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Comparative Effects of Continuous and Interval Aerobic Training on Cellular Respiration Indices (OCR) and ATP Content in Skeletal Muscle of Rats with Type 2 Diabetes

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A B S T R A C T

Type 2 diabetes impairs skeletal muscle energy metabolism, partly through mitochondrial respiratory dysfunction and reduced ATP-generating capacity. This experimental study compared the effects of continuous and interval aerobic training on cellular respiration indices and ATP content in the gastrocnemius muscle of male Wistar rats with type 2 diabetes. Male Wistar rats were allocated to six groups: healthy control, healthy + continuous training, healthy + interval training, diabetic control, diabetic + continuous training, and diabetic + interval training (n = 8 per group). Type 2 diabetes was induced using a high-fat diet followed by intraperitoneal streptozotocin injection. The exercise interventions were performed for eight weeks. After the intervention, gastrocnemius muscle samples were collected for analysis of mitochondrial respiratory indices, including basal respiration, ATP-linked respiration, maximal respiration, spare respiratory capacity, proton leak, coupling efficiency, and tissue ATP content. Data were analyzed using two-way analysis of variance followed by Tukey post hoc tests. Diabetes reduced mitochondrial respiratory indices, spare respiratory capacity, coupling efficiency, and ATP content. Both aerobic training protocols improved respiratory function and ATP content in diabetic rats. The interval training protocol produced larger improvements than continuous training in maximal respiration, spare respiratory capacity, and ATP content. Eight weeks of aerobic training partially restored diabetes-related impairments in skeletal muscle mitochondrial function. Interval aerobic training appeared to produce stronger respiratory adaptations than continuous training. These findings provide preclinical evidence for the role of exercise intensity structure in mitochondrial adaptation under diabetic conditions.

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

1. Introduction

Type 2 diabetes is one of the most important metabolic health challenges worldwide. It is characterized not only by chronic hyperglycemia but also by impaired substrate utilization, insulin resistance, and progressive disruption of tissue-level energy metabolism. Skeletal muscle is central to glucose disposal and whole-body metabolic regulation; therefore, diabetes-related alterations in skeletal muscle mitochondrial function can substantially affect metabolic control and physical performance (1, 2).

Mitochondrial function depends on coordinated electron transport, oxygen consumption, oxidative phosphorylation, and ATP synthesis. In skeletal muscle, oxygen consumption rate (OCR) provides functional information about basal respiration, ATP-linked respiration, maximal respiratory capacity, spare respiratory capacity, proton leak, and coupling efficiency. In diabetes, impaired mitochondrial respiration may reduce ATP production, increase oxidative stress, and limit the capacity of skeletal muscle to meet energy demands during rest and activity (3-5).

Aerobic exercise is a major non-pharmacological strategy for improving metabolic health. Continuous moderate-intensity training usually provides a sustained oxidative stimulus, whereas interval training alternates higher-intensity bouts with recovery periods and may generate stronger fluctuations in energetic stress. These differences in exercise structure may lead to different mitochondrial adaptations. Previous experimental and clinical studies have shown that interval training can improve glycemic control, insulin-related signaling, and mitochondrial-associated markers; however, the comparative effects of continuous and interval aerobic training on OCR-related respiratory indices and ATP content in diabetic skeletal muscle remain insufficiently clarified (6-9).

The present study was designed to compare the effects of eight weeks of continuous and interval aerobic training on mitochondrial respiration indices and ATP content in the gastrocnemius muscle of male Wistar rats with type 2 diabetes. It was hypothesized that both exercise protocols would improve OCR-related indices and ATP content, but that the interval training protocol would produce stronger adaptations in maximal respiration and spare respiratory capacity (9, 10).

2. Methods and Materials

2.1. Study Design

This was an experimental laboratory study with a comparative interventional design. The study examined the main effects of metabolic condition (healthy vs. diabetic), exercise condition (control, continuous aerobic training, and interval aerobic training), and their interaction on skeletal muscle mitochondrial respiration and ATP content.

