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Long-term Hypogeomagnetic Field Exposure Reduces Muscular Mitochondrial Function and Exercise Capacity in Adult Male Mice*

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Abstract The hypogeomagnetic field (HMF) is one of the key risk factors for manned deep space missions. It has been reported that HMF exposure affects multiple exercise-related behaviors of animals, but the long-term effects of HMF on the exercise capacity of adult humans need further investigation to determine the potential risk of HMF exposure during deep space missions. In this study, we reared adult male C57BL/6 mice in an HMF for one month, simulated by a 3-axis Helmholtz coil system, and examined the effects on their exercise capacities at behavioral, tissue, cellular, and molecular levels. Compared with that of the control mice reared in the geomagnetic field, the endurance of the HMF-exposed mice was significantly reduced. Decreases were also observed in the citric acid level and the number of subsarcolemmal mitochondria in skeletal muscles, and morphological alterations of skeletal mitochondria were noted. These results indicate an HMF-induced inhibition of muscular mitochondrial function and an endurance-related decline in the process of energy metabolism. Our findings provide direct *in vivo* evidence to demonstrate that mitochondria can respond to HMF exposure and that mitochondrion-related indices could be used for risk evaluation and the development of methods of counteraction.

Key words geomagnetic field, hypogeomagnetic field, exercise capacity, skeletal muscle, energy metabolism, mitochondria
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Accumulating evidence suggests that a hypomagnetic field (HMF, also called hypogeomagnetic field, HGMF, defined as <5 μT, see Mo *et al*.[1]) affects the normal functioning of animals in many ways, such as embryonic development[2-4], emotion[5], learning and memory[6-9], cognition[10-11], circadian rhythm[12-13], and bone metabolism[14-15]. Given that the environmental magnetic fields in outer space, including the Moon and Mars, are hypogeomagnetic (much weaker than the Earth's geomagnetic field; GMF ~ 50 μT), the potential adverse effect on living organisms, especially on the behavior of human beings, has been a serious concern since the beginning of manned space projects[10-11]. Recently, the HMF has been accepted as a risk factor for long-term, manned deep space missions. In ground-based simulations, the adverse effects of long-term HMF exposure on the general activities of animals have been recorded and confirmed[13]. Early in 1968, Busby[16] reported that Halpern and Van Dyke had observed Swiss/Webster white mice and their progeny, reared in the HMF simulated by μ-metal

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cylinders (<100 nT), were less active than GMF controls. Kopanev *et al*. [17] and Li *et al*. [18] independently observed similar effects in rabbits (~83 nT) and hamsters (~50 nT), respectively. In these investigations, the behavior responses of animals to HMF exposure were recorded, along with any HMF-induced alterations to embryonic development. Newborn animals also were used[17] or HMF exposures were applied over several generations[16,18]. Further studies on the effect of long-term HMF exposure on adult animals are worthwhile, for the evaluation of potential risk to humans.

In adult animals, nervous and uneasy behavior was observed after short-term HMF exposure (10 min[19], 1 day[20], or 3 days[5]). In 1989, Levina *et al*.[21] reared adult male rats in an HMF (<300 nT) for 3 months and found a significant decrease in the work capacity, endurance, and behavioral activities in the HMF-exposed rats. However, the responses of animals to short-term HMF exposure are most likely to be considered as stress reactions[5]. Recently, we found that a month of HMF exposure inhibits the general activities of adult, male C57BL/6 mice, without depression-like or anxiety-related behaviors[13]. Further systematic investigations would add to our understanding of the underlying mechanism of long-term bio-HMF interaction.

In this study, taking advantage of our previously constructed HMF-exposure system[13,22], we examined the effects of one month of HMF exposure (<500 nT) on adult C57BL/6 mice by evaluating parameters of exercise capacity, such as endurance, coordination, and grip capacity. Our results showed that one-month HMF-exposure significantly reduced locomotion, especially the endurance of the mice. Further analysis of the function and structure of the skeletal muscle revealed a significant decrease in citric acid level, as well as alterations in the number and morphology of mitochondria. Our findings suggest that an HMF could induce the inhibition of sustained activity in adult animals and that the HMF-induced change to mitochondrial function is a key part of this process.

1 Materials and Methods

1.1 HMF simulation

The HMF for animal rearing was simulated using a 3-axis Helmholtz coil system (HCS, diameter=2 m), which compensated for the local GMF in three directions, as previously described[13](Figure 1a). The GMF-control animals were reared on a wooden table in the same room, 1.5 m away from the HCS (Figure 1b). The static(SMF) and alternative(AMF) magnetic fields was measured by a 3-axis Fluxgate Magnetometer and an AC induction magnetometer, constructed by the National Space Science Center, Chinese Academy of Sciences(CAS) [23] (Beijing, China). The HMF-exposed groups were reared in a region with a residue SMF intensity of <500 nT, and the average SMF intensity was (53.0±2.0) μT for the GMF controls (Figure 1c, d, Table 1).

