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编者按 2022年北京冬奥会是促进我国体育事业发展的一次盛会，向世界展现了新时代中国的国家形象；也是振奋民族精神的重要契机，再次掀起了全民运动的热潮。运动对预防疾病和改善生活方式都具有重要的作用。合理的运动能够改善健康状况，增强体质，促进生长发育和新陈代谢，增强人体各器官的功能。对运动和健康相关的生物学原理和分子机制的研究有助于更好地选择合适的运动项目和方式。本期《生物化学与生物物理进展》刊出了7篇论文，从不同的角度探讨了运动对健康的影响以及其中的分子机制，包括运动促进健康衰老的内质网相关机制的研究，运动诱发镇痛效应、运动经外泌体防治肌少症、运动调节生物钟缓解代谢性疾病、运动调节蛋白质酰化修饰、膜感受器GPCRs在运动改善骨代谢、ESCRT系统在骨骼肌等细胞膜损伤修复机制的综述。论文选题和内容属于当前运动与健康研究领域的热点，在本期集成《运动与健康专题》，以飨读者。

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Exercise Alleviates ER Reductive Stress and Promotes Healthy Aging*

WANG Yuan-Yuan[1,2)**], QIAO Xin-Hua[1)**], SHI Chang[1,2)**], YE Ao-Jun[1,2)**], GUO Miao-Miao[1,2)], ZHAO Yu-Zheng[3)***], CHEN Chang[1,2)***]

([1)]*National Laboratory of Biomacromolecules, CAS Center for Excellence in Biomacromolecules, Institute of Biophysics, Chinese Academy of Sciences, Beijing* 100101, China;
[2)]*University of Chinese Academy of Sciences, Beijing* 100049, China;
[3)]*School of Pharmacy, East China University of Science and Technology, Shanghai* 200237, China)

Abstract **Objective** Exercise has been approved as an effective anti-aging approach. However, how exercise affects the organelle-specific redox status of the endoplasmic reticulum (ER) and whether it contributes to ER function and healthy aging are still unknown. **Methods** We constructed an ER-specific reductive stress *C. elegans* model that overexpresses *ctl-1*, a homolog of the mammalian catalase gene, to research the effect of ER reductive stress on aging at the organismal level. We then used the $Hyperion_{ER}$ probe which responds well to hydrogen peroxide to evaluate the redox status in the ER of body wall muscle during swimming and during aging. **Results** Our results show that H_2O_2 in the ER was markedly reduced during aging and the number of body bending, the life span and the stress response ability in *Pnfya-1*::*ctl-1*$_{ER}$::*mCherry C. elegans* was markedly decreased compared with that in *Pnfya-1*::*ctl-1-M*$_{ER}$::*mCherry*, indicating that ER reductive stress occurs during the aging process and ER reductive stress promotes aging at the organismal level. Both short-term and long-term exercise can increase the oxidative power of the ER in *C. elegans*, and exercise alleviates the age-related ER reductive stress and promotes healthy aging. **Conclusion** Our results demonstrate the effect of exercise on ER redox status at the organelle level for the first time and uncover a new mechanism for exercise in delaying aging at the organismal level from the redox point of view, suggesting that maintaining the oxidation power of the ER may be a valuable geroprotective strategy.

Key words exercise, endoplasmic reticulum (ER), reductive stress, aging, *C. elegans*, stress response
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Highlights

1. Endoplasmic reticulum (ER) reductive stress accelerates aging in *C. elegans*.
2. Exercise increases the oxidation power of the ER.
3. Exercise alleviates the age-related ER reductive stress and promotes healthy aging.

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** These authors contributed equally to this work.
*** Corresponding author.
CHEN Chang. Tel: 86-10-64888406, E-mail: changchen@moon.ibp.ac.cn
ZHAO Yu-Zheng. Tel: 86-21-64251287, E-mail: yuzhengzhao@ecust.edu.cn
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There are accumulated studies on the relationship between redox stress and aging. According to the free radical theory of aging, excessive free radicals and reactive oxygen species (ROS) cause direct damage to biomacromolecules and tissues, leading to aging[1]. Therefore, many antioxidant defense approaches have been explored for anti-aging. However, successful antioxidant intervention is far from expected. The free radical theory of aging is a plausible theory of aging based on oxidative stress[2]. The recently published paper emphasizes the precise nature of redox regulation and points out that redox status must be considered in the context of species, time, place, level and target. Precision redox is the key for antioxidant pharmacology; in other words, antioxidant pharmacology should apply the "5R" principle (right species, right place, right time, right level and right target) rather than nonspecific antioxidant treatments[3]. One good example is that mitochondria and cytoplasm become more oxidized, while the endoplasmic reticulum (ER) becomes more reduced in the aging process[4-5]. Reductive stress refers to the state of redox imbalance, in which reducing equivalents, such as GSH/GSSG, NADH/NAD^+, NADPH/$NADP^+$ or cysteine extremely elevated exceeded the self-equilibrium system, possibly in conjunction with extensive activation of the antioxidant system or suppression of oxidative activity[4, 6]. Our previous study shows that ER presents reductive stress in senescent human fibroblasts and ER reductive stress promotes cell senescence. More importantly, enhancement of ER oxidizing power delays cell senescence[4]. However, the role of ER oxidation power on individual aging is unclear.

