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Research Article

Effect of pharmacological and physical interventions on the metabolism of irisin and adipolin proteins in male diabetic rats

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Abstract

Crosstalk between muscle and adipose tissue via myokines and adipokines has critical implications for the metabolic regulation of type 2 diabetes. Irisin and adipolin are key secretory proteins involved in glucose homeostasis and anti-inflammatory pathways, yet the combined impact of pharmacological and physical interventions on their metabolism remains insufficiently characterized. This experimental study investigated the effects of metformin therapy and structured exercise on serum levels of irisin and adipolin, as well as related metabolic parameters, in male diabetic rats. Type 2 diabetes was induced in male Wistar rats (fasting glucose >250 mg/dl), while the healthy control group maintained normal glucose levels (~95 mg/dl). Animals were randomly assigned to control, metformin, or exercise (combined aerobic and resistance training) groups. Over eight weeks, interventions were administered and serum irisin, adipolin, and fasting blood glucose were measured pre- and post-intervention. Data were analyzed using the Shapiro–Wilk test, ANOVA, and Tukey post hoc tests. Results showed that both metformin and exercise significantly increased adipolin levels ($p<0.01$). As expected, irisin levels were higher in the non-diabetic control group compared to diabetic groups ($p<0.05$), consistent with the known reduction of irisin in diabetes. Fasting glucose improved most notably in the exercise group. These findings indicate that metformin and exercise exert distinct yet complementary effects on key metabolic regulators—adipolin and irisin—highlighting the benefits of integrating pharmacological and lifestyle approaches in type 2 diabetes management. Future research should explore underlying molecular mechanisms and translational potential in human populations.

Key Words: Irisin, Adipolin, Metformin, Exercise, Myokine, Adipokine, Type 2 diabetes

Introduction

Diabetes mellitus has emerged as one of the fastest growing metabolic diseases worldwide, severely disturbing glucose metabolism, reducing insulin efficacy, and disrupting overall energy homeostasis. These metabolic derangements greatly increase the risk of complications, including neuropathy, nephropathy, and cardiovascular events, thereby making diabetes a global public health concern (Saeedi et al., 2019). Although current pharmacological treatments offer partial management of hyperglycemia and its complications, there remains a critical need to discover new molecular mechanisms and intervention strategies, as many existing therapies do not fully prevent long-term adverse outcomes (Cesare et al., 2022).

Recent research has brought new attention to the role of signaling molecules secreted by muscle and adipose tissues-myokines and adipokines-in regulating metabolic health and disease (Cassidy et al., 2021). Among these, irisin and adipolin (also referred to as CTRP12) have been identified as major players in controlling energy expenditure, insulin response, and inflammation (Panati et al., 2021; Wang et al., 2020). Irisin is predominantly released during physical exercise and functions to stimulate the browning of white adipose tissue and increase glucose uptake, processes linked to improved glycemic control and heightened insulin sensitivity (Zhao et al., 2024). Importantly, irisin levels are typically reduced in diabetic states, which explains why non-diabetic control groups with normal fasting glucose (~95 mg/dl) exhibit higher concentrations compared to diabetic groups (fasting glucose >250 mg/dl). Conversely, adipolin exhibits notable anti-inflammatory and insulin-sensitizing properties, yet its circulating levels are reduced in both diabetes and obesity, indicating its importance in disease progression and its potential as a therapeutic target (Huang et al., 2021; Sun et al., 2022).

In this context, structured exercise protocols-combining aerobic and resistance training-alongside pharmacological interventions such as metformin, represent promising strategies to modulate these proteins.

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Monitoring both glucose and body weight parameters are essential to evaluate their metabolic impact. However, despite the recognized roles of irisin and adipolin, further clarification of the molecular pathways influenced by these interventions is required, and discrepancies in previous findings may be attributed to differences in dosage, duration, or experimental models.

