Short Communication

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Caveolin-3 regulates slow oxidative myofiber formation in female mice

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Abstract

Objectives: Caveolin-3 (Cav-3) plays a pivotal role in maintaining skeletal muscle mass and function. Mutations or deletions of Cav-3 can result in the development of various forms of myopathy, which affect the integrity and repair capacity of muscle fiber membranes. However, the potential effect of Cav-3 on myofiber type composition remains unclear.

Methods: To investigate the effect of Cav-3 on muscle strength and running capacity, we examined the grip force test and the low/high-speed running test. Oxidative and glycolytic myofiber-related genes, proteins, and skeletal muscle fiber composition were measured to determine the role of the Cav-3 in oxidative myofiber formation.

Results: We report the impact of Cav-3 on enhancing muscle endurance performance in female mice, and the discovery of a new physiological function to increase the proportion of slow-twitch oxidative muscle fiber by analyzing the gastrocnemius and soleus. Skeletal muscle-specific ablation of Cav-3 in female mice increased oxidative myofiber-related gene expression and type I oxidative myofiber composition, with resultant improvements in endurance performance. In male mice, the absence of Cav-3 in skeletal muscle reduced in the expression of glycolytic fiber-related genes and proteins.

Conclusions: This study identified Cav-3 as a regulator of slow-twitch oxidative muscle fiber formation acting on female mice, which may provide a potential target for improving muscle oxidative function.

Keywords: caveolin-3; oxidative myofiber; skeletal muscle; female mice

Introduction

Skeletal myofibers, depending on their metabolic and contractile properties, can be classified as slow-twitch (type I, oxidative) and fast-twitch (type II, glycolytic) [1]. Based on the variance of myosin heavy chain (MyHC), it can be classified into slow-twitch fibers constituted by MyHC-1 and fast-twitch fibers composed of MyHC-2A, MyHC-2B or MyHC-2X [2]. The slow-twitch oxidative muscle fiber is rich in mitochondria and exhibits aerobic metabolism as well as exceptional anti-fatigue capability [1], whereas the fast-twitch glycolytic muscle fiber exhibits poor oxidative capacity and is prone to fatigue [3, 4]. The caveolin protein gene family, which is made up of the proteins Cav-1, Cav-2, and Cav3, is engaged in several critical physiological functions, including signal transduction, vesicle transport, cholesterol homeostasis, and tumor suppression. Cav-3 is an integral membrane protein and its dysfunction is associated with various diseases, such as muscle atrophy, diabetes, and insulin resistance [5]. Cav-3 is also known to be associated with the polyprotein dystrophin glycoprotein complex (DGC), which plays an important role in maintaining muscle fiber integrity, and DGC dysfunction leads to muscular dystrophy, such as Limb-Girdle Muscular Dystrophy (LGMD-1C) [6, 7]. Previous studies reported that loss of Cav-3 in male mice results in a decrease in muscle fiber size and glucose utilization [8, 9]. However, the effect of Cav-3 on skeletal muscle fiber type composition remains unknown. To investigate this effect, we examined the body composition, exercise performance, and myofiber composition in a muscle-specific Cav-3 knockout in both sexes of a mouse model. The summary of this article is presented in Figure 1.

Materials and methods

Detailed description of the Materials and Methods is presented in the Supplementary Material.