Exercise has been demonstrated to be an effective anti-aging approach[7]. The anti-aging benefits of exercise manifest in multilevel aspects, including attenuating neurodegeneration, increasing numerous cardiovascular functions and bone density, improving respiratory function, and improving muscle strength and endurance[8-10]. Exercise increases the generation of reactive oxygen species and nitrogen species (RONS), which can induce antioxidants[11], DNA repair[12] and protein degradation to cope with oxidative damage[13]. H_2O_2 produced by NADPH oxidase during exercise stimulates PGC-1α activation and mitochondrial biogenesis by activating AMPK[14]. Exercise activates the ER unfolded protein response (UPR) in the skeletal muscle of mice[15], and cell death induced by ER stress could be blocked by physical exercise by elevating the UPR response[16]. ER stress-related gene and protein expression (p-PERK, XBP-1s, p-eIF2α) in individuals with endothelial dysfunction or obesity and type 2 diabetes decreases after exercise, and ER stress-mediated apoptosis and inflammatory responses are altered by exercise training[17]. However, the ER-specific characterization of redox status during exercise and whether it affects ER function and the aging process are open to be answered.

In view of the above scientific questions, we focused on the role and function of ER reductive stress in aging and whether exercise could reverse ER reductive stress and promote healthy aging. We intend to explore the specific effect of exercise on ER oxidation power and the role of ER oxidation power on individual aging. By using the ER-specific genetically encoded fluorescent H_2O_2 probe Hyperion, we observed that the ER undergoes reductive stress during aging, while exercise could alleviate reductive stress. Then, we constructed an ER-specific reductive stress *C. elegans* model with overexpression of *ctl-1*, a homolog of the mammalian catalase gene, and found that ER reductive stress promotes aging and that enhancement of ER oxidizing power by long-term exercise promotes healthy aging in *C. elegans.*

1 Materials and methods

1.1 *C. elegans* strains and culture

The *C. elegans* strains used in this study were Bristol N2 (obtained from the *Caenorhabditis elegans* Genetics Center), *oraIs001* (*Pmyo-3*:: $Hyperion_{ER}$), *oraEx001* (*Pnfya-1*:: $ctl\text{-}1_{ER}$ :: *mCherry*) and *oraEx002* (*Pnfya-1*:: $ctl\text{-}1\text{-}M_{ER}$ :: *mCherry*). *oraEx001* (*Pnfya-1*:: $ctl\text{-}1_{ER}$:: *mCherry*) and *oraEx002* (*Pnfya-1*:: $ctl\text{-}1\text{-}M_{ER}$:: *mCherry*) are abbreviated as ctl-1_{ER} and ctl-1-M_{ER} in this study. Transgenic strains were obtained as follows. $Hyperion_{ER}$ and $ctl\text{-}1_{ER}$ were generated using *Hyperion* cDNA or *C. elegans ctl-1* cDNA as templates by adding an N-terminal signal sequence of ERp44(1 – 30), a KDEL ER retention sequence was added at the C-terminus, and $ctl\text{-}1_{ER}$ was followed by a mCherry tag. The *ctl-1* inactive mutant $ctl\text{-}1\text{-}M_{ER}$ (H71A, N144A, Y354F) was generated by site-directed mutagenesis using PCR. Genes encoding $Hyperion_{ER}$ and $ctl\text{-}1_{ER}$ and $ctl\text{-}1\text{-}M_{ER}$ were cloned into L2534 vector or pPD49.26 vector (Addgene, 1686) respectively. Extrachromosomal transgenic strains were obtained by microinjection. A total of 100 mg/L transgene plasmid was injected into the gonads of *C. elegans*. The extrachromosomal arrays were integrated by exposing the animals to γ-irradiation that were subsequently backcrossed three times. The *C. elegans* strains used in this study were maintained

at 20℃ on standard nematode growth media seeded with the OP50 strain of *Escherichia coli* as their food source.

1.2 Determination of the *C. elegans* redox state by a plate reader or confocal microscopy

Redox states were detected using a microplate reader (Thermo Scientific Varioskan LUX). We measured approximately 120 *Pmyo-3*:: $Hyperion_{ER}$ *C. elegans* at 525 nm after excitation at 405 and 488 nm in a plate reader, and the ratio of 488/405 nm indicated the relative level of H_2O_2. For imaging, nematodes with the redox reporter $Hyperion_{ER}$ were mounted on 3% agarose pads on glass slides and immobilized with 2 mmol/L levamisole (Sigma). Images were taken on a Zeiss LSM710 confocal microscope using a 63×objective. Live nematodes were excited with 405 and 488 nm lasers, and the emission was detected from 500 nm to 530 nm. Individual cells of 20 animals were analyzed for each condition. Images were analyzed using Zen (Zeiss) and ImageJ (National Institutes of Health) software. Nematodes with the redox reporter $Hyperion_{ER}$ were treated with 10 mmol/L DTT or 1 mmol/L H_2O_2 as a positive control of the probe response to redox.

1.3 Confocal microscopy confirmation of subcellular localization

Worms (*Pmyo-3*:: $Hyperion_{ER}$, *Pnfya-1*:: $ctl\text{-}1_{ER}$:: *mCherry* and *Pnfya-1*:: $ctl\text{-}1\text{-}M_{ER}$ :: *mCherry*) were exposed for 24 h in combination to 10 μmol/L ER-Tracker Green or Red at 20℃ . Following 10 min intestinal clearance of fluorescent dyes on NGM agar plates, living nematodes were reversibly paralyzed on glass slides with levamisole, and confocal microscopy was used to confirm subcellular fluorescence localization.

1.4 Swim exercise protocol

Short-term swim exercise mode: the worms in the L4 stage were divided into two groups. One group was placed on a 3.5 cm unseeded NGM plate for 90 min as the control group, while the other group was placed on a 3.5 cm unseeded NGM plate flooded with 1 ml of M9 buffer to allow the worms to swim for 90 min. After 90 min, worms in the two groups were transferred to seeded NGM plates to recover.

Long-term swim exercise mode: swim exercise was performed according to the swim session 3+3+2+2 regimen as described in the *C. elegans* exercise protocol[18], which could induce key features of mammalian exercise. The 3+3+2+2 regimen was as follows: 9:00 AM, 3:00 PM, and 9:00 PM on the first two days, 9:00 AM and 9:00 PM on the last two days (90 min/session).

