Aerobic Exercise Inhibits Inflammatory Response in Atherosclerosis via Sestrin1 Protein

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Abstract

Background Aerobic exercise plays an important role in prevention and treatment of atherosclerosis but its role in inflammatory response is not completely clear. Inflammatory response is the main pathological process during occurrence and development stage of atherosclerosis. SESNs are considered as anti-inflammation protein in atherosclerosis.

Results In current study, a high expression level of SESN1 is identified under the condition of aerobic exercise, further investigation shows levels of IL-1β/IL-6/TNF-α are significantly suppressed compared to those atherosclerosis mice with no aerobic training. Besides, we find that the activation of NK-κB signaling is impeded. Combine with our previous study, SESN1 is considered as the downstream factor of aerobic exercise which tend to inhibit the activation of inflammatory signaling and result in suppress the expression level of inflammatory factors. Another exciting finding is that MMP9/13 are also suppressed but the potential mechanism is unclear.

Conclusion Overall, present study sheds light on the significance of aerobic exercise for inflammation and stability of plaque through SESN1 may help developing new clinical treatments of atherosclerosis.

1. Introduction

Atherosclerosis-related diseases own the highest mortality and morbidity worldwide [1]. It is well-established that inflammatory response plays critical role in atherosclerosis and resulted in controlling the disease progress and plaque stability [2]. Aerobic exercise is a main primary and secondary prevention method in atherosclerosis which contributed to improving cardiac function and structure [3]. Besides preventing diseases, aerobic exercise is the most popular exercise in the world which strongly recommended by AHA, ESC, and other authorized academic society worldwide [4–5]. Last decades, abundant studies had revealed aerobic exercise contributed to improve life quality and reduce cardiac related mortality [5–6], but the underline mechanism remained uncertain.

SESNs (sestrin proteins) are highly conserved family proteins include Sestrin1/Sestrin2/Sestrin3 three isoforms which mainly induced by stress, injury, DNA damage, inflammatory response, and other disorders [7]. Study has proved that SESN1 (sestrin1) protein is upregulated and exerted anti-inflammatory response biological functions in atherosclerosis [7]. SESNs are considered to exerted protective functions during cardiovascular diseases such as atherosclerosis, AMI (acute myocardial infarction), CHD (chronic heart disease), cardiac hypertrophy, hypertension and other age-related diseases [8–10]. Previous studies revealed SESN1 regulated the level of IL-1β, NLRP3, and activation of NF-Kb mainly through AMPK(AMP-activated protein kinase)-mTROC pathway in atherosclerosis [11–12]. Yang kp et al demonstrate that up-regulating expression level of SESN1 inhibited inflammatory response and silence of SESN1 turned to aggravated this progress opposite in atherosclerosis murine [13]. Some studies have proved expression level of SESN1 protein regulated by aerobic exercise to some extent [14]. All above evidences indicated that SESN1 probably is the potential regulatory point during the

inflammatory response. Combine with the importance of aerobic exercise, SESN1 and inflammatory response in atherosclerosis, we made a hypothesis that aerobic exercise may control the inflammatory response and alleviate the immune injury by regulating the expression level of SESN1. The study report is as follows.

2. Materials And Methods

2.1 Animal model

Animal experiment was performed at Yangtze University which supervised by the Guidelines for the Use of Animals, and was approved by Yangtze University of Medicine Animal Care and Use Committee. Six- to eight-week-old male apolipoprotein E–deficient (ApoE−/−, B6.129P2-Apoetm1Unc) mice were achieved from the Animal Laboratory Center of Wuhan University. The ApoE−/− mice were fed with high-cholesterol diet (0.2% cholesterol; 21% fat weight^{-1}) for 24 weeks to establish the atherosclerosis model. All mice were divided into aerobic exercise group (AEG) and control group equally (CG) when atherosclerosis model were established successfully. All mice were fed with high-cholesterol diet while aerobic exercise group was treated with exercise daily and routinely.

2.2 Aerobic exercise protocol

We conducted an exercise capacity testing first. Graded treadmill tests were performed as previously described [15]. Exercise tolerance was assessed by graded treadmill running tests after adaptation to treadmill exercises over 1 week (10 min per day) [16]. Speed started at 6 m/min and was increased by 3 m/ min every 3 minutes until exhaustion, while mice were no longer able to continue [16]. Mice were ordered to execute an 8 weeks of moderate-intensity exercise continuous, 5 days/week. Each training consisted of 60-minute with 60% of maximal workload which achieved in graded treadmill running test [16–17]. Training mice were reevaluated for running performance at the 4th week in order to adjust aerobic exercise intensity.

