Aerobic Exercise Promotes the Expression of ATGL and Attenuates Inflammation to Improve Hepatic Steatosis via lncRNA SRA

Baoai Wu
Shanxi University
Yiming Tian
Shanxi University
Chong Xu
Shanxi University
Yu Zeng
Shanxi University
Feng Yan
Shanxi University
AnPing Chen
Shanxi University
Jinfeng Zhao (zhaojinfengzjf@126.com)
Shanxi University
Longchang Chen
Wuhan University of Bioengineering

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Abstract

The role of aerobic exercise in preventing and improving non-alcoholic fatty liver has been widely established. SRA is a long non-coding RNA, which has received increasing attention due to its important role in lipid metabolism. However, it is unclear whether aerobic exercise can prevent and treat hepatic lipid accumulation via SRA. The mice were randomly divided into three groups as follows, normal control group (NC), high-fat diet group (HFD), and high-fat diet plus aerobic exercise (8weeks, 6 days/ week, 18 m/ min for 50 min, 6% slope) group (HAE). After 8 weeks, the mice in the HAE group showed significant improvement in hepatic steatosis. Body weight as well as blood TC, LDL-C, and liver TG levels were significantly lower in the HAE group than in the HFD group. Compared with the HFD group, the expression of SRA was markedly suppressed and the expression of ATGL was significantly increased in the HAE group. Additionally, the JNK/P38 signaling was inhibited, the pro-inflammatory factors were down-regulated, and the anti-inflammatory factor was increased. The results of this study provided new support for aerobic exercise to improve hepatic lipid metabolism via lncRNA.

New Findings

What is the central question of this study?

SRA is a key long non-coding RNA for lipid metabolism, however, the effect of aerobic exercise on SRA has not been reported. In this study, we verified whether aerobic exercise could improve hepatic steatosis by modulating SRA.

What is the main finding and its importance?

We proved that aerobic exercise promoted the expression of ATGL and attenuated inflammation to improve hepatic steatosis via SRA. This study provides new insights into the mechanism by which aerobic exercise regulates liver lipid metabolism through lncRNA.

1. Introduction

Non-alcoholic fatty liver disease (NAFLD) begins with hepatic steatosis, a clinicopathological alteration characterized by the over-accumulation of lipids in hepatocytes [1,2] Due to the high prevalence globally (approximately 25% of adults) and its serious complications, including hyperlipidemia, type 2 diabetes, insulin resistance (IR), and obesity, NAFLD has become the primary health issue [3,4]. In addition, in the occurrence and development of hepatic steatosis, disturbances in lipid metabolism are usually associated with an inflammatory state [5,6].

Long non-coding RNAs (lncRNAs) are defined as transcripts over 200nt, lacking any coding capacity [7], and are emerging as an important new regulator that affect a variety of biological processes and pathogenesis of metabolic diseases [8,9]. One notable lncRNA closely related to lipid metabolism is the steroid receptor RNA activator（SRA), which has been shown to affect diet-induced obesity, glucose

tolerance, and hepatic steatosis [10,11]. Adipose triglyceride lipase (ATGL), a lipid droplet surface protein, is the major cytoplasmic triglycerides (TG) lipase in the process of lipolysis [12,13]. Forkhead box protein O1 (FoxO1) is a central regulator of metabolism in a variety of cell types and tissues [14], and ATGL expression is controlled by FoxO1 as a target gene [15]. Notably, a separate study confirmed that SRA reduced ATGL expression independently of insulin signaling by inhibiting the transcriptional activity of FoxO1 [16].

The activation of the JNK/P38 MAPK signaling pathway can increase the production of hepatic inflammatory cytokines, leading to hepatic steatosis [17]. Previous studies have established that SRA modulates phosphorylation of the P38/JNK signaling pathway to regulate adipogenesis [18]. In addition, SRA inhibits the production of inflammatory factors in both in vivo and in vitro knockout experiments [18,19]. Therefore, SRA is likely to be a key potential lncRNA for improving the inflammatory response to hepatic steatosis via the MAPK signaling pathway.

Although extensive research is ongoing, the first-line of therapy is still only exercise and dietary interventions, with no approved pharmacological interventions or surgical treatments at this time. Aerobic exercise is a low-risk, non-pharmaceutical intervention that is readily available to the vast majority of the general public [20]. It effectively reduces variables associated with NAFLD, such as body weight, serum cholesterol levels, and intrahepatic fat, by inhibiting the expression of lipogenic genes [21,22]. It can also ameliorate hepatic inflammation by inhibiting pro-inflammatory mediators such as tumor necrosis factor-α (TNF-α) and interleukin-1 (IL-1) [23]. Several recent studies have reported alterations in lncRNA expression by exercise in the metabolic syndrome [24,25], but the potential mechanisms by which aerobic exercise prevents and ameliorates lipid accumulation in hepatocytes via lncRNA remain unknown.

In this study, we examined whether aerobic exercise could affect SRA expression and how SRA could promote ATGL expression and suppress inflammation to improve hepatic steatosis in mice on a high-fat diet.

2. Methods

2.1 Ethical approval

This protocol was approved by the Scientific Research Ethics Committee of Shanxi University. All experiments conformed to local and international guidelines for the ethical use of animals, and every effort was made to reduce the number and suffering of animals used. This study adhered to the ARRIVE guidelines.

