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RAPID COMMUNICATION

# miR-378a-3p responds to physical activity by modulating insulin-mediated glucose uptake and tricarboxylic acid cycle in skeletal muscle

Aerobic exercise training alters gene expression in skeletal muscle, and miRNAs significantly contribute to motion response. However, the molecular mechanisms by which miRNAs regulate the response to exercise training are not well understood. Here, we found that the abundance of miR-378a-3p in skeletal muscle significantly decreased after exercise training. miR-378a-3p knockout mice showed increased insulin sensitivity, insulin-mediated glucose uptake, and an oxidative phenotype, recapitulating the role of miR-378a-3p in exercise-induced adaptations of skeletal muscle. Mechanistically, miR-378a-3p modulates insulin-mediated glucose uptake through the IGF1/IGF1R-AKT-GLUT4 axis by targeting *Igf1r* and *Igf1*. Additionally, loss of miR-378a-3p promotes the tricarboxylic acid (TCA) cycle by promoting the process of oxaloacetic acid synthesis by targeting *Car5b*. Moreover, deletion of miR-378a-3p alleviates obesity and improves glucose metabolism in mice fed a high-fat diet, suggesting its potential as a therapeutic target for metabolic diseases. Overall, our findings highlight the crucial role of miR-378a-3p in regulating insulin-mediated glucose uptake and TCA cycling in skeletal muscle, offering promising therapeutic implications for metabolic diseases.

In this study, we compiled a set of 60 exercise training-responsive miRNAs from previous research (Table S1) and assessed their expression levels and tissue specificity using the TissueAtlas database (Fig. S1A). Among these miRNAs, hsa-miR-378a-3p, hsa-miR-206, and hsa-miR-451a were identified as muscle-specific miRNAs (Fig. 1A) which are conserved across species (Fig. S1B). Following exercise, miR-378a-3p and miR-206 showed decreased expression, while miR-451 exhibited increased expression in mouse tibialis anterior (TA) muscles (Fig. S1C). miR-378a-3p and miR-206 were predominantly expressed in skeletal muscle, whereas miR-451 was primarily found in the stomach (Fig. S1D). Notably, miR-378a-3p displayed the highest expression among the three miRNAs in skeletal muscles with varying exercise capacities (Fig. S1E), leading us to focus on it for subsequent experiments.

To investigate the impact of miR-378a-3p on the response to exercise training, we utilized Cre-Loxp technology to generate miR-378a-3p knockout (KO) mice (Fig. S2). Despite knocking out miR-378a-5p, we observed no changes in its expression levels in the TA, quadriceps (QUA), soleus (SOL), gastrocnemius (GS), and extensor longus digitorum (EDL) muscles after physical training (Fig. S3A). In contrast, miR-378a-3p expression was significantly down-regulated (Fig. 1B). Notably, miR-378a-3p was found to be dispensable for normal skeletal muscle development (Fig. S3B, C). Interestingly, the loss of miR-378a-3p in mice replicated the effects induced by exercise training in skeletal muscle. This included an increase in slow-twitch oxidative muscle composition (Fig. 1C; Fig. S3D–F) and the promotion of an oxidative phenotype within the skeletal muscle (Fig. 1D). As anticipated, we also observed elevated mRNA levels of PGC1-$\alpha$, PLIN5, and PDH, which are key genes involved in oxidative metabolism, along with an increased abundance of NADPH and citric acid in the TA muscles of miR-378a-3p KO mice (Fig. S3G, H) and C2C12 myotubes transfected with miR-378a-3p inhibitors (Fig. S4).

To elucidate the underlying biological mechanism of miR-378a-3p's response to exercise training, we performed proteomic profiling analysis and identified metabolic pathway-enriched proteins regulated by miR-378a-3p (Table S2, 3 and Fig. S5A). The glucose tolerance test (GTT) and insulin tolerance test (ITT) showed that KO mice exhibited improved glucose tolerance compared with their