2.3 Murine aorta immune cell enrichment

Aorta immune cells were enriched following published protocol with minor modifications [13, 18, 19]. Generally, mice were anesthetized with 2% halothane (Sigma-Aldrich) and perfused via cardiac puncture with ice-cold PBS. The whole aorta including branches was collected and incubated in 50μl of digestion buffer (RPMI1640 medium containing 450 U/mL collagenase type I, 125 U/mL collagenase type XI, 60 U/Ml hyaluronidase type I-s, 20mM Hepes and 60 U/mL DNase-I) for 45min. The tissue was then pressed through a 70-μm cell strainer and the resultant single-cell suspension was centrifuged at 300rcf for 5min at 4^{o}C. The cells were re-suspended in 500μl of PBS on ice before further processing.

2.4 RNA purification and rt-PCR

Cellular RNAs were prepared using the Arcturus PicoPure RNA Isolation Kit (Thermo Fisher). Aorta RNAs were prepared using the TRIzol reagent (Thermo Fisher) following the vendor's manual. cDNAs were

synthesized using the High-Capacity RNA-to-cDNATM Kit (Thermo Fisher). Real-time PCR was set using Fast SYBR Green Master Mix (Thermo Fisher) on a 7300 Real-Time PCR System (Invitrogen). Primer sequences are shown in Supporting Information (SESN1 Forward 5’ to 3’ TCAGACGGACTTTCAAACGC Reverse 5’ to 3’ CTTGGTCCGTGTCCTAGTGG).

2.5 Immunoblotting

Total cellular proteins were extracted by incubating cells in RIPA buffer (Thermo Fisher) on ice for 30min. The nuclear proteins were isolated using the Nuclear Extraction Kit (Abcam). Antibody anti-SESN1 (ab134091) was purchased from Abcam. Anti-β-actin (sc-47778) and anti-NF-κB p50 (sc-8414) were bought from Santa Cruz Biotechnology. IκBα antibody (4814), NF-κB p65 antibody (8242), phospho-IKKα/β (Ser176/180) antibody (2694), and IKKβ antibody (8943) were purchased from Cell Signaling Technology. The densitometry was done on a Biospetrum 500 imaging system (UVP, LLC) and quantified with ImageJ. The target proteins were normalized to β- actin. The normalized target proteins in treatment groups were then divided by normalized target proteins in control groups. To quantify IKKβ phosphorylation, the phosphorylated IKKβ was normalized to total IKKβ, and then normalized phosphorylated IKKβ in treatment groups were then divided by normalized phosphorylated IKKβ in control groups.

2.6 ELISA

ELISA was performed with the mouse IL-1β/IL-6/TNF-α/MMP Quantikine Kits which achieved from R&D Systems, all experiment process were followed the operating instruction.

2.7 Statistical analysis

Experimental data were presented as mean ± SD. Student’s *t*-test or one-way ANOVA was used to compare the differences among groups. $p < 0.05$ was considered significant.

3. Result

3.1 SESN1 is up-regulated after 8 weeks of aerobic exercise.

To study the effects of aerobic exercise on SESN1, we isolated the monocytes and macrophages of aorta in both two groups respectively according to lineage markers and monocyte/macrophage specific markers. mRNA of SESN1 were quantified by real-time PCR. Level of SESN1 in AEG mice were significantly elevated compared to CG as showed in Fig. 1. Our previous study had confirmed that SESN1 was up-regulated in atherosclerosis mice [13], combined with this, we supposed that aerobic exercise up-regulated SESN1 level further on.

3.2 IL-1β/IL-6/TNF-α are dramatically inhibited in AEG mice

We detected the level of IL-1β/IL-6/TNF-α by ELISA. Pro-inflammatory factors were the main cytokines in atherosclerosis which was the key regulated point in regulating inflammatory injury. Just as we expected, IL-1β expression level in AEG was significantly depressed while IL-6/TNF-α were moderated suppressed compared to CG ($p < 0.001$, $p < 0.05$ Fig. 2). Aerobic exercise down-regulated IL-1β/IL-6/TNF-α via SESN1 indirectly, contributed to exert anti-inflammatory function eventually. Since IL-1β/IL-6/TNF-α were the downstream cytokines of NF-kb, another result showed activation of NF-kb inflammatory pathway was depressed in aerobic exercise mice.