1.5 Egg-laying

Ten gravid adult worms were transferred to pure NGM with bacterial lawns, and the lawn was changed every day until the end of pregnancy. The hatched larvae were counted the following day.

1.6 Measurement of the level of H_2O_2 in *C. elegans*

Approximately 300 worms were collected to measure H_2O_2 using a H_2O_2 assay kit (Beyotime Biotechnology, S0038). The assays were performed according to the manufacturer's protocols. In brief, the supernatant of lytic nematodes was diluted with H_2O_2 detection buffer, and the same volume of H_2O_2 detection solution was added. The reaction was kept at 25℃ for 30 min, and the absorption at 560 nm was detected immediately by a microplate reader.

1.7 Lifespan assay

Lifespan analysis was conducted at 20℃ . A synchronized population of L1 worms was seeded onto standard nematode growth media (NGM) plates and allowed to grow until young adult worms. Approximately 100 young adult worms from each group were picked onto 10 plates containing 0.1 g/L fluorodeoxyuridine (FuDR) to suppress progeny production, and the maximum lifespan was calculated.

1.8 Motility assay

Different strains of *C. elegans* were placed in a drop of M9 and allowed to recover for 30 s, after which the number of body bends was counted for 1 min; 15 animals were counted per experiment, and the data from one representative experiment are shown.

1.9 Oxidative stress, heat-shock stress and reduction resistance assays of *C. elegans*

Synchronous young adult worms were transferred to S-basal buffer containing 200 mmol/L paraquat for 6 h at 20℃. We shook the worms every 1 h to avoid hypoxia in liquid buffer and then counted the worm number of death and survival. For heat-shock stress, young adult worms were cultured for 7 h on NGM plates at 35℃, and then the survival rate was determined. The synchronized L1 nematodes were seeded on NGM plates containing 0, 2, 4 μmol/L DTT (dithiothreitol) or 0, 2, 4 mg/L TG (thapsigargin), and then the *C. elegans* that developed into the young adult period were counted.

2 Results

2.1 Exercise alleviated ER reductive stress during aging in *C. elegans*

To study the ER redox state of the endoplasmic reticulum in *C. elegans*, we used the Hyperion probe, which has a stronger fluorescence intensity than

HyPer1-3 and responses well to hydrogen peroxide, to evaluate the redox status in the ER. We obtained a stable *C. elegans* strain *Pmyo-3*::*Hyperion*$_{ER}$, and found that Hyperion$_{ER}$ colocalized with the ER-specific tracker (Figure 1a), indicating its localization in the ER. Hyperion$_{ER}$ probes also responded well to reductive challenge with DTT and oxidative challenge with H_2O_2 (Figure 1b). We first investigated the ER redox change of the body wall muscle during aging of *C. elegans*. To address this, we synchronized *C. elegans* expressing the Hyperion sensor in the ER and determined the 488/405 nm ratios representing relative H_2O_2 level during Day 4 (young adults) and aging (Days 8, 10, and 12). The redox state of *C. elegans Pmyo-3*::*Hyperion*$_{ER}$ in different periods was measured by a microplate reader, and the ratio 488/405 nm of the Hyperion$_{ER}$ probe showed that H_2O_2 was markedly reduced on Day 8, Day 10 and Day 12 compared with that on Day 4, indicating that ER is under reductive stress during aging (Figure 1c), which is consistent with a previous report that ER shifts toward reducing conditions during aging in *C. elegans*[5]. Our previous work demonstrated that reductive stress occurs during replicative senescence[4], implying that the redox transition during aging is conserved among species.

Swim pattern was performed according to the *C. elegans* exercise protocol[18] as shown in Figure 1d. We then imaged *Pmyo-3*::*Hyperion*$_{ER}$ using confocal microscopy to test the redox state of the ER of the body wall muscle right after the short-term (90 min) swimming training. The results showed that the ratio of 488/405 nm of Hyperion$_{ER}$ was markedly increased in the swimming group compared with the control group, indicating that ER is in a more oxidized state after exercise (Figure 1e), showing that exercise alleviates ER reductive stress during aging in *C. elegans*.