2.2 Animals study

Forty 8-week-old male C57BL/6J mice weighing 21.4±0.92g were purchased from Beijing Vital River Laboratory Animal Technology Biotech (Beijing, China). The animal license number is SCXK (Beijing) 2016-0006. After one week of adaptive feeding, the mice were randomly divided into four groups: normal

control group (NC, n = 10), normal aerobic exercise group (NE, n=10), high-fat diet (60% fat, 20% carbohydrate, 20% protein) group (HFD, n = 10) [16], and high-fat diet with aerobic exercise group (HAE, n = 10). The NE group received a normal diet for four weeks followed by aerobic exercise intervention for 8 weeks, the HFD group received a 12-week high-fat diet, and the HAE group was given aerobic exercise intervention for 8 weeks after a high-fat diet for 4 weeks. The animal groups are shown in Figure 1.

To specifically overexpress lnc RNA-SRA in mice, we used a mouse lnc RNA-SRA sequenced adenovirus (Sangon Biotech, Shanghai, China). The virus was assayed in 293A cells and the virus titer was measured. Lnc RNA-SRA sequence was correct and the virus was successfully packaged at a titer of 9×10^{11} PFU/ml. Meanwhile, the packaging of an unrelated sequence control adenovirus was assayed and the virus was successfully packaged at a titer that met the experimental requirements. Adenoviral vectors (100 μL) were injected into the tail vein of mice for five weeks and divided into three groups: virus empty vector as a negative control (EV, n=10), SRA overexpression group (SRA+, n=10), SRA overexpression and combined with 5 weeks aerobic exercise group (SRA+AE, n=10).

All mice were housed in standard laboratory conditions (12h dark-light cycle, 20~26°C, 40~60% relative humidity) at the China Institute of Radiation Protection and had free access to standard food and water. The body weight of the mice was recorded weekly. After the final treadmill exercise program was completed, the mice were fasted overnight, and were anesthetized intraperitoneally with sodium pentobarbital at a dose of 80 mg/Kg, followed by spinal dislocation method of execution. The blood samples were collected in tubes containing EDTA, centrifuged at 3500 rpm at 4 ℃ for 10 min, and then the supernatant was transferred to new tubes and stored at -20℃. Mice livers were quickly removed and washed with cold phosphate-buffered saline (PBS), then part of the liver tissue was cut into the fixing solution, and the remaining liver tissues were frozen in liquid nitrogen, stored at -80 ºC until extraction.

2.3 Exercise protocol

Aerobic exercise in mice was performed using an animal treadmill (SA101, Jiangsu Saionce Biotechnology Co., China). After the mice had completed a week of acclimatisation, the adjusted aerobic exercise protocol [26]. was determined to be 18m/min × 50min, 6% incline, six days a week (starting at 7pm), 8 weeks of intervention for the HAE group and 5 weeks for the SRA+AE group, with mice having a 5-minute warm-up before each exercise session. The specific exercise protocol is shown in Table 1.

2.4 Hepatic histology

The fresh livers were laid flat in the embedding box and immersed in the prepared 10% formalin solution; after 24-48h the box was removed, rinsed in 50% alcohol solution and immersed in 70% alcohol overnight. The cassette was transferred from the 70% alcohol solution to the 80% alcohol solution for 2h. The wax was dehydrated and preserved for embedding, then sliced, spread, retrieved and dried in a warm oven. Filter hematoxylin for HE staining. The tissue was completely coated with drops of neutral gum, covered with a coverslip and spread out to dry naturally. The degree of hepatic steatosis and the lipid accumulation was observed with a light microscope (Zeiss，German).

2.5 Blood analysis

Serum total cholesterol (TC), triglyceride (TG), LDL cholesterol (LDL-C), HDL cholesterol (HDL-C) were assayed by spectrophotometer (UV-6100s, Mapada, Shanghai, China) according to the kit's instructions (Nanjing Jian cheng biotech, China). The content of TG and TC were measured at 510nm, LDL-C and HDL-C were measured at 546nm.

2.6 Liver triglyceride analysis

Liver tissue was weighed at a ratio of weight (g): volume (ml) = 1:9, added to 9 times the volume of homogenising medium (saline for non-high-fat samples, anhydrous ethanol for high-fat samples), homogenised under an ice-water bath, centrifuged at 2500 rpm for 10 minutes and the supernatant was removed. 10μl of 10 % mouse liver homogenate was mixed and incubated at 37°C for 10 minutes according to the TG assay kit (Sangon Biotech, Nanjing, China) instruction sheet. The absorbance of each tube was measured at a wavelength of 510 nm and an optical diameter of 0.5 cm.

2.7 RNA extraction and quantitative real-time PCR

The RNA was extracted by the Trizol method [27], and the air-dried RNA was precipitated for 5-10 min after discarding the supernatant, and the precipitate was dissolved in 20 μl of DEPC water. Take 2μl of the dissolved RNA and measure the OD260, OD280 , and OD260/OD280 values by micro spectrophotometer (Allsheng Instruments Co., Ltd, Hangzhou, China) to calculate the purity and concentration of RNA. The concentration of sample RNA was calculated according to the following formula based on the absorbance value: Total RNA concentration (μg/μl) = $OD260\times40\times10^{-3}$. Quantitative PCR was performed on a LightCycler480 system (Roche, Switzerland) using TB Green premix Ex Taq mix (TaKaRa, Dalian, China). mRNA expression was calculated by the $2^{-\triangle\triangle CT}$ method. Gene-specific primers were listed in Table2，β-actin was used as an internal control.