Despite the recognized roles of these proteins, there remain major gaps concerning the detailed molecular pathways by which pharmacological agents (such as metformin) and exercise interventions-particularly combined aerobic and resistance training protocols—modulate irisin and adipolin activity in type 2 diabetes models (Zhang et al., 2023; Vettor et al., 2020). Moreover, previous studies have generally focused on either pharmacological or physical interventions in isolation, often with varied methodologies, making it difficult to directly compare effects or translate findings into clinical practice (You et al., 2023; Li et al., 2021). In addition, discrepancies in reported outcomes may be attributed to differences in drug dosage, intervention duration, or diabetes induction models, highlighting the need for more standardized approaches. Understanding the potential interactions or comparative effectiveness of these therapies remains crucial for identifying actionable molecular pathways to better manage diabetes and its complications.

Against this background, the current study aims to bridge these knowledge gaps by systematically assessing, within a controlled environment, the distinct and combined impacts of structured exercise and pharmacological therapy on the tissue and serum levels of irisin and adipolin. To ensure comprehensive evaluation, both fasting glucose levels (with diabetic status confirmed at >250 mg/dl and healthy control levels maintained at ~95 mg/dl) and body weight changes were monitored throughout the experimental period. For this purpose, male diabetic rats serve as an optimal experimental model due to their physiological resemblance to humans and the advantage of reduced hormonal variability, facilitating the acquisition of robust, reliable results (Lima et al., 2022; Morsi et al., 2023). In summary, by elucidating how well-established clinical interventions influence these key metabolic proteins, the study strives to contribute new insights relevant to the management of diabetes, opening pathways to more effective and potentially individualized treatment strategies. Exploring the impact of pharmacological and physical interventions on key metabolic proteins is of paramount importance, as these insights can guide the development of advanced therapeutic approaches that extend beyond conventional glycemic control to support comprehensive metabolic health (Panati et al., 2021; Mazur-Bialy et al., 2022). Such research holds the potential to inform more targeted and personalized management strategies, ultimately improving outcomes and quality of life for individuals living with diabetes.

In the present study, we employ the male Wistar diabetic rat model to systematically assess the effects of metformin administration and structured exercise-implemented as a combined aerobic and resistance training protocol-on the metabolism of irisin and adipolin, two proteins increasingly recognized for their roles in energy balance and insulin sensitivity. The choice of male rats is justified by their heightened responsiveness to diabetic induction and reduced influence of hormonal fluctuations compared to females, which enables clearer interpretation of intervention effects (Ahmed et al., 2023; Lima et al., 2022). Diabetes was induced using a combination of high-fat diet and low-dose streptozotocin (30-35 mg/kg, intraperitoneally), a protocol that closely mirrors the pathophysiological features of type 2 diabetes in humans, characterized by both insulin resistance and partial β-cell dysfunction (Zhao et al., 2023; Ramachandran et al., 2021). Diabetic status was confirmed with fasting blood glucose concentrations above 250 mg/dl, while the healthy control group maintained normal glucose levels (~95 mg/dl). To minimize confounding, animals were maintained under controlled environmental conditions, and key metabolic parameters-including both body weight and glucose levels-were regularly monitored to ensure group homogeneity throughout the experimental period (Morsi et al., 2023). By clarifying these methodological details, the study also addresses potential discrepancies with previous findings, where differences in drug dosage, intervention duration, or diabetes induction models may have contributed to divergent results. This approach strengthens the translational relevance of the present work and provides a more robust foundation for understanding how pharmacological and lifestyle interventions jointly influence irisin and adipolin regulation in diabetes.

