



Regulation of the GLUT4 Translocation Pathway in Diabetic Skeletal Muscle: Comparative Effects of Aerobic and Resistance Training on AS160 and GLUT4 Gene Expression in Type 2 Diabetic Mice

Reyhaneh Alizadeh Behbahani¹, Saeed Keshavarz^{1*}, Leila Sarrami¹, Jamshid Banaei Borojeni¹

1. Department of Sport Sciences, Na.C., Islamic Azad University, Najafabad, Iran

* Corresponding author email address: saeed.keshavarz@iau.ac.ir

Article Info

Article type:

Original Research

How to cite this article:

Alizadeh Behbahani, R., Keshavarz, S., Sarrami, L., & Banaei Borojeni, J. (2026). Regulation of the GLUT4 Translocation Pathway in Diabetic Skeletal Muscle: Comparative Effects of Aerobic and Resistance Training on AS160 and GLUT4 Gene Expression in Type 2 Diabetic Mice. *Health Nexus*, 4(3), 1-8.

<https://doi.org/10.61838/kman.hn.5562>



© 2026 the authors. Published by KMAN Publication Inc. (KMANPUB), Ontario, Canada. This is an open access article under the terms of the Creative Commons Attribution-NonCommercial 4.0 International (CC BY-NC 4.0) License.

ABSTRACT

Background: Impaired glucose transport into skeletal muscle is a central mechanism in insulin resistance and type 2 diabetes mellitus. AS160 (TBC1D4) and glucose transporter type 4 (GLUT4) are key molecular components of the insulin-dependent glucose transport pathway. Exercise training is an established non-pharmacological intervention for improving glucose homeostasis; however, the comparative effects of aerobic and resistance training on AS160 and GLUT4 gene expression in diabetic skeletal muscle remain insufficiently clarified. **Objective:** This study compared the effects of aerobic and resistance training on AS160 and GLUT4 gene expression in the soleus and extensor digitorum longus (EDL) muscles of type 2 diabetic mice. **Methods:** Fifty male C57BL/6 mice were assigned to five groups: healthy control, sham, diabetic control, diabetic plus aerobic training, and diabetic plus resistance training. Type 2 diabetes was induced using a high-fat diet followed by low-dose streptozotocin injection. The training protocols were performed for 8 weeks, 5 sessions per week. AS160 and GLUT4 gene expression in the soleus and EDL muscles was measured by real-time polymerase chain reaction and normalized to GAPDH using the $2^{-\Delta\Delta Ct}$ method. Data were analyzed using two-way mixed analysis of variance with Bonferroni post hoc comparisons. **Results:** Diabetes reduced AS160 and GLUT4 expression in both muscles. Both exercise modalities increased the expression of the two genes compared with the diabetic control group. The GLUT4 response was stronger than the AS160 response, and aerobic training produced a larger increase in GLUT4 expression than resistance training. The soleus muscle showed a stronger metabolic response than the EDL muscle. **Conclusion:** Exercise training, particularly aerobic training, partially restored defects in the skeletal muscle glucose transport pathway in type 2 diabetic mice. These findings support structured exercise as a molecularly relevant strategy for improving insulin sensitivity in diabetes.

Keywords: type 2 diabetes mellitus; aerobic training; resistance training; AS160; GLUT4; glucose transport; skeletal muscle

1. Introduction

Impaired glucose transport into skeletal muscle is one of the defining pathophysiological features of type 2 diabetes mellitus (T2DM). Even when circulating insulin is

available, skeletal muscle cells in insulin-resistant states show a reduced capacity to take up glucose, which contributes to chronic hyperglycemia and wider metabolic dysfunction. Skeletal muscle is the largest glucose-

consuming tissue in the body and therefore plays a major role in systemic energy balance and glucose homeostasis. Disturbances in glucose transport in this tissue can accelerate the progression of T2DM and impair functional capacity.