3.3 The NF-kb signaling is impeded in AEG mice

It was well-established that the activation of inflammatory reaction in atherosclerosis mainly through NF-κB pathway [21, 22]. Previous study suggested that NF-kb was regulated by SESN1 appropriately [13, 21]. In order to clarify the relationship between aerobic exercise, SESN1/NF-kb/ IκBα and phosphorylation of IKKβ were quantified. Immunoblotting images showed that IKKβ phosphorylation was significantly inhibited in aerobic exercise mice while IκBα owned a higher expression level. Nuclear NF-κB p50 and p65 were decreased which in keeping with IKKβ. Results revealed that aerobic exercise contributed to inhibiting the NF-κB pathway via SESN1 (Fig. 3).

3.4 Aerobic exercise down-regulates the level of MMP9/13

MMP (matrix metalloprotinase) has critical role in atherosclerosis plaque, especially in the pathogenesis of atherosclerosis and related vascular and cardiac conditions such as atherosclerotic plaque rupture leading to myocardial infarction [23]. Regulation the level of MMP contributed to improving the stability of plaque. Considered the importance of MMP, we performed the study in order to clarify the function of aerobic exercise and SESN1 more comprehensive. As showed in Fig. 4, level of MMP9/13 in AEG were both decreased compared with control group, it suggested that aerobic exercise may inhibit the MMP level through SESN1. Previous study indicated that SESN1 promoted phosphorylation of threonine (Thr172) resulted in activation of AMKP and contributed to depress the expression level of MMP [7, 24].

4. Discussion

Present study aims at the effects of aerobic exercise on atherosclerosis. Aerobic exercise is the crucial prevention method in atherosclerosis which recommended from growing body of evidences, but few study demonstrated the effects of aerobic exercise on inflammatory response. SESNs are a highly conserved family proteins which induced by ER, DNA damage, inflammation and others. Jun Hee Lee et al also suggested SESNs is a feedback inhibitor of TOR that prevents age-related pathologies [25].

Level of SESN1 was up-regulated prominently after 8 weeks of aerobic exercise. The exciting result showed aerobic exercise is a vivo practical method to up-regulated SESN1 which contributed to alleviating inflammatory response. Our study disclose IL-1β/IL-6 and TNF-α are suppressed in aerobic exercise mice, and overexpression of SESN1 supposed to be the main reason according to our previous study [13]. Regarding to the potential mechanism: previous study reports that SESN1 as an activator of AMPK resulted in inhibiting mTORC1 [2, 26], mTORC1 enhanced the activation of NLRP3 inflammasome

by various pathways [27, 28]. Other studies showed that AMPK modulated the inhibition of NLRP3 via mitochondrial biogenesis and the induction of autophagy [27]. Aerobic exercise activated the AMPK tend to inhibiting mTORC which eventually Inhibited the activation of NLRP3 inflammasome.

Another interesting consequence is MMP. MMP mainly resulted in plaque rupture which caused acute myocardial infarction. Despite present result showed MMP9/13 were both suppressed in aerobic exercise mice, but it still remained uncertain whether SESN1 participates the regulation of MMP since no evidence demonstrated the relationship between SESNs and MMP. The formation and structure of atherosclerotic plaque own some complex reasons such as infiltration and migration of monocytes, the expression of chemokines and the uptake of lipids. Further study need be performed in order to clarify the effects of SESN1 on the plaque.

Last intriguing finding of this study is that NF-κB signaling diminished in aerobic exercise mice, the underline mechanism due to the overexpression of SESN1 which caused by aerobic training. As far as we know, there is not exact evidence showing the regulatory role of SESN1 in NF-κB. However, SESN2 which belongs to the family proteins with SESN1, it had been proved that SESN2 positively regulated autophagy via binding to p62 or physically associating with ULK1 [13, 29]. Autophagy renders degradation of NIK and IKKs, leading to devitalization of NF-κB [30]. SESN1 may enhance autophagy in the same way as SESN2 did. Cardiovascular diseases are associated with oxidative stress strongly [31]. ROS are important biological molecules for maintaining homeostasis in the human body. Studies have proved excessive level of ROS accelerates the development of cardiovascular disorders by causing oxidative stress in the body [32]. Our previous study has revealed SESNs can be localized on the mitochondria and can control mitochondrial function via mTORC1-independent mechanisms, resulting in reduced production of ROS and cell death [7]. SESNs act as a peroxidase reductase, and SESN induction promotes the cyclic absorption of over-oxidized peroxidase, protecting cells from ROS accumulation [33].

Several limitations need be clarified. First of all, we performed 8 weeks of aerobic training without revealing a specific logical relationship between elevated protein levels and training duration. Second, as with training duration, the logical relationship between training intensity and protein expression levels was unclear. Third, the effect of aerobic training on foam cell formation, especially on lipid infiltration in the aorta, was not investigated in this study. Last, the regulatory effect of exercise on other SESNs subtypes has not been clarified in this study. In order to perfect therapeutic strategies of SESN1, above questions need be resolved. In addition to aerobic exercise, disclose the upstream and downstream pathways that underlie SESN1's multi-beneficial effects are indispensable.