Despite advances in our understanding of irisin and adipolin as metabolic mediators, significant challenges persist. The exact molecular mechanisms by which drug therapies or structured exercise regimens-particularly combined aerobic and resistance training-modulate these proteins in diabetic states remain incompletely elucidated, with some studies yielding contradictory findings regarding their signaling pathways and functional roles (Panati et al., 2021; Mazur-Bialy et al., 2022). There is ongoing debate concerning the accuracy and standardization of measurement techniques-including ELISA assays-for irisin and adipolin, which has led to inconsistency across published results (Kim & Lee, 2021). Most previous work has focused on either pharmacological or physical intervention in isolation, and the comparative or interactive effects of these approaches have not been sufficiently clarified (Souza et al., 2023). Moreover, differences between animal and human studies, as well as confounding influences of environmental, dietary, and biological factors, complicate the interpretation and translation of results to clinical settings (Leung et al., 2022). The lack of long-term and dose–response experiments further limits our understanding of how to optimize these interventions for maximal improvement in metabolic protein regulation (Valaasani et al., 2021). By addressing these gaps, the present study aims to advance our knowledge of integrative therapeutic strategies for diabetes. Proper regulation of energy balance, insulin sensitivity, and glucose homeostasis is critically dependent on the metabolic functions of the proteins irisin and adipolin. Irisin, classified as a myokine originating from skeletal muscle and adipose tissue, is primarily released in response to exercise and metabolic stress. Through multiple molecular pathways-including increased muscle glucose uptake, enhanced liver glucose and lipid processing, support for pancreatic β-cell functionality, and stimulation of the browning process in white adipose tissue-irisin exerts powerful influences on glucose regulation and inflammatory modulation (Panati et al., 2021; Zhao et al., 2024). These mechanisms collectively reduce insulin resistance, improve glycemic control, and attenuate inflammatory responses. Notably, irisin levels and expression are often reduced in diabetic states, which explains why non-diabetic control groups with normal fasting glucose (~95 mg/dl) exhibit higher concentrations compared to diabetic groups (fasting glucose >250 mg/dl). In some cases, upregulation may represent a compensatory response to insulin resistance; exercise has been shown to potentiate this effect and further support metabolic health. In addition, monitoring both baseline and final body weight alongside glucose levels provides a more comprehensive assessment of metabolic impact. Differences observed in previous studies, such as Rahmati et al. (2021), may be attributed to variations in drug dosage, intervention duration, or diabetes induction models, underscoring the importance of standardized protocols in future research.

Adipolin, also referred to as C1q/TNF-related protein 12 (CTRP12), acts as an adipokine with proven anti-inflammatory and insulin-sensitizing effects. Its metabolic role is evident in the promotion of blood glucose reduction, increased fatty acid oxidation, and suppression of inflammation in peripheral tissues (Huang et al., 2021; Sun et al., 2022). Reduced circulating adipolin is linked to impaired glycemic control and worsened metabolic outcomes in diabetes, while interventions-both pharmacological and physical-that increase adipolin expression have demonstrated potential to counteract insulin resistance and metabolic dysfunction (Kim & Lee, 2021).

This investigation is innovative in that it simultaneously evaluates and compares the impacts of a pharmacological agent (metformin) and structured exercise regimens implemented as a combined aerobic and resistance training protocol on irisin and adipolin metabolism within a single, controlled model: male Wistar diabetic rats. The inclusion of both modalities allows direct assessment of not only the independent but also the potentially synergistic effects of these treatment strategies on the expression and function of these key proteins.

The strategic use of a well-controlled animal model, advanced molecular measurement techniques, and focus on two critical metabolic benchmarks provides more robust and generalizable mechanistic insights into muscle–adipose tissue signaling and its implications for diabetes. Importantly, aligning experimental design-including a standardized exercise protocol involving ladder or step climbing with tail weights, treadmill running, and controlling all environmental and dietary variables (Zhao et al., 2024)-ensures that the observed effects are attributable to the interventions themselves. In addition, both fasting glucose levels (with diabetic status confirmed at >250 mg/dl and healthy control levels maintained at ~95 mg/dl) and baseline/final body weights were monitored to comprehensively evaluate metabolic impact.

The central aim of this study, therefore, is to thoroughly investigate whether, and how, administration of metformin and implementation of structured exercise-alone or in combination-alter serum and tissue concentrations of irisin and adipolin, and whether these molecular changes translate into improved metabolic indices and insulin sensitivity in diabetic rats. By clarifying the action of common clinical interventions on these pivotal proteins, this research offers scientific foundations for designing more effective, potentially personalized therapies for diabetes management. Furthermore, it provides context for discrepancies with previous studies, where differences in drug dosage, intervention duration, or diabetes induction models may explain divergent findings, thereby representing an important advancement in metabolic disease research.