2.8 Western blot

Three groups of mice liver tissues were lysed in RIPA lysis buffer, protein concentration was determined by the BCA method (Beyotime, Shanghai, China). Proteins (40μg) were separated by SDS-polyacrylamide gel electrophoresis (SDS-PAGE) and transferred onto PVDF membranes (Millipore, Billerica, USA). Soak PVDF membranes in TBST (sealing solution) containing 5% skimmed milk powder and sealed with shaking for 2h at room temperature. Phosphorylated proteins were closed with 1% BSA. After overnight incubation with FoxO1 (1:1000, 18592-1-AP, Wuhan Sanying Biotechnology Co., Ltd.), P-FoxO1 (1:1000, ab131339, Abcam), ATGL (1:1000, 55190-1-AP, Wuhan Sanying Biotechnology Co., Ltd.), P38 (1:1000, 8690T, CST), P-P38 (1:1000, 9211S, CST), JNK (1:1000, 24164-1-AP, Wuhan Sanying Biotechnology Co., Ltd.), P-JNK (1:1000, 24164-1-AP, Wuhan Sanying Biotechnology Co., Ltd.), P-P38 (1:1000, 9211S, CST), JNK (1:1000, 24164-1-AP, Wuhan Sanying Biotechnology Co., Ltd.), P-JNK (1:2000, Sc-6254, Santa Cruz), and β-actin (1:500, BM0627, Wuhan PhD Bioengineering Co., Ltd.) primary antibody. The membranes were washed and incubated with HRP-conjugated secondary antibody (1:5000) (Boster Biotech, Wuhan,

China). Then the ECL reagent (Applygen Technologies Inc, Beijing, China) was mixed with the working solution and added dropwise to the PVDF film, the excess substrate solution was blotted off with filter paper, the film was pressed, the developer and fixer were put in sequence, and the film was rinsed.

2.9 Image processing

All figures in the experiment were processed with the software Origin 2019 and Photoshop 2021 for image processing.

2.10 Statistical Analysis

All experimental data were expressed as mean±standard deviation (mean±SD). Statistical analysis was performed using SPSS 25.0 software (SPSS, Seattle, WA, USA), and one-way ANOVA was performed after testing for normality and chi-square. The SNK-q test was used to compare two groups and values of $P < 0.05$ were regarded as statistically significant.

3. Results

3.1 Aerobic exercise alleviated hepatic steatosis in mice

Morphological observations of the liver showed no abnormal changes in the livers of mice in the NC and NE groups, which were bright red with smooth envelopes and sharp margins (Figure 2A). In HFD group mice, the liver was yellowish-brown with blunt-edged edges, swollen volume, soft texture, and oily cut surface. The liver of the HAE group returned to a state close to that of the NC group. To study the effects of aerobic exercise on liver pathology, we performed liver H&E staining analysis. As shown in the lower panel of Figure 2A, compared with NC mice, the NE group was normal and unchanged, mice in the HFD group showed severe histopathological changes in the liver after 12 weeks of the high-fat diet, with a significant increase in the number and size of hepatic lipid droplets and accompanied by punctate necrosis of hepatocytes and infiltration of inflammatory cells. In contrast, we found that in the HAE group of mice subjected to aerobic exercise intervention, the number and size of hepatic lipid droplets were reduced, the hepatocytes were arranged neatly and hepatic steatosis was significantly improved. The body weight of mice in the HFD group showed a rapid increase and was markedly higher than that of the NC group at the end week of the experiment ($P<0.05$). However, from 5 weeks to 12 weeks of aerobic exercise intervention, the body weight of mice in the HAE group was significantly reduced compared with that in the HFD group ($P<0.05$; Figure 2B), the NE group showed the steadiest trend with the lowest body weight at the end of the last week of exercise but no significant difference compared to the NC group ($P>0.05$). We also investigated the effects of aerobic exercise on blood lipid and liver triglyceride levels. After 8 weeks of aerobic exercise, compared to the HFD group, HAE group mice had significantly depressed levels of TC and LDL-C($P<0.05$), but not TG and HDL-C($P>0.05$), in their plasma (Figure 2C). Liver TG levels were shown to be reduced by nearly half in the HAE group compared to the HFD group ($P<0.01$; Figure 2D). In addition, blood HDL-C and liver TG levels were significantly altered in the NE group

compared to the NC group. These observations show that 8 weeks of aerobic exercise training alleviated hepatic steatosis caused by a high-fat diet in mice and improved blood lipid levels.

3.2 Aerobic exercise inhibited SRA expression.

Given the important role of SRA in liver lipids metabolism, we next studied the effects of aerobic exercise on hepatic SRA expression. The results showed that SRA expression was significantly induced in the liver of the HFD group compared to the NC group ($P< 0.05$), while the expression of SRA in the liver of HAE mice under the intervention of aerobic exercise was significantly suppressed ($P<0.05$; Figure 2E). In addition, to further verify the inhibitory effect of aerobic exercise on SRA, we overexpressed SRA by tail vein injection using a mouse lncRNA SRA sequenced adenovirus. Compared to the empty vector EV group, the SRA+ group showed significant lipid accumulation and inflammatory cell infiltration in the liver and a significant increase in intrahepatic TG levels ($P<0.05$, Figure 2F, 2G), whereas the SRA+AE group returned to normal liver lipid accumulation and a significant decrease in TG levels after five weeks of aerobic exercise intervention ($P<0.05$). Furthermore, the findings of RT-qPCR analysis showed that SRA expression was significantly increased in the SRA+ group and significantly suppressed in the SRA+AE group($P<0.05$; Figure 2H). These data indicate that aerobic exercise plays an important role in improving hepatic steatosis by inhibiting SRA expression.

