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The Effect of Exercise Training Timing on *SREBP* Gene Expression in Adipose Tissue of Diabetic Mice

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Abstract

Objective: Sterol regulatory element-binding protein-1c (*SREBP-1c*) regulates lipid metabolism, and its disruption in diabetes leads to lipid accumulation, insulin resistance, and inflammation. BMAL1, a core circadian clock gene, modulates physiological rhythms. This study aimed to examine the effect of aerobic exercise timing on BMAL1 and SREBP-1c expression in adipose tissue of diabetic mice.

Materials and Methods: Thirty male mice were randomly assigned to two temporal groups: ZT3 (light phase) and ZT15 (dark phase). Within each time point, animals were divided into three subgroups: Healthy Control (HC), Diabetic Control (DC), and Diabetic + Exercise Training (TD). Diabetes was induced using a high-fat diet and streptozotocin injections. After confirmation, the TD groups performed treadmill running for eight weeks (60–80 min/day, 50–60% Vmax, 5 days/week) at their respective time points. Gene expression of BMAL1 and SREBP-1c was analyzed using Real-time PCR, and data were statistically evaluated using SPSS.

Results: Exercise during the dark phase significantly increased BMAL1 ($P< 0.0001$) and SREBP1 ($P< 0.05$) levels in the diabetic group compared to the diabetic control group. No significant differences were observed in BMAL1 or SREBP1 levels between the diabetic control group and the diabetic group with exercise during the light phase.

Conclusion: The results show that by upregulating BMAL1 and *SREBP-1c* in adipose tissue, evening exercise improves metabolic status in diabetic mice. These findings lay the groundwork for further translational research in humans and point to possible uses in developing time-specific exercise interventions for the management of diabetes.

Keywords: Diabetes mellitus, Exercise training, *SREBP-1c*, *BMAL2* Protein, Adipose tissue

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Introduction

Type 2 diabetes (T2D) is a prevalent chronic metabolic disease that has raised significant public health concerns due to its increasing prevalence in recent decades. Obesity a major risk factor, for T2D, is closely linked to insulin resistance and the development of the disease. Effective therapeutic interventions are needed to manage obesity and prevent T2D by enhancing insulin sensitivity through mechanisms such as, the regulation of metabolism by key transcription factors like Sterol regulatory element-binding proteins (*SREBPs*). *SREBP-1c*, a crucial regulator of carbohydrate and fat metabolism plays a key role in mediating insulin's effects on target genes (1). Strategies such as physical activity and calorie restriction have been shown to reduce the risk of T2D. Circadian rhythms, which regulate vital physiological processes, including metabolism. Specific genes in tissues such as the liver and adipose tissue control these rhythms. While primarily synchronized by light, factors such as temperature, diet, and exercise also influence them. Disruptions of circadian rhythms can impair lipid metabolism by altering the rhythmic regulation of proteins involved in lipid transport, synthesis, and degradation (2).

The timing of exercise training significantly modulates gene expression in adipose tissue, particularly in animal models of diabetes (3). Circadian clocks are present in nearly all mammalian cells and coordinate gene expression and biological rhythms, thereby influencing cellular metabolism. Mutations in core clock genes such as *CLOCK* and *BMAL1* can lead to obesity, impaired glucose metabolism, and decreased body temperature (4). Peripheral clocks in organs like the liver, pancreas, and adipose tissue are crucial for regulating energy and glucose metabolism. For example, *BMAL1* plays a role in insulin secretion and adipogenesis, the formation of new fat cells (5). These findings emphasize the critical role of circadian clocks in maintaining metabolic health and energy balance and indicate how their disruption may contribute to chronic diseases.

This study aimed to investigate how the timing of exercise affects the expression of *BMAL1* and *SREBP-1c* in the adipose tissue of diabetic mice. Understanding how exercise modulates these molecular pathways could help inform time-specific interventions and guide future therapeutic strategies for T2D.