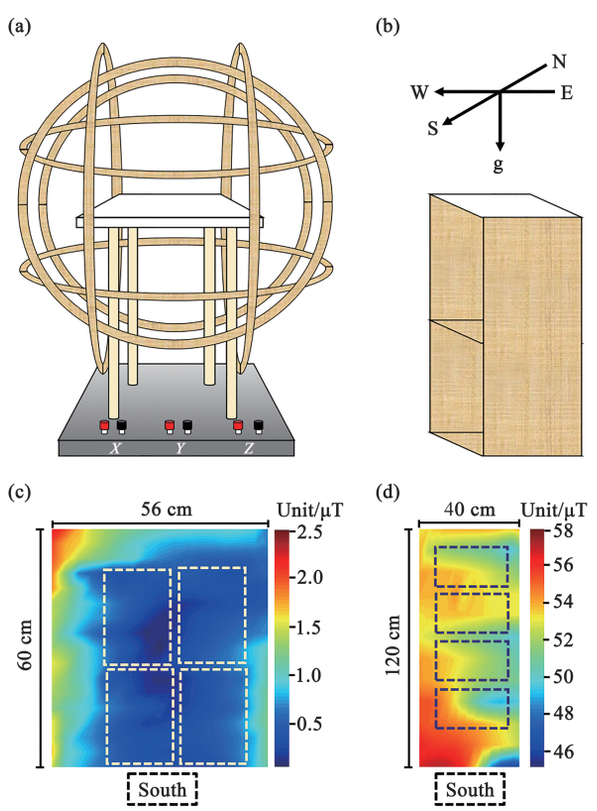

Fig. 1 Experimental setups

（a） The Helmholtz coil system （HCS） simulates the HMF condition. （b） The wooden table for the GMF-control groups. Directions of the magnetic fields: S, southward; N, northward; E, eastward; W, westward; g, downward. （c） The HMF-exposed animals were reared on the central plane of the HCS with residual magnetic field <500 nT, as indicated by the dashed white rectangles. （d） The GMF controls were reared on top of the wooden table as indicated by the dashed blue rectangles.

Table 1 The magnetic field conditions[1)]

		GMF	HMF
SMF	|B| [2)]（μT）	53.0±2.0	0.69±0.43[3)]
AMF	|B|（μT）	0.69±0.0	0.23±0.0
	Dominant frequency（Hz）	120	120

[1)] Data are shown as mean±SD. SMF: *n*=6, AMF: *n*=6. [2)] The vector sum of the magnetic field in three directions. [3)] The average SMF of the central plane in the HCS. Animals were reared where SMF< 500 nT, as indicated in Figure 1b.

1.2 Animal rearing

Adult (4−6 weeks old) male C57BL/6 mice were obtained from Vital River Laboratory Animal Technology (Beijing, China). Four mice were housed in one standard "shoebox" cage. The metal covers of the animal cages were replaced with plastic covers. The animal rearing conditions were maintained at (21±1)℃ and 40%−60% humidity with a 12 h∶12 h light∶dark rhythm (light on at 9:00 a.m.). Food and water were provided *ad libitum* throughout the experiment. Bedding was replaced once a week. Animals were acclimated in the experiment room for 7 days before being randomly assigned to the GMF or HMF groups.

For behavior tests and biochemical assays, animals were exposed to the HMF or GMF for 30 days. The tissue samples for real-time PCR, biochemical assays, and electron-microscopy were dissected from a hind limb. All procedures were performed in accordance with the principles and guidelines established by the Animal Welfare and Research Ethics Committee of the Institute of Biophysics, CAS (Permit Number: SYXK2014-32).

1.3 Behavior tests

Animals were habituated in the test room for at least 2 h before the tests. After each test, the whole apparatus was cleaned thoroughly with 70% alcohol, to eliminate the odor or trace of the animals. The data were analyzed by experimenters who were blind to the experimental designs.

1.3.1 Rotarod test

Motor coordination of the mice was measured with a rotarod apparatus (Panlab Harvard Apparatus, MA, USA). Mice were trained to keep balance on the rotarod for 3 days in the test room(GMF) at a low speed of 4 r/min). The training procedure was set as 10 min/trial, three trials per day, with a 2 h interval between trials (training time: 9:00−10:00, 12:00−13:00, 15:00−16:00). On the fourth day, the mice were tested on the rotarod at a speed uniformly accelerated from 4 to 40 r/min in 5 min. The latency time for each mouse to fall from the rod was recorded.

1.3.2 Treadmill test

Animals were forced to run until exhausted on a 5-lane treadmill with no inclination (Panlab Harvard Apparatus, MA, USA). Animals were habituated in the lanes for 15 min with the power off. The rolling belt was set at 5 cm/s to warm up the animals for 5 min and the shock grid was set at 0.2 mA. Animals kept running on the belt to escape from the shock grid. In the test session, the speed of the rolling belt was accelerated from a starting speed of 5 cm/s, increasing by 1 cm/s every 10 s, to a maximum speed of 50 cm/s. Animals were identified as exhausted when any one of the following three criteria was met: (1) staying on the shock grid for 5 s without any attempt to escape; (2) staying on the shock grid for 2−5 s, three times; (3) staying on the shock grid <2 s, but the time in the shock grid was longer than the time running on the belt. The distance travelled before exhaustion was recorded.

1.3.3 Forelimb grip strength test

The forelimb grip strength was measured with an automatic grip strength meter(BIOSEB, FL, USA), as previously described[24-25]. The mouse was lifted by the tail, close to the device's grid, and lowered until it grasped the grid with its forepaws. Then the experimenter carefully pulled the mouse away from the grid until it let go. The maximum forelimb grip strength was measured 5 times consecutively, and the mean was calculated as the outcome of one trial. One trial was executed per mouse.