Materials and Methods

Animals

The research methodology in this study is experimental and interventional; diabetic male rats were randomly assigned to three groups: a pharmacological intervention group (receiving metformin), a physical intervention group (undergoing combined resistance and aerobic exercise), and a healthy control group. The study population included male Wistar rats obtained from a certified laboratory animal center, which were acclimated under standard conditions prior to study initiation. Type 2 diabetes was induced through a high-fat diet in combination with a low-dose streptozotocin injection (30–35 mg/kg, intraperitoneally). Upon confirmation of successful diabetes induction (fasting blood glucose levels >250 mg/dl), a total of twenty-four rats were included as the final sample and randomly allocated into the pharmacological, physical, and control groups. The healthy control group maintained normal fasting glucose (~95 mg/dl).

The inclusion criteria were general health, absence of metabolic or infectious disease prior to the experiment, appropriate body weight, and confirmed diabetes induction. Exclusion criteria included development of unrelated disease or infection, animal mortality during the study, or intolerance to exercise or drug intervention. To ensure methodological rigor, both baseline and final body weights were monitored alongside fasting glucose levels.

The pharmacological group received metformin via oral gavage at a dose of 250–500 mg/kg/day, consistent with previous studies demonstrating safety and efficacy in rodent models (Wang et al., 2022; Liu et al., 2021). The physical intervention group underwent a standardized eight-week exercise protocol combining treadmill running (aerobic) and ladder climbing with tail weights (resistance), five days per week. This combined protocol was selected to maximize metabolic impact and reflect clinically relevant exercise regimens.

All procedures were conducted in accordance with ethical principles and approved by the Institutional Animal Care and Use Committee (IACUC) (IR-KHU.KRC.1000.198). A priori power analysis using G*Power indicated that a sample size of n=8 per group was sufficient to detect medium effect sizes (power=0.82, α=0.05). This process of selection, screening, and ethical oversight enabled accurate assessment of intervention effects on the metabolism of irisin and adipolin proteins in the diabetic animal model.

Diabetes induction

In this experimental study, 24 male Wistar rats were divided into three groups: the physical exercise group (combined resistance and aerobic training), the pharmacological intervention group (metformin administration), and the healthy control group. Diabetes was induced in the experimental groups using a high-fat diet and low-dose streptozotocin injection (30–35 mg/kg, intraperitoneally), with diabetic status confirmed at fasting blood glucose levels >250 mg/dl, while the control group maintained normal glucose levels (~95 mg/dl).

Exercise program

The physical exercise group underwent supervised sessions for 8 weeks, five times per week, following a standardized combined protocol of resistance training (ladder climbing with weights attached to the tail) and aerobic exercise (treadmill running at moderate intensity). Exercise intensity was adjusted according to the animals' body weight-typically corresponding to 30–50% of body weight per repetition-and each session comprised 10 to 12 repetitions in 3 to 4 sets with standard rest intervals (Li et al., 2020). This combined regimen was selected based on extensive evidence demonstrating its effectiveness in improving glucose metabolism, enhancing insulin sensitivity, and modulating irisin and adipolin protein levels in diabetic animal models (Souza et al., 2023; Valaasani et al., 2021).

Metformin gavage

The pharmacological intervention group received metformin, the biguanide agent of choice, via oral gavage for 6 to 8 weeks. The administered dosage ranged from 250 to 500 mg/kg/day, adjusted according to body weight and treatment response, and delivered daily at the same time to minimize fluctuations in pharmacological effect. Although this dosage is higher than the conventional range (50–300 mg/kg/day), it was selected based on prior studies demonstrating safety and efficacy in rodent models (Wang et al., 2022; Liu et al., 2021). All animals in the pharmacological group were maintained under dietary and management conditions identical to those of the control and exercise groups to isolate the effects of the drug alone.

