (fasting glucose: E = 95.7 ± 8.2 mg/dL, C = 96.7 ± 7.6 mg/dL, M = 99.4 ± 10.8 mg/dL, $P = 0.25$; HbA1c: E = 5.9 ± 0.1%, C = 6.0 ± 0.2%, M = 6.1 ± 0.2%; $P = 0.16$). By week 12, fasting glucose and HbA1c levels significantly decreased in the exercise and metformin groups compared to the control group (fasting glucose: E = 88.7 ± 8.3 mg/dL, M = 90.8 ± 10.2 mg/dL, C = 97.9 ± 12.0 mg/dL, $P = 0.01$; HbA1c: E = 5.7 ± 0.2%, M = 5.6 ± 0.4%, C = 6.1 ± 0.2%, $P < 0.001$).

Table 4 outlines the blood lipid parameters at baseline and week 12. No significant changes were observed in the control or metformin groups at week 12 compared to baseline. However, the exercise group showed significant reductions in total cholesterol (from 205.6 ± 45.3 to 191.3 ± 41.7 mg/dL, $P = 0.01$), LDL cholesterol (from 127.0 ± 38.0 to 119.3 ± 33.8 mg/dL, $P = 0.01$), and triglyceride levels (from 138.3 ± 91.7 to 110.0 ± 47.7 mg/dL, $P = 0.01$).

Table 5 summarizes the changes in BMI, weight, and blood pressure. Both the exercise group (BMI: from 29.6 ± 4.6 to



Control	$P = 0.069$
Metformin	**$P < 0.0001$**
Exercise	**$P < 0.0001$**

Figure 2 | While GDF-15 levels were similar in the control, metformin, and exercise groups at baseline, GDF-15 levels were lower in the exercise group and higher in the metformin group at week 12 compared to the control group.

Table 3 | Comparison of groups in terms of blood glucose and insulin parameters at baseline and the 12th week

Variables	Groups	Baseline		12 weeks		*z/t*	*P* value
		Mean ± SD	Med (Q25–Q75)	Mean ± SD	Med (Q25–Q75)		
Glucose (mg/dL)	Control (1)	96.7 ± 7.6	95.0 (91.0–101.0)	97.9 ± 12.0	97.4 (88.0–104.0)	–0.93^{z}	0.35
	Metformin (2)	99.4 ± 10.8	99.0 (91.0–108.0)	90.8 ± 10.2	91.0 (84.0–97.0)	**4.21^{t}**	**<0.001**
	Exercise (3)	95.7 ± 8.2	96.5 (91.0–101.0)	88.7 ± 8.3	87.5 (84.0–92.0)	**4.96^{t}**	**<0.001**
F/KW test		1.43^{F}		**10.35^{KW}**			
P value		0.25		**0.01**			
Post-hoc				**1 > 3 = 2**			
Insülin (μIU/mL)	Control	10.9 ± 7.1	8.9 (6.7–13.9)	11.7 ± 7.6	10.3 (6.5–17.0)	–1.15^{z}	0.25
	Metformin	13.6 ± 7.9	11.5 (8.7–16.3)	11.1 ± 6.3	10.2 (7.0–14.7)	**–3.06^{z}**	**0.01**
	Exercise	10.9 ± 6.5	9.3 (6.5–12.1)	9.7 ± 8.2	8.2 (4.2–10.9)	**–2.13^{z}**	**0.03**
KW test		5.20		2.51			
P value		0.07		0.29			
HOMA-IR (mmol/L)	Control	2.6 ± 1.7	2.1 (1.5–3.7)	2.9 ± **2**.00	2.5 (1.4–4.1)	–1.31^{z}	0.19
	Metformin	3.4 ± 2.3	2.9 (2.0–4.3)	2.6 ± 1.6	2.5 (1.4–3.2)	**–3.23^{z}**	**<0.001**
	Exercise	2.6 ± 1.7	2.1 (1.6–3.0)	1.9 ± 1.0	1.9 (0.9–2.4)	**–3.20^{z}**	**<0.001**
KW test		4.86		1.61			
P value		0.89		0.45			
C-peptid (ng/mL)	Control	2.4 ± 1.0	2.2 (1.9–2.9)	2.6 ± 1.0	2.5 (1.9–2.9)	–1.48^{t}	0.15
	Metformin	2.8 ± 1.2	2.6 (2.0–3.4)	2.6 ± 0.9	2.4 (2.0–3.0)	–1.11^{z}	0.27
	Exercise	2.5 ± 0.8	2.3 (2.0–2.7)	2.3 ± 1.0	2.3 (1.7–2.7)	–1.55^{z}	0.12
KW test		2.94		2.45			
P value		0.23		0.29			
HbA1c (mmol/mol)	Control (1)	41.1 ± 1.5	41.0 (39.9–41.0)	42.8 ± 2.4	42.1 (41.0–44.5)	**–3.93^{z}**	**<0.001**
	Metformin (2)	41.9 ± 2.4	41.0 (39.9–44.3)	38.2 ± 4.3	37.7 (36.6–41.0)	**–4.02^{z}**	**<0.001**
	Exercise (3)	41.1 ± 1.20	41.0 (39.9–41.3)	38.3 ± 2.4	38.8 (36.6–39.9)	**–4.48^{z}**	**<0.001**
F test		2.38		**21.72**			
P value		0.10		**<0.001**			
Post-hoc				**2 = 3 < 1**			
HbA1c (%)	Control (1)	5.9 ± 0.1	5.9 (5.8–5.9)	6.1 ± 0.2	6.0 (5.9–6.2)	**–3.93^{z}**	**<0.001**
	Metformin (2)	6.0 ± 0.2	5.9 (5.8–6.2)	5.64 ± 0.39	5.60 (5.50–5.90)	**–4.023^{z}**	**<0.0001**
	Exercise (3)	5.9 ± 0.1	5.9 (5.8–5.9)	5.7 ± 0.2	5.7 (5.5–5.8)	**–4.48^{z}**	**<0.001**
*F*test		3.71		**21.72**			
P değeri		0.16		**<0.001**			
Post-hoc				**2 = 3 < 1**			

