Yang xin shi tablet enhances adaptability to exercise training by relieving statin-induced skeletal muscle injury

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To the Editor: Widely used statins lower the risk of death from cardiovascular diseases. However, statin-associated skeletal muscle complaints hinder their clinical application to some extent. *Yang xin shi* tablet (YXST), a traditional Chinese medicine formula, can effectively improve the clinical symptoms of patients with cardiovascular diseases when combined with conventional drugs. However, the synergistic effects of YXST combined with a statin and the potential mechanisms remain unknown. Hence, exploring the effects and mechanisms of YXST in improving statin-induced muscle injury can be beneficial for the exercise rehabilitation and prognosis of patients with cardiovascular disease.

High-fat diet-fed apolipoprotein E knockout ($ApoE^{-/-}$) mice (Vital River, Beijing, China) were raised in the specific pathogen-free housing. After 4 weeks, the $ApoE^{-/-}$ mice were randomly divided into four groups of six mice each: exercise, exercise + simvastatin (SMV), exercise + SMV + low dose of YXST (LYXST), and exercise + SMV + high dose of YXST (HYXST). Experimental mice were given running exercise and administrated 20 mg/kg SMV (Merck, St. Louis, MO, USA), SMV + 750 mg/kg YXST (Growful, Qingdao, China), or SMV + 1500 mg/kg YXST by gavage for 8 weeks. Exercise capacity was evaluated at the end of the treatment using the running tolerance test (ZhiShuDuoBao, Beijing, China). Moreover, total cholesterol and creatine kinase concentrations were measured at the end of treatment. Muscle morphology was assessed by section staining of the gastrocnemius (Solarbio, Beijing, China). Energy metabolism was assessed by glycogen reserves in the gastrocnemius (Solarbio). Mitochondrial complex III activity (Beyotime, Shanghai, China) was measured to evaluate mitochondrial function. Western blot assays were performed using the following antibodies: anti-adenosine 5′-monophosphate-activated protein kinase (AMPK; Abcam, Cambridge, UK), anti-*p*-AMPK (Abcam), anti-PGC-1α (Abcam), and anti-*p*-PGC-1α (Abcam) antibodies. The real-time quantitative polymerase chain reaction kits (Yeasen, Shanghai, China) were used to determine the expression levels of nuclear respiratory factor 1 (NRF1) and mitochondrial transcription factor A (TFAM). The murine primer sequences are as follows: NRF1, forward primer 5′-GGTGGGGGACAGATAGTCCT-3′, reverse primer 5′-GCTGTCCGATATCCTGGTGG-3′; TFAM, forward primer 5′-TCACCCTATCTTGGGGTCATC-3′, reverse primer 5′-GGGATTTGCCAGCTCAAAGTG-3′; β-actin, forward primer 5′-CTGTCCCTGTATGCCTCTG-3′, reverse primer 5′-ATGTCACGCACGATTTCC-3′. Statistical analysis was performed using SPSS 17.0 software (SPSS Inc., Chicago, IL, USA). Data were expressed as mean ± standard deviation (SD). The significance level was tested using a one-way analysis of variance. A $P < 0.05$ was considered statistically significant.

We found that YXST neither affected the plasma lipid levels nor interfered with the hypolipidemic effects of SMV on $ApoE^{-/-}$ mice (41.68 ± 9.62 *vs.* 22.54 ± 2.76, $P < 0.05$; 41.68 ± 9.62 *vs.* 25.75 ± 3.21, $P < 0.05$; 41.68 ± 9.62 *vs.* 22.51 ± 2.01, $P < 0.05$). Our results also showed that YXST improved simvastatin-induced exercise intolerance (652.50 ± 169.40 *vs.* 1095.25 ± 237.10, $P < 0.05$; 652.50 ± 169.40 *vs.* 1030.80 ± 150.28, $P < 0.05$). To confirm the protective effects of YXST on muscle, we observed changes in histomorphology and found that $ApoE^{-/-}$ mice in the exercise + SMV + HYXST groups had more dense muscle fibers than those in the exercise + SMV

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group. In addition, YXST could remove the elevation of plasma creatine kinase induced by SMV (0.91 ± 0.20 *vs.* 0.38 ± 0.06, $P < 0.05$; 0.91 ± 0.20 *vs.* 0.35 ± 0.06, $P < 0.05$), indicating that YXST can effectively inhibit the myolysis caused by statin.