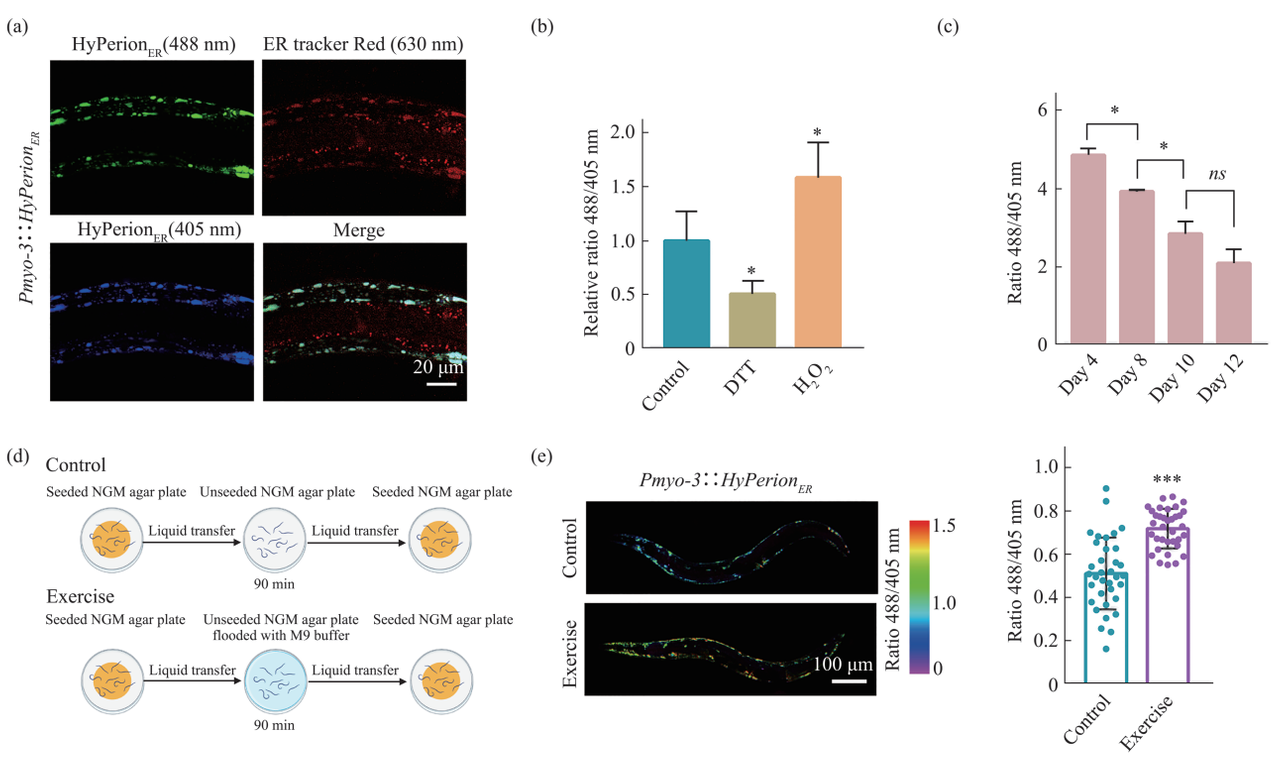

Fig. 1　Exercise alleviated ER reductive stress during aging in *C. elegans*

(a) Colocalization of Hyperion$_{ER}$ and ER tracker Red. Confocal imaging of Hyperion$_{ER}$ probe (488 nm and 405 nm) and ER tracker Red (630 nm) in *myo-3*::*Hyperion*$_{ER}$. (b) Ratiometric analysis of *myo-3*::*Hyperion*$_{ER}$ treated with 1 mmol/L H_2O_2 and 10 mmol/L DTT upon excitation at 488/405 nm by a plate reader, relative ratio 488/405 nm indicated relative H_2O_2 level. H_2O_2 and DTT treatment were used as positive controls of the probe response to redox. Data are shown as the mean±SEM (n=3). *P<0.05 by unpaired t test. (c) Ratiometric analysis of *myo-3*::*Hyperion*$_{ER}$ on Day 4, Day 8, Day 10 and Day 12 by a plate reader. Data are shown as the mean±SEM (n=3). ns means no significance. *P<0.05 by unpaired t test. (d) Schematic diagram of the 90-min swimming training program for each exercise round. Control *C. elegans* was transferred to an unseeded NGM plate for 90 min, while exercise *C. elegans* was transferred to an unseeded NGM plate filled with M9 buffer for the same 90 min to swim. (e) Left panel, confocal imaging of *Pmyo-3*:: *Hyperion*$_{ER}$ in the control and swimming groups. Right panel, ratiometric analysis of the redox status in the ER with the Hyperion$_{ER}$ probe upon excitation at 488/405 nm. Data are shown as the mean±SEM (30 *C. elegans*), n=3. ***P<0.001 by unpaired t test.

2.2　ER reductive stress decreased lifespan and body bending in *C. elegans*

To understand the function of ER reductive stress on individual aging, we constructed an ER-specific reductive stress model in *C. elegans*. *ctl-1*, a homolog to *catalase* in mammals, was overexpressed in the ER of the whole body of *C. elegans* to decrease H_2O_2 levels in the ER. Ectopically expressed catalase in the ER is enzymatically active[19]. The catalytically inactive *ctl-1* mutant ctl-1-M_{ER} (H71A, N144A, Y354F) was selected as the mock control for CAT_{ER} and described as ctl-1-M_{ER}[20]. We obtained *Pnfya-1*:: *ctl-1*$_{ER}$:: *mCherry* and *Pnfya-1*:: *ctl-1-M*$_{ER}$:: *mCherry* stains. CTL-1_{ER} and CTL-1-M_{ER} colocalized well with the ER tracker (Figure 2a). The amount of H_2O_2 generated by *Pnfya-1*:: *ctl-1*$_{ER}$:: *mCherry* was significantly lower than that generated by *Pnfya-1*::*ctl-1-M*$_{ER}$::*mCherry* (Figure 2b), showing that a reductive stress model in *C. elegans* was constructed successfully.

The effect of ER reductive stress on the lifespan of *C. elegans* was examined. There was no significant difference between *Pnfya-1*:: *ctl-1*$_{ER}$:: *mCherry* and *Pnfya-1*:: *ctl-1-M*$_{ER}$:: *mCherry C. elegans* in mortality within 20 days; however, the mortality in *Pnfya-1*::*ctl-1*$_{ER}$::*mCherry* was significantly higher than that in the control group from the 20th day. The maximum lifespan in *Pnfya-1*:: *ctl-1*$_{ER}$:: *mCherry C. elegans* was 29 days, and that in *Pnfya-1*:: *ctl-1-M*$_{ER}$:: *mCherry C. elegans* and N2 was approximately 35 days (Figure 2c). This result showed that ER reductive stress shortened the maximum lifespan in *C. elegans*.