5. Conclusion

Aerobic exercise up-regulates the level of SESN, contributes to suppressing levels of IL-1β, IL-6, TNF-α and NF-κb activation. Another finding was that MMP9 and MMP13 are down-regulated in aerobic exercise mice, but the role of SESN1 during this process remained query. Above biological function alleviated the inflammatory response in atherosclerosis.

Declarations

Ethics approval and consent to participate

Not applicable

Consent for publication

All authors read and approved the final manuscript. All authors agree to publish.

Availability of data and materials

All data generated or analyzed during this study are included in this published article.

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Competing interests

The authors declare that they have no competing interests.

Authors' contributions

Sun Yunfeng and Ke Li conducted most experiments and analyzed the data; Yawei Wu performed mouse model and cell culture; Yang Keping designed the study and wrote the manuscript.

Acknowledgements

Yunfeng Sun and Ke Li contribute equally to this study.

References

1. Yao B, Wan X, Zheng X, et al. Critical roles of microRNA-141-3p and CHD8 in hypoxia/reoxygenation-induced cardiomyocyte apoptosis. Cell Biosci. 2020;10:20. https://doi.org/10.1186/s13578-020-00384-5.
2. Back M, Yurdagul A Jr, Tabas I, Oorni K, Kovanen PT. Inflammation and its resolution in atherosclerosis: mediators and therapeutic opportunities. Nat Rev Cardiol. 2019;16:389–406.
3. Smith Sidney C, Benjamin Emelia J, Bonow Robert O, et al. AHA/ACCF secondary prevention and risk reduction therapy for patients with coronary and other atherosclerotic vascular disease: 2011 update: a guideline from the American Heart Association and American College of Cardiology Foundation endorsed by the World Heart Federation and the Preventive Cardiovascular Nurses Association[J]J Am Coll Cardiol, 2011, 58: 2432–46.

4. Thomas Randal J,Balady, Gary,Banka Gaurav, et al. 2018 ACC/AHA Clinical Performance and Quality Measures for Cardiac Rehabilitation: A Report of the American College of Cardiology/American Heart Association Task Force on Performance Measures.[J].Circ Cardiovasc Qual Outcomes, 2018, 11: e000037.
5. Padilha Camila S, Figueiredo Caique,Minuzzi Luciele Guerra. et al. Immunometabolic responses according to physical fitness status and lifelong exercise during aging: New roads for exercise immunology.[. J]Ageing Res Rev. 2021;68:101341.
6. Strath Scott J, Kaminsky Leonard A, Ainsworth Barbara E, et al. Guide to the assessment of physical activity: Clinical and research applications: a scientific statement from the American Heart. Association[J]Circulation. 2013;128:2259–79.
7. Sun Yunfeng,Wu Yawei,Tang Shuting. et al. Sestrin proteins in cardiovascular disease.[. J]Clin Chim Acta. 2020;508:43–6.
8. Lee JH,Budanov Andrei V, Park Eek, Joong, et al. Sestrin as a feedback inhibitor of TOR that prevents age-related pathologies.[J].Science, 2010, 327: 1223-8.
9. Dong Bin,Xue Ruicong,Sun Yu. et al. Sestrin 2 attenuates neonatal rat cardiomyocyte hypertrophy induced by phenylephrine via inhibiting ERK1/2.[J]. Mol Cell Biochem. 2017;433:113–23.
10. Tayyar AT, Tayyar A, Kozali S, Karakus R, Eser A, Yayla CA, Yalcin ET, Dag I. & Mustafa Eroglu (2018): Maternal serum sestrin 2 levels in preeclampsia and their relationship with the severity of the disease, Hypertension in Pregnancy, DOI: 10.1080/10641955.2018.1540702.
11. Parmigiani Anita,Nourbakhsh Aida,Ding Boxiao. et al. Sestrins inhibit mTORC1 kinase activation through the GATOR complex.[. J]Cell Rep. 2014;9:1281–91.
12. Liao Hai-Han,Ruan Jie-Yun,Liu Huang-Jun et al. Sestrin family may play important roles in the regulation of cardiac pathophysiology.[J].Int J Cardiol, 2016, 202: 183–4.
13. Keping Yang,Yunfeng Sun,Pengzhuo Xiao. et al. Sestrin1 inhibits oxidized low-density lipoprotein-induced activation of NLRP3 inflammasome in macrophages in a murine atherosclerosis model.[. J]Eur J Immunol. 2020;50:1154–66.
14. Sanchis-Gomar F. Sestrins: novel antioxidant and AMPK-modulating functions regulated by exercise? [J]. J Cell Physiol. 2013;228:1647–50.
15. Ferreira JC, Rolim NP, Bartholomeu JB, Gobatto CA, Kokubun E, et al. Maximal lactate steady state in running mice: effect of exercise training. Clin Exp Pharmacol Physiol. 2007;34:760–5.
16. Cunha Telma F, Bacurau Aline VN, Moreira Jose BN, et al. Exercise training prevents oxidative stress and ubiquitin-proteasome system overactivity and reverse skeletal muscle atrophy in heart failure.[. J]PLoS One. 2012;7:e41701.
17. Ferreira JC, Rolim NP, Bartholomeu JB, Gobatto CA, Kokubun E, et al. Maximal lactate steady state in running mice: effect of exercise training. Clin Exp Pharmacol Physiol. 2007;34:760–5.
18. Gjurich BN, Taghavie-Moghadam PL, Galkina EV. Flow Cytometric Analysis of Immune Cells Within Murine Aorta. Methods Mol Biol. 2015;1339:161–75.