The public health relevance of this problem is substantial. The World Health Organization describes type 2 diabetes as a condition in which the body no longer uses insulin effectively and notes that insufficient physical activity and excess body weight are important modifiable contributors to its development (1). Experimental and clinical studies similarly indicate that physical inactivity and reduced skeletal muscle metabolic capacity are closely linked to insulin resistance. Consequently, interventions that restore skeletal muscle glucose uptake are central to the prevention and management of T2DM.

At the molecular level, glucose entry into skeletal muscle requires activation of signaling cascades that culminate in the translocation of GLUT4-containing vesicles to the plasma membrane. AS160, also known as TBC1D4, is a downstream Akt substrate involved in the regulation of GLUT4 trafficking. In response to insulin signaling, AS160 phosphorylation facilitates GLUT4 vesicle mobilization and membrane insertion. In insulin-resistant conditions, impairment of the IRS1/Akt/AS160 axis limits GLUT4 translocation and reduces muscle glucose uptake. Mann et al. (2022) reviewed evidence showing that Akt substrates, including TBC1D4/AS160, are responsive to muscle contraction and participate in exercise-related regulation of glucose uptake (2).

Exercise training is among the most effective non-pharmacological strategies for improving insulin sensitivity. Aerobic training can increase oxidative capacity, mitochondrial function, and substrate utilization, whereas resistance training can increase muscle mass, mechanical loading, and insulin-mediated glucose disposal. Han et al. (2023) showed that aerobic exercise improved insulin resistance in C57BL/6J mice through mechanisms involving Sestrin3, mTORC2/Akt signaling, and GLUT4 expression (3). Wang et al. (2018) similarly reported that exercise improved glucose uptake in murine myotubes through AMPK α 2-mediated induction of sestrins (4). In humans with T2DM, strength training has also been shown to increase GLUT4 content and insulin signaling in skeletal muscle (5).

The response to exercise may differ according to muscle phenotype. The soleus muscle is dominated by slow oxidative fibers, whereas the EDL muscle contains a higher proportion of fast glycolytic fibers. Because oxidative and glycolytic muscles differ in mitochondrial density, insulin responsiveness, and substrate use, their molecular responses to training may not be identical. Recent proteomic evidence supports the idea that skeletal muscle protein relationships differ across normal glucose metabolism, prediabetes, and T2DM states (6). This raises the possibility that exercise-induced regulation of AS160 and GLUT4 may depend not only on the training mode but also on the muscle type examined.

Previous studies have examined parts of this pathway. Garavandpour et al. (2024) reported that 8 weeks of aerobic training increased GLUT4 and AS160 protein levels and improved insulin resistance in the EDL muscle of type 2 diabetic rats (7). Banaeifar et al. (2019) reported that resistance training increased muscle GLUT4 gene expression and improved blood glucose and insulin resistance in type 2 diabetic rats (8). Other studies have shown that aerobic exercise can improve skeletal muscle inflammation and insulin resistance by regulating inflammatory and microRNA-related signaling pathways (9). However, fewer studies have directly compared aerobic and resistance training in the same experimental design while examining both AS160 and GLUT4 expression in muscles with different fiber-type characteristics.

Accordingly, the present study aimed to compare the effects of aerobic and resistance training on AS160 and GLUT4 gene expression in the soleus and EDL muscles of type 2 diabetic mice. It was hypothesized that both training modalities would improve the expression of genes involved in glucose transport, but that the magnitude and pattern of response would differ between aerobic and resistance training and between the two skeletal muscles.

2. Methods and Materials

2.1. Study design and animals

This study was designed as an experimental laboratory investigation using a mouse model of T2DM. Fifty male C57BL/6 mice were used. After arrival at the animal facility, the mice were allowed to acclimatize for 1 week under

controlled environmental conditions: temperature 22-24 °C, relative humidity 50-55%, and a 12:12 h light-dark cycle. Water and food were provided ad libitum. After acclimatization, the animals were randomly allocated to five groups: healthy control, sham, diabetic control, diabetic plus aerobic training, and diabetic plus resistance training. All procedures related to housing, diabetes induction, exercise training, anesthesia, and tissue sampling were conducted in accordance with ethical principles for the use of laboratory animals.