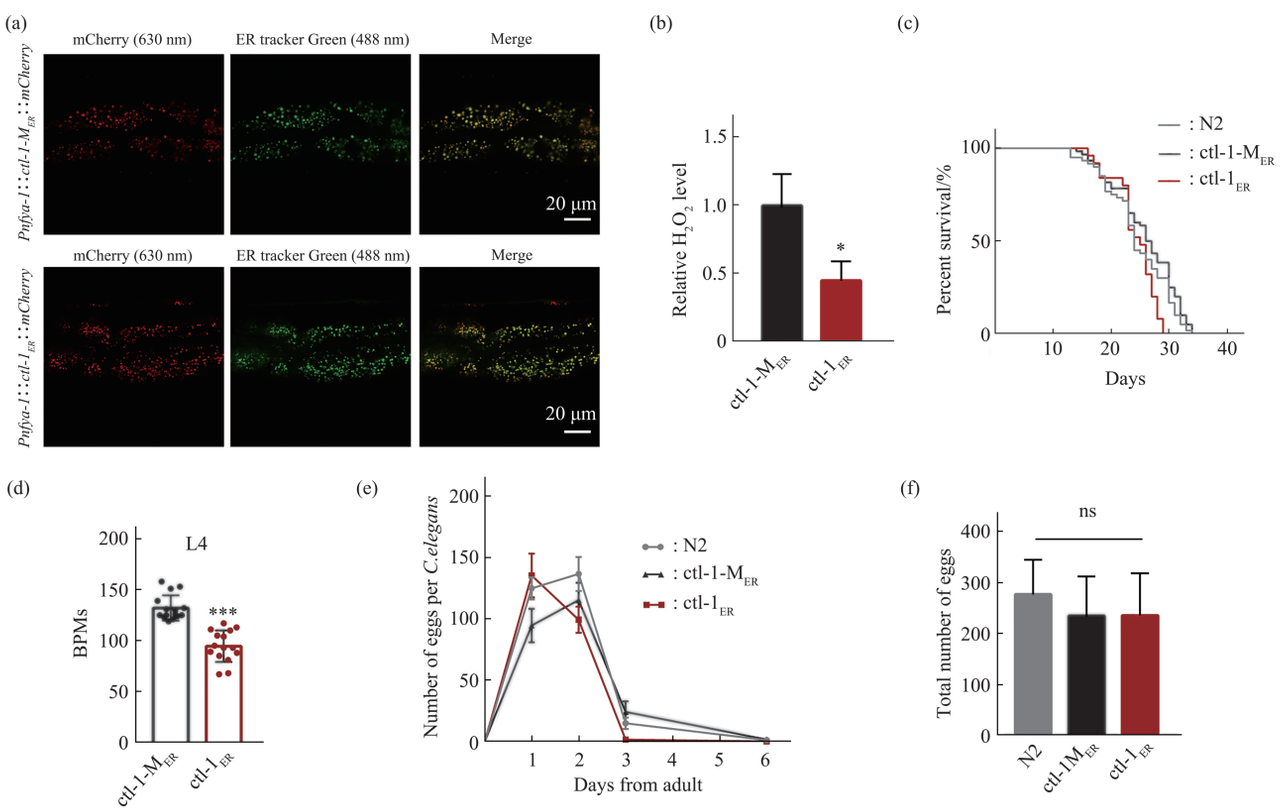

Fig. 2　ER reductive stress decreased lifespan and body bending in *C. elegans*

(a) Colocalization of CTL-1_{ER} or CTL-$1M_{ER}$ with ER tracker Green. Confocal imaging of *Pnfya-1*::*ctl-1*$_{ER}$::*mCherry* or *Pnfya-1*::*ctl-1-M*$_{ER}$::*mCherry* and ER tracker Green (488 nm). (b) H_2O_2 levels in *C. elegans* overexpressing CTL-1_{ER} and CTL-$1M_{ER}$ were measured by a hydrogen peroxide assay kit. Data are shown as the mean±SEM (200 *C. elegans*), *n*=3. **P*<0.05 by unpaired *t* test. (c) Survival curves of *Pnfya-1*::*ctl-1*$_{ER}$::*mCherry*, *Pnfya-1*::*ctl-1-M*$_{ER}$::*mCherry* and N2 *C. elegans*, and *Pnfya-1*::*ctl-1*$_{ER}$::*mCherry, C. elegans* exhibited a significant decrease in lifespan. Data are shown as the mean±SEM (100 *C. elegans*), *n*=3. *Pnfya-1*::*ctl-1*$_{ER}$::*mCherry vs*. *Pnfya-1*::*ctl-1-M*$_{ER}$::*mCherry.* (d) The frequency of body bends per minute (BPMs) of *Pnfya-1*::*ctl-1*$_{ER}$::*mCherry* and *Pnfya-1*::*ctl-1-M*$_{ER}$::*mCherry* was calculated. Data are shown as the mean±SEM (15 *C. elegans*), *n*=3. ****P*<0.001 by unpaired *t* test. (e) The daily number of eggs laid from Day 3 to Day 10 was counted (10 *C. elegans*). *n*=3. (f) The total number of eggs laid from Day 3 to Day 10 was counted (10 *C. elegans*). *n*=3. ns means no significance by unpaired *t* test.

The number of body bends per minute (BPMs) during crawling behavior is a good index to evaluate motility[21] , which partially reflects the health status of *C. elegans*[22]. The number of body bends per minute in *Pnfya-1*:: *ctl-1*$_{ER}$:: *mCherry C. elegans* was markedly decreased compared with that in *Pnfya-1*:: *ctl-1-M*$_{ER}$:: *mCherry* (Figure 2d), which demonstrated that ER reductive stress weakened the motility of *C. elegans* and that the *Pnfya-1*:: *ctl-1*$_{ER}$:: *mCherry* animals were in a suboptimal health state.

We also detected egg laying, including daily number and total number, throughout the whole life of *C. elegans*[23] and found that there was no significant difference between *Pnfya-1*:: *ctl-1*$_{ER}$ :: *mCherry* and *Pnfya-1*:: *ctl-1-M*$_{ER}$ :: *mCherry C. elegans* in the number of eggs laid (Figure 2e and 2f), showing that ER reductive stress has no obvious effect on the reproduction of *C. elegans*.