19. Bonanno E, Mauriello A, Partenzi A, Anemona L, Spagnoli LG. Flow cytometry analysis of atherosclerotic.
20. Bonanno E, Mauriello A, Partenzi A, Anemona L, Spagnoli LG. Flow cytometry analysis of atherosclerotic plaque cells from human carotids: a validation study. Cytometry. 2000;39:158–65.
21. Moore KJ, Sheedy FJ, Fisher EA. Macrophages in atherosclerosis: a dynamic balance. Nat Rev Immunol. 2013;13:709–21.
22. Jiang Y, Wang M, Huang K, Zhang Z, Shao N, Zhang Y, Wang W, Wang S. Oxidized low-density lipoprotein induces secretion of interleukin-1beta by macrophages via reactive oxygen species-dependent NLRP3 inflammasome activation. Biochem Biophys Res Commun. 2012;425:121–6.
23. Ye Shu,Putative targeting of matrix metalloproteinase-8 in atherosclerosis.[J].Pharmacol Ther, 2015, 147: 111–22.
24. Chen Z, Wei Huafang,Zhao Xiaoling. et al. Metformin treatment alleviates polycystic ovary syndrome by decreasing the expression of MMP-2 and MMP-9 via H19/miR-29b-3p and AKT/mTOR/autophagy signaling pathways.[. J]J Cell Physiol. 2019;234:19964–76.
25. Science. 2010 March 5; 327(5970): 1223–1228. doi:10.1126/science.1182228.
26. Budanov AV, Karin M. p53 target genes sestrin1 and sestrin2 connect genotoxic stress and mTOR signaling. Cell. 2008;134:451–60.
27. Cordero MD, Williams MR, Ryffel B. AMP-Activated Protein Kinase Regulation of the NLRP3 Inflammasome during Aging. Trends Endocrinol Metab. 2018;29:8–17.
28. Ali MF, Dasari H, Van Keulen VP, Carmona EM. Canonical Stimulation of the NLRP3 Inflammasome by Fungal Antigens Links Innate and Adaptive B-Lymphocyte Responses by Modulating IL-1beta and IgM Production. Front Immunol. 2017;8:1504.
29. Cordani M, Sanchez-Alvarez M, Strippoli R, Bazhin AV, Donadelli M, Sestrins at the Interface of ROS Control and Autophagy Regulation in Health and Disease. *Oxid Med Cell Longev* 2019. 2019: 1283075.
30. Trocoli A, Djavaheri-Mergny M. The complex interplay between autophagy and NF-kappaB signaling pathways in cancer cells. Am J Cancer Res. 2011;1:629–49.
31. Cervantes Gracia K, Llanas-Cornejo D, Husi H. CVD and Oxidative Stress. J Clin Med. 2017;6:22.
32. Li Huige., Xia Ning. The role of oxidative stress in cardiovascular disease caused by social isolation and loneliness. Redox Biol. 2020;37(undefined):101585. doi:10.1016/j.redox.2020.101585.
33. S, E., et al., Role of sestrin2 in peroxide signaling in macrophages, FEBS Lett., 583 (2009) 3531–3535.