1.4 The body composition test

The body composition of each mouse was tested with EchoMRI 100 (EchoMRI, TX, USA), as described previously[26]. The machine was calibrated with pure water and vegetable oil.

1.5 High performance liquid chromatography（HPLC）

HPLC was used to determine the concentration of citric and lactic acids in the muscle tissue. The freshly dissected hindlimb muscles were homogenized by a grinder in RIPA lysis buffer (Beyotime, China) with a weight/volume ratio of 1 kg/10 L. The mixtures were then incubated on ice for 20 min and centrifuged at 15 000 *g*, at 4℃ for 10 min. The supernatant was collected and heated at 85℃ for

5 min to break down the proteins or any other sediments. Following a 15 000 *g* centrifugation at 4℃ for 10 min, the supernatants were collected for the preparation of HPLC detection samples for citric and lactic acids (LC-20A, Shimadzu, Japan; HC-C18 column (5 μm), Agilent Technologies, CA, USA) according to the following protocol: 10 min incubation at 95℃, centrifuge at 15 000 *g* for 10 min, collect the supernatant, filter with pore size 0.22 μm. The mobile phase was phosphate buffer solution with 5% methanol(pH adjusted to 2.5 with phosphoric acid). The HPLC system was operated at a flow rate of 1 ml/min with 10 min detection time. The column temperature was set to 35℃ . The detection wavelength was 210 nm. The retention time, and concentration of citric acid and lactic acid were determined according to the standard curves, as described previously[27-28].

1.6 Mitochondrial quantification

The total number of mitochondria in the muscle samples were determined by mitochondrial DNA quantification by real-time PCR, as described previously[29]. Total genomic DNA was extracted from the muscle samples using the DNeasy Blood & Tissue Handbook (Qiagen, Germany), according to the manufacturer's instructions. The DNA concentration was measured with Nanodrop 2000 (Thermo Fisher scientific, MA, USA), and then adjusted to 10 mg/L.

The real-time PCR examination was performed using the EvaGreen 2× qPCR MasterMix kit (Abm, Canada) on a Rotor-Gene Q (Qiagen, Germany). The reactions were performed using the following protocol: pre-incubation at 95°C for 10 min (1 cycle); then 35 cycles with denaturation at 95°C for 3 s, and annealing at 60°C for 30 s. Data was calculated by the $2^{-\triangle\triangle Ct}$ method. The primers used for the real-time PCR reactions are listed in Table S1.

1.7 Transmission electron microscopy（TEM）

The muscle samples were dissected and immediately fixed with 2.5% glutaraldehyde at 4℃ overnight, followed by a post-fixation in 1% osimium tetroxide for 2 h at 4℃ . Then the samples were sequentially dehydrating with graded ethanol washes and immersed in SPI-PON812 resin. The ultrathin sections were counterstained. Images were scanned using a FEI Tecnai spirit transmission electron microscope(Tecnai Spirit, Netherlands).

Details of image capture and processing procedures are available in the work of Jean-Philippe Leduc-Gaudet[30]. Briefly, the subsarcolemmal (SS) and intermyofibrillar(IMF) mitochondria in the soleus muscles of 3 mice from each group were captured with a Morada G3 CCD digital camera (EMSIS, Germany) at 11 000× magnification. Each mitochondrion was manually traced in the longitudinal orientation using ImageJ(NIH, USA), then the area, perimeter, aspect ratio, circularity, form factor and Feret's diameter of each mitochondrion were measured, as previously reported[30]. The morphology and shape descriptors were calculated as: area(μm^2), perimeter(μm), circularity (4π(area/perimeter^2)), Feret's diameter(μm), aspect ratio(major axis/minor axis), and form factor ((perimeter)/(4π · area)).

The length of sarcomeres was measured on the TEM images with ImageJ. The numbers of the SS and IMF mitochondria were counted on the TEM images separately, with ImageJ, as the number of mitochondria present in each field of view (FOV) (10 μm×10 μm).

1.8 Statistical analyses

The data from total mitochondrial quantification, citric acid and lactic acid assays and behavioral tests were analyzed with student's *t*-test. The length of sarcomere, the body compositions, and the number of the IMF and SS mitochondria were evaluated using the Mann-Whitney test. The distributions of the mitochondrial shape descriptor values were analyzed with the Kolmogorov-Smirnov test. The means of mitochondrial shape parameters were compared with one-way ANOVA, with Sidak's multiple comparison test. All statistical analyses were performed using Prism 7 (GraphPad Software, CA, USA) [30]. Values were expressed as mean±standard error mean(S. E. M.). The differences were considered significant when $P<0.05$.

2 Results

2.1 One−month HMF exposure inhibits animal exercise capacity

To examine the effects of long-term HMF exposure (<500 nT) on the exercise capacity of adult male mice, the grip strength, rotarod and treadmill tests were applied to evaluate grip strength, coordination and endurance. In the treadmill test, the total distance traveled by the HMF-exposed mice was

significantly smaller than that of the GMF-controls (P =0.0173), indicating a reduction of exercise capacity, especially the endurance of the adult mice (Figure 2a). The motor coordination and forelimb grip strengths of the HMF-exposed mice were at the same levels as those of the GMF control mice(Figure 2b, c). Meanwhile, the indices of diet intake, such as the body weight, food and water intakes of the HMF-exposed and GMF-control mice were at the same level during the 30-day exposure (Figure S1), and body composition(fat/lean) was also similar between the two groups (Figure 2d, e).