F, One ANOVA test; HbA1c, Glycated Haemoglobin; HOMA-IR, Homeostatic Model Assessment-Insulin Resistance; KW, Kruskal Wallis *H* Test; *t*, Dependent Sample *t* Test; *z*, Wilcoxon Signed Rank Test.

27.9 ± 4.3 kg/m^2, $P < 0.001$; weight: from 79.9 ± 13.1 to 75.3 ± 12.0 kg, $P < 0.001$) and the metformin group (BMI: from 30.7 ± 5.2 to 29.8 ± 5.2 kg/m^2, $P < 0.001$; weight: from 78.6 ± 14.0 to 76.1 ± 13.7 kg, $P < 0.001$) experienced significant BMI and weight reductions by week 12. Regarding systolic blood pressure, no changes were observed in the control and metformin groups, whereas the exercise group demonstrated a significant reduction (from 120.7 ± 15.7 to 111.8 ± 10.4 mmHg, $P < 0.001$).

Table 6 presents the correlation between changes in GDF-15 levels and changes in weight within the exercise and control groups. A significant correlation was found between the increase in GDF-15 levels and the decrease in weight in the metformin group ($r = 0.45$, $P = 0.04$), whereas no such correlation was observed in the exercise group.

Figures 3 and 4 illustrate the comparison of the exercise group with the control group and the metformin group with the control group, respectively, across all parameters.

DISCUSSION

In this study examining the effect of exercise and metformin on GDF-15 in individuals with prediabetes, it was determined that GDF-15 decreased with the implementation of a 12-week guideline-based aerobic exercise program and increased with metformin use. It was observed that BMI, weight, HbA1C, glucose, insulin, and HOMA-IR values decreased with exercise and metformin. It was determined that total cholesterol, LDL, triglycerides, and systolic blood pressure decreased with exercise, whereas these values did not change with metformin. These results indicate the beneficial effects of metformin and

Table 4 | Comparison of groups in terms of blood lipid parameters at baseline and 12 weeks

Variables	Groups	Baseline		12 week		*z/t*	*P* value
		Mean ± SD	Med (Q25–Q75)	Mean ± SD	Med (Q25–Q75)		
Total cholesterol (mg/dL)	Metformin	195.3 ± 38.6	197.0 (172.0–211.0)	201.9 ± 40.0	192.5 (181.1–223.0)	-1.64^{t}	0.11
	Exercise	199.8 ± 34.0	197.0 (175.0–218.0)	197.8 ± 35.2	188.3 (172.0–218.0)	-0.51^{z}	0.61
	Exercise	205.6 ± 45.3	208.6 (175.2–236.0)	191.3 ± 41.7	191.4 (162.1–213.0)	**2.92^{t}**	**0.01**
F test		0.51		0.69			
P value		0.60		0.71			
LDL cholesterol (mg/dL)	Control	118.3 ± 31.6	117.4 (98.0–134.0)	122.3 ± 28.4	117.6 (104.0–142.0)	-1.17^{t}	0.25
	Metformin	116.3 ± 26.4	118.0 (95.0–133.0)	114.4 ± 29.39	108.0 (90.0–131.0)	0.44^{t}	0.67
	Exercise	127.0 ± 38.0	128.6 (95.0–157.0)	119.3 ± 33.8	116.2 (99.0–140.5)	**2.61^{t}**	**0.01**
F test		0.94		0.52			
P value		0.40		0.60			
HDL cholesterol (mg/dL)	Control	53.4 ± 15.9	51.0 (42.0–63.0)	54.1 ± 17.6	53.5 (41.0–62.0)	-0.17^{z}	0.87
	Metformin	56.1 ± 16.3	53.0 (43.0–71.6)	55.8 ± 16.4	53.0 (42.0–67.0)	-0.90^{z}	0.37
	Exercise	51.4 ± 12.9	51.5 (41.5–58.0)	51.7 ± 14.4	49.9 (43.8–59.3)	-1.29^{z}	0.20
KW test		1.20		0.80			
P value		0.52		0.67			
Triglyceride (mg/dL)	Control	125.8 ± 79.8	110.8 (79.5–137.0)	125.7 ± 71.0	107.9 (83.0–145.0)	-0.20^{z}	0.85
	Metformin	133.3 ± 67.9	129.3 (74.2–175.2)	133.1 ± 73.8	112.0 (84.0–154.0)	-0.48^{z}	0.63
	Exercise	138.3 ± 91.7	108.7 (89.8–157.4)	110.0 ± 47.7	107.7 (70.0–128.7)	**-3.25^{z}**	**0.01**
KW test		0.43		1.39			
P value		0.81		0.50			