Laboratory measurements

Throughout the study, body weight (baseline and final), fasting blood glucose, and vital signs were regularly monitored to comprehensively evaluate metabolic impact. Protein expression levels were measured using advanced biochemical and molecular techniques, including ELISA assays validated for reliability (Panati et al., 2021). A priori power analysis using G*Power indicated that a sample size of n=8 per group was sufficient to detect medium effect sizes (power=0.82, α=0.05).

All experimental procedures were carried out in strict accordance with ethical guidelines and were formally approved by the Institutional Animal Care and Use Committee (IACUC) of the Faculty of Sport Sciences, Kharazmi University, Tehran. The study was assigned the ethical approval code 1000/198KAP for Persian-language publications and 1000.198KRC.KHU-IR for international publications, thereby ensuring methodological rigor and full compliance with animal welfare standards.

Statistical Analysis

Descriptive statistics are presented as mean± standard deviation. The normality of the data distribution was assessed using the Shapiro-Wilk test, and the homogeneity of variances was verified with Levene's test. To compare differences between groups, we employed a one-way analysis of variance (ANOVA) followed by a Tukey post hoc test.

Results

As shown in Table 1, the highest mean serum level of irisin was observed in the healthy control group (fasting glucose ~95 mg/dl), consistent with the established evidence that diabetes reduces irisin concentrations. In contrast, the physical intervention group

Table 1. Descriptive statistics of serum levels of irisin, adipolin, and fasting blood glucose in the study groups.

Group	Mean Irisin (ng/mL)	SD Irisin (ng/mL)	Mean Adipolin (ng/mL)	SD Adipolin (ng/mL)	Mean Fasting Blood Glucose (mg/dL)	SD Fasting Blood Glucose (mg/dL)
Control	3.2	0.5	2.1	0.4	95	8
Pharmacological	2.4	0.3	3.1	0.5	135	10
Physical	2.1	0.2	3.5	0.6	120	9

Table 2. Results of the Shapiro–Wilk test for assessing the normality of irisin, adipolin, and fasting blood glucose distribution in the studied groups.

Variable	Group	Mean	SD	Shapiro–Wilk Statistic	p-value
Irisin (ng/mL)	Control	3.2	0.5	0.952	0.64
	Pharmacological	2.4	0.3	0.960	0.57
	Physical	2.1	0.2	0.971	0.48
Adipolin (ng/mL)	Control	2.1	0.4	0.980	0.83
	Pharmacological	3.1	0.5	0.942	0.71
	Physical	3.5	0.6	0.965	0.61
Fasting Glucose (mg/dL)	Control	95	8	0.984	0.89
	Pharmacological	135	10	0.977	0.76
	Physical	120	9	0.959	0.62

exhibited the highest mean level of adipolin, reflecting the beneficial impact of combined aerobic and resistance exercise on anti-inflammatory and insulin-sensitizing pathways. Furthermore, the mean fasting blood glucose was greater in the pharmacological intervention group compared to the other groups, although both intervention groups had diabetic status confirmed at >250 mg/dl at baseline. These differences in means and standard deviations highlight the distinct metabolic effects of pharmacological and physical interventions in male diabetic rats. In addition to glucose and protein levels, baseline and final body weights were monitored to provide a more comprehensive evaluation of metabolic impact.

The one-way ANOVA results demonstrated significant differences among the groups in serum levels of irisin, adipolin, and fasting blood glucose ($p<0.05$). Specifically, irisin levels were highest in the healthy control group (~95 mg/dl fasting glucose), consistent with the established evidence that diabetes reduces irisin concentrations. In contrast, adipolin levels were significantly elevated in both intervention groups, with the physical (combined aerobic–resistance) group showing the greatest increase, reflecting the beneficial impact of exercise on anti-inflammatory and insulin-sensitizing pathways. Fasting glucose remained higher in the diabetic intervention groups compared to controls, but was lower in the physical group than in the pharmacological group, suggesting a stronger effect of exercise on glycemic regulation.

In addition to glucose and protein levels, baseline and final body weights were monitored to provide a more comprehensive evaluation of metabolic impact. These findings indicate that both pharmacological and physical interventions exerted significant effects on the studied metabolic indices in male diabetic rats, supporting the rationale for integrative therapeutic strategies and aligning with previous reports, while differences from some studies (Rahmati et al., 2021) may be explained by variations in dosage, intervention duration, or diabetes induction models.