2.2. Animals and Housing Conditions

The experimental sample consisted of male Wistar rats aged approximately 8-10 weeks and weighing 200-250 g at baseline. After transfer to the animal laboratory, the animals were acclimatized for one week under controlled environmental conditions: temperature 22 ± 2°C, 12:12 h light-dark cycle, controlled humidity, and free access to water and standard chow, except during the high-fat diet phase for diabetic groups. After acclimatization, the animals were randomly allocated to six groups, with eight animals in each group: healthy control, healthy + continuous aerobic training, healthy + interval aerobic training, diabetic control, diabetic + continuous aerobic training, and diabetic + interval aerobic training.

2.3. Induction of Type 2 Diabetes

Type 2 diabetes was induced using a combined high-fat diet and streptozotocin (STZ) protocol, consistent with previously used experimental approaches in diabetic rodent models (9, 10). Rats allocated to diabetic groups received a high-fat diet for 12 weeks. After this phase, they received a single intraperitoneal injection of STZ at 100 mg/kg body weight dissolved in citrate buffer (pH 4.4). Sham/healthy animals received citrate buffer only. Seven days after injection, fasting blood glucose was measured from tail blood. Animals with fasting blood glucose values above 13.8 mmol/L were considered diabetic and were included in the subsequent intervention phase. Body weight and food intake were monitored throughout the study.

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2.4. *Treadmill Familiarization*

Before the main training interventions, rats in the exercise groups completed a five-day treadmill familiarization period to reduce handling and treadmill-related stress. During this phase, the animals walked or ran at low speed for approximately 10-15 min per day.

2.5. *Continuous Aerobic Training Protocol*

The continuous training protocol was designed to provide a progressive sustained oxidative stimulus. After a low-intensity warm-up, rats performed continuous treadmill running. Exercise intensity progressed from approximately 55% of aerobic capacity in the early phase to approximately 75% in the final weeks. In the first week, rats ran at approximately 15 m/min for 20 min. Duration and speed were gradually increased across the intervention until the final weeks, when exercise duration reached approximately 50-60 min and speed reached approximately 25 m/min. Training was performed continuously without planned high-intensity intervals. Animals were monitored for fatigue and exercise intolerance during each session.

2.6. *Interval Aerobic Training Protocol*

The interval training protocol was based on repeated higher-intensity treadmill bouts separated by recovery periods. Each session began with a 10-min low-intensity warm-up, followed by 10 repeated intervals. Each interval consisted of 4 min of high-intensity running followed by 2 min of active or passive recovery. Running speed increased gradually from approximately 16 m/min in the early weeks to 26 m/min in the final weeks. Treadmill inclination was set at approximately 20-25 degrees to increase metabolic demand. Exercise tolerance, fatigue signs, and reliance on the end grid of the treadmill were monitored, and intensity was adjusted if excessive fatigue occurred.

2.7. *Tissue Collection*

Forty-eight hours after the final training session and after overnight fasting, the animals were anesthetized using ketamine and xylazine. The gastrocnemius muscle was rapidly excised, washed in cold medium, frozen in liquid nitrogen, and stored at -80°C until analysis. The gastrocnemius was selected because of its frequent use in studies of skeletal muscle oxidative capacity, mitochondrial function, and energy metabolism.

2.8. *Mitochondrial Respiration and ATP Assessment*

Cellular oxygen consumption-related respiratory indices were assessed using a mitochondrial respiration analysis procedure on prepared muscle samples. The measured indices included basal respiration, ATP-linked respiration, maximal respiration, spare respiratory capacity, proton leak, and coupling efficiency. Skeletal muscle ATP content was measured using a biochemical ATP assay kit according to the manufacturer's protocol. Because the source manuscript did not specify the respiration platform, normalization strategy, ATP kit manufacturer, or measurement units, these methodological details must be completed from the laboratory records before journal submission.

2.9. *Statistical Analysis*

Data were reported as mean ± standard deviation. Normality and homogeneity of variances were evaluated using the Shapiro-Wilk and Levene tests, respectively. Two-way analysis of variance was used to examine the effects of metabolic condition, exercise condition, and their interaction. Tukey post hoc tests were used for between-group comparisons when appropriate. The significance threshold was set at $p < 0.05$. Analyses were performed using SPSS version 27.

3. Findings and Results

3.1. *Baseline and Metabolic Characteristics*

Table 1 summarizes body weight, fasting blood glucose, and food intake. The diabetic control group showed higher fasting blood glucose and food intake and a lower increase in body weight compared with the healthy control group. Among diabetic rats, both training protocols were associated with lower fasting blood glucose than the diabetic control condition, with the lowest fasting blood glucose observed in the diabetic + interval training group.