F, one way ANOVA test; HDL, high density lipoprotein; KW, Kruskal Wallis *H* test; LDL, low density lipoprotein; *t*, dependent sample *t* test; *z*, Wilcoxon signed rank test.

Table 5 | Comparison of groups in terms of BMI, weight and blood pressure at baseline and 12 weeks

Variables	Groups	Baseline		12 week		*z/t*	*P* value
		Mean ± SD	Med (Q25–Q75)	Mean ± SD	Med (Q25–Q75)		
BMI (kg/m^2)	Control	30.5 ± 5.6	28.6 (26.8–34.0)	30.4 ± 5.5	29.0 (26.8–33.6)	0.33^{t}	0.74
	Metformin	30.7 ± 5.2	30.1 (27.3–33.8)	29.8 ± 5.2	28.6 (26.2–32.0)	**6.30^{t}**	**<0.001**
	Exercise	29.6 ± 4.6	29.0 (26.6–33.1)	27.9 ± 4.3	27.6 (25.1–31.7)	**6.99^{t}**	**<0.001**
F test		0.40^{F}		2.01^{F}			
P value		0.67		0.14			
Weight (kg)	Control	77.7 ± 15.6	76.8 (66.8–86.0)	77.6 ± 15.5	75.5 (69.0–88.0)	0.34^{t}	0.73
	Metformin	78.6 ± 14.0	75.9 (72.9–85.9)	76.1 ± 13.7	74.6 (66.7–83.3)	**6.20^{t}**	**<0.001**
	Exercise	79.9 ± 13.1	77.0 (71.0–89.0)	75.3 ± 12.0	73.1 (69.0–81.9)	**6.75^{t}**	**<0.001**
F test		0.40		0.19		0.20	
P value		0.67		0.83		0.82	
Systolic blood pressure (mmHg)	Control (1)	125.1 ± 18.1	126.0 (111.0–139.0)	127.4 ± 18.0	126.5 (111.0–143.0)	-0.83^{t}	0.42
	Metformin (2)	128.3 ± 14.9	125.0 (117.0–142.0)	125.1 ± 12.6	121.0 (116.0–134.0)	1.66^{t}	0.11
	Exercise (3)	120.7 ± 15.7	120.0 (111.0–129.0)	111.8 ± 10.4	112.0 (105.0–117.0)	**3.93^{t}**	**<0.001**
F test		1.66		**13.92**			
P value		0.20		**<0.001**			
Post-hoc				**3 < 1 = 2**			
Diastolic blood pressure (mmHg)	Control	78.9 ± 13.4	81.0 (70.0–91.0)	78.6 ± 11.7	78.5 (68.0–87.0)	0.22^{t}	0.82
	Metformin	80.9 ± 8.3	80.0 (74.0–88.0)	78.6 ± 11.0	80.0 (71.0–86.0)	1.15^{t}	0.26
	Exercise	76.1 ± 6.5	75.0 (72.0–80.0)	73.7 ± 7.3	74.0 (70.0–78.0)	-1.60^{z}	0.11
KW test		5.18		4.83			

BMI, body mass index; *F*, one way ANOVA test; KW, Kruskal Wallis *H* test; *t*, dependent sample *t* test; *z*, Wilcoxon signed rank test.