TEM image analysis showed that the length of sarcomeres, a key structural marker of skeletal muscle, was not changed by the HMF exposure (Figure 2f). Therefore, the 30-day consecutive HMF-exposure-induced exercise reduction(endurance) is not correlated with the changes in general metabolic process(diet intake), motor related tissue structure or the control of general locomotor behavior(motor coordination).

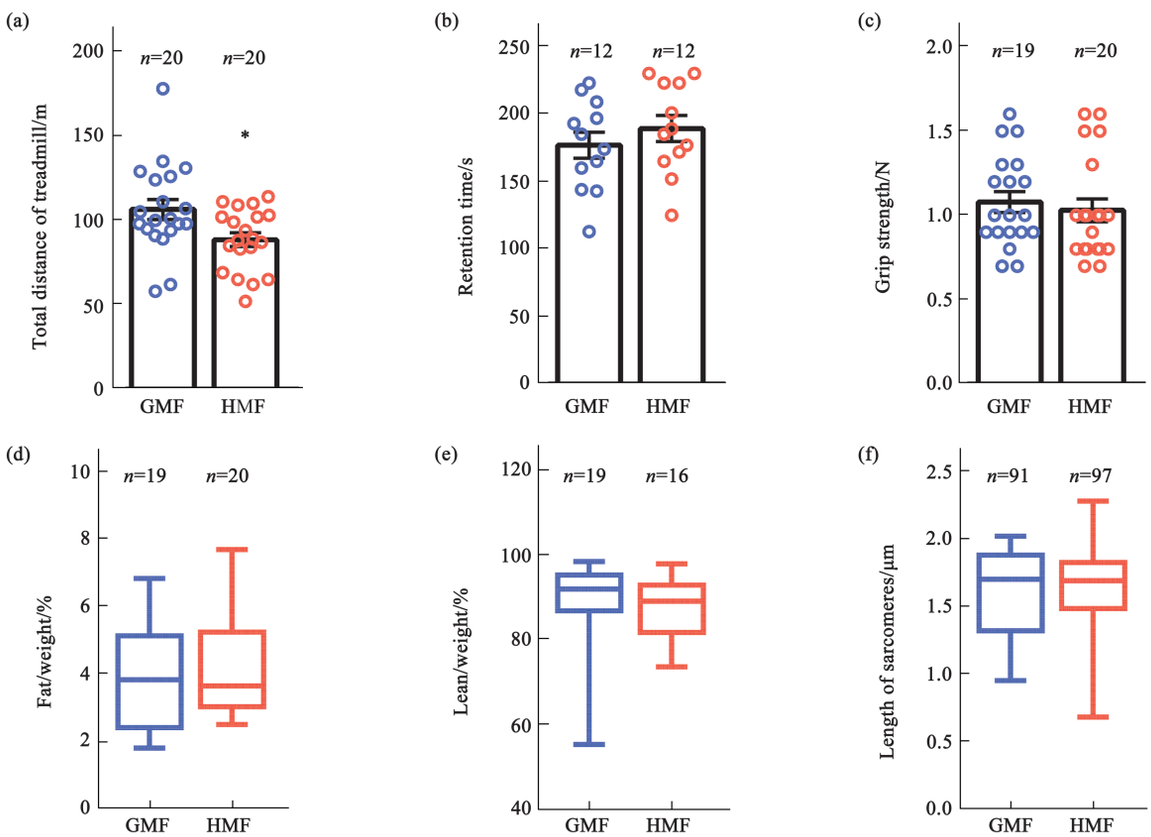

Fig. 2　HMF−induced reduction in the exercise capacity of adult male mice

(a) Treadmill test showed that 30-day HMF exposure reduced the endurance of the mice. (b, c) The HMF exposure did not change the motor coordination(b) and forelimb grip(c) strength. (d,e) HMF exposure did not change the body composition of the mice. (f) The length of the sarcomeres in the soleus muscle of the HMF-exposed mice was at the same level as that of the GMF controls. n is the number of animals in (a−e), and the section number in (f). (a−c): Data is shown as mean±S.E.M. Means were compared with unpaired bilateral student's t-test. * P<0.05. (d−f): Data is shown as boxes (25% to 75%) and whiskers(min to max) and compared with Mann-Whitney test.

2.2　HMF−induced citric acid level reduction in skeletal muscle

Since the endurance of an animal is closely related to energy metabolism, in skeletal muscle, and levels of citric and lactic acids are key biochemical indices for the metabolic condition[31-33], we collected skeletal muscle tissues from the HMF-exposed and the GMF-control mice at rest and measured the citric acid and lactic acid levels in their tissues. The results showed that the citric acid level in the muscle of the

HMF-exposed mice ((3.65±0.16) mmol/L) was significantly reduced (P<0.0001) compared with the controls ((5.50±0.20) mmol/L) (Figure 3a); while the lactic acid level in the HMF group ((2.40±0.12) mmol/L) was the same with that of the control mice((2.28±0.06) mmol/L)(Figure 3b). As the level of citric acid is positively correlated with mitochondrial aerobic respiration in muscle[32,34-36], the results indicate that 30-day HMF has probably altered the mitochondrial function in skeletal muscle, which could explain the shorter distance traveled by the HMF-exposed mice, in the treadmill test.