Statin reduces the substrate energy metabolism during aerobic exercise.[1] The PAS staining showed a significantly reduced content of muscle glycogen upon YXST treatment in ApoE$^{-/-}$ mice with exercise training with SMV gavage. Mitochondrial complex III was the off-target of statin, and its activity was reduced in patients with statin-induced myopathy.[2] Thus, our results showed that the complex III activity of SMV-treated mice decreased, which was restored by YXST (5.72 ± 0.80 *vs.* 2.06 ± 0.85, $P < 0.05$; 2.06 ± 0.85 *vs.* 11.51 ± 1.97, $P < 0.05$; 2.06 ± 0.85 *vs.* 8.21 ± 1.77, $P < 0.05$).

On the basis of the above results, we further explored the potential mechanisms involved. We found that YXST

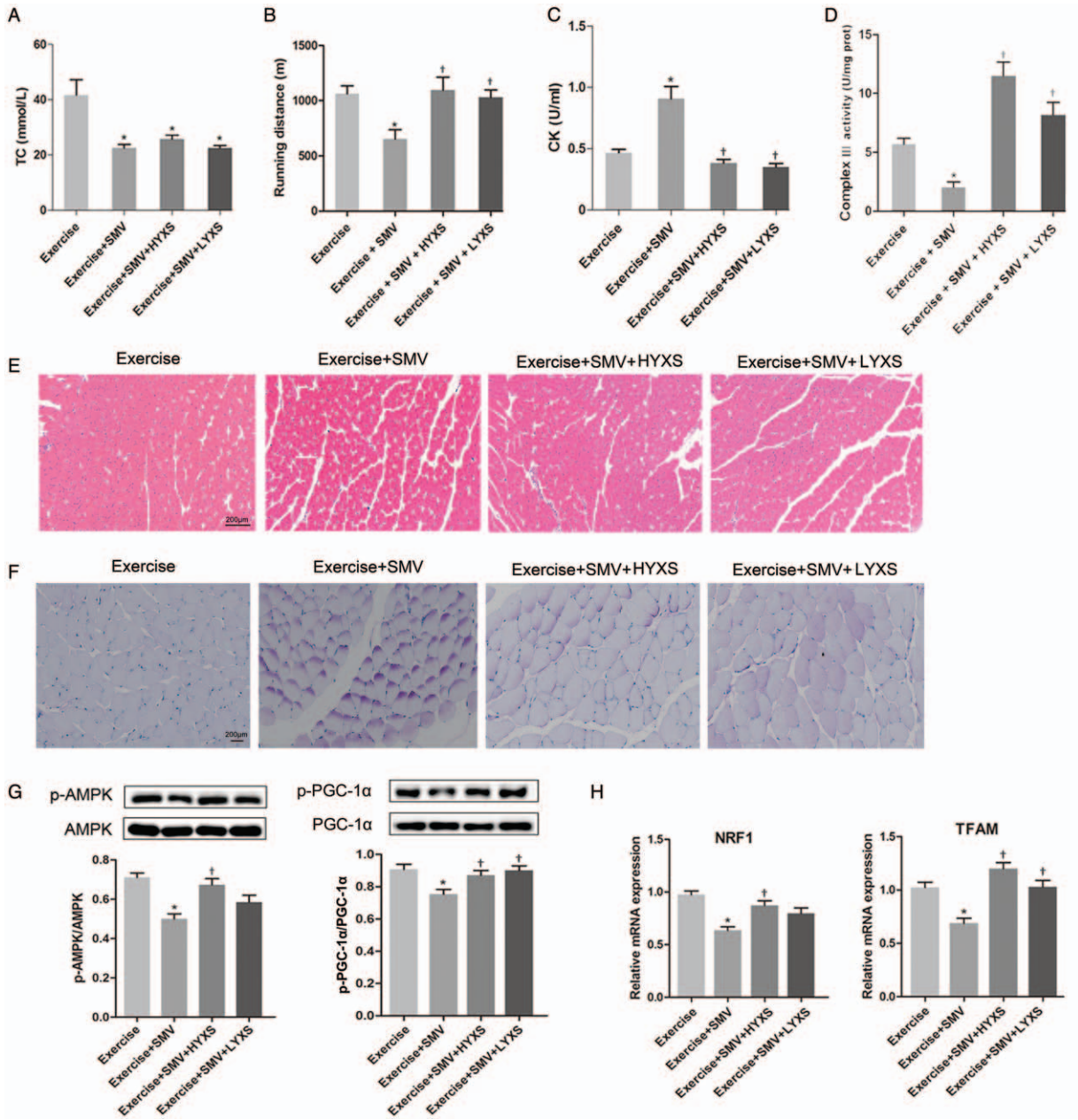

Figure 1: ApoE$^{-/-}$ mice were assigned to four groups: exercise, exercise + simvastatin (SMV), exercise + SMV + high dose of *Yang xin shi* (HYXS), exercise + SMV + low dose of *Yang xin shi* (LYXS). (A) Total clolesterol (TC) was measured before sacrifice. (B) Exercise capacity tests were performed at the end of experiment. (C) Plasma creatine kinase (CK) concentrations were measured before sacrifice. (D) Enzymatic activity of the complex III in the mitochondria isolated from gastrocnemius. (E) The morphologic changes of gastrocnemius were presented by hematoxylin and eosin (HE) staining (original magnification ×100). (F) The content of glycogen was determined by periodic acid-Schiff (PAS) staining (original magnification ×200). (G) Total proteins were extracted from gastrocnemius, and equal amounts