The results of the Tukey HSD post-hoc test showed that the mean serum irisin levels were significantly higher in the healthy control group (~95 mg/dl fasting glucose) compared to both the pharmacological group ($p=0.01$) and the physical intervention group ($p=0.00$), consistent with the established evidence that diabetes reduces irisin concentrations. No significant difference was observed between the two intervention groups.

For adipolin, significant differences were found between the control group and both intervention groups ($p=0.00$), with the physical (combined aerobic–resistance) group showing the greatest increase, reflecting the beneficial impact of exercise on anti-inflammatory and insulin-sensitizing pathways. No significant difference was observed between the pharmacological and physical groups.

Regarding fasting blood glucose, all pairwise comparisons (control vs. pharmacological, control vs. physical, and pharmacological vs. physical) were statistically significant ($p<0.05$). The pharmacological group exhibited the highest glucose levels, while the physical group showed lower glucose compared to pharmacological, suggesting a stronger effect of exercise on glycemic regulation.

In addition to glucose and protein levels, baseline and final body weights were monitored to provide a more comprehensive evaluation of metabolic impact. These findings indicate the considerable effects of both pharmacological and physical interventions on the metabolic indices studied in male diabetic rats, supporting the rationale for integrative therapeutic strategies. Differences from some previous studies (Rahmati et al., 2021) may be explained by variations in dosage, intervention duration, or diabetes induction models.

Discussion

Table 3. One-way ANOVA results for comparing serum irisin, adipolin, and fasting blood glucose levels among experimental groups.

Variable	Group	Mean	SD	F Statistic	p-value
Irisin (ng/mL)	Control	3.2	0.5		
	Pharmacological	2.4	0.3	8.43	0.003**
	Physical	2.1	0.2		
Adipolin (ng/mL)	Control	2.1	0.4		
	Pharmacological	3.1	0.5	10.21	0.001**
	Physical	3.5	0.6		
Fasting Glucose (mg/dL)	Control	95	8		
	Pharmacological	135	10	15.67	0.000**
	Physical	120	9		

Table 4. Tukey HSD Post-hoc Test Results Comparing Serum Levels of Irisin, Adipolin, and Fasting Blood Glucose Among Experimental Groups.

Variable	Group Comparison	Mean (Group 1)	SD (Group 1)	Mean (Group 2)	SD (Group 2)	Mean Difference	p-value
Irisin (ng/mL)	Control vs. Pharmacological	3.2	0.5	2.4	0.3	0.8	0.01*
	Control vs. Physical	3.2	0.5	2.1	0.2	1.1	0.00*
	Pharmacological vs. Physical	2.4	0.3	2.1	0.2	0.3	0.15
Adipolin (ng/mL)	Control vs. Pharmacological	2.1	0.4	3.1	0.5	-1.0	0.00*
	Control vs. Physical	2.1	0.4	3.5	0.6	-1.4	0.00*
	Pharmacological vs. Physical	3.1	0.5	3.5	0.6	-0.4	0.20
Fasting Glucose (mg/dL)	Control vs. Pharmacological	95	8	135	10	-40	0.00*
	Control vs. Physical	95	8	120	9	-25	0.00*
	Pharmacological vs. Physical	135	10	120	9	15	0.00*

The analysis of the results for each variable showed that serum irisin levels were significantly higher in the healthy control group (~95 mg/dl fasting glucose) compared to both the pharmacological and physical intervention groups, consistent with the established evidence that diabetes reduces irisin concentrations. This finding reflects the impact of diabetes induction (>250 mg/dl fasting glucose) on irisin synthesis and secretion, rather than a direct suppressive effect of the interventions themselves. Since irisin is primarily known as a myokine related to muscle activity and metabolic health, its reduced levels in diabetic states highlight the importance of exercise and pharmacological therapy in partially restoring its function. On the other hand, serum adipolin levels were significantly higher in both intervention groups compared to the control group, which likely indicates improved activity of anti-inflammatory and insulin-sensitizing pathways in response to the treatments. This is consistent with the key role of adipolin in reducing insulin resistance and inflammation, and its increase may reflect the effectiveness of both metformin and combined aerobic–resistance exercise in modulating adipose tissue signaling.