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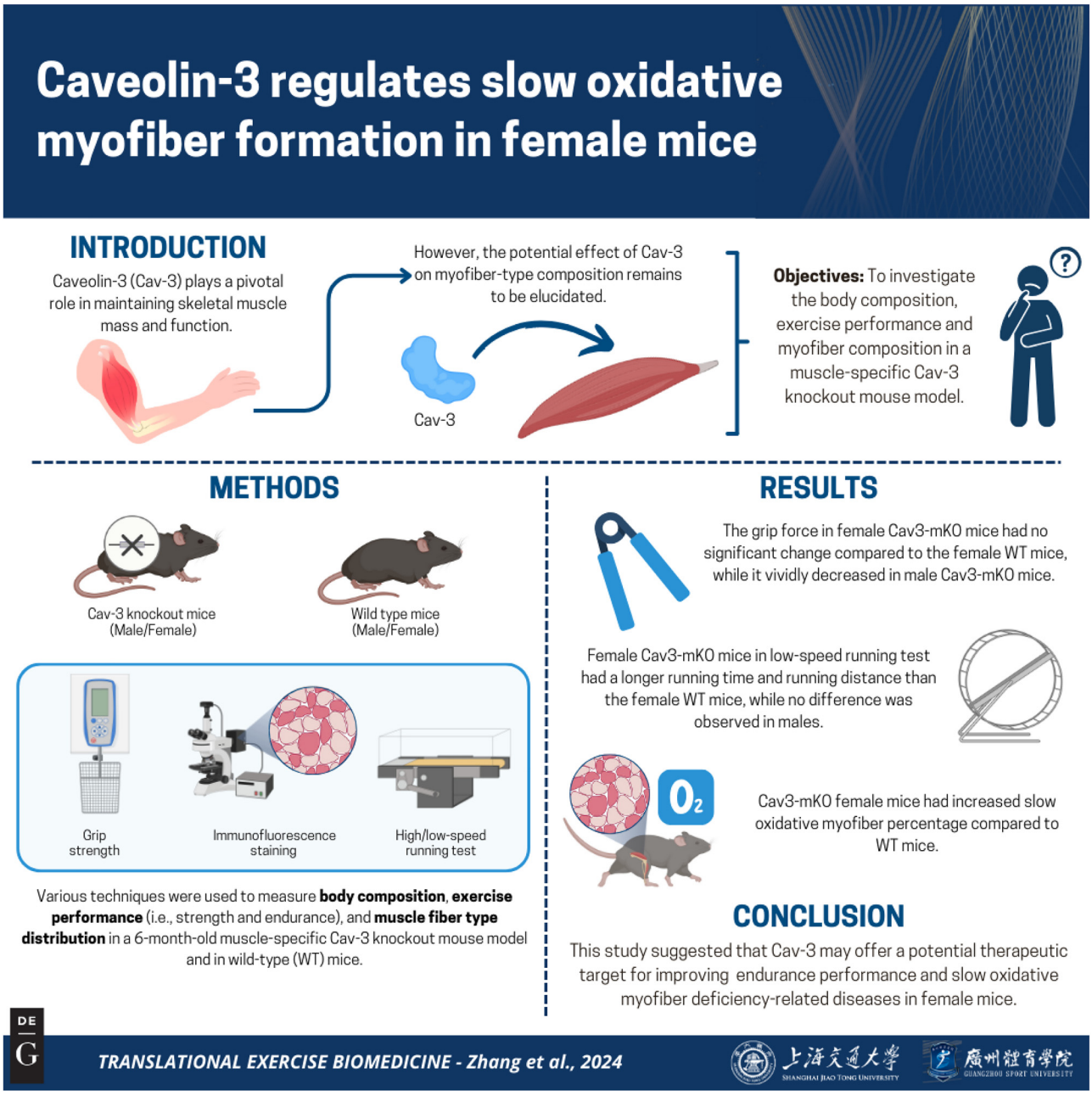

Figure 1: Graphical representation of the study. Figure created with biorender.

Animals

Caveolin-3$^{flox/flox}$ mice (wild-type, WT) with a C57BL/6J genetic background were obtained from Guangzhou Saiye Baimu Biotechnology Co., Ltd. The animals maintained until they reached six months of age for the purpose of conducting experimental analysis. To create mice with muscle conditional deletion of the Cav-3 allele, Cav-3$^{flox/flox}$ mice were crossed with mice carrying Cre recombinase to obtain muscle conditional knockout of the Cav-3 (Cav3-mKO). All procedures involving the use and care of laboratory animals are licensed by the Zhengzhou University Life Sciences Institutional Review Board (ZZUIRB2024-01).