Figures

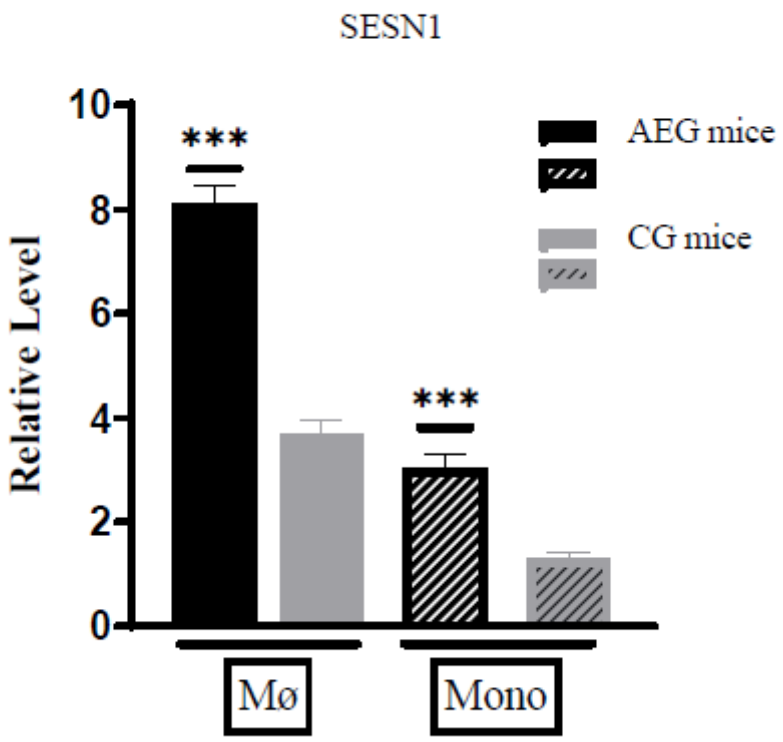

Figure 1

SESN1 was up-regulated after 8 weeks of aerobic exercise. N=7 samples per group. (A) mRNA level of sestrin1 in aortal immune cells. (B) Representative flow cytometry histograms showing the staining of SESN1 in aorta macrophages and monocytes. The data were achieved from 2 respective experiments with 1 mice each group. Mø: macrophages, Mono: monocytes. *: $p<0.05$; **: $p<0.01$; ***: $p<0.001$.