Table 6 | The correlation between changes in GDF-15 levels and weight within the exercise and control groups

	Difference of weight (kg)	
	P	*r*
In metformin group		
Difference of GDF-15 (ng/L)	0.04	0.45
In exercise group		
Difference of GDF-15 (ng/L)	0.74	0.06

Spearman correlation analysis was used. *r*, correlation coefficient.

exercise in the management of prediabetes and emphasize that exercise has more metabolic benefits compared to metformin. Our dataset has reached sufficient saturation to obtain statistically significant results in our analyses, and the study findings are supported by the current sample size. Nonetheless, further studies with larger sample groups are anticipated to enhance the generalizability of our findings and extend these results to broader populations.

The differing effects of exercise and metformin on GDF-15 are intriguing. The decreasing effect of exercise on GDF-15 is consistent with the anti-inflammatory, metabolic regulatory, and insulin-sensitizing roles of exercise. On the other hand, the mechanism by which metformin treatment increases GDF-15 levels may be attributed to the drug's effects on energy homeostasis. The observed differences in GDF-15 levels between exercise and metformin highlight the intricate regulatory pathways underlying its role in metabolic health. These differences likely stem from the distinct cellular mechanisms activated by each intervention.

Prediabetes is characterized by a chronic low-grade inflammatory state, which significantly contributes to its pathophysiology and progression to type 2 diabetes[16–18]. This inflammatory burden disrupts glucose metabolism, increases insulin resistance, and exacerbates metabolic dysfunction. Both aerobic exercise and metformin, the primary interventions evaluated in this study, are known to modulate inflammation through distinct mechanisms. Exercise exerts its anti-inflammatory effects by reducing pro-inflammatory cytokines and increasing anti-inflammatory mediators, as well as improving mitochondrial function and oxidative stress regulation[19]. This aligns with our finding of decreased GDF-15 levels following the 12-week exercise intervention, suggesting that the resolution of chronic inflammation may underlie the observed metabolic improvements. Conversely, metformin's anti-inflammatory actions are mediated via AMP-activated protein kinase (AMPK) activation and the integrated stress response (ISR), both of which regulate cellular energy homeostasis and reduce metabolic stress[20, 21]. The increase in GDF-15 levels observed with metformin in this study likely reflects an adaptive response to inflammatory and metabolic stress, facilitating appetite regulation and energy balance through the gut-brain axis. In fact, in our research, an increase in GDF-15 was found to be associated with weight loss. These findings underscore the role of inflammation in the divergent effects of exercise and metformin on GDF-15 levels, highlighting its context-dependent functions as both a biomarker and mediator of metabolic adaptation. This perspective

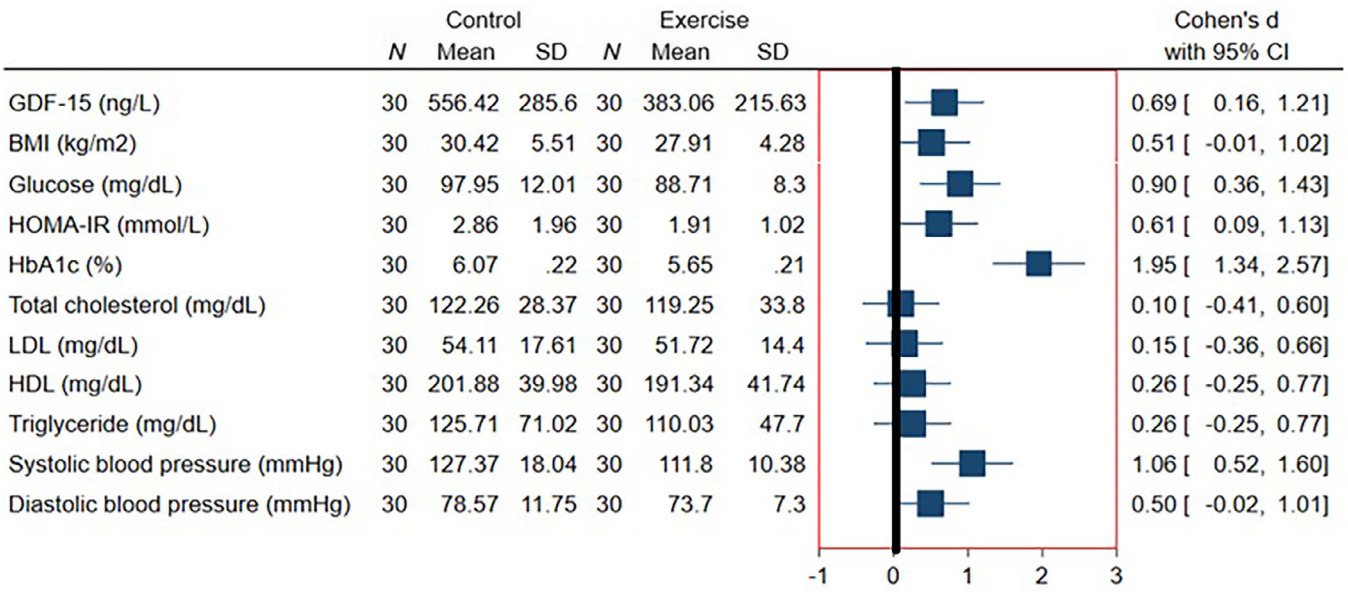


Figure 3 | According to the comparison of the exercise and control groups at week 12, it is seen that GDF-15, glucose, HbA1c, and systolic blood pressure levels are higher in the control group compared to the exercise group.