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Table 1

Baseline characteristics, metabolic indices, and confirmation of the diabetic model

Group	n	Initial weight (g)	Final weight (g)	Weight change (%)	Fasting blood glucose (mmol/L)	Food intake (g/day)
HC	8	221.6 ± 9.4	302.8 ± 11.9	+35.9	5.3 ± 0.5	24.7 ± 1.8
H+CT	8	222.4 ± 10.1	289.7 ± 10.8	+29.6	5.0 ± 0.4	25.1 ± 2.0
H+IT	8	221.2 ± 8.8	286.4 ± 10.6	+29.2	4.8 ± 0.4	24.5 ± 1.7
DC	8	223.1 ± 9.7	246.5 ± 13.4	+10.8	18.9 ± 1.8	31.8 ± 2.5
D+CT	8	222.3 ± 10.0	267.8 ± 12.2	+20.6	14.1 ± 1.4	28.5 ± 2.3
D+IT	8	223.5 ± 9.5	275.1 ± 11.6	+22.8	12.9 ± 1.3	27.3 ± 2.1

Note. Values are presented as mean ± SD where applicable. Abbreviations: HC, healthy control; H+CT, healthy + continuous training; H+IT, healthy + interval training; DC, diabetic control; D+CT, diabetic + continuous training; D+IT, diabetic + interval training. Data were translated and reformatted from the submitted manuscript.

3.2. Mitochondrial Respiratory Indices

Diabetes was associated with lower basal respiration, ATP-linked respiration, maximal respiration, spare respiratory capacity, and coupling efficiency, together with higher proton leak. Both exercise interventions improved respiratory indices in diabetic rats. The interval training group showed larger increases in maximal respiration and spare respiratory capacity than the continuous training group.

Table 2

Cellular respiration indices and mitochondrial respiratory function in the study groups

Group	Basal respiration	ATP-linked respiration	Maximal respiration	Spare respiratory capacity	Proton leak	Coupling efficiency (%)
HC	132.6 ± 9.7	118.4 ± 8.9	189.5 ± 12.3	58.7 ± 4.6	12.4 ± 1.2	84.6 ± 4.1
H+CT	141.2 ± 10.1	126.3 ± 9.1	203.1 ± 13.4	64.8 ± 5.1	11.9 ± 1.1	86.2 ± 4.3
H+IT	154.6 ± 11.4	139.8 ± 10.2	228.7 ± 15.2	78.3 ± 5.8	10.8 ± 1.0	89.4 ± 4.7
DC	81.2 ± 7.4	67.5 ± 6.3	108.4 ± 9.2	26.5 ± 3.1	24.7 ± 2.0	61.8 ± 3.5
D+CT	109.3 ± 8.5	96.8 ± 7.4	154.7 ± 11.6	43.6 ± 4.2	18.1 ± 1.5	74.9 ± 3.9
D+IT	127.5 ± 9.4	115.4 ± 8.3	183.9 ± 12.7	57.9 ± 4.8	14.2 ± 1.3	81.7 ± 4.2

Note. Values are presented as mean ± SD. Group abbreviations are defined in Table 1. OCR units and normalization method were not specified in the source manuscript and should be completed before submission.

Figure 1

Mitochondrial respiratory function across study groups. HC, healthy control; H+CT, healthy + continuous training; H+IT, healthy + interval training; DC, diabetic control; D+CT, diabetic + continuous training; D+IT, diabetic + interval training.