2.2. Induction of type 2 diabetes

Diabetes was induced using a high-fat diet followed by low-dose streptozotocin injection. The approach was adapted from experimental models in which a high-fat diet is used to produce insulin resistance before pharmacological induction of hyperglycemia. The diabetic groups received a high-fat diet for 6 weeks. Thereafter, low-dose streptozotocin was administered intraperitoneally to complete the T2DM model. The sham group underwent the injection procedure but received vehicle instead of streptozotocin. After 72 h, fasting blood glucose was measured from tail blood. Animals with fasting blood glucose levels greater than 250 mg/dL were considered diabetic and continued in the study.

2.3. Aerobic training protocol

The aerobic training protocol was performed on a treadmill for 8 weeks, 5 days per week. Before the main intervention, mice in the diabetic plus aerobic training group completed short familiarization sessions to reduce stress and avoidance behavior. The protocol began at a low speed and duration and progressed gradually. Running speed was increased to approximately 12-15 m/min, which corresponds to moderate-intensity aerobic exercise in this model. Training intensity was estimated to be approximately 60-75% of aerobic capacity, and progressive overload was applied by controlled increases in speed and session duration.

2.4. Resistance training protocol

Resistance training was performed using a ladder-climbing model, a common animal model for inducing

resistance-type muscular and metabolic adaptations. Mice in the diabetic plus resistance training group were first familiarized with ladder climbing without additional load. The main protocol was then performed for 8 weeks. The ladder was approximately 1 m high with an angle of about 85°. Load was attached to the tail and increased progressively according to body weight and performance. Each session included several climbs separated by short rest intervals. The protocol was designed to provide sufficient mechanical and metabolic stimulus while minimizing excessive fatigue.

2.5. Tissue sampling

Forty-eight hours after the final training session, the animals were kept free from exercise to reduce acute exercise effects on gene expression. After controlled fasting, mice were anesthetized with ketamine and xylazine. The soleus and EDL muscles were rapidly excised from the hindlimb. Samples were washed with cold physiological saline, trimmed of excess tissue, frozen immediately in liquid nitrogen, and stored at -80 °C until molecular analysis. The soleus and EDL muscles were selected because they represent different fiber-type profiles and metabolic capacities.

2.6. Real-time PCR analysis

Total RNA was extracted from skeletal muscle samples according to the kit manufacturer's protocol under RNase-free conditions. RNA concentration and purity were assessed using a NanoDrop spectrophotometer. Samples with adequate quality were used for cDNA synthesis. Real-time polymerase chain reaction was performed for AS160 and GLUT4. GAPDH was used as the reference gene. Relative gene expression was calculated using the $2^{-\Delta\Delta Ct}$ method and expressed as fold change.

2.7. Statistical analysis

After Ct values were extracted, AS160 and GLUT4 expression values were normalized to GAPDH and calculated as relative expression. Data were examined for normality using the Shapiro-Wilk test and for homogeneity of variance using Levene's test. A two-way mixed analysis of variance was used to examine the effects of group, muscle

type, and their interaction on AS160 and GLUT4 expression. The study groups were treated as the between-group factor, and muscle type (soleus and EDL) was treated as the within-subject factor. Bonferroni post hoc tests were used for pairwise comparisons. Partial eta squared was reported as the effect size. The level of significance was set at $p < 0.05$. Analyses were performed using SPSS version 27.

3. Findings and Results

Table 1 presents the animal characteristics and confirms successful induction of the diabetic model. Fasting blood glucose levels in the diabetic groups were greater than 250 mg/dL, indicating successful establishment of T2DM. Final body weight increased more in the diabetic control group than in the healthy control and sham groups, whereas both exercise interventions partially attenuated this increase.