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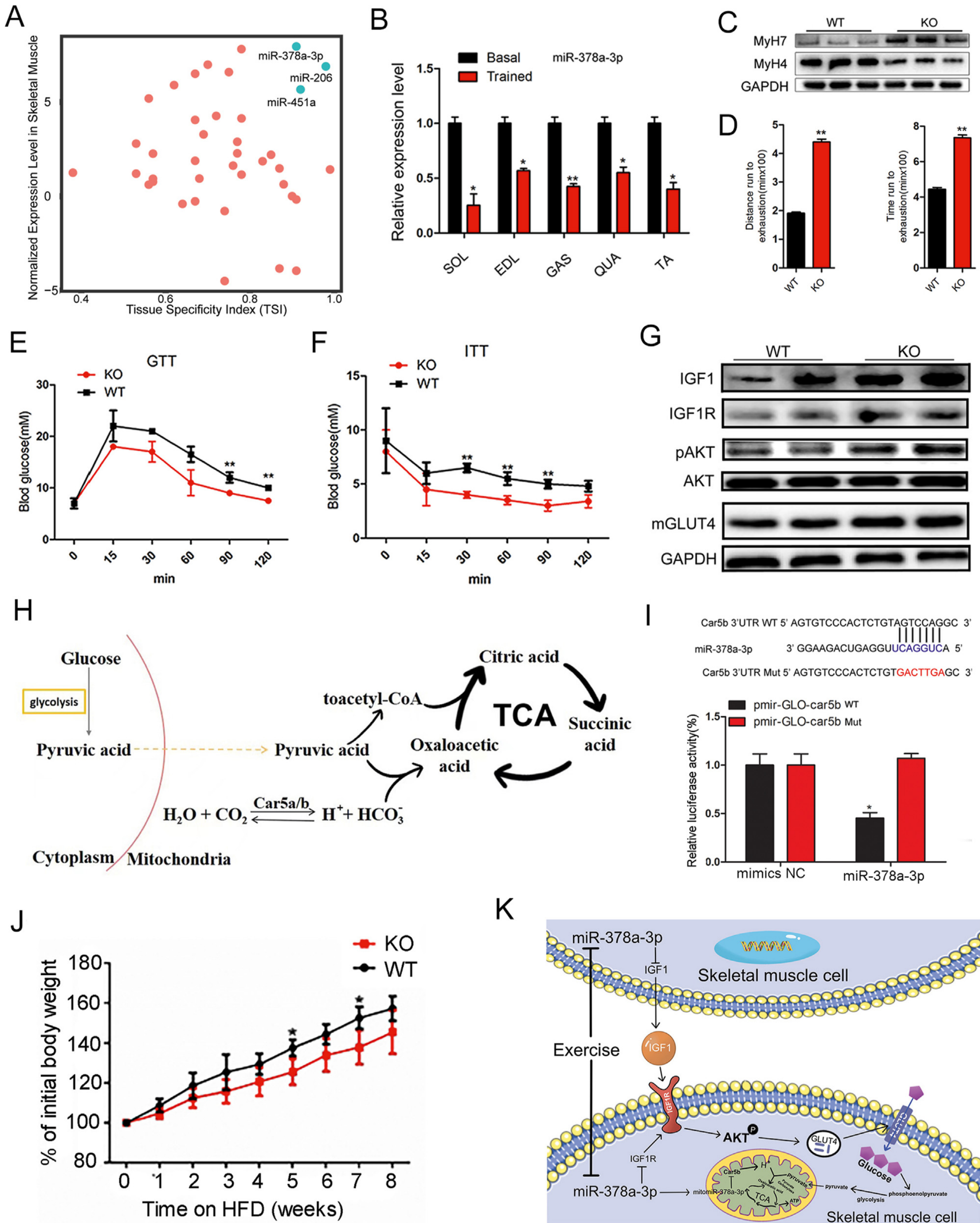


**Figure 1** Physical exercise-responsive miR-378a-3p regulates insulin-mediated glucose uptake by controlling the activity of the IGF1/IGF1R-AKT-GLUT4 axis via targeting *Igf1*/*Igf1r*, while also participating in the TCA cycle by modulating oxaloacetic acid synthesis via targeting *Car5b* gene in skeletal muscle. **(A)** The scatter plot showing the tissue specificity index (*x*-axis) and VSN expression level in skeletal muscle tissue (*y*-axis) of exercise-training responsive miRNAs. **(B)** qRT-PCR analysis of relative miR-378a-3p in muscles (soleus (SOL), extensor digitorum longus (EDL), gastrocnemius (GAS), quadriceps (QUA), and tibialis anterior (TA)) from untrained (basal) and endurance exercise-trained (trained) mice ($n = 6$). **(C)** Western blot analysis of MHC isoforms in the GAS

WT counterparts (Fig. 1E, F). Additionally, we observed that the absence of miR-378a-3p in KO mice or its down-regulation in C2C12 myotubes increased insulin-stimulated glucose uptake (Fig. S5B, C), suggesting a role for miR-378a-3p in insulin-mediated glucose uptake and insulin sensitivity in skeletal muscle. Additionally, palmitate treatment which induces insulin resistance in cells, up-regulated the expression of miR-378a-3p (Fig. S5D), suggesting a link between miR-378a-3p and insulin resistance.