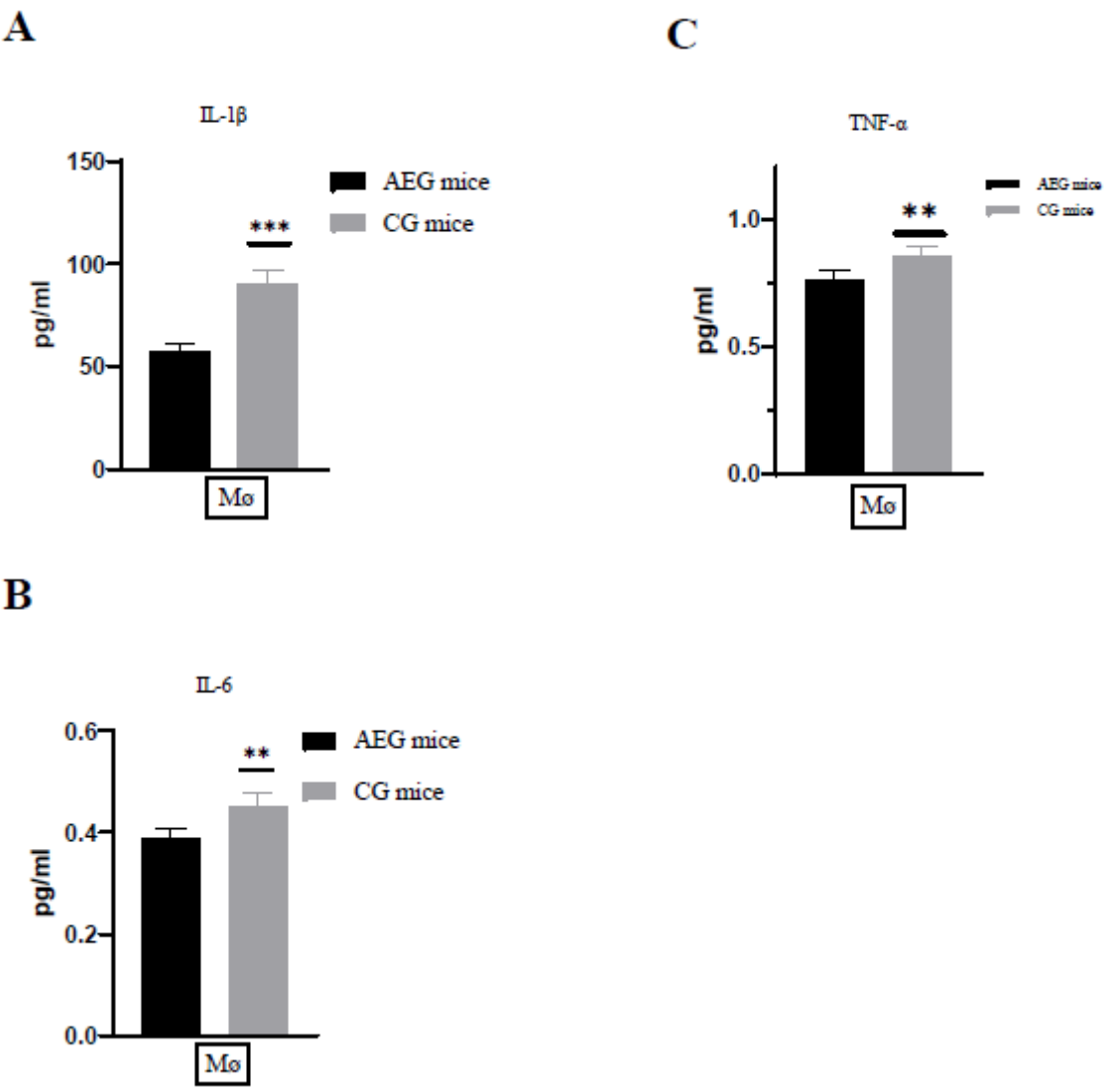

Figure 2

IL-1β/IL-6/TNF-α were dramatically inhibited in AEG mice. (A-C) Concentration of IL-1β,IL-6 and TNF-α in mice aorta macrophages. Quantified by ELISA, N=10 samples per group. *:p<0.05; **:p<0.01; ***:p<0.001.