Table 1

Animal characteristics and confirmation of the diabetic model

Group	n	Initial weight (g)	Final weight (g)	Fasting blood glucose (mg/dL)	Metabolic status
Healthy control	10	19.79 ± 1.15	27.36 ± 1.98	100.92 ± 8.21	Healthy
Sham	10	20.16 ± 1.22	27.19 ± 2.03	103.74 ± 8.06	Sham
Diabetic control	10	19.88 ± 1.10	34.87 ± 2.71	320.13 ± 25.02	Diabetic
Diabetic + aerobic training	10	20.08 ± 1.14	29.01 ± 2.16	302.78 ± 20.95	Diabetic
Diabetic + resistance training	10	20.31 ± 1.27	30.24 ± 2.21	309.46 ± 23.11	Diabetic

Table 2 shows the expression of AS160 and GLUT4 in the soleus muscle. Diabetes reduced the expression of both genes. Aerobic and resistance training increased AS160 and

GLUT4 expression compared with the diabetic control group, with a stronger response for GLUT4. Aerobic training produced the largest increase in GLUT4 expression.

Table 2

AS160 and GLUT4 gene expression in the soleus muscle

Gene	Healthy control	Sham	Diabetic control	Diabetic + aerobic training	Diabetic + resistance training
AS160	1.00 ± 0.08	1.01 ± 0.09	0.52 ± 0.07	1.61 ± 0.21	1.43 ± 0.18
GLUT4	1.00 ± 0.07	0.98 ± 0.10	0.43 ± 0.06	2.54 ± 0.33	2.11 ± 0.27

Table 3 presents gene expression in the EDL muscle. The diabetic control group showed the lowest levels of AS160 and GLUT4. Both exercise protocols increased expression

values, but the increase was greater after aerobic training than after resistance training, especially for GLUT4.

Table 3

AS160 and GLUT4 gene expression in the EDL muscle

Gene	Healthy control	Sham	Diabetic control	Diabetic + aerobic training	Diabetic + resistance training
AS160	1.00 ± 0.09	0.97 ± 0.08	0.48 ± 0.06	1.54 ± 0.20	1.37 ± 0.17
GLUT4	1.00 ± 0.08	1.00 ± 0.09	0.39 ± 0.05	2.39 ± 0.31	1.96 ± 0.24

The two-way mixed ANOVA results are shown in Table 4. The group effect was significant for both AS160 and GLUT4, indicating that diabetes and exercise training altered gene expression. The muscle effect and group-by-

muscle interaction were also significant, showing that the soleus and EDL muscles differed in the magnitude of their response.

Table 4

Two-way mixed ANOVA for AS160 and GLUT4 gene expression

Gene	Effect	F	df	p	Partial eta squared	Dominant pattern
AS160	Group	26.83	4	0.001	0.69	Moderate increase; incomplete normalization
AS160	Muscle	4.52	1	0.036	0.17	Higher response in soleus
AS160	Group × muscle	3.76	4	0.043	0.14	Muscle-dependent response
GLUT4	Group	38.16	4	0.001	0.81	Strong increase; aerobic superiority
GLUT4	Muscle	5.41	1	0.024	0.22	Higher response in soleus
GLUT4	Group × muscle	4.28	4	0.031	0.18	Muscle-dependent response

Figure 1 illustrates the overall pattern of AS160 and GLUT4 expression in the soleus muscle. The diabetic control group showed reduced expression of both genes.

Both training protocols increased expression, but GLUT4 responded more strongly than AS160, and aerobic training produced the highest GLUT4 expression.