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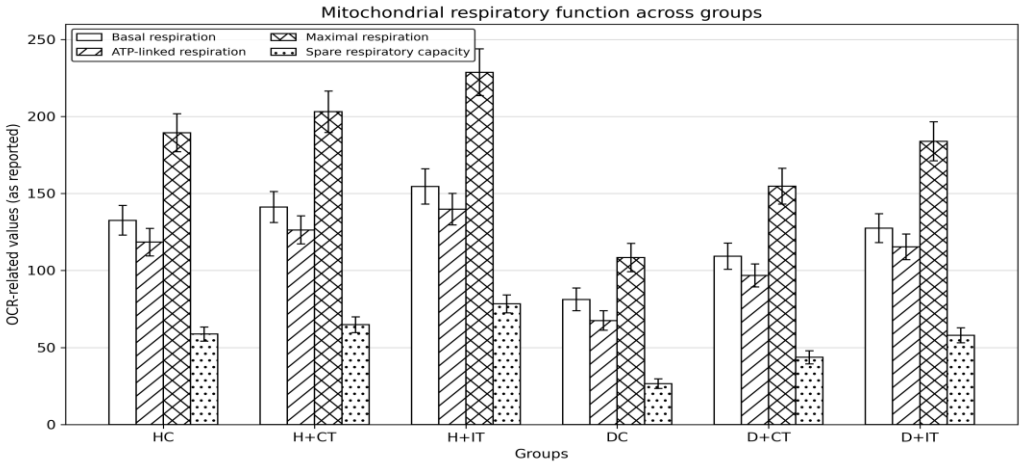

3.3. *Skeletal Muscle ATP Content*

Diabetes reduced skeletal muscle ATP content compared with the healthy control condition. Both continuous and interval training increased ATP content in diabetic rats, with the largest improvement observed after interval training.

Table 3

Skeletal muscle ATP content across study groups

Group	ATP content	% change vs. healthy control	% change vs. diabetic control
HC	8.74 ± 0.61	0	+22.8
H+CT	9.36 ± 0.66	+7	+31.5
H+IT	10.14 ± 0.73	+16	+42.4
DC	7.12 ± 0.54	-18	0
D+CT	8.06 ± 0.58	-7	+13
D+IT	8.86 ± 0.63	+2	+24

Note. Values are presented as mean ± SD. Group abbreviations are defined in Table 1. ATP assay units were not specified in the source manuscript; the analytical unit should be inserted from the assay kit/laboratory record before submission.

Figure 2

ATP content and coupling efficiency across study groups. Group abbreviations are defined in Figure 1.

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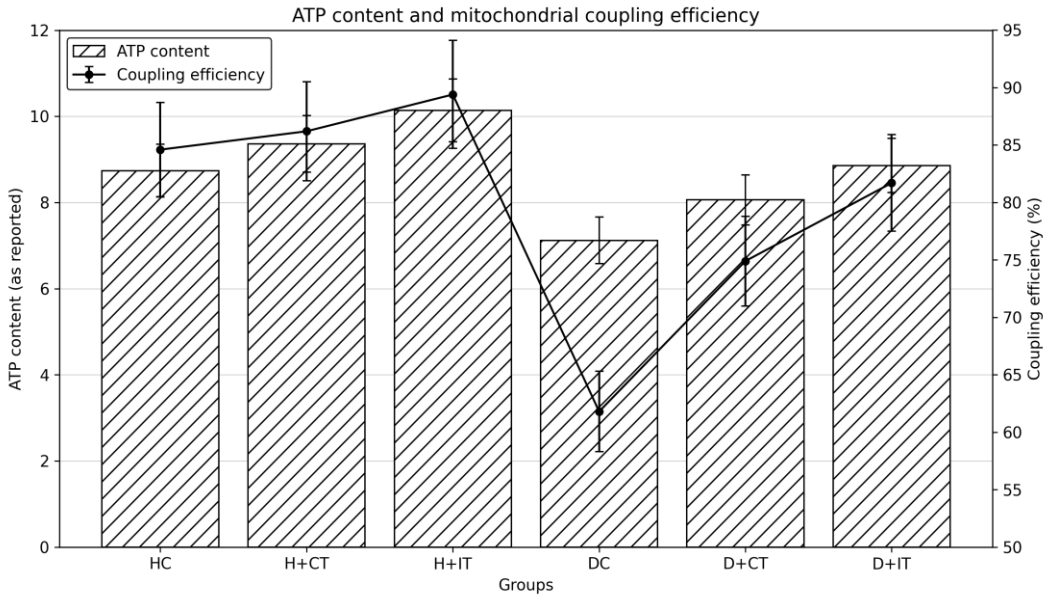

3.4. Post Hoc Comparisons

Tukey post hoc comparisons indicated that diabetes reduced OCR-related indices and ATP content, whereas both exercise protocols improved these variables. The magnitude of improvement was larger in the diabetic + interval training group than in the diabetic + continuous training group.