Results

Cav-3 knockout improved exercise tolerance in female mice

To investigate the effect of Cav-3 on skeletal muscle function, we examined muscle strength and running capacity in WT and Cav3-mKO mice. To do this, we used non-invasive tests: the grip force test, which is widely used to evaluate muscle strength, and the low/high-speed running tests, which are also widely carried out to estimate running capacity. Body weight and composition analysis showed that the body weight and fat mass in male and female Cav3-mKO mice did not differ statistically from male and female WT mice (Figure 2A–C). Interestingly, lean mass was not altered in

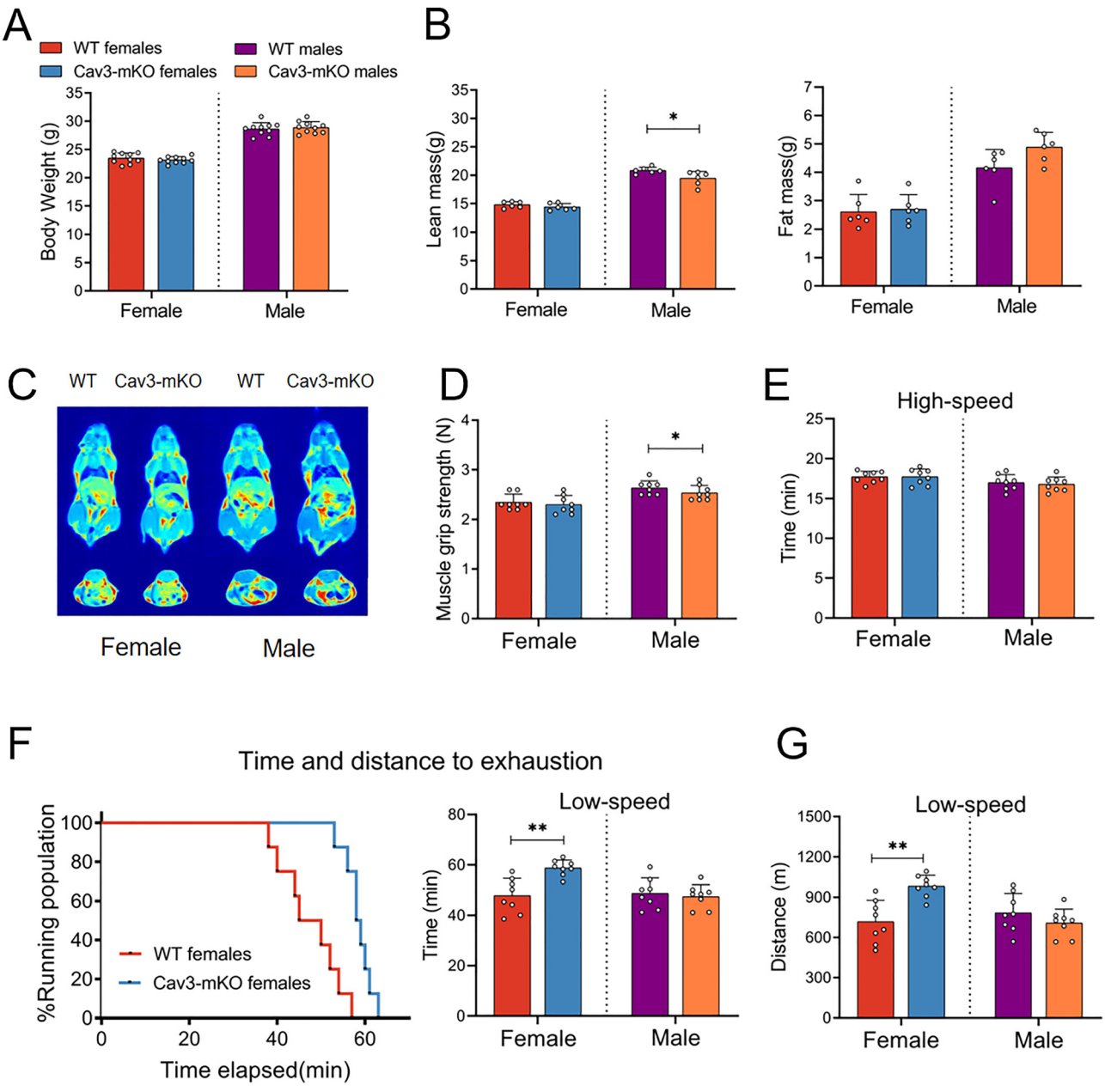

Figure 2: Cav-3 knockout improved exercise tolerance in female mice. (A) Body weight changes in Cav3-mKO and WT mice, n=10 for each group. (B) Lean (left) and fat mass (right) analyzed using nuclear magnetic resonance, n=6 for each group. (C) Pseudo-color images visualized the distribution of body composition in mice, n=3 for each group. (D) Extremity grip strength was measured three times per mouse to determine the maximum muscle strength in mice, n=8 for each group. (E) High-speed run time for assessment of aerobic capacity, n=8 for each group. (F) In the endurance fatigue test, the survival plot displays the percentage of mice running at a particular moment (left), and the bar graph shows the average time to exhaustion (right), n=8 for each group. (G) Running distance for low-speed run fatigue, n=8 for each group. *p<0.05, **p<0.01 for WT vs. Cav3-mKO mice, by independent sample *t*-test.

female Cav3-mKO mice compared to female WT mice, while lean mass in male Cav3-mKO mice was slightly reduced compared to male WT mice (Figure 2B). Although the difference in fat mass was not statistically significant, the percentage of fat increased significantly (Supplementary Figure 1A). Correspondingly, the grip force in female Cav3-mKO mice had no significant difference compared to the female WT mice and the grip force had vividly decreased in male Cav3-mKO mice (Figure 2D). Further, compared to the male and female WT mice, there was no difference between Cav3-mKO and WT mice in high-speed running time for either male or female mice (Figure 2E). However, female Cav3-mKO mice in low-speed running test had a longer running time and distance than the female WT mice, whereas the running time and distance in male Cav3-mKO mice were unchanged (Figure 2F and G). These findings suggest that muscle-specific Cav-3 deletion improves endurance capacity in female mice.