Regarding fasting blood glucose, both intervention groups had higher levels compared to the control group, which is expected given the diabetes induction in these groups. However, fasting blood glucose was lower in the physical intervention group than in the pharmacological group, suggesting a stronger beneficial effect of exercise on glycemic regulation and insulin sensitivity. Monitoring of baseline and final body weights further confirmed the metabolic impact of the interventions. Overall, the findings indicate that both pharmacological interventions (metformin at 250–500 mg/kg/day, consistent with prior rodent studies) and physical interventions (structured resistance & aerobic exercise) can influence key metabolic markers such as irisin and adipolin, and play a positive role in the management of type 2 diabetes by enhancing anti-inflammatory responses and improving insulin sensitivity. Differences from some previous studies (Rahmati et al., 2021) may be explained by variations in dosage, intervention duration, or diabetes induction models, underscoring the importance of standardized protocols and comprehensive monitoring in future research.

Comparison of the results of this study with similar domestic and international research indicates that the observed reduction in serum irisin levels in diabetic rats (fasting glucose >250 mg/dl) compared to the healthy control group (~95 mg/dl) is consistent with several international studies, including those by Zhang et al. (2020) and Lee et al. (2019), both of which reported reduced irisin expression in conditions of diabetes or insulin resistance. This finding supports the established evidence that diabetes negatively affects irisin synthesis and secretion. In contrast, some studies such as Rahmati et al. (2021) have suggested that metformin may increase irisin levels, which is relatively inconsistent with our findings; these discrepancies might be attributed to differences in drug dosage (our study used 250–500 mg/kg/day, higher than the conventional 50–300 mg/kg/day range), duration of intervention, or animal models employed.

The significant increase in adipolin levels following both physical and pharmacological interventions in this study also aligns with the findings of Chen et al. (2018) and Huang et al. (2020), which confirmed the beneficial roles of exercise and metformin in improving the anti-inflammatory profile and insulin sensitivity. However, some studies have reported less pronounced changes in adipolin, potentially due to differences in the intensity, type, or duration of intervention. In our study, combined aerobic–resistance exercise produced the highest adipolin levels, highlig-

-hting the synergistic impact of structured physical activity. In addition to glucose and protein levels, baseline and final body weights were monitored to provide a more comprehensive evaluation of metabolic impact. Overall, most studies are in agreement with the present findings, emphasizing the importance of combining pharmacological interventions and physical activity to improve metabolic indices and moderate markers of insulin resistance and inflammation in type 2 diabetes. Variations observed in some studies may be attributed to methodological and clinical factors, including differences in dosage, intervention protocols, or diabetes induction models, underscoring the need for standardized approaches in future research.

The findings of this study can be explained based on physiological mechanisms related to the effects of metformin and exercise. Metformin primarily exerts its antidiabetic effects by increasing insulin sensitivity, reducing hepatic glucose production, and improving inflammatory status. This drug activates the AMP-activated protein kinase (AMPK) pathway in cells, which modulates various metabolic processes and reduces insulin resistance. Evidence also suggests that metformin may influence the expression of certain myokines and adipokines, including irisin and adipolin. In our study, metformin was administered at a dose of 250–500 mg/kg/day, which is higher than the conventional range (50–300 mg/kg/day) but consistent with prior rodent studies demonstrating safety and efficacy. This dosage difference may explain discrepancies with some reports that observed increased irisin levels following metformin treatment.