The results above proved that we successfully constructed an ER-specific reductive stress model in *C. elegans,* and ER reductive stress decreased lifespan and body bending in *C. elegans*, indicating that ER reductive stress was harmful to health and accelerated aging.

2.3 ER reductive stress decreased the stress response in *C. elegans*

The stress response capacity declines with aging[24]. The ability to respond to ER stress was compared in the control and reductive stress models. The synchronized L1 nematodes were placed on NGM plates containing DTT (2 μmol/L, 4 μmol/L) or TG (2 mg/L, 4 mg/L) to grow (including OP50), and the proportion of *C. elegans that* developed to adults was compared. The proportion of *Pnfya-1*:: *ctl-1*$_{ER}$ :: *mCherry C. elegans* that developed to adults was lower than that of *Pnfya-1*:: *ctl-1-M*$_{ER}$:: *mCherry C. elegans* (Figure 3a and 3b). This result indicated that the response to ER stress was decreased in the ER reductive stress model. Meanwhile, the antioxidative stress ability of nematodes was studied. When facing acute oxidative stress, the survival ability of *Pnfya-1*:: *ctl-1*$_{ER}$:: *mCherry C. elegans* was significantly lower than that of *Pnfya-1*:: *ctl-1-M*$_{ER}$:: *mCherry C. elegans* (Figure 3c). The survival rate under heat-shock stress (35℃ , 7 h) had no significant difference between *Pnfya-1*:: *ctl-1*$_{ER}$:: *mCherry* and *Pnfya-1*:: *ctl-1-M*$_{ER}$::

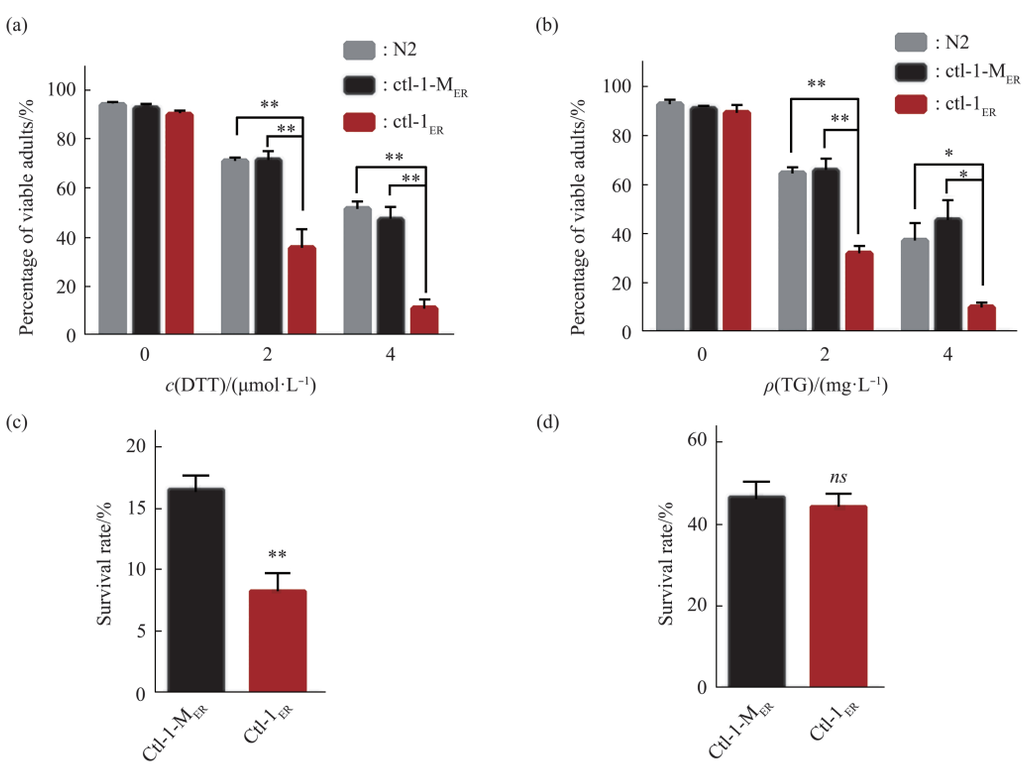

Fig. 3 ER reductive stress decreased the stress response in *C. elegans*

(a, b) The ratio of adults/L4 in *C. elegans* overexpressing CTL-1$_{ER}$ and CTL-1-M$_{ER}$. *C. elegans* were treated with different concentrations of DTT (0, 2, 4 μmol/L) or TG (0, 2, 4 mg/L) at L1, and the number of worms that developed into adults was calculated. Data are shown as the mean±SEM (200 *C. elegans*), *n*=3. **P*<0.05, ***P*<0.01 by unpaired *t* test. (c) Survival rate of *C. elegans* overexpressing CTL-1-M$_{ER}$ and CTL-1$_{ER}$ in the presence of 200 mmol/L paraquat (PQ). Data are shown as the mean±SEM (200 *C. elegans*), *n*=3. ***P*<0.01 by unpaired *t* test. (d) Survival rate of *C. elegans* overexpressing CTL-1-M$_{ER}$ and CTL-1$_{ER}$ under heat stress (35℃) for 7 h. *n* = 3, *ns* means no significance by unpaired *t* test.

mCherry C. elegans (Figure 3d).

Taken together, these results indicate that the stress response ability is compromised in the *C. elegans* under ER reductive stress.