The knockout of Cav-3 in skeletal muscle promotes the formation of slow-twitch oxidative muscle fiber in female mice

Increased endurance and oxidative capacity are associated with increased slow oxidative myofiber proportion [3]. Based on the above results, we speculated that Cav-3 deletion in skeletal muscle may promote the transformation to slow oxidative myofibers in female mice. As expected, in the gastrocnemius muscle of female Cav3-mKO mice, the oxidative myofiber-related genes such as Tnni1, Tnnt1, and MyHC-1 were significantly up-regulated in comparison to female WT mice, while glycolytic myofiber-related genes such as Tnnt3 and MyHC-2B were significantly down-regulated (Figure 3A). In keeping with this, the Western Blot revealed that the expression of MyHC-1 protein was increased and the expression of MyHC-2B protein was decreased in gastrocnemius muscle of female Cav3-mKO mice (Figure 3C). Further, the Immunofluorescence staining also revealed that the slow oxidative myofiber percentage was increased and the fast glycolytic myofiber percentage was decreased in the gastrocnemius muscle of female Cav3-mKO mice (Figure 3E–I). These positive effects on regulating myofiber-related protein expression and myofiber proportion were observed in the soleus muscle of female Cav3-mKO mice (Figure 3B–D, F, and J). On the other hand, in male mice, the expression of glycolytic myofiber-related markers was decreased in both gastrocnemius and soleus muscles compared to the WT mice (Figure 3A–D). However, muscle-specific Cav-3 deletion failed to activate oxidative myofiber-related markers expression and increase oxidative myofiber percentage when compared to WT mice (Figure 3G–I). In summary, the above results indicate that Cav-3 deletion in skeletal muscle may promote slow oxidative myofiber formation by regulating the glycolytic to oxidative type transformation in female mice.