Material and methods

This experimental study investigated the effects of exercise timing on the expression of *SREBP-1c* and *BMAL1* genes in the adipose tissue of diabetic mice. Thirty adult male NMRI mice were randomly assigned to six groups (n= 5 per group), based on their initial body weight to ensure uniformity: Healthy Control Light Phase (CH-ZT3), Healthy Control Dark Phase (CH-ZT15), Diabetic Control Light Phase (CD-ZT3), Diabetic Control Dark Phase (CD-ZT15), Diabetic Exercise Training Light Phase (TD-ZT3), and Diabetic Exercise Training Dark Phase (TD-ZT15). The sample size (n= 5) was selected based on previous studies using similar experimental models to ensure adequate statistical power (6,7). Diabetes was induced in the four diabetic groups through a high-fat diet for five weeks followed by a low-dose streptozotocin injection (20 mg/kg). Mice with fasting blood glucose >126 mg/dL were considered diabetic.

Determination of maximal speed (Vmax)

Aerobic capacity was evaluated using a maximal speed test on a treadmill during the fourth and eighth weeks of the training period, following a one-week acclimation period. Exercise intensity was adjusted according to the initial Vmax and the Vmax measured at week four. After a five-minute warm-up at 6 m/min, treadmill speed was incrementally increased by 2 m/min every two minutes until the mice reached exhaustion. The highest speed achieved was recorded as Vmax (8,9).

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All tests were performed at consistent times for each group, and environmental conditions including temperature and light exposure were carefully controlled to minimize variability.

Moderate-Intensity aerobic exercise training

The TD groups underwent aerobic training for 60-80 minutes at 50–60% of Vmax, conducted during the early light phase (ZT3, 9:00 AM) and early dark phase (ZT15, 9:00 PM) over eight weeks. Body weight and fasting blood glucose were measured weekly throughout the training period (8).

Tissue sampling and preparation

Real-time PCR was employed to assess the expression of *SREBP-1c* and *BMAL1* genes in visceral adipose tissue. A list of primers used in this study, including their sequences, amplicon lengths, and GenBank accession numbers, is presented in Table 1. Primer design was performed using Primer3 software. Total RNA was extracted from visceral adipose tissues using a Total RNA Extraction Kit (Pars Tous, Iran) and reverse-transcribed into cDNA. Quantitative real-time PCR (qRT-PCR) was performed using a SYBR Green Real-Time PCR Kit (Pars Tous, Iran) and a Lava96 Real-Time PCR Detection System (DaAnGene Co. Ltd.). The reaction mixture consisted of 25.6 µL of SYBR Green Master Mix, 2.5 µL of each primer (10 µM), 3 µL of cDNA (200 ng), and 7.52 µL of DNase-free water, for a final volume of 38.6 µL. The thermal cycling protocol included an initial denaturation at 94 °C for 5 min, followed by 50 cycles of denaturation at 94 °C for 15 s, annealing at 60 °C for 15 s, and extension at 72 °C for 30 s. Gene expression levels were quantified using the comparative ΔΔCt method with *GAPDH* as the internal control. Amplification efficiency for both target and reference genes were confirmed by generating standard curves and calculating the slope and efficiency. Results were reported based on the $2^{-\Delta\Delta Ct}$ formula (10).

Statistical analysis

Statistical analysis for blood factors and visceral adipose tissue gene expression was performed using GraphPad Prism 9.0.0 and SPSS-23 software. Data normality was assessed using the Shapiro-Wilk test, and homogeneity of variance was evaluated using Leven's test. One-way ANOVA was employed to compare means between groups, followed by Bonferroni post-hoc tests. Data are presented as mean± SD, with significance set at $P< 0.05$.

Ethical considerations

The study was approved by the Ethics and Research Committee of Shahid Chamran University of Ahvaz, Ahvaz, Iran (Code:EE/1401.2.24.173079/scu.ac.ir).

Results

SREBP-1c (F = 26.24, *P*= 0.0001), *BMAL1* (F= 33.83, *P*= 0.0001), maximum running speed (F= 40.95, *P*< 0.0001), glucose levels (F= 22.22, *P*< 0.0001), and body weight (F= 5.48, *P*= 0.008) all showed significant differences between groups, according to analysis of variance. Diabetic mice had significantly higher body weight (*P*= 0.0075) and glucose (*P*= 0.0064) than the healthy control group, according to post-hoc analysis (Table 2). When compared to the diabetic control group, aerobic exercise training significantly decreased body weight (*P*= 0.007) and glucose (*P*= 0.0031) in the exercise

Table 1. Primer sequences used for qRT-PCR analysis of *SREBP-1c*, *BMAL1*, and *GAPDH* in adipose tissue of diabetic mice.