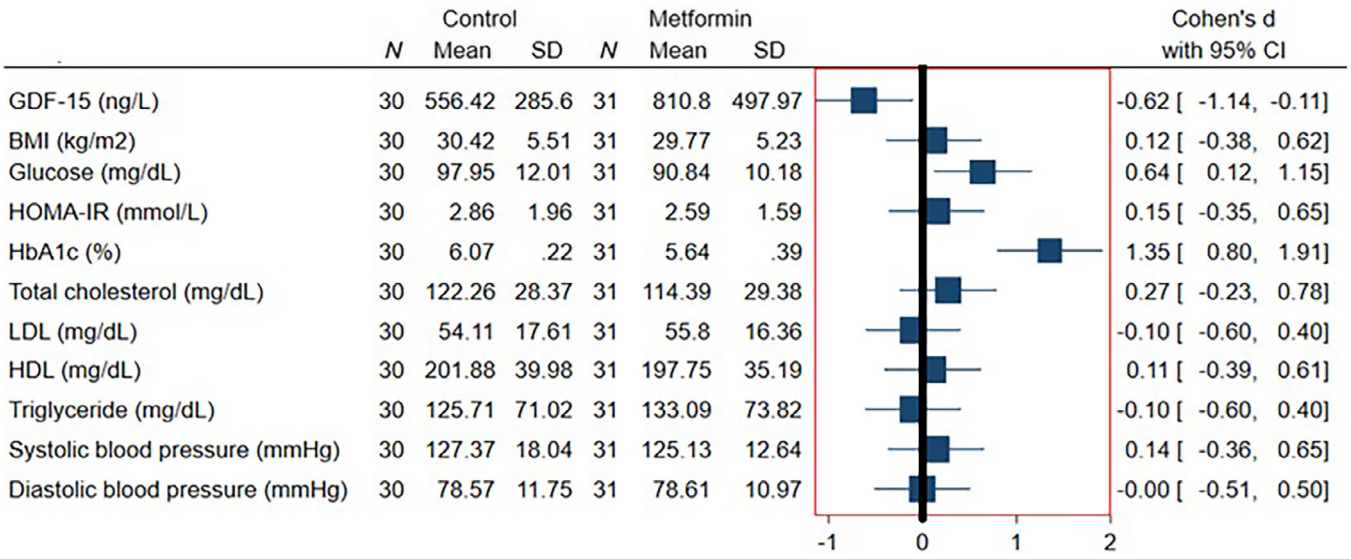


Figure 4 | According to the comparison of the metformin and control groups at week 12, it is seen that GDF-15 levels are lower, glucose, and HbA1c levels are higher in the control group compared to the metformi group.

suggests that targeting inflammation may enhance the therapeutic efficacy of interventions in prediabetes.

Exercise-induced changes in GDF-15 levels

Chronic aerobic exercise reduces GDF-15 levels, possibly by attenuating systemic inflammation and improving mitochondrial efficiency. Exercise reduces oxidative stress through enhanced mitochondrial biogenesis and function, which diminishes the need for adaptive cytokines like GDF-15[22]. This aligns with findings showing that exercise improves glucose metabolism and insulin sensitivity by increasing GLUT4 translocation in skeletal muscle and promoting mitochondrial respiration[23]. Additionally, exercise may influence GDF-15 via its effects on anti-inflammatory cytokines and transcriptional regulators. For instance, regular aerobic exercise has been shown to upregulate peroxisome proliferator-activated receptor gamma coactivator-1 alpha (PGC-1α), which regulates mitochondrial gene expression and decreases reactive oxygen species (ROS) production[24]. These effects likely reduce the activation of stress pathways that stimulate GDF-15 expression. Interestingly, while acute exercise induces transient increases in GDF-15 due to mechanical and oxidative stress on skeletal and cardiac muscle, these elevations are not sustained with chronic training. Instead, the long-term effect of exercise appears to downregulate GDF-15, reflecting reduced metabolic and inflammatory stress and improved metabolic homeostasis[25].