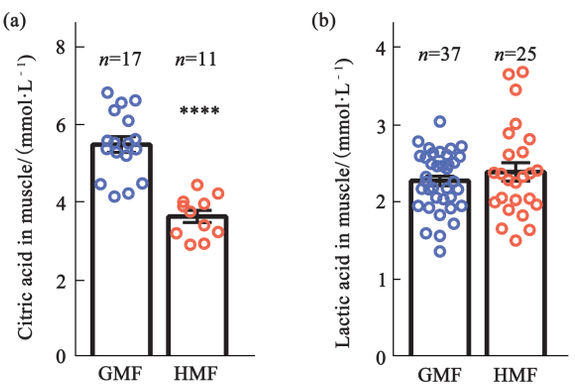

Fig. 3 Citric and lactic acid levels in skeletal muscle

(a) HMF exposure significantly reduced the citric acid level in the rectus femoris muscle of the mice; (b) HMF exposure did not change the lactic acid level. n is the number of animals from 3 individual experiments. Data is shown as mean±S. E. M, and means were compared with the student's t-test. **** P<0.0001.

2.3 HMF−induced numerical reduction of SS mitochondria in skeletal muscle

Previously, we have shown that mitochondria can directly respond to HMF exposure and exhibit functional changes in skeletal muscle cells cultured in the HMF *in vitro*[37]. Thus, we propose that the reduction seen here is also related to changes in the mitochondria in the skeletal muscles. The number of mitochondria is directly related to the metabolic function of muscle cells. By using mitochondrial DNA quantification, we found that our long-term HMF exposure did not change the total mitochondrial count in skeletal muscle(Figure 4a).

There are two types of mitochondria in skeletal muscle: the spheroidic, SS mitochondria[30] which are of high metabolic plasticity[38], and the branched, tubulose, IMF mitochondria[30] which exhibit high efficiency of respiration and ATP production[39-43]. Although the volume of an SS mitochondrion is larger than that of an IMF mitochondrion, the SS mitochondria make up only a small numerical proportion (~10%) of the total mitochondria in muscle[44]. Thus, we hypothesized that it is possible

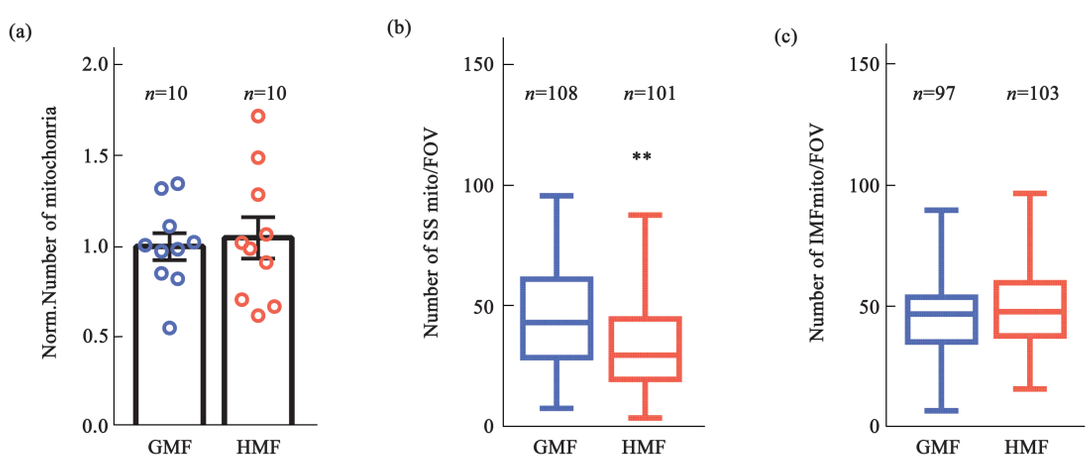

Fig. 4 Effects of HMF on the number of mitochondria in skeletal muscle

(a) The HMF exposure did not change the number of total mitochondria in the gastrocnemius muscle. (b) The HMF exposure reduced the number of SS mitochondria in the HMF-exposed mice. (c) The HMF exposure did not change the number of IMF mitochondria. Total number of muscle mitochondria was determined by real-time PCR. The numbers of SS and IMF mitochondria are quantified with TEM images. n is the number of animals for each group in (a), the difference was tested with unpaired bilateral student's t-test, data is presented as mean±S.E.M. n is the number of TEM fields of view(FOV) (10 μm×10 μm) from 3 mice of each group used for counting in (b, c), the differences were compared with Mann-Whitney test, data are presented as boxes (25% to 75%) and whiskers (min to max). ** P<0.01.

that any numerical changes in SS mitochondria would be masked by the overwhelming number of IMF mitochondria in the muscle tissue. To further evaluate the effect of the HMF on the number of each type of muscular mitochondrion, we manually counted the SS and IMF mitochondrial numbers in the TEM images. The results confirmed our hypothesis that fewer SS mitochondria were present in the muscle images of the HMF-exposed mice(P=0.0011) compared with the GMF controls(Figure 4b); but the number of IMF mitochondria was unchanged(Figure 4c). Therefore, the results indicate that SS mitochondria are more sensitive to HMF exposure, in our experimental conditions.