3.3 Aerobic exercise upregulated the hepatic ATGL levels via SRA

To determine whether the marked reduction in lipids observed in the livers of mice in the HAE and SRA+ groups was the result of increased lipolysis, we compared the expression of ATGL by PCR and WB. After 12 weeks of high-fat diet feeding in the HFD group, the ATGL mRNA and protein levels in the liver were significantly reduced, compared with the NC group ($P< 0.05$), and aerobic exercise markedly increased the levels of liver ATGL in HAE group mouse livers (Figure 3A, 3B). SRA is known to inhibit FoxO1 transcriptional activity independently of insulin signaling [16]. To understand the mechanism of how aerobic exercise regulates ATGL expression through SRA, we next assessed the expression and phosphorylation levels of FoxO1, a well-known transcription factor that positively regulates ATGL [28]. The analysis of FoxO1 showed that a high-fat diet inhibited phosphorylation in the liver of mice($P< 0.05$; Figure 3C), which may be closely related to IR [29], and its phosphorylation was significantly up-regulated in the HAE group ($P< 0.05$). This is further evidence that SRA inhibits the transcriptional activity of FoxO1 independent of the known insulin signaling. Furthermore, the SRA+AE group also showed a significant upregulation of ATGL expression as well as FoxO1 phosphorylation levels in response to the aerobic exercise intervention ($P< 0.05$; Figure 3D-F). These results together suggest that aerobic exercise inhibits FoxO1 phosphorylation via SRA to upregulate hepatic ATGL levels to promote lipolysis, thereby reducing intrahepatic TG levels.

3.4 Aerobic exercise improved inflammation through SRA

SRA shows profound regulation of P38/JNK MAPK signaling, which is closely related to inflammation and metabolic disorders [19,30]. This relationship prompted us to investigate whether the P38/JNK

pathway is involved in SRA regulated hepatic steatosis and its related pathology. As expected, we determined that aerobic exercise reduced liver mRNA levels of pro-inflammatory factors including IL-6, TNF-α, and increased levels of the anti-inflammatory factor IL-10 in mice ($P< 0.05$; Figure 4A). Compared with mice under normal conditions, the phosphorylation levels of P38 and JNK1/2 were significantly increased in liver samples from the HFD group ($P< 0.05$; Figure 4B-D). After aerobic exercise intervention, the phosphorylation level in the liver of HAE group mice was significantly reduced ($P< 0.05$). In addition, the pro-inflammatory markers IL-6 and TNF-α were significantly increased and the anti-inflammatory marker IL-10 was greatly decreased and JNK/P38 phosphorylation levels were increased in the SRA overexpressing mice (Figure 4E-H). In contrast, in the SRA+AE group, the levels of pro-inflammatory IL-6 and TNF-α were obviously suppressed and the levels of anti-inflammatory IL-10 were markedly increased and the levels of JNK/P38 phosphorylation were downregulated after five weeks of aerobic exercise. Based on these findings, we inferred that aerobic exercise improves P38/JNK mediated inflammation by inhibiting the expression of SRA in hepatic steatosis.

4. Discussion

NAFLD is one of the most common chronic liver diseases and its pathogenesis involves abnormal activation of hepatic lipogenesis and inflammation production [3,31]. However, the regulatory networks that regulate the physiological and pathological activation of hepatic lipogenic programs are not fully understood. Recently, increasing numbers of studies have revealed the importance of lncRNAs in the regulation of diverse biological processes and have been shown to play an important role in lipid metabolism [32,33]. In this study, we found that aerobic exercise suppressed the expression of SRA in the liver of mice, thereby improving hepatic steatosis (Figure 1). The decrease in intrahepatic lipid aggregation may be due to increased expression of ATGL, which promotes the breakdown of TG.

FoxO1 acts as a transcription factor that directly regulates ATGL expression [28]. Phosphorylated FoxO1, which is regulated by insulin, binds to the chaperone 14-3-3 protein for transport from the nucleus to the cytoplasm and downregulates transcriptional activity to suppress target gene expression [29]. The phosphorylation of FoxO1 and ATGL expression were significantly reduced in mouse liver under a high-fat diet (Figure 5), which may be related to the inhibition of FoxO1 activity by PI3K/AKT signaling in insulin resistance, which also indirectly suggests that SRA alters the transcriptional activity of FoxO1 independently of insulin to inhibit ATGL expression.

Chronic inflammation is an important factor in hepatic steatosis [17]. The deficiency of SRA reduced the expression of inflammatory genes in vivo and in vitro [19,30]. Our results suggested that aerobic exercise inhibition of SRA decreases the expression of pro-inflammatory factors genes. This may be a result of the inhibition of inflammatory genes by P38/JNK signaling, a signal associated with the inflammatory response. These results reflected a possible close connection between SRA and established signaling and metabolic networks.

It is well established that many lncRNAs associated with lipid metabolism are involved in transcriptional regulation, acting through specific protein-binding partners. For instance, lncRNA Blnc1 is a core component of the LXR/SREBP1c protein pathway that is required for lipogenic induction [34]; lncRNA LSTR binds to the TDP-43 protein, resulting in a decrease in Cyp8b1 gene expression and substantial changes in bile acid composition [33]. Previous studies have indicated that SRA exerts its diverse biological functions and roles in the development of cancer and other diseases by interacting with the protein chaperones in the activation and inhibition complexes. For example, SRA regulates NR transcriptional activity by directly or indirectly interacting with NR [11]. SRA also forms complexes with core regulators of NRs and TFs, such as SRC-1, P68, PUS1/3, components of RISC complex proteins (PACT, TRBP, Dicer, and Argonaut2), and inhibitory core regulatory proteins SHARP and SLIRP [35,36]. Here, although aerobic exercise has been shown to enhance the transcriptional activity of FoxO1 through SRA to regulate ATGL expression in the liver, how SRA binds to its corepressor to promotes this level of change needs to be further investigated and confirmed.