Discussion

Previous studies have reported that reduced Cav-3 expression leads to skeletal muscle myostatin signaling activation and muscle dysfunction [10]. Our research revealed that the absence of Cav-3 reduced lean mass and muscle strength in male mice. This may be related to mitochondrial mass/function, whose decline leads to impaired protein synthesis in skeletal muscle, which may lead to muscle degeneration and weakness [11]. From the molecular mechanism, it was also found to affect the generation of glycolytic type MyHC-2B genes and proteins. Although these results have been previously observed in male mice, the present study yielded different findings when the same experiment was conducted in female Cav3-mKO mice.

By further examination, we found that the expression of fast type MyHC-2B was significantly decreased in female Cav3-mKO mice, while the expression of slow type MyHC-1 was markedly increased. Compared to fast muscle fibers, slow muscle fibers exhibit a higher mitochondrial content and cristae density [12]. For females, the advancement of slow muscle fibers is beneficial because it could increase oxidative capacity, enhancing muscular endurance and recovery. Notably, it has been demonstrated that the estrogen nuclear receptor ERRγ, a pro-oxidative transcription factor, is significantly expressed in slow-twitch oxidative muscle fibers and that its overexpression results in the transformation of glycolytic muscle fibers into slow-twitch oxidative muscle fibers [13]. Estrogen can up-regulate the expression of Cav-3 in skeletal muscle cells through ERα, potentially elucidating the different phenotypes observed in male and female mice upon deletion of the same gene [14]. Furthermore, testosterone plays an important role in muscle fiber type composition and fiber size [2]. In addition to the impact of sex hormones on muscle fiber composition, insulin resistance plays a crucial role in driving muscle fiber transformation. Recent research has demonstrated that the loss of Cav-3 results in systemic insulin resistance and decreased adiponectin levels in mice, indicating a close association between insulin resistance and adiponectin levels [15]. Adiponectin, a regulatory factor, plays a crucial role in modulating muscle