2.4 Exercise improves oxidation power in the ER and healthy span in *C. elegans*

Next, we explored whether long-term exercise could improve the oxidation power of ER of the body wall muscle and improve their health span. We used exercise mode "3+3+2+2" to train *C. elegans* persistently for 4 days, and the results showed that the H_2O_2 level in the exercise group was markedly higher than that in the control group on Day 10 (Figure 4a), proving that ER oxidation power is increased after long-term exercise and that both immediate and long-term exercise could increase the oxidative power of ER. The motility of *C. elegans* on Day 8 was also evaluated using the frequency of BPMs, and we found that BPMs in the exercise group were markedly increased compared with those in the nonexercised group but similar to those in the young group on Day 4 (Figure 4b), indicating that motility in young *C. elegans* can be maintained through exercise. In order to prove that the improvement of motility after swim exercise is dependent on the increase of ER oxidative power, we tested the effect of swim exercise on ctl-1-M_{ER} and ctl-1_{ER} *C. elegans.* The BPMs on Day 10 were evaluated after long-term exercise training in "3 + 3 + 2 + 2" mode. The results showed that in the *Pnfya-1*:: *ctl-1-*M_{ER}:: *mCherry C. elegans* strain, BPMs were markedly increased in the exercise group compared to the nonexercised control group, which is similar to the effect in the wild type *C. elegans,* while in the *Pnfya-1*:: *ctl-*1_{ER}:: *mCherry C. elegans* strain, BPMs had no significant difference in the exercise group compared to the nonexercised control group (Figure 4c). These results suggest that the exercise benefit depends on the ER oxidation power improvement.

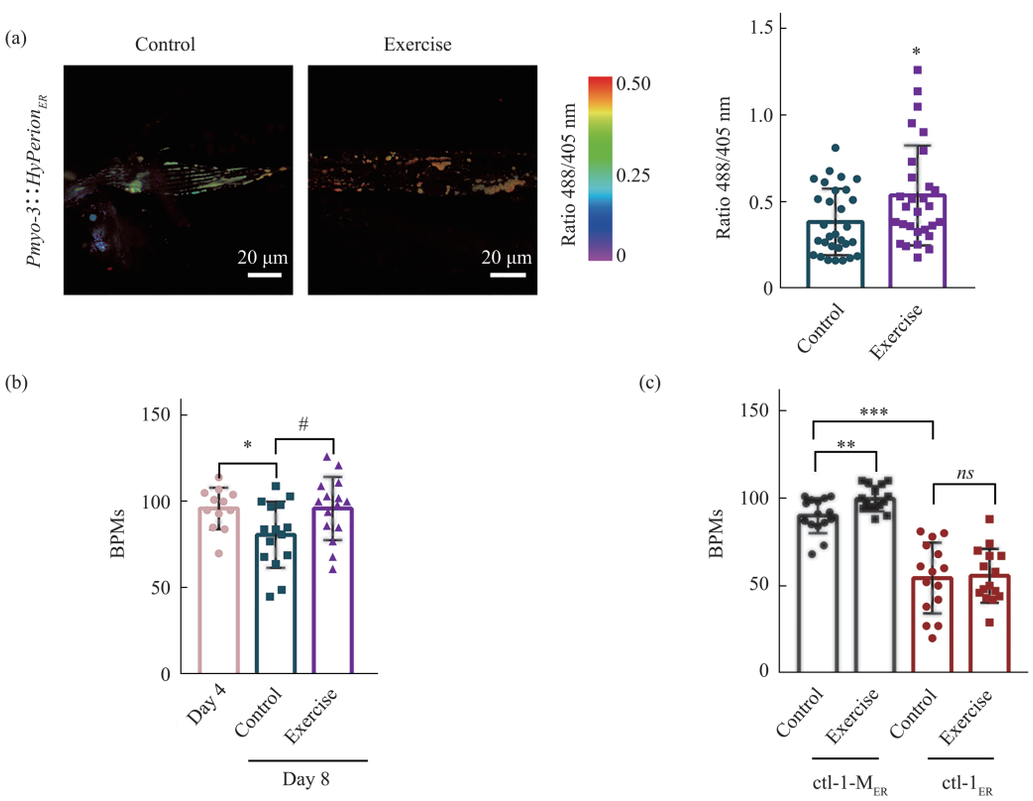

Fig. 4　Exercise improves oxidation power in the ER and healthy span in *C. elegans*

(a) ER redox states in *myo-3*::*Hyperion*$_{ER}$ in the control group and the long-term (3+3+2+2 regimen) exercise group on Day 10. Data are shown as the mean±SEM (15 *C. elegans*), two data points were collected per nematode, n=3, **P*<0.05 by unpaired *t* test. (b) The frequency of body bends per minute (BPMs) of *myo-3*::*Hyperion*$_{ER}$ in the control group and the long-term exercise group at Day 8 was calculated, and no-exercise group at Day 4 as a positive control. Data are shown as the mean±SEM (15 *C. elegans*), n=3. **P*<0.05 by unpaired *t* test. (c) The frequency of BPMs of ctl-1-M_{ER} or ctl-1_{ER} in the control group and the long-term exercise group at Day 10 was calculated. Data are shown as the mean±SEM (15 *C. elegans*), n=3. ***P*<0.01, ****P*<0.001, ns means no significance by unpaired *t* test.

3 Discussion

Exercise plays a positive anti-aging role. Understanding the mechanism of redox regulation is still to be elucidated. How exercise affects ER redox status and whether it contributes to ER function and healthy aging are still unknown. In this study, we found that in *C. elegans*, the ER presents in a more oxidized state after a period of swimming exercise, as indicated with the ER-specific H_2O_2 probe Hyperion. The ER suffers reductive stress during the aging process in *C. elegans* which is consistent with previous study that ER presents reductive stress in senescent human fibroblasts[4] and aged *C. elegans*, showing that the redox state change of ER during aging is conservative in humans and *C. elegans*[5]. ER reductive stress accelerates the aging of *C. elegans*, as evidenced in the constructed ER-specific reductive stress model ctl-1_{ER}. The delighting results showed that long-term exercise markedly hindered age-related ER reductive stress and promoted healthy aging. We first demonstrate that ER reductive stress promotes individual aging and that the oxidative power in the ER endowed by exercise indeed contributes to healthy aging.