of proteins were subjected to Western blot analysis for the indicated proteins, followed by densitometric quantification by ImageJ software. (H) The mRNA levels of nuclear respiratory factor 1 (NRF1) and mitochondrial transcription factor A (TFAM) in gastrocnemius. The data are expressed as mean ± standard deviation ($n = 5$). $^{*}P < 0.05$ *vs.* exercise; $^{\dagger}P < 0.05$ *vs.* exercise + SMV.

increased the phosphorylation of AMPK (0.46 ± 0.08 *vs.* 0.64 ± 0.09, $P < 0.05$) and PGC-1α (0.70 ± 0.10 *vs.* 0.87 ± 0.09, $P < 0.05$; 0.70 ± 0.10 *vs.* 0.88 ± 0.07, $P < 0.05$) and up-regulated the expression of NRF1 (0.64 ± 0.07 *vs.* 0.87 ± 0.09, $P < 0.05$). Ultimately, it acted on some factors, such as TFAM (0.69 ± 0.09 *vs.* 1.20 ± 0.11, $P < 0.05$; 0.69 ± 0.09 *vs.* 1.03 ± 0.13, $P < 0.05$), to increase energy metabolism. These results indicated that YXST enhanced energy metabolism by activating the AMPK-PGC-1α signaling pathway [Figure 1A–1H].

Statins reduce exercise capacity, and long-term use can lead to decreased skeletal muscle strength and increased risk of falling in elderly patients.[3] We found that YXST can effectively inhibit the myolysis induced by SMV. Mitochondrial activities are closely related to energy production.[4,5] We found that YXST significantly increased the activity of mitochondrial complex III and glycogen utilization to counteract the side effects of statin in the skeletal muscle.

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Conflicts of interest

None.

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