2.4　HMF changes mitochondrial morphology in skeletal muscle

The morphological characteristics of IMF and SS mitochondria are closely related to their functions[45-46]. We examined the morphologies of both types in skeletal muscle, using TEM images (Figure 5). The area, perimeter, circularity, Feret's diameter, aspect ratio, and form factor of each mitochondrion were measured. Compared with the controls, both types of mitochondria from the HMF-exposed skeletal muscle samples were of a smaller size (smaller area and perimeter value), thinner (higher aspect ratio), and were more irregular in shape (larger form factor value). Specifically, after the 30-day HMF exposure, the SS mitochondria exhibited less circularity and the IMF mitochondria were reduced in size (smaller Feret's diameter) (Figure 5, 6; Table 2). Generally, our observations confirmed that the mitochondria in skeletal muscles underwent shrinkage, accompanied by a tendency toward irregularity of shape, after a 30-day HMF exposure.

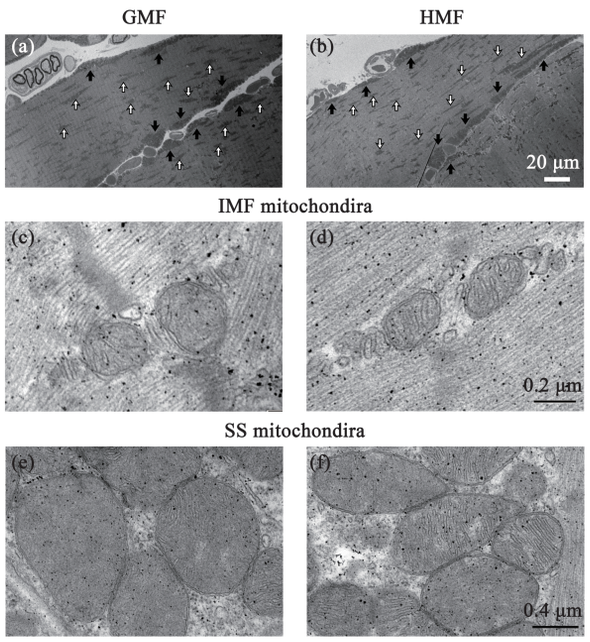

Fig. 5　Morphology of mitochondria in skeletal muscles of mice after one−month exposure in the GMF and HMF

The representative TEM images for the morphology of the IMF and SS mitochondria in lower magnification (×890) (a, b), white and black arrows represent the IMF and SS mitochondria respectively, and in higher magnification (×49 000). (c, d), IMF mitochondria; (e − f), SS mitochondria.

Table 2　The morphology parameters of mitochondria in skeletal muscle

	SS		IMF	
	GMF	HMF	GMF	HMF
Area/μm^2	0.41±0.34	0.39±0.35*	0.20±0.25	0.15±0.19****
Perimeter/μm	2.40±0.98	2.3±1.05*	1.64±1.05	1.42±0.87****
Circularity	0.82±0.12	0.81±0.12***	0.81±0.12	0.81±0.12
Feret's Dia/μm	0.91±0.39	0.89±0.42	0.62±0.40	0.54±0.34****
Aspect Ratio	1.69±0.57	1.74±0.63**	1.71±0.62	1.75±0.64**
Form Factor	0.59±0.22	0.65±0.30****	0.95±0.37	1.08±0.40****
N	4 795	3 528	4 451	5 160

N is the number of mitochondria measured in each group. Data, shown as Mean±SD, were obtained from 3 mice in each group. Data were analyzed with one-way ANOVA, with Sidak's multiple comparison. *P<0.05; **, P<0.01; *** P<0.001; **** P<0.0001.