Aerobic exercise is not only for weight loss but also for improving body adaptability and function. The data presented here show that aerobic exercise successfully improved hepatic steatosis in mice fed with a high-fat diet, it also significantly improved body weight and blood lipid levels. Our previous studies found that aerobic exercise reduced the expression of PCSK9, an important gene for cholesterol metabolism [26]. Recent studies have confirmed that exercise affects the level of lncRNA in the body. For example, the GW29-e0223 LncRNA gene chip analyzes the effect of simultaneous exercise on aortic insulin resistance [24]; exercise can reduce insulin resistance in type 2 diabetes by mediating the lncRNA MALAT1/microRNA-382-3p/ resistin axis [25]. These studies suggest that exercise can exert multiple physiological modulatory effects on related metabolic diseases through LncRNAs, but the deeper mechanisms remain unclear. Therefore, exploring the mechanisms of lncRNA-related diseases and combining them with exercise to provide new therapeutic strategies would be an area of interest.

In summary, as shown in Figure 5, we first demonstrated that aerobic exercise reduces intrahepatic lipids and inflammation by inhibiting SRA levels, thereby improving hepatic steatosis. Mechanistically, aerobic exercise inhibited the transcriptional activity of FoxO1 by suppressing the expression of SRA, which resulted in the upregulation of ATGL expression. In addition, aerobic exercise may also alleviate inflammation by regulating SRA to inhibit P38/JNK signaling. Our results revealed that SRA plays an important role in aerobic exercise to improve hepatic fat metabolism.

Declarations

Acknowledgment

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Competing interests

The authors claim that they have no conflict of interest.

Author contributions

Conception or design of the work: Chen, L.C., Wu, B.A.,Tian, Y.M and Zhao, J.F. Conduct of experiments and data acquisition: Chen, L.C., Yan, F., Zeng Y., Xu, C., Chen, A.P., Data analysis or interpretation: Chen, L.C., and Zhao, J.F.

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Tables

Table 1 8-week-treadmill exercise protocol

Week	Time	Velocity ($m \cdot min^{-1}$)	Duration (min)	Slope (°)
1 week	1	8	10	0
	2	10	20	2
	3	12	30	3
	4	14	40	4
	5	16	50	5
	6	18	50	6
	7	Rest		
2-9 eeks	1-6	18	50	6

Table 2 Primers for mRNA expression analysis in RT-qPCR

Gene	Forward primer	Reverse primer
SRA	GGCGGGCTGGTGGTACTCG	GCGTCGGCTGATATCATCACATACC
ATGL	TTCACCATCCGCTTGTTGGAG	AGATGGTCACCCAATTTCCTC
1L-6	CAAGTCGGAGGCTTAATTACAC	TGCAAGTGCATCATCGTTG
TNF-α	CATCTTCTCAAAATTCGAGTGACAA	TGGGAGTAGACAAGGTACAACCC
1L-10	CCAAGCCTTATCGGAAATGA	TTTTCACAGGGGAGAAATCG
β-actin	GGAAAGACAACGGACAAATCAC	TACGGATCGAAACTGGCAC

Figures

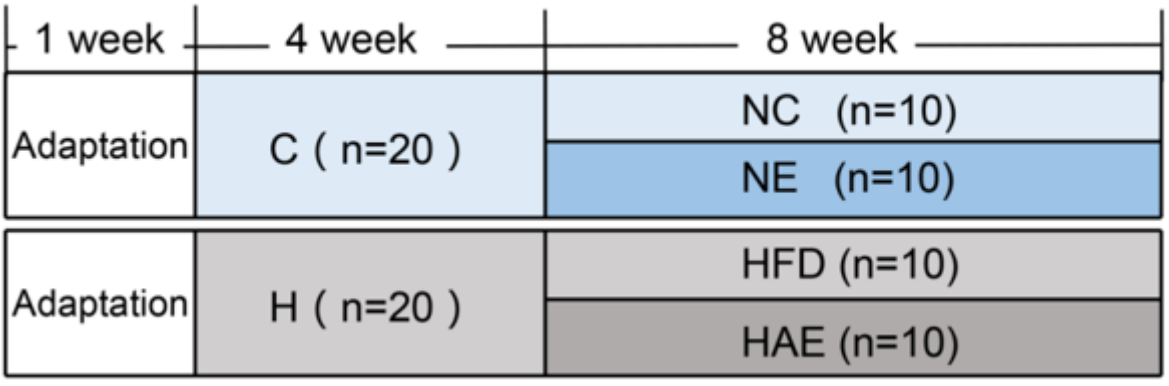

Figure 1

Animal groups Abbreviations: NC, normal control group; NE,normal aerobic exercise group; HFD, high-fat diet group and HAE, high-fat diet with aerobic exercise group.