Metformin-induced increases in GDF-15 levels

Metformin's ability to increase GDF-15 is mediated through the ISR, triggered by mitochondrial dysfunction and activation of unfolded protein response (UPR) pathways[26]. Metformin induces the transcription of activating transcription factor 4 (ATF4) and C/EBP homologous protein (CHOP), which together upregulate GDF-15 expression[13]. This response is thought to mitigate mitochondrial stress and maintain cellular energy balance. In addition to its effects on the ISR, metformin activates AMPK, a key regulator of energy metabolism. AMPK enhances lipid oxidation and glucose uptake while reducing gluconeogenesis. Recent studies suggest that AMPK activation also plays a role in stimulating GDF-15 production, linking metformin's metabolic effects to its modulation of GDF-15[27,28]. GDF-15 acts on the glial-derived neurotrophic factor receptor alpha-like (GFRAL) receptor in the brainstem, a critical component of the gut-brain axis. Activation of this pathway suppresses appetite and reduces energy intake, contributing to the weight loss and improved metabolic outcomes observed with metformin[29].

Context-dependent roles of GDF-15

The divergent effects of exercise and metformin on GDF-15 underscore its context-dependent roles. Exercise-induced reductions in GDF-15 likely reflect a resolution of metabolic stress and inflammation, while metformin-induced elevations align with its therapeutic effects on appetite regulation and weight control. This duality suggests that GDF-15 serves both as a biomarker of metabolic stress and as a functional mediator of metabolic adaptation.

Understanding how exercise and metformin interact to influence GDF-15 could uncover novel therapeutic strategies for metabolic disorders. For example, combining these interventions may yield complementary benefits, with exercise addressing systemic inflammation and insulin resistance and

metformin enhancing appetite control and energy balance. Further research is needed to delineate the molecular interplay between these pathways and assess their long-term effects on GDF-15 dynamics and metabolic outcomes.

In a study conducted by Myong-Won *et al.*, 12 women with sarcopenia were instructed to perform resistance exercises for 16 weeks, and 10 women were assigned to the control group. The study revealed an increase in GDF-15 levels in the control group, whereas no change was observed in the exercise group[30]. GDF-15 levels are known to increase with aging[31]. Although 16 weeks is relatively short to expect this increase, it is possible that aging occurs more rapidly in individuals with sarcopenia, and the lack of increase in the exercise group suggests that exercise slows this increase. In a study involving 10 individuals with polycystic ovary syndrome and 9 healthy individuals, a quantitative decrease in GDF-15 levels was observed with the implementation of a chronic exercise program; however, this decrease did not reach statistical significance[32]. In three patients who underwent percutaneous coronary intervention, an increase in GDF-15 was observed at the post-intervention 30th minute. After these individuals were assigned to the exercise and control groups, it was determined that GDF-15 levels did not change with the chronic exercise program[33]. In our current study, a decrease in GDF-15 levels was observed with the chronic exercise program. The lack of such a decrease in these studies was attributed to the small number of patients included and the data not reaching statistical significance. In our study, 30 participants each were included in the exercise and control groups, and the data were subjected to robust analyses. A standardized exercise program was applied to participants following detailed assessments, and equality between groups was ensured for confounding factors such as habits and nutritional status.

In a study conducted on elderly individuals, it was revealed by the multiple regression analysis method that higher physical activity levels in 1 year were associated with lower GDF-15 levels in the first year[34]. Furthermore, higher levels of GDF-15 were associated with rapid age-related body weight loss[34]. In another study conducted on elderly individuals, basal GDF-15 elevation was found to be associated with decreased physical function[35]. Hirano *et al.*[36] found that GDF-15 was higher in individuals with chronic obstructive pulmonary disease compared to healthy individuals, that GDF-15 was increased in individuals with a sedentary lifestyle, and that GDF-15 elevation was even associated with cognitive loss.

GDF-15 is known to increase in a range of different pathologies and diseases, including chronic inflammatory diseases, prediabetes, diabetes, cardiovascular and kidney diseases, as well as malignancies, many of which are age-related[37]. GDF-15 was reported to be considered a general disease marker that increases with advanced disease and is associated with all-cause mortality[38]. Metabolic disorder in individuals with prediabetes triggers inflammatory processes, and this period, when the first disturbances in glucose metabolism occur, is a key period to achieve metabolic regulation. During this period, exercise interventions can help individuals return to a healthy metabolic state, and this is expected to result in the regression of inflammatory processes. In the present study, the decrease in GDF-15 levels with exercise in individuals with prediabetes was attributed to improved metabolic health and regression of inflammatory processes.