Figure 1

Overall pattern of AS160 and GLUT4 gene expression in the soleus muscle

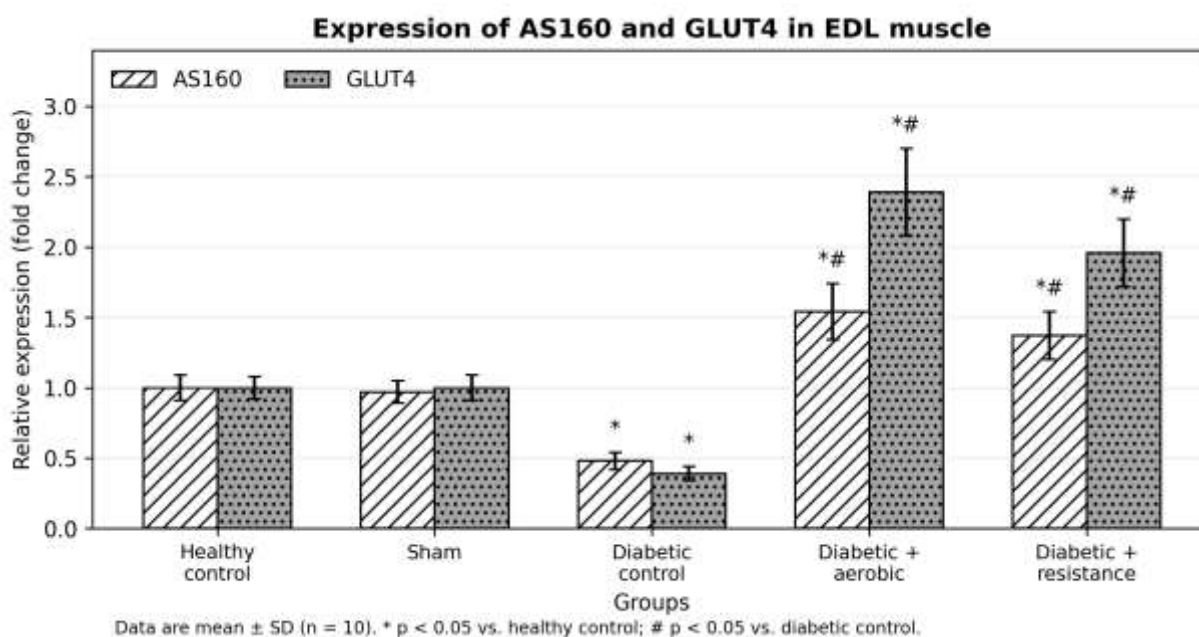


Figure 2 shows the expression pattern in the EDL muscle. Similar to the soleus muscle, diabetes reduced AS160 and GLUT4 expression. Aerobic and resistance training

increased expression, but the magnitude of the response was lower than that observed in the soleus muscle.

Figure 2

Overall pattern of AS160 and GLUT4 gene expression in the EDL muscle



Overall, T2DM reduced the expression of AS160 and GLUT4 in skeletal muscle. Both aerobic and resistance training increased the expression of these genes, but the GLUT4 response was clearer and stronger than the AS160 response. Aerobic training had the greatest effect on GLUT4 expression, with values increasing to approximately 2.39-2.54 fold depending on muscle type. AS160 also increased after exercise training, but the magnitude of change was smaller.

4. Discussion

This study examined the effects of aerobic and resistance training on two key genes involved in skeletal muscle glucose transport, AS160 and GLUT4, in a mouse model of T2DM. The main findings were that diabetes reduced the expression of both genes in soleus and EDL muscles, while both exercise modalities partially restored their expression. The response was stronger for GLUT4 than for AS160, and aerobic training produced a larger increase in GLUT4 than resistance training. The soleus muscle showed a stronger response than the EDL muscle, suggesting that training-induced regulation of the glucose transport pathway is influenced by muscle fiber characteristics.

The reduction in AS160 expression in the diabetic control group is consistent with impairment of downstream insulin signaling. AS160/TBC1D4 is a key Akt substrate that contributes to the trafficking of GLUT4 vesicles toward the

plasma membrane. When insulin signaling is disrupted, impaired AS160 phosphorylation and related downstream events may reduce GLUT4 translocation and limit glucose uptake. The increase in AS160 after training suggests that exercise may partially reactivate components of insulin signaling in diabetic skeletal muscle. This interpretation is consistent with the review by Mann et al. (2022), which emphasized the involvement of Akt substrates such as TBC1D4/AS160 in contraction- and exercise-related glucose transport (2).