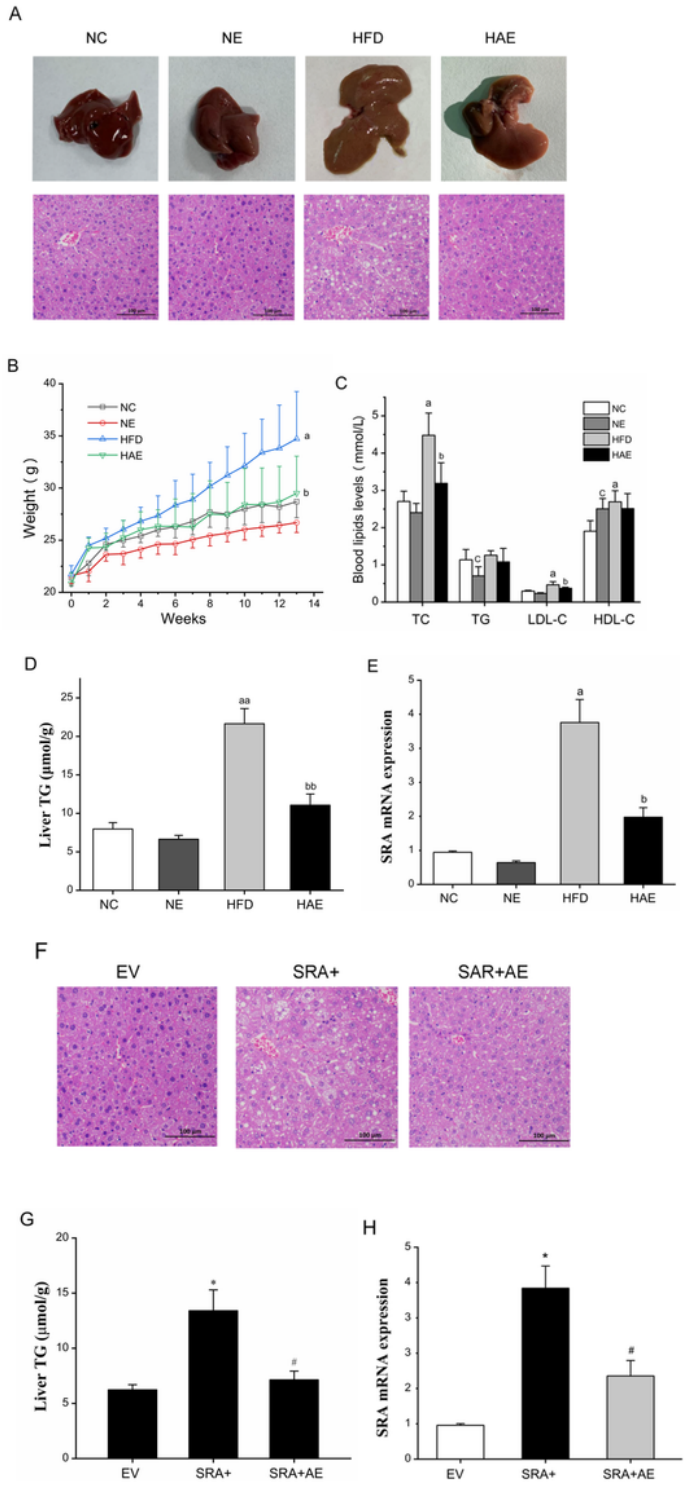

Figure 2

Aerobic exercise inhibits SRA expression to attenuate hepatic steatosis in mice. (A) Visual observation (Upper) and HE staining (Lower) to observe liver steatosis, n=6/group. (B) The trend of body weight change of mice in each group, n=10/group. (C) Blood lipids levels of mice in each group, n=6/group. (D) Triglyceride content in mice liver, n=6/group. a P0.05, aa P0.01 vs. NC group; b P0.05, bb P0.01 vs. HFD group; c P0.05 vs. NC group. (E) SRA expression in the liver of each group measured by RT-qPCR, n=6. (F) H&E stained liver sections of EV, SRA+, SRA+AE mice, n=5/group. (G) Hepatic triglyceride levels in EV, SRA+, SRA+AE mice, n=6/group. (H) SRA mRNA expression in the liver of each group measured by RT-qPCR, n=6/group. The values represent the means ± SD. * P0.05 vs. EV group; # P0.05 vs. SRA+ group.