Considering the acute effects of exercise, a study involving seven healthy males found that exercising at 67% of their VO_{2max} for 1 h resulted in a 34% increase in GDF-15 immediately after exercise and a 64% increase compared to baseline levels at the 2nd hour post-exercise[25]. It was determined that in professional rugby players, GDF-15 levels increased after 35 days of intensive training[39]. This increase in GDF-15 levels in healthy athletes may be attributed to cardiac stress. An important exercise-induced myokine, GDF-15, mediates glucose-stimulated insulin secretion in skeletal muscle contraction[40]. Quist *et al.*[41] found that GDF-15 increased acutely with exercise but was unchanged at 6 months, considering the chronic effect, also associating higher GDF-15 concentrations with a more unfavorable cardiometabolic profile. In marathon runners, GDF-15 was reported to increase acutely 1.8-fold with exercise, and this increase was associated with muscle damage[42]. When all these data were evaluated together with the data obtained in the present study, it was concluded that the effects of exercise on GDF-15 may be in different directions acutely and chronically. While cardiac and muscular-induced GDF-15 increases in the short term after exercise, it is believed to decrease with the improvement of the metabolic profile in individuals with metabolic disorders due to its negative effects on the regulation of glucose metabolism. GDF-15 regulates the peripheral interaction between skeletal muscle and adipose tissue in multiple ways.

In our study, a correlation was found between the change in GDF-15 levels and the decrease in weight in the metformin group. Metformin affects energy balance and weight control by increasing GDF-15 levels. GDF-15 binds to the GFRAL receptor in the brainstem, activating neural networks that reduce energy intake and increase feelings of satiety[13]. This process enables metformin to lead to weight loss in both diabetic and non-diabetic individuals[13]. GDF-15 is defined as a metformin biomarker in the literature[43]. Approximately 60% of metformin's effect in reducing the risk of progression to type 2 diabetes has been attributed to weight loss[26]. Maintaining weight loss achieved through lifestyle changes in individuals with prediabetes is challenging due to physiological adaptations that suppress energy expenditure. In rodents fed a high-fat diet, metformin-induced GDF-15 was found to suppress food intake via a GFRAL-dependent mechanism, reducing obesity and improving glycemic control[28]. GDF-15 was discovered to prevent reductions in energy expenditure, thus promoting greater weight loss and regression of metabolically associated fatty liver disease compared to caloric restriction alone[28]. The effect of GDF-15 is dependent on the GFRAL-beta-adrenergic signaling axis, which supports energy expenditure by enhancing fatty acid

oxidation and calcium-mediated energy cycling in skeletal muscle and is induced by metformin[28]. These findings suggest that therapeutically targeting the GDF-15/GFRAL pathway could be beneficial for maintaining skeletal muscle energy expenditure during caloric restriction.

The effects of metformin on body weight have been evaluated in two independent randomized controlled clinical trials. The results of these studies demonstrate that oral administration of metformin to wild-type mice increases GDF-15 expression, particularly in the distal intestine and kidneys[26]. While metformin prevented weight gain associated with a high-fat diet in normal mice, this effect was absent in GFRAL-deficient mice[26]. In obese mice, the effects of metformin on weight loss were suppressed by a GFRAL antagonist antibody[26]. Although metformin exerts GDF-15-dependent effects on energy intake and expenditure, it maintained its capacity to lower blood glucose levels even in the absence of GDF-15 activity[26]. Furthermore, a study in obese children found that metformin use at 6 and 12 months was associated with increased GDF-15 levels, and higher increases in GDF-15 were linked to greater reductions in body weight and visceral fat[44]. Consistent with the literature, our current study in individuals with prediabetes observed an increase in GDF-15 levels with metformin use.

The association of metformin-induced GDF-15 elevation with reduced appetite raises the question of whether exercise-induced increases in GDF-15 also affect appetite. Klein *et al.*[45] found that while pharmacological increases in GDF-15 were associated with appetite suppression, physiological changes in GDF-15 related to exercise were not associated with feeding behavior or exercise motivation.

The secretion of GDF-15 by various malignant tumors and its role in mediating tumor-associated cachexia and weight loss were first identified in 2007[46]. Studies on the potential benefits of GDF-15 blockade for managing side effects of platinum-based chemotherapy have shown that GDF-15 neutralization mitigates chemotherapy-induced anorexia and weight loss in both mice and non-human primates[47]. In a study by Lu *et al.*, the effect of ketogenic diets on obesity management was explained through the critical role of the GDF-15/GFRAL signaling pathway. According to this study, hepatic PPARγ (peroxisome proliferator-activated receptor gamma) activation increases GDF-15 production, mediating the effects of ketogenic diets on obesity, whereas suppression of hepatic PPARγ expression reduces this[48]. GDF-15 regulates food intake, energy expenditure, and body weight in response to metabolic and toxin-induced stress. GFRAL-knockout mice exhibit hyperphagia under stress conditions and are resistant to chemotherapy-induced anorexia and weight loss[49]. A newly developed GLP-1/GDF-15 fusion protein, QL1005, has demonstrated superior performance compared to semaglutide in vitro[50]. Experiments in obese mice and cynomolgus monkeys have shown that QL1005 leads to dose-dependent reductions in body weight, food intake, insulin, and glucose levels, with minimal gastrointestinal side effects[50].