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Table 4

Tukey post hoc comparisons for selected OCR and ATP outcomes

Outcome	Comparison	Mean difference	95% CI	p value	% change
OCR index	Healthy control vs. diabetic control	35.6	21.8 to 49.4	0.001	+38
OCR index	Diabetic control vs. diabetic + continuous	22.5	11.4 to 31.7	0.004	+34
OCR index	Diabetic control vs. diabetic + interval	43.2	28.6 to 57.9	0.001	+57
OCR index	Diabetic + continuous vs. diabetic + interval	13.8	4.7 to 21.5	0.019	+18
ATP content	Healthy control vs. diabetic control	1.37	0.66 to 2.11	0.008	-18
ATP content	Diabetic control vs. diabetic + continuous	0.94	0.21 to 1.67	0.021	+13
ATP content	Diabetic control vs. diabetic + interval	1.74	0.72 to 2.41	0.006	+24
ATP content	Diabetic + continuous vs. diabetic + interval	0.80	0.08 to 1.22	0.037	+9

Note. The ambiguous OCR/ATP or ATP/OCR ratio column from the source manuscript was not retained because the numerator, denominator, and unit normalization were not clearly defined.

4. Discussion

The present study compared the effects of continuous and interval aerobic training on mitochondrial respiratory function and ATP content in skeletal muscle of rats with type 2 diabetes. The main finding was that diabetes impaired multiple indices of mitochondrial respiration, including maximal respiration, spare respiratory capacity, and coupling efficiency. These changes were accompanied by lower ATP content, suggesting reduced oxidative energy-generating capacity in diabetic skeletal muscle. Both exercise protocols improved these outcomes, but the interval training protocol produced the larger improvement in respiratory capacity.

The reduction in OCR-related indices in the diabetic control group is consistent with the concept that diabetes can disrupt mitochondrial electron transport, reduce oxidative phosphorylation efficiency, and lower the ability of skeletal muscle to generate ATP. Lower spare respiratory capacity is particularly relevant because it reflects a reduced reserve to respond to increased energetic demand. In skeletal muscle, this limitation may contribute to fatigue, reduced exercise tolerance, and impaired metabolic flexibility.

Aerobic training may improve mitochondrial function through repeated energetic stress, activation of energy-sensing pathways, and enhanced oxidative enzyme activity. Interval training may impose a stronger mitochondrial stimulus because the repeated transitions between high-intensity work and recovery create large fluctuations in substrate demand, oxygen use, and redox state. In the present study, this pattern was reflected in greater improvement in maximal respiration and spare respiratory capacity in the diabetic + interval training group. Continuous training also improved mitochondrial function, but the response appeared smaller and more gradual.

The ATP response was directionally consistent with the OCR findings, but the magnitude of ATP change appeared smaller than the change in respiratory indices. This is physiologically plausible because improved respiratory capacity does not necessarily translate linearly into tissue ATP accumulation. ATP content reflects a balance between production, utilization, turnover, and sampling conditions. Therefore, the combined assessment of OCR-related variables and ATP content provides a more informative picture of mitochondrial adaptation than either outcome alone.

A key methodological issue is that the original manuscript did not specify the exact OCR measurement platform, tissue preparation method, normalization strategy, or ATP assay unit. These details are essential for reproducibility, especially because functional respiration measurements depend strongly on whether the analysis used fresh fibers, permeabilized fibers, isolated mitochondria, cells, or homogenized tissue. These details should be completed before journal submission.

4.1. Limitations

This study used an animal model, and direct clinical recommendations for humans should therefore be made cautiously. Although the high-fat diet plus STZ protocol is commonly used to model type 2 diabetes-like metabolic dysfunction, additional confirmation using insulin concentration, HOMA-IR, or glucose tolerance testing

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would strengthen the characterization of the model. The study was limited to OCR-related indices and ATP content and did not measure complementary molecular pathways such as AMPK, PGC-1α, mitochondrial fusion/fission markers, oxidative stress indices, or inflammatory mediators. Finally, details of the OCR platform, ATP assay, and unit normalization require completion for full reproducibility.

5. Conclusion

Eight weeks of aerobic training improved diabetes-related impairments in skeletal muscle mitochondrial respiration and ATP content in male Wistar rats. Interval aerobic training produced stronger improvements in maximal respiration, spare respiratory capacity, and ATP content than continuous aerobic training. These findings support the importance of exercise intensity structure in mitochondrial adaptation under diabetic conditions, while also emphasizing the need for complete methodological reporting before publication.