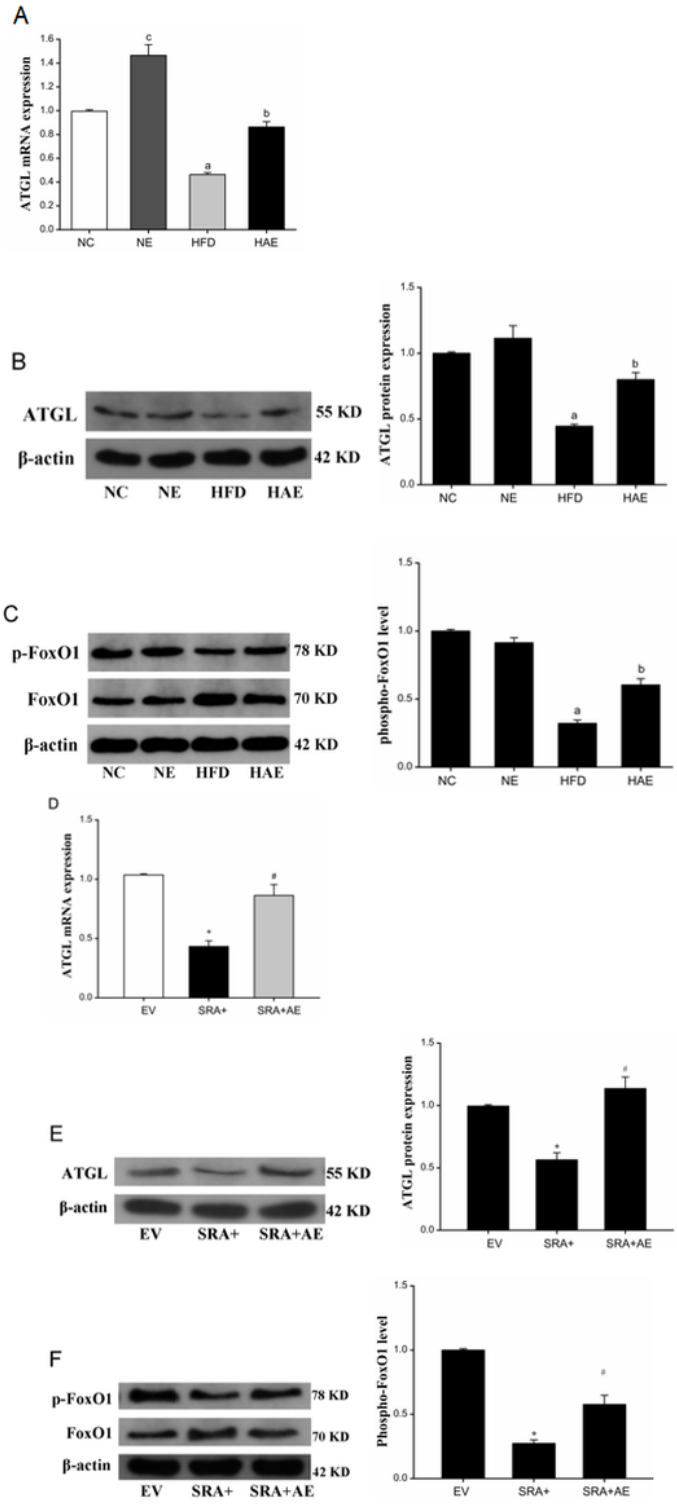

Figure 3

Aerobic exercise upregulates the hepatic ATGL levels via SRA. (A) The ATGL mRNA levels in livers of mice were measured by RT-qPCR, n=6/group. (B) Protein levels of ATGL were determined by western blot. (C) The protein expression and phosphorylation levels of FoxO1 in mice liver assessed by western blot. a P 0.05 vs. NC group; b P0.05 vs. HFD group; c P0.05 vs. NC group. (D) Hepatic ATGL mRNA levels in EV, SRA+, SRA+AE groups of mice, n=6. (E) Protein levels of ATGL were determined by western blot. (F) The

protein expression and phosphorylation levels of FoxO1 in mice liver assessed by western blot. The values represent the means ± SD. *P<0.05 vs. EV group, # P<0.05 vs. SRA+ group.