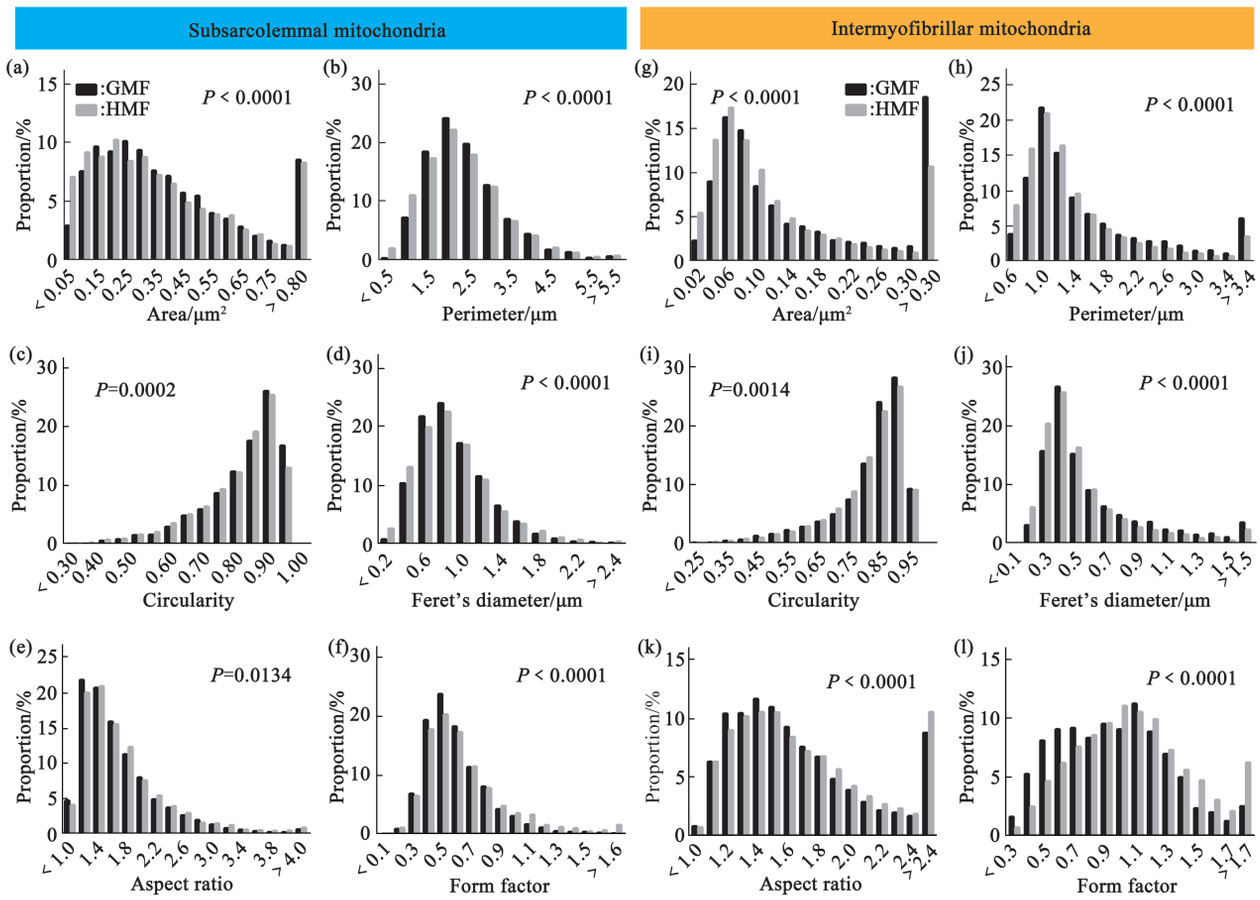

Fig. 6　HMF−induced morphological changes in the SS and IMF mitochondria in skeletal muscle

Frequency distribution of the morphology and shape descriptors for SS(a−f) and IMF(g−i) mitochondria. Differences in frequency distributions were compared with Kolmogorov-Smirnov test. The shape descriptors were calculated as: area(μm²), perimeter(μm), circularity($4\pi \cdot (\text{area}/\text{perimeter}^2)$), Feret's diameter(μm), aspect ratio(major axis)/(minor axis), and form factor((perimeter)/($4\pi \cdot$area)). Data were obtained from 3 mice in each group. * P<0.05; ** P<0.01; *** P<0.001; **** P<0.0001.

3　Discussion

Our previous work showed that a one-month HMF(<500 nT) exposure led to a decrease in general activity in adult mice[13], but more details of the underlying mechanism of this adverse effect were necessary to evaluate the risk of HMF exposure to humans. In this study, by using a simulated long-term HMF exposure on adult male mice, we provided further evidence to show the relation of mitochondrial functioning to the inhibition of the animals' capacity to exercise, specifically endurance (Figure 2a). Moreover, these effects are accompanied by a reduction in citric acid, as well as fewer SS mitochondria and morphological changes to the mitochondria in skeletal muscles. Given the reported adverse effects of HMF on learning and memory[9], our findings support that HMF is a risk factor for manned space missions, and might physically and mentally, decrease the work capacity of astronauts. Our results showed that mitochondrial function is a potential target of long-term HMF exposure. Further studies, focusing on the mitochondria, would facilitate the development of risk-evaluation indices and strategies for counteraction.

Citric acid is a key metabolite in the tricarboxylic acid cycle (TCA), for producing ATP, and is involved in energy production in mitochondria. Previously, we have shown that HMF exposure could reduce the ATP production of mitochondria from primary cultured mouse muscle cells[37]. The observed decrease in the citric acid level in the skeletal muscle of HMF-exposed mice(Figure 3a) would probably correlate

with the alteration of ATP production in muscle mitochondria, during the metabolic process. Fitts and colleagues found that, as strength/magnitude of treadmill training increased, rats showed an increase in malate-pyruvate oxidation, citrate synthase, level of cytochrome C, and endurance capacity[47]. Many other researchers also found that endurance training increased the activity of citrate synthase[48-50] and the concentration of citric acid in the resting state[51]. Furthermore, supplementation with citric acid has been found to enhance an individual's endurance[52]. These reports show that endurance is closely correlated to the concentration of citric acid. In addition, succinate, another key metabolite downstream of citric acid in the TCA, has been reported to play an important role in maintaining endurance[53-55]. In order to elucidate the underlying molecular mechanisms of an HMF on energy metabolism, other TCA-related metabolites and their processes, especially succinate, are worth examining in HMF conditions. It is probable that the observed reduction in exercise capacity (endurance) was due to HMF-induced inhibition of energy production in muscle mitochondria, and that the citric acid level and other citric acid-related metabolites would serve as biomarkers to monitor physical changes.