The larger GLUT4 response after aerobic training suggests that aerobic exercise may be particularly effective in enhancing the capacity of skeletal muscle for glucose uptake. Aerobic exercise promotes mitochondrial biogenesis, oxidative enzyme activity, and substrate utilization, all of which can create a cellular environment favorable to improved insulin sensitivity. Han et al. (2023) showed that aerobic exercise improved skeletal muscle insulin sensitivity in C57BL/6J mice through signaling mechanisms involving Sestrin3, mTORC2/Akt activation, and GLUT4 expression (3). Li et al. (2025) also reported that aerobic exercise improved inflammation and insulin resistance in skeletal muscle through regulation of miR-221-3p and JAK/STAT-related pathways (9). These mechanisms may help explain why GLUT4 expression was particularly responsive to aerobic training in the present study.

Resistance training also improved AS160 and GLUT4 expression, although the magnitude of change was generally lower than that observed with aerobic training. This finding does not diminish the therapeutic relevance of resistance training. Resistance training can increase muscle mass, improve insulin signaling, and enhance glucose disposal through mechanical and metabolic loading. In patients with T2DM, Holten et al. (2004) showed that strength training increased insulin-mediated glucose uptake, GLUT4 content, and insulin signaling in skeletal muscle. Similarly (5), Banaeifar et al. (2019) reported increased GLUT4 gene expression and improved glycemic indices after resistance training in diabetic rats (8). Therefore, resistance training may act as a complementary intervention, especially where preservation of muscle mass and strength is also a clinical priority.

The stronger response observed in the soleus muscle than in the EDL muscle is physiologically plausible. The soleus has a higher proportion of slow oxidative fibers, greater mitochondrial density, and higher baseline insulin sensitivity. These characteristics may make it more responsive to metabolic training stimuli. In contrast, the EDL muscle is more glycolytic and may show a smaller adaptive response in gene expression related to oxidative metabolism and insulin action. Czajkowska et al. (2024) demonstrated that skeletal muscle proteomic relationships vary according to metabolic status, which supports the concept that muscle phenotype and metabolic condition shape exercise responsiveness (6).

The present results are broadly consistent with previous Iranian and international studies. Garavandpour et al. (2024) found that aerobic training increased GLUT4 and AS160 protein levels and improved insulin resistance in the EDL muscle of type 2 diabetic rats (7). The present study extends that line of evidence by comparing aerobic and resistance training and by examining both soleus and EDL muscles. The findings also align with broader evidence showing that exercise improves glucose uptake and insulin sensitivity through multiple interacting pathways, including inflammatory regulation, oxidative stress modulation, mitochondrial adaptation, and insulin signaling restoration.

The clinical implication is that structured exercise can influence skeletal muscle glucose transport at the molecular level. Aerobic training appears especially relevant for

increasing GLUT4 expression, whereas resistance training may provide complementary benefits through muscular adaptation and partial restoration of AS160/GLUT4 signaling. For individuals with T2DM, combined exercise programs may therefore be advantageous, although this study did not directly test combined training. Translation from animal models to human practice should be cautious, but the molecular findings support the biological plausibility of exercise-based interventions for improving insulin sensitivity.

4.1. Limitations

Several limitations should be considered. First, this study used an animal model, and direct generalization to humans requires caution. Second, the intervention lasted 8 weeks; longer protocols may reveal additional or more stable adaptations. Third, the study focused on AS160 and GLUT4 gene expression and did not include parallel assessment of protein expression, protein phosphorylation, AMPK, mTOR, inflammatory markers, or oxidative stress indices. Fourth, combined aerobic and resistance training was not examined. Future studies should evaluate longer interventions, combined training models, and integrated transcriptomic, proteomic, and metabolic outcomes.

5. Conclusion

Type 2 diabetes reduced AS160 and GLUT4 gene expression in the soleus and EDL muscles of C57BL/6 mice. Both aerobic and resistance training partially restored the expression of these genes, but aerobic training produced a stronger GLUT4 response. The soleus muscle showed a greater adaptive response than the EDL muscle. These findings indicate that exercise training, especially aerobic training, can improve key components of the skeletal muscle glucose transport pathway and may contribute to better insulin sensitivity in T2DM.