Accession No.	Gene symbol	Forward	Reverse	product length(bp)
NM_011480	*SREBP-1C*	5'-CGACTACATCCGCTTCTTGCAG-3'	5'-CCTCCATAGACACATCTGTGCC-3'	143
NM_007489	*BMAL1*-mice	5'-ACCTCGCAGAATGTCACAGGCA-3'	5'-CTGAACCATCGACTTCGTAGCG-3'	115
NM_008084	*GAPDH*	5'-CATCACTGCCACCCAGAAGACTG-3'	5-ATGCCAGTGAGCTTCCCGTTCAG-3'	

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training groups.

Nevertheless, no group's glucose levels showed any discernible variations between the light and dark phases (*P*= 0.4140).

The exercise training groups showed a significant increase in maximum running speed when compared to the other groups (*P*= 0.0087), but there were no significant differences between the light and dark phases (*P*= 0.9996) (Table 2).

The healthy control group (CH-ZT3) had significantly higher levels of *BMAL1* protein than any other group (*P*< 0.0001). At ZT3 and ZT15, *BMAL1* levels were significantly lower in the diabetic control group (CD) than in the healthy control group (*P*< 0.0001). The diabetic control group and the diabetic group with light-phase exercise training (TD-ZT3) did not differ significantly (*P*> 0.05), but the diabetic group with dark-phase exercise training (TD-ZT15) had significantly higher *BMAL1* levels than the diabetic control group (*P*< 0.0001). Additionally, in all groups, *BMAL1* levels were significantly higher during the light phase (ZT3) than during the dark

Table 2. Comparison of mean values of assessed variables post-intervention across different groups.

Groups	**Weight** (g)	**Glucose**(mg/dL)	**Maximum speed** (m/min)
CH-ZT3	33.00±3.60^{b}	101.18±4.10^{e}	15.16±1.04^{b}
CH-ZT15	34.00±2.64^{b}	116.40±4.22^{d}	15.50±1.32^{b}
CD-ZT3	33.41±1.52^{a}	143.58±5.09^{b}	14.83±1.60^{b}
CD-ZT15	40.66±3.05^{a}	157.33±5.47^{a}	15.60±0.91^{b}
TD-ZT3	34.33±1.52^{b}	143.20±5.33^{b}	24.66±1.15^{a}
TD-ZT15	36.33±1.52^{b}	127.40±3.36^{c}	24.80±1.70^{a}

Significant differences between the groups were found in the Bonferroni post-hoc test Different Latin letters above each column indicate that the mean value of that column is significantly different from the means of columns labeled with different letters, while columns sharing the same letters show no significant difference between their means.

CH: Control Healthy group, CD: Control Diabetic group, TD: Training Diabetic group, ZT3: Light phase, ZT15: Dark phase.