High levels of GDF-15 are often used in epidemiologic studies to predict adverse health outcomes, leading researchers to speculate that GDF-15 directly affects these adverse outcomes. However, it should be noted that such studies are only correlational and cannot establish causality. This assumption contradicts studies conducted on animal models, which demonstrate that overexpression of GDF-15 can improve health outcomes and that the absence of the gene can worsen outcomes. Therefore, it may be a more plausible interpretation that elevated levels of GDF-15 are induced as an adaptive response that can sometimes be inadequate against physiological stress, disease, or other harmful stimuli. It was reported that GDF-15, which is chronically elevated by cell stress, would return to its previous levels by a feedback mechanism after metabolic recovery[38]. The reduction in GDF-15 levels observed in our study with a chronic exercise program, as opposed to its increase during acute exercise noted in previous studies, is attributed to a decrease in physiological stress associated with dysglycemia and a reduction in the adaptive response.

This study examined the impact of lifestyle changes and pharmacological interventions in the management of prediabetes. The biological foundations of the effects of exercise and metformin treatment on metabolic health are being elucidated through the biomarker GDF-15 levels. This approach helps to understand how therapies work at the molecular level and identifies the contribution of specific biological pathways to treatment. Furthermore, this study contributes to the development of comprehensive and effective strategies for the management of prediabetes. Elucidating the effects of exercise and metformin at the cellular level provides valuable data for clinical applications and contributes to the literature.

Our study has various limitations. First, the fact that the sample was limited to only three hospitals in Istanbul, Turkiye, limits the generalizability of the study results. There is no ethnic diversity in the study. Second, blinding is not possible in exercise interventions. In this study, blinding was applied only for the study of blood samples and statistical analyses. Third, the inclusion of relatively healthy prediabetics, excluding those with diseases such as cardiovascular disease and heart failure, may have caused healthy selection bias. Fourth, the 12-week exercise duration limits the ability to observe the long-term effects of exercise. Fifth, moderate-intensity aerobic exercises were performed. It is not clear whether greater differences would be achieved with high-intensity exercise. Sixth, the initial motivation levels of the participants may have an impact on the results of the study. Individuals with high levels of motivation may follow the program more regularly than those with low levels of motivation, which may affect the results. Seventh, the limits of sensitivity and specificity of the methods used to measure biomarkers such as GDF-15 may affect the accuracy of the results. Furthermore, the lack of standardization of measurements made in different laboratories may also limit the comparability of data. Eighth, the metformin group was not randomized but rather observational, which limits the ability to

make direct comparisons between the metformin group and the other groups. Another significant limitation of this study is the relatively small sample size (a total of 91 participants). Studies with larger sample sizes would allow for subgroup analyses based on demographic variables such as gender and age. Although the findings of this study reached sufficient statistical power within the designated sample, replication of these findings in broader and demographically diverse populations is essential to confirm the results. Recognizing this limitation, future research should aim to test the generalizability of these findings by utilizing larger and more diverse populations. In this study, the effects of gender, age, and other demographic factors could not be examined in detail. Future studies employing larger and more heterogeneous samples for subgroup analyses may provide a more comprehensive understanding of the effects on GDF-15 levels. This would enable a more detailed analysis of the impacts of exercise and metformin on specific subgroups within prediabetic individuals.

In conclusion, it was determined that in individuals with prediabetes aged 18–60, GDF-15, which serves both as a biomarker of metabolic disorder and has a negative regulatory effect on appetite, decreased with 12 weeks of aerobic exercise and increased with metformin administration. This study elucidates the mechanisms of GDF-15, a current biomarker, in the management of prediabetes. Broad, long-term studies are necessary to determine the therapeutic potential of GDF-15.

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DISCLOSURE

The authors declare no conflict of interest.

Approval of the research protocol: Clinical Research Ethics Committee of Istanbul Medeniyet University Goztepe Training and Research Hospital (approval number: 2021/0255, date: 07.04.2021).

Informed consent: Approved by the ethics committee and signed by all participants.

Registry and the registration no. of the study/trial: This study was registered in the Protocol Registration and Results System (Clinicaltrials.gov PRS) with registration number NCT04799938 (16.03.2021).

Animal studies: N/A.

AUTHOR CONTRIBUTIONS

All authors contributed significantly and approved the final version of the manuscript.

CONTENT

Content has not been published elsewhere.

ETHICS STATEMENT

The protocol for the research project has been approved. A detailed informed consent form approved by the ethics committee was signed by all participants.

DATA AVAILABILITY STATEMENT

The datasets generated during and/or analyzed in the current study are available from the corresponding author upon reasonable request.

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