Authors' Contributions

R.A.B. contributed to manuscript preparation and data organization. S.K. contributed to study supervision, methodology, and critical revision. L.S. and J.B.B. contributed to scientific review and interpretation of

findings. All authors reviewed and approved the final manuscript.

Declaration

AI-assisted language editing and translation support were used during editorial preparation of the English manuscript. The authors remain responsible for the scientific content, data accuracy, interpretation, and reference integrity.

Transparency Statement

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

Acknowledgments

The authors acknowledge the institutional support of the Department of Sport Sciences, Najafabad Branch, Islamic Azad University, Najafabad, Iran.

Declaration of Interest

The authors report no conflict of interest.

Funding

This research received no specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Ethics Considerations

This study was approved by the Ethics Committee of Islamic Azad University, Najafabad Branch (approval code: IR.IAU.NAJAFABAD.REC.1405.088). All animal procedures were conducted according to ethical principles for laboratory animal care and use.

References

1. World Health O. Diabetes. 2024.
2. Mann G, Riddell MC, Adegoke OAJ. Effects Of Acute Muscle Contraction On The Key Molecules In Insulin And Akt Signaling In Skeletal Muscle In Health And In Insulin Resistant States. *Diabetology*. 2022;3(3):423-46. [DOI]
3. Han X, Yang Y, Liu S, Niu Y, Shao H, Fu L. Aerobic Exercise Ameliorates Insulin Resistance In C57Bl/6J Mice Via Activating Sestrin3. *Biochimica et Biophysica Acta (BBA) - Molecular Basis of Disease*. 2023;1869(1):166568. [PMID: 36220588] [DOI]
4. Wang T, Niu Y, Liu S, Yuan H, Liu X, Fu L. Exercise Improves Glucose Uptake In Murine Myotubes Through The

5. Ampka2-Mediated Induction Of Sestrins. *Biochimica et Biophysica Acta (BBA) - Molecular Basis of Disease*. 2018;1864(10):3368-77. [PMID: 30048751] [DOI]
6. Holten MK, Zacho M, Gaster M, Juel C, Wojtaszewski JFP, Dela F. Strength Training Increases Insulin-Mediated Glucose Uptake, Glut4 Content, And Insulin Signaling In Skeletal Muscle In Patients With Type 2 Diabetes. *Diabetes*. 2004;53(2):294-305. [PMID: 14747278] [DOI]
7. Czajkowska A, Czajkowski M, Szczerbinski L, Jurczuk K, Reska D, Kwedlo W, et al. Exploring Protein Relative Relations In Skeletal Muscle Proteomic Analysis For Insights Into Insulin Resistance And Type 2 Diabetes. *Scientific Reports*. 2024;14:17631. [PMID: 39085321] [PMCID: PMC11292014] [DOI]
8. Garavandpour F, Valipour Dehno V, Eslami R. The Effect Of Eight Weeks Of Aerobic Training On Glut4 And As160 Protein Levels And Insulin Resistance In The Edl Muscle Of Type 2 Diabetic Rats. *Feyz Journal of Kashan University of Medical Sciences*. 2024;28(4):335-43.
9. Banaeifar A, Ebrahimpor S, Tabatabaei H, Ebadi Ghahremani M. The Effect Of Resistance Training On Muscle Glut4 Gene Expression, Blood Glucose And Insulin Resistance In Type 2 Diabetic Rats. *Journal of Ilam University of Medical Sciences*. 2019;26(6):46-57. [DOI]
10. Li N, Zhang L, Guo Q, Shi H, Gan Y, Wang W, et al. Aerobic Exercise Improves Inflammation And Insulin Resistance In Skeletal Muscle By Regulating Mir-221-3P Via Jak/STAT Signaling Pathway. *Frontiers in Physiology*. 2025;16:1534911. [PMID: 40070461] [PMCID: PMC11893602] [DOI]