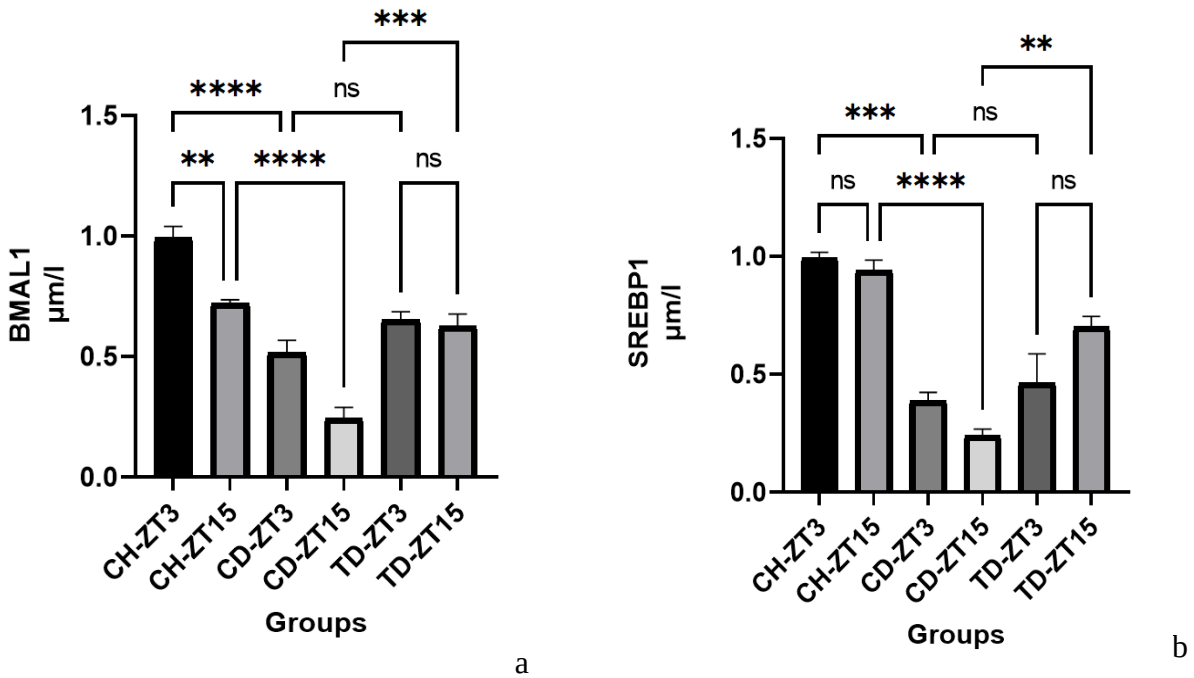


Figure 1. Gene expression levels of *BMAL1* and *SREBP-1c* in different groups. Data are presented as mean ± standard deviation. Pairwise comparisons between bars indicate significant differences at the *P*< 0.05 level. CH-ZT3: Healthy control group tested during the light phase. CH-ZT15: Healthy control group tested during the dark phase. CD-ZT3: Diabetic control group tested during the light phase. CD-ZT15: Diabetic control group tested during the dark phase. TD-ZT3: Diabetic group with exercise training tested during the light phase. TD-ZT15: Diabetic group with exercise training tested during the dark phase

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phase (ZT15) ($P< 0.0001$) (Figure 1a).

The diabetic control group (CD-ZT3, CD-ZT15) had significantly lower levels of *SREBP-1c* protein than the healthy control group (CH-ZT3, CH-ZT15) ($p < 0.001$). While light-phase exercise training (TD-ZT3) did not result in a significant difference when compared to the diabetic control group ($P> 0.05$), dark-phase exercise training (TD-ZT15) significantly raised *SREBP-1c* levels in the diabetic group when compared to the diabetic control group ($P< 0.05$). Within any group, there were no discernible variations in *SREBP-1c* levels between the light phase (ZT3) and the dark phase (ZT15) ($P> 0.05$) (Figure 1b).

Conclusion

Aerobic exercise conducted during the dark phase enhanced metabolic health and upregulated the expression of *SREBP-1c* and *BMAL1* genes in the adipose tissue of diabetic mice. However, it is important to exercise caution when applying to humans should be approached with caution. Large-scale human studies using diverse exercise protocols are needed to clarify the underlying mechanisms and evaluate clinical efficacy. Moreover, investigating the interaction among genetic, environmental, and lifestyle factors is essential for designing personalized exercise interventions in diabetes management. This study highlights the importance of exercise timing in regulating key metabolic genes and provides a foundation for developing innovative therapeutic strategies.

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Conflict of Interest

None

Authors' contributions

M.J: Conceived and designed the study; collected and curated the data; contributed data and analysis tools; performed the analysis; developed the methodology; validated the results; and contributed to writing-original draft.

M.H: Supervised the study; contributed analysis tools; provided project administration; and participated in writing-review and editing, as well as data interpretation.

Z.H.F**:** Contributed data and analysis tools; performed statistical analyses; participated in writing-original draft, review, and editing; and provided methodological supervision.

M.Sh: Conceived and designed the analysis; collected data; validated experimental procedures; and contributed to the study methodology.

All authors critically revised the manuscript for important intellectual content, agree to be accountable for all aspects of the work, and have read and approved the final version of the manuscript.

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