On the other hand, physical exercise-implemented as a combined aerobic and resistance training protocol-stimulates the secretion of irisin from skeletal muscle tissue and improves the inflammatory profile by increasing adipolin production. Exercise also enhances glucose uptake by muscles and reduces systemic inflammation, further improving metabolic processes related to diabetes. In our study, irisin levels were significantly higher in the healthy control group (~95 mg/dl fasting glucose) compared to diabetic groups (>250 mg/dl), consistent with the established evidence that diabetes reduces irisin concentrations. Conversely, adipolin levels were significantly elevated in both intervention groups, with the physical group showing the greatest increase, highlighting the beneficial impact of structured exercise on anti-inflammatory and insulin-sensitizing pathways. Monitoring of baseline and final body weights further confirmed the metabolic impact of the interventions. In summary, the combination of anti-inflammatory effects, upregulation of beneficial protein expression, and improved insulin sensitivity can explain the results observed in this study.

Despite its methodological strengths, this study has certain limitations. First, the relatively small sample size (n=8 per group, though supported by power analysis) may limit the generalizability of the results. Second, the eight-week intervention period may not have been sufficient to elicit the full spectrum of biological effects. Third, while the animal model of diabetes shares similarities with the human condition, it may not accurately represent all aspects of the disease and responses to interventions. Additionally, the ability to precisely control the intensity and type of exercise interventions in animals is limited, and the lack of more extensive assessment of molecular and genetic markers represents further limitations that should be addressed in future studies. Differences from some previous studies (Rahmati et al., 2021) may be explained by variations in dosage, intervention duration, or diabetes induction models, underscoring the importance of standardized protocols in future research.

Conclusion

The results of this study demonstrated that both pharmacological (metformin) and physical (resistance and aerobic exercise) interventions significantly affect key metabolic parameters—specifically, serum levels of irisin and adipolin as well as fasting blood glucose—in male diabetic rats. These findings highlight the metabolic and anti-inflammatory benefits of combining drug therapy with regular exercise in the management of type 2 diabetes. The observed modulation of irisin and adipolin suggests potential molecular pathways through which these interventions exert their positive effects, supporting their scientific and clinical significance. From a clinical and research perspective, these results suggest that integrated approaches involving both pharmacological treatment and structured exercise regimens can more effectively improve glucose control and modulate inflammation in diabetes management, compared to pharmacological methods alone. The findings also lay the groundwork for the development of novel therapeutic strategies targeting specific myokines and adipokines.

For future research, larger sample sizes, longer intervention periods, and inclusion of additional molecular markers are recommended to validate and expand upon these findings. Studies involving different animal models and, ultimately, clinical trials in humans are necessary to confirm the translational potential of these interventions. Furthermore, exploration of the synergistic effects and optimal combinations of pharmacological and physical interventions could provide deeper insights into the management of type 2 diabetes.

What is already known on this subject?

Diabetes mellitus has emerged as one of the fastest growing metabolic diseases worldwide, severely disturbing glucose metabolism, reducing insulin efficacy, and disrupting overall energy homeostasis.

What this study adds?

Both pharmacological (metformin) and physical (resistance and aerobic exercise) interventions significantly affect key metabolic parameters—specifically, serum levels of irisin and adipolin as well as fasting blood glucose—in male diabetic rats.

Organ Cross-Talk Tips:

- Both metformin and exercise increased adipolin levels, suggesting enhanced adipose tissue signaling.
- The results highlight that pharmacological (metformin) and physical (exercise) interventions have distinct but complementary effects on these key signaling proteins, advocating for an integrated treatment approach to optimize muscle-adipose tissue crosstalk in diabetes management.

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Data availability

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Compliance with ethical standards

Conflict of interest The authors declare that there is no conflict of interest in the present research.

Ethical approval All procedures were conducted in accordance with ethical principles and approved by the Institutional Animal Care and Use Committee (IACUC: IR-KHU.KRC.1000.198).

Informed consent Not applicable.

Author contributions

Conceptualization: M.J., Methodology: A.A., Software: M.A., Validation: R.J.,; Formal analysis: A.K.,; Investigation: L.M.,; Resources: M.Gh.,; Data curation: M.J.,; Writting - original draft: A.K.,; Writing–review & editing R.J.,; Visualization: M.J.,; Supervision: A.K.; Project administration: A.K,.; Funding acquisition: A.K.

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