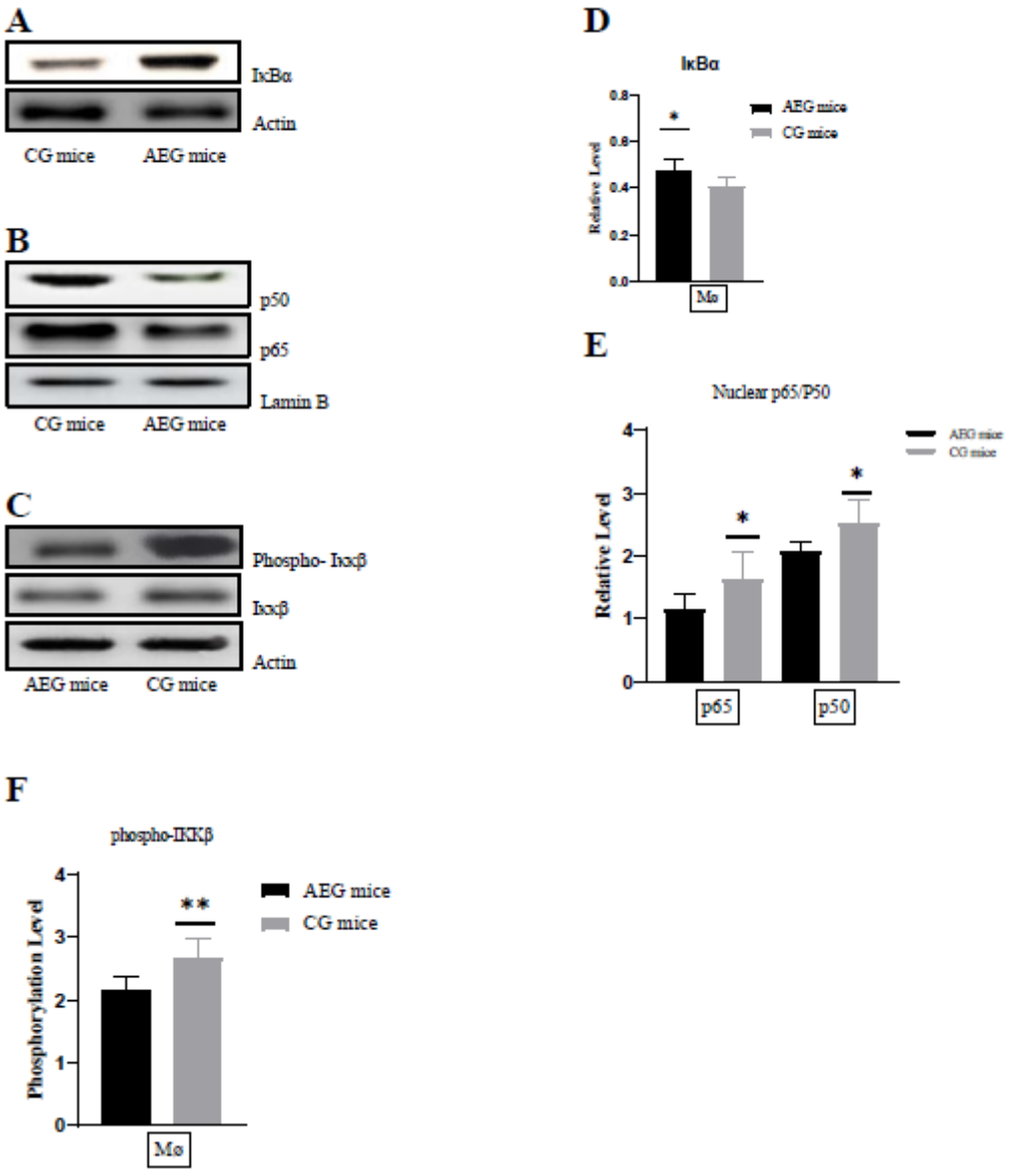

Figure 3

The NF-kb signaling impeded in AEG mice. (A) Immunoblotting images of IκBα expression in macrophages. (B) Immunoblotting images the expression of nuclear NF-κB p50 and p65 in macrophages. (C) Immunoblotting images of Iκκβ phosphorylation in macrophages. (D-F) Statistics of IκBα, p65, p50 and phosphor-Iκκβ level which presented by OD. N=10 samples in each group. *: p<0.05; **: p<0.01; ***: p<0.001.

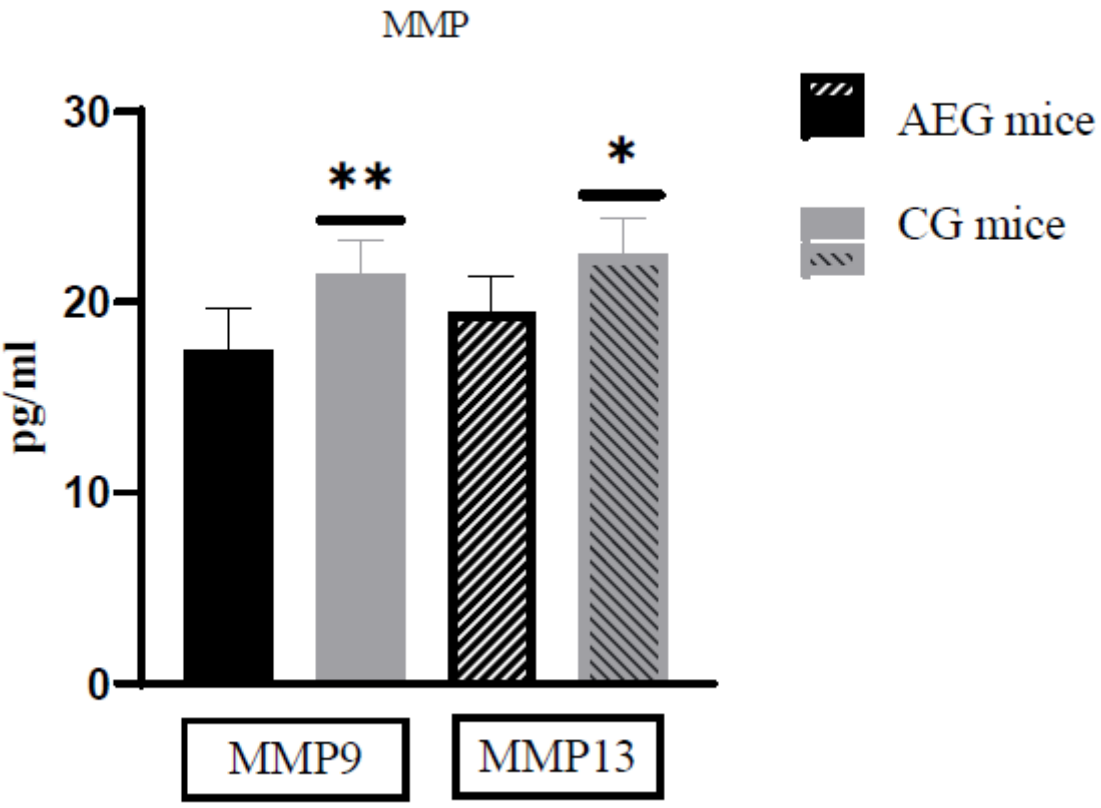

Figure 4

Aerobic exercise down regulated the level of MMP9/13. N=10 samples per group. The data are pooled from 20 independent experiments with 1 sample per group per experiment. *: $p<0.05$; **: $p<0.01$; ***: $p<0.001$. Both MMP9/13 were depressed under aerobic exercise.