The IGF1/IGF1R signaling pathway reportedly regulates the activation of the PI3K-AKT/PKB cascades by stimulating the intrinsic tyrosine kinase activity[1]; and phosphorylation of AKT (pAKT) allows the translocation of GLUT4 to the sarcolemma, thus facilitating glucose entry into the cell.[2] Our results showed that the protein level of IGF1, IGF1R, pAKT, and mGLUT4 increased significantly in the TA muscles of KO mice compared with the WT mice (Fig. 1G). *In vitro* results showed that miR-378a-3p modulates insulin-mediated glucose uptake through the IGF1/IGF1R-AKT-GLUT4 axis in C2C12 myotube (Fig. S6A–D). Mechanistically, *Igf1* and *Igf1r* were identified as the direct target genes of miR-378a-3p (Fig. S6E, F; Fig. S7–10).

A previous study identified miR-378a-3p as a mitomiRNA, capable of entering mitochondria and affecting the expression of mitochondrial mRNAs.[3] Additionally, the absence or inhibition of *Car5b*, an isozyme of carbonic anhydrase, has been shown to disrupt oxaloacetate synthesis and subsequently impair the function of the TCA cycle within mitochondria 4 (Fig. 1H).[4] In this study, we found that miR-378a-3p participates in the TCA cycle through the regulation of oxaloacetic acid synthesis by targeting *Car5b* gene (Fig. 1I; Fig. S11).

Since obesity and type 2 diabetes are strongly linked to insulin resistance and glucose utilization, we checked whether miR-378a could serve as a therapeutic target for metabolic diseases. Interestingly, increasing the dimethylbiguanide (metformin) concentration, an oral medicine for diabetes,[5] in the culture medium significantly decreased the expression level of miR-378a-3p (Fig. S12A). The KO mice were resistant to obesity induced by a high-fat diet (HFD) compared with the WT mice (Fig. 1J; Fig. S12B–E). Furthermore, the expression of miR-378a-3p increased significantly in skeletal muscles from ob/ob mice (Fig. S12F).

Collectively, our findings demonstrate that miR-378a-3p regulates glucose uptake in skeletal muscle by modulating the activity of the IGF1/IGF1R-AKT-GLUT4 axis via targeting the *Igf1*/*Igf1r* genes. Furthermore, miR-378a-3p also plays a role in the mitochondria by influencing the TCA cycle through its regulation of oxaloacetic acid synthesis via targeting the *Car5b* gene (Fig. 1K). Overall, our study enhances our understanding of the involvement and mechanisms of miRNAs in response to physical exercise. These findings provide valuable insights into the potential therapeutic targeting of miRNAs for enhancing exercise capacity, particularly in individuals with obesity.

## Ethics declaration

The study was approved by the ethics committee of the Institute of Animal Science of CAAS and performed according to the guidelines of the China Biological Studies Animal Care and Use Committee.

## Author contributions

Z.L.T. developed the study concept and experimental design. A.A.A. and K.L. provided key advice. Y.Y.L., Z.S.W., D.Y.F., Y.W.L., and C.J.H. conducted molecular, cellular, and animal experiments. Z.S.W. performed bioinformatic analysis. Z.S.W. and Y.Y.L. wrote the manuscript and the other co-authors provided comments and revisions.

## Conflict of interests

The authors declare that they have no conflict of interests.

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muscles of WT and KO mice ($n = 4$). GAPDH was used as the loading control. **(D)** Statistic of the running time and running distance of the WT and KO mice. Running time represented the duration from initiation to exhaustion on the treadmill after 6 h of fasting time ($n = 6$). **(E)** Glucose tolerance test (GTT) and **(F)** insulin tolerance test (ITT) in WT and KO mice ($n = 8$). **(G)** Western blot analysis of IGF1, IGF1R, AKT, phosphorylation of Akt (p-Akt, ser473), and GLUT4 protein expression in the TA and SOL muscles of WT and KO mice. GAPDH was used as the loading control. **(H)** The mechanism graph of the oxaloacetic acid synthesis. **(I)** Sequence alignment of miR-378a-3p and the *Car5b* 3′UTR/*Car5b* 3′UTR mutated (upper panel) and analysis of the effect of miR-378a-3p mimics on the activity of the reporter containing *Car5b* 3′UTR (pmir-GLO-Car5b-WT) or *Car5b* 3′UTR mutated at the miR-378a-3p binding sites (pmir-GLO-Car5b-WT) by luciferase assay in C2C12 myoblasts ($n = 4$). **(J)** Body weight of miR-378a KO and WT mice after 8 weeks of normal chow. **(K)** Schematic diagram of the working model of miR-378a-3p in skeletal muscle.

## Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.gendis.2023.06.021.

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