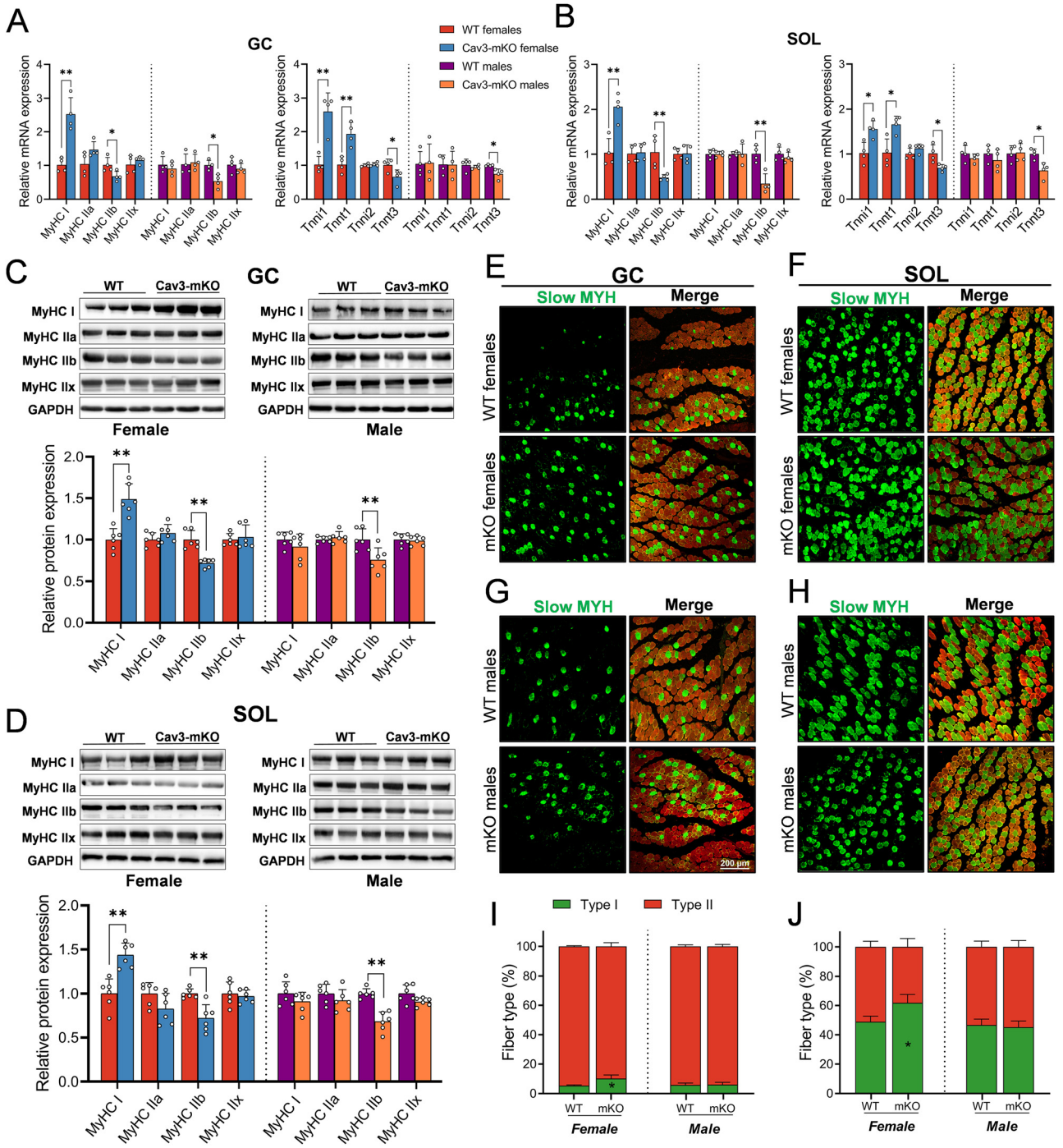

Figure 3: The transformation from glycolytic to oxidative muscle fibers were enhanced in female mice with Cav-3 knockout. (A–B) The MyHC (left) and representative fast/slow twitch troponin (right) gene expressed in GC and SOL of female and male mice, n=4 for each group (abbreviations: GC, gastrocnemius; SOL, soleus.). (C–D) Representative immunoblotting was performed for GC and SOL lysates (top). The quantification (bottom) of myosin heavy chain expression in muscles was shown, n=6 for each group. (E–H) Cross-section of muscles (E-G for GC; F-H for SOL) from female and male mice immunostained for slow-twitch (green) and merge (red and green) using specific antibodies staining. Scale bars: 200 μm. n=3 for each group (I–J) Immunofluorescence quantitative data (I for GC, J for SOL). *p<0.05, **p<0.01 for WT vs. Cav3-mKO mice, by independent sample *t*-test.

fiber transformation [16]. The underlying mechanism for the myofiber transformation from fast to slow type in female Cav3-mKO mice is unknown, and we will examine it in future research.

Conclusions

Overall, our findings showed that muscle-specific Cav-3 deletion can impact the transformation of fast to slow muscle fibers in female mice. This study suggested that Cav-3 may offer a potential therapeutic target for improving endurance performance and slow oxidative myofiber deficiency-related diseases in female mice.

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Research ethics: All procedures involving the use and care of laboratory animals are licensed by the Zhengzhou University Life Sciences Institutional Review Board (ZZUIRB2024-01).

Informed consent: Not applicable.

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Data availability: The raw data can be obtained on request from the corresponding author.

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