Considering the effect of exercise on redox status, there are many reports that exercise increases the generation of reactive oxygen species and nitrogen species (RONS), which is a global description of the change in cell redox, rather than organelle-specific evaluation. ROS levels in mitochondria have been shown to increase during exercise[25-26]. However, the effect of exercise on ER is unknown. Since oxidative protein folding is processed in the ER, the relatively oxidizing environment in the ER is beneficial for disulfide bond formation in secretory and membrane proteins and avoids unfolded protein aggregation. From our results, we can see that ER oxidation power is significantly increased after 4 days of exercise, as indicated by the elevated H_2O_2 level, and exercised *C. elegans* behaved more actively, with a much higher rate of body bending, than nonexercised *C. elegans*. More strikingly, the exercised *C. elegans* at Day 8 behaved as young as those at Day 4, as evidenced by the similar BPMs. Our results confirmed the importance and effectiveness of the “5R” principle of precision redox regulation and suggested that strategies for improving the oxidative power of the ER might be considered in geroprotective strategies in the future.

Following the concept of precision redox[3], we used ER-specific genetically encoded redox fluorescent probe-$Hyperion_{ER}$ as the precision tool to sense H_2O_2 in the ER in the process of aging and exercise and constructed an organelle-specific reductive stress model. The previous reports are most global reductive stress models, for example, with antioxidant treatment, or interfering small HSPs, G6PD and Nrf2 pathways[27-28]. One exception is an ER-specific reductive stress model we previously constructed by ER-specific overexpression of catalase in human fibroblast cells[4]. Animal model of Alzheimer’s disease (APP/PS1 transgenic mice) was regarded as a reductive stress model because individuals at high risk for Alzheimer’s disease suffer reductive stress before the onset of the disease, as indicated by an increase in GSH/GSSG levels in serum[29]. Chronic proteotoxic stress caused by expressing the aggregation-prone and disease-associated proteins β23-mCherry, $A\beta_{1-42}$ and Q40-RFP in muscle tissue of *C. elegans* leads to a shift toward reducing conditions in the ER and a shift toward more oxidizing conditions in the cytosol, in which more than one organelle was affected. Therefore, the organelle-specific reductive stress models are encouraged for future relevant studies.

4 Conclusion

Our study revealed that ER reductive stress accelerates the aging of *C. elegans* and exercise increases the oxidation power of the ER and alleviates the age-related ER reductive stress and promotes healthy aging. We provide a new mechanism for exercise to delay aging from the redox view. Since this study is in the *C. elegans* model, the ER redox states improved by exercise need to be validated in other mammal or primate models or human beings in the future. Moreover, strategies to specifically increase ER oxidation power are of great significance for application. It is also worth testing this mechanism and intervention in other ER reductive stress models. More precision geroprotective strategies are expected to be implemented.

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锻炼缓解内质网还原应激从而促进健康衰老*

王圆圆[1,2)**]　乔新华[1)**]　时　畅[1,2)**]　叶傲君[1,2)**]　郭苗苗[1,2)]　赵玉政[3)***]　陈　畅[1,2)***]

（[1)] 中国科学院生物物理研究所，生物大分子国家重点实验室，北京 100101；
[2)] 中国科学院大学，北京 100049；[3)] 华东理工大学药学院，上海 200237）

摘要　目的　锻炼是延缓衰老的有效策略，本工作的目的在于探索锻炼是如何在细胞器水平影响内质网的氧化还原状态，以及内质网氧化还原状态是否影响个体衰老。**方法**　利用定位于内质网响应过氧化氢的Hyperion探针检测线虫衰老过程中及经过游泳运动后体壁肌肉内质网的氧化还原状态。通过在线虫内质网中特异过表达哺乳动物过氧化氢酶的同源基因 *ctl-1* 构建内质网特异的还原应激模型，研究了内质网还原应激对个体衰老的影响。线虫的健康状态以线虫寿命、身体摆动次数及对压力的响应能力为判断指标进行表征。**结果**　用$Hyperion_{ER}$探针检测发现，衰老线虫的内质网中过氧化氢水平相比与年轻线虫显著降低，表明内质网在衰老过程中发生了还原应激。线虫经过短时90 min游泳运动及长时期4 d（3次+3次+2次+2次，90 min/次）的游泳运动都可以增加内质网的氧化力。相比于对照，内质网还原应激的线虫寿命缩短，身体摆动次数降低，应对压力的响应能力下降，表明内质网还原应激加速线虫衰老。进一步研究发现，长时期的锻炼可以提高内质网的氧化力，缓解衰老相关的内质网还原应激，经过锻炼的第8天的线虫运动活力显著高于未锻炼的第8天的线虫，而与第4天的线虫相似，表明锻炼延缓了线虫衰老。**结论**　本工作揭示了锻炼对内质网氧化还原状态的调控，在个体水平发现内质网还原应激促进衰老，发现锻炼能增加内质网氧化力，改善衰老相关的内质网还原应激，促进健康衰老。本研究阐明了锻炼在细胞器水平对氧化还原的精准调控，从氧化还原角度揭示了锻炼促进健康衰老的新机制，提示维持内质网的氧化力是延缓衰老的潜在策略。

关键词　锻炼，内质网，还原应激，衰老，线虫，应激反应
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** 并列第一作者。
*** 通讯联系人。
陈畅　Tel：010-64888406，E-mail：changchen@moon.ibp.ac.cn
赵玉政　Tel：021-64251287，E-mail：yuzhengzhao@ecust.edu.cn
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