Authors' Contributions

Elham Fanaei contributed to investigation, data collection, and manuscript preparation. Elham Eftekhari Gheinani contributed to supervision, conceptualization, methodology, and critical revision. Jamshid Banaei Borojeni contributed to methodology, scientific supervision, and critical revision.

Declaration

Language editing and formatting assistance was used to improve clarity. The scientific content, data, and final responsibility remain with the authors.

Transparency Statement

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

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Declaration of Interest

The authors report no conflict of interest.

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Ethics Considerations

The study was approved by the Ethics Committee of Islamic Azad University, Najafabad Branch (IR.IAU.NAJAFABAD.REC.1405.086). Animal handling, housing, anesthesia, and tissue collection procedures were conducted according to institutional animal welfare principles and the general principles of humane animal research. Reporting was aligned with the ARRIVE 2.0 recommendations where information was available (11, 12).

References

1. International Diabetes F. IDF Diabetes Atlas. 11th ed: International Diabetes Federation; 2025.
2. World Health O. Diabetes. 2024.
3. Fealy CE, Mulya A, Axelrod CL, Kirwan JP. Mitochondrial Dynamics In Skeletal Muscle Insulin Resistance And Type 2 Diabetes. Translational Research. 2018;202:69-82. [PMID: 30153426] [DOI]
4. Liesa M, Shirihai OS. Mitochondrial Dynamics In The Regulation Of Nutrient Utilization And Energy Expenditure. Cell Metabolism. 2013;17(4):491-506. [PMID: 23562075] [PMCID: PMC5967396] [DOI]
5. Pinti MV, Fink GK, Hathaway QA, Durr AJ, Kunovac A, Hollander JM. Mitochondrial Dysfunction In Type 2 Diabetes Mellitus: An Organ-Based Analysis. American Journal of Physiology-Endocrinology and Metabolism. 2019;316(2):E268-E85. [PMID: 30601700] [PMCID: PMC6397358] [DOI]
6. Colberg SR, Sigal RJ, Yardley JE, Riddell MC, Dunstan DW, Dempsey PC, et al. Physical Activity/Exercise And Diabetes: A Position Statement Of The American Diabetes Association. Diabetes Care. 2016;39(11):2065-79. [PMID: 27926890] [PMCID: PMC6908414] [DOI]
7. Little JP, Gillen JB, Percival ME, Safdar A, Tarnopolsky MA, Punthakee Z, et al. Low-Volume High-Intensity Interval Training Reduces Hyperglycemia And Increases Muscle Mitochondrial Capacity In Patients With Type 2 Diabetes. Journal of Applied Physiology. 2011;111(6):1554-60. [PMID: 21868679] [DOI]
8. Wang N, Liu Y, Ma Y, Wen D. High-Intensity Interval Versus Moderate-Intensity Continuous Training: Superior Metabolic Benefits In Diet-Induced Obesity Mice. Life Sciences. 2017;191:122-31. [PMID: 28843495] [DOI]
9. Zheng L, Rao Z, Guo Y, Chen P, Xiao W. High-Intensity Interval Training Restores Glycolipid Metabolism And Mitochondrial Function In Skeletal Muscle Of Mice With Type 2

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E-ISSN: 2981-2569

Diabetes. Frontiers in Endocrinology. 2020;11:561. [PMID: 32922365] [PMCID: PMC7456954] [DOI]
10. Chavanelle V, Boisseau N, Otero YF, Combaret L, Dardevet D, Montaurier C, et al. Effects Of High-Intensity Interval Training And Moderate-Intensity Continuous Training On Glycaemic Control And Skeletal Muscle Mitochondrial Function In Db/Db Mice. Scientific Reports. 2017;7:204. [PMID: 28303003] [PMCID: PMC5427962] [DOI]
11. National Research C. Guide for the care and use of laboratory animals. 8th ed: National Academies Press; 2011. [DOI]
12. Percie Du Sert N, Hurst V, Ahluwalia A, Alam S, Avey MT, Baker M, et al. The Arrive Guidelines 2.0: Updated Guidelines For Reporting Animal Research. PLOS Biology. 2020;18(7):e3000410. [PMID: 32663219] [PMCID: PMC7360023] [DOI]

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