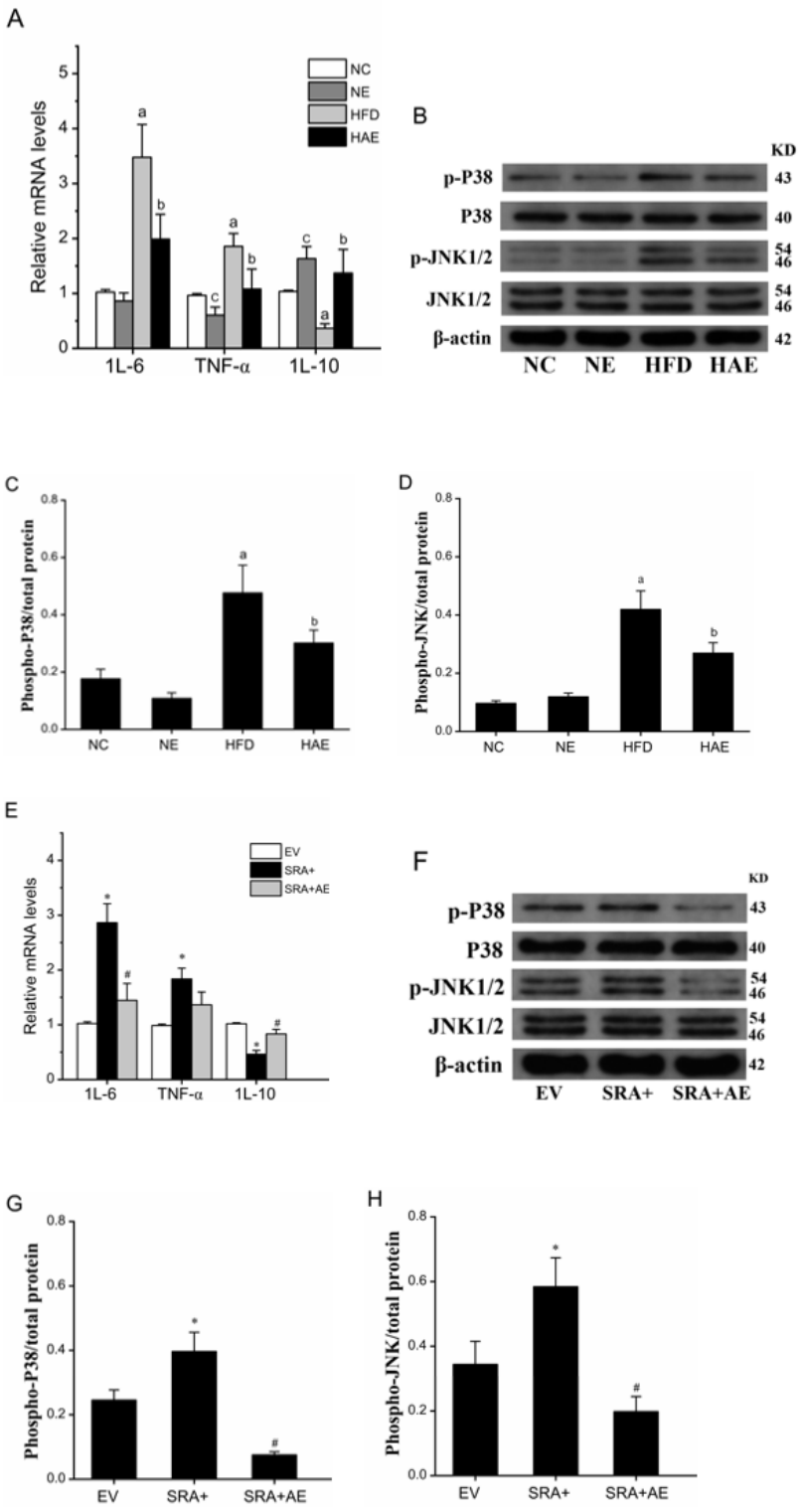

Figure 4

Aerobic exercise improves P38/JNK-mediated inflammation through SRA (A) The mRNA levels of inflammatory factors in mice liver assessed by RT-qPCR, n=6/group. (B) Western blot analysis of protein expression and phosphorylation of P38 and JNK. (C) The phosphorylated P38/ total protein levels in the

liver of each group of mice. (D) The Phosphorylated JNK/ total protein levels in the liver of each group of mice. a P0.05 vs. NC group; b P0.05 vs. HFD group; c P0.05 vs. NC group. (E) The mRNA levels of inflammatory factors in the liver of EV, SRA+, SRA+AE mice were assessed by RT-qPCR, n=6/group. (F) Western blot analysis of protein expression and phosphorylation of P38 and JNK in EV, SRA+, SRA+AE mice. (G) The phosphorylated P38/ total protein levels in the liver of each group of mice. (H) The Phosphorylated JNK/ total protein levels in the liver of each group of mice. The values represent the means ± SD. *P0.05 vs. EV group, # P0.05 vs. SRA+ group.

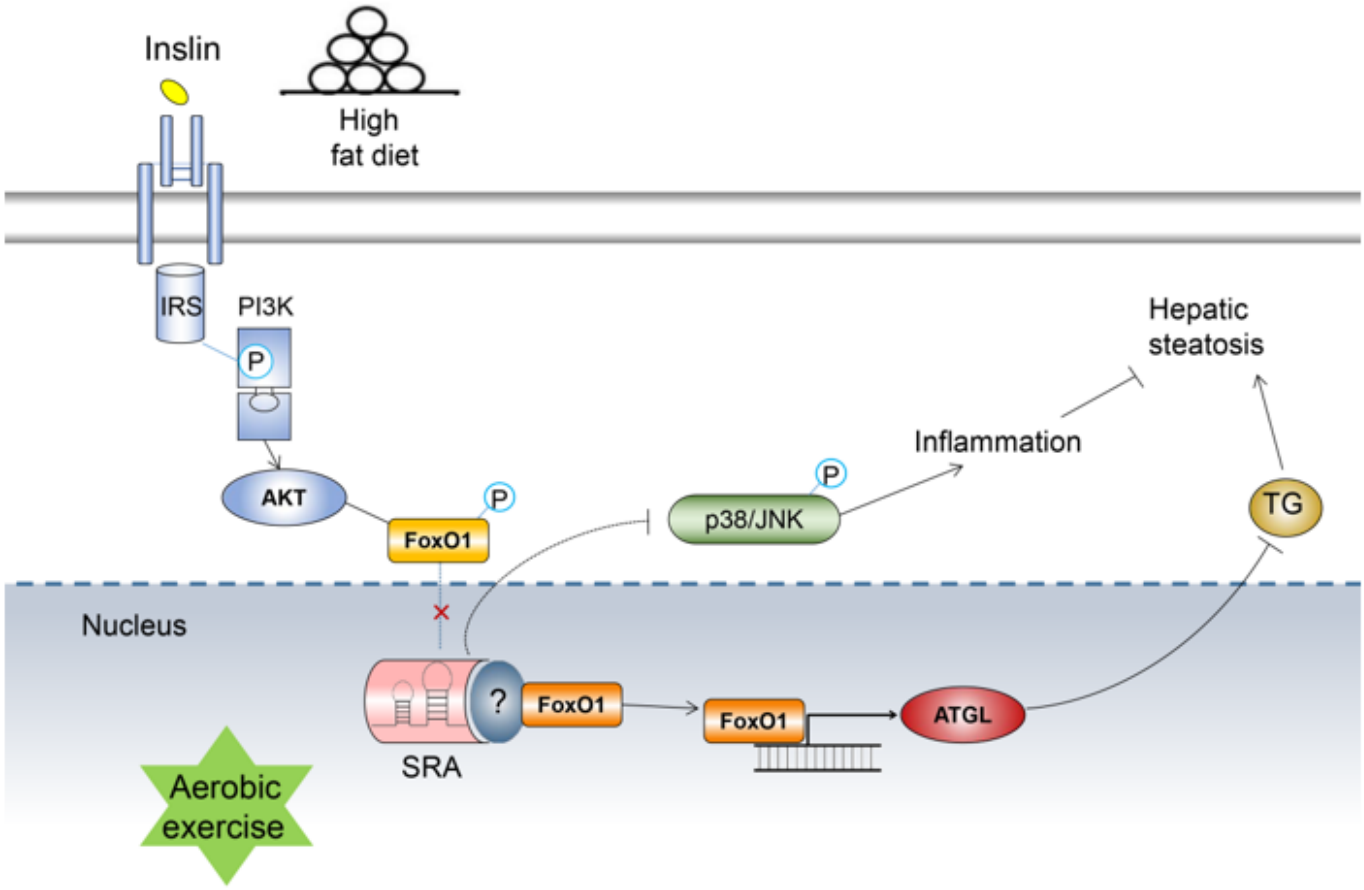

Figure 5

Aerobic exercise via SRA improves hepatic steatosis description model.