We also noticed that the level of muscular lactic acid, another molecule closely related to animal motility, was not changed by our experimental conditions or in our previous *in vitro* study with primary cultured muscle cells[37]. It has been well established that the level of lactic acid would quickly accumulate in muscle during acute exercise activity when lactic acid clearance was exceeded by lactic acid production in the muscle, which leads to exhaustion when the concentration of lactic acid exceed the fatigue threshold[56]. Keeping a sustained balance between the production and clearance of lactic acid in the muscle would guarantee the maintenance of endurance capacity during exercise. Since sampling was carried out at rest, in this study, to further understand the effect of the HMF on the endurance of adult animals, it would be necessary to investigate the role of lactic acid by collecting samples from the HMF-exposed animals, immediately after the exercise, which would provide dynamic details of the effect on regulation and function of the motion system.

We showed that long-term HMF exposure induced significant numerical reduction in the SS mitochondria in skeletal muscle (Figure 4b). The SS mitochondria located close to the cell membrane[44,57] are important for metabolite production[41,58] and active transportation of metabolites[41]. Due to their lower membrane potential, SS mitochondria show faster ATP synthesis and turnover than the IMF mitochondria[41]. The IMF mitochondria located between myofibrils[44,57] are more likely to support myofibrillar contraction[41], and have a higher activity for many enzymes related to the respiratory chain, such as succinate dehydrogenase and citrate synthase[57], and a higher efficiency of respiration[39] and ATP production[42]. These studies would suggest that SS mitochondria play an assistant role to the IMF mitochondria, in ATP production. Therefore, a decrease in the number of SS mitochondria, would probably reduce the citric acid concentration in the tissue.

In addition, we found long-term HMF exposure-induced morphological changes in both the SS and IMF mitochondria: shrinkage and decrease in regularity of shape (Figure 6). Reduced mitochondrial size is an adverse factor for endurance. It has been reported that the volume of mitochondria will increase with endurance training[59-62], mitochondrial oxygen consumption[63], and with the levels of mitochondrial markers such as citrate synthase and cytochrome C[47]. Müller found that rats, after endurance training, had larger SS mitochondria in muscles, which suggesting that the larger SS mitochondria benefit to endurance[58]. Our finding of a smaller mitochondrial size in skeletal muscles of the HMF-exposed mice (Figure 6, Table 2) is in correlation with their reduced endurance. In addition, Picard *et al*. [64]reviewed the relationship between mitochondrial morphology transition and function, and showed that morphological changes affect oxygen consumption and ATP synthesis, *via* the TCA cycle, and fragmented mitochondria show a decrease in respiration efficiency and ATP synthesis. Therefore, changes to mitochondrial morphology may also alter the concentration of citric acid. Taken together, it is reasonable to propose that long-term HMF exposure reduces endurance in adult male mice by inhibition of the energy metabolism in mitochondria (citric acid level), accompanied by an alteration in mitochondrial structure and production. Interestingly, it has been reported that the morphology, as well as the

distribution of mitochondria in muscle, varies during endurance exercise[65-67], suggesting that the plasticity of mitochondria during exercise is also important to endurance. As mentioned above, further studies on the effects of HMF on mitochondrial function should carefully consider the sampling strategy and include detailed analyses of the dynamic changes to mitochondrial number, size, and distribution during exercise training.

Previously, we have shown that HMF exposure inhibited the metabolic activity of murine skeletal muscle cells and that mitochondria can respond to an HMF directly, *in vitro*[37]. This current study provides *in vivo* evidence to support the effects on mitochondria and suggests that they are key organelles in the biological response to the HMF. It will be interesting to see whether the mitochondrion is a magnetic responder in muscle only, or if the response is common to other tissues. Moreover, we have previously reported that cytoskeletal proteins, actin and tubulin, can also respond to HMFs *in vitro*[68-69], although we did not observe changes to the structure of skeletal muscle cells in this work. The role of cytoskeletal proteins is worthy of further investigation, in order to reveal the underlying mechanism of the effect of HMFs on mitochondria.

4 Conclusion

In this work, we found that long-term HMF exposure reduces the exercise capacity(endurance) of adult male mice, and adversely affects the function, number, and morphology of the muscle mitochondria. Further investigations focusing on the mitochondrial function-related animal response to long-term HMF exposure would add to our understanding of the underlying cellular and molecular mechanisms. This would benefit the evaluation and prevention of adverse effects of the HMF on the health of astronauts, in the planning of manned, deep-space missions.

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Supplementary material (20200047_Suppl. pdf (including Figure S1, Figure S2, Table S1) is available online (http://www.pibb.ac.cn, http://www.cnki.net).

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长期亚磁场处理降低成年雄性小鼠肌肉组织的线粒体功能和运动能力*

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摘要　亚磁场是深空载人航天任务中的一个关键风险因素. 研究表明，亚磁场影响动物多种运动相关行为，但长期亚磁场处理对成年个体运动能力的影响还需要进一步的研究，以评估深空飞行任务中亚磁场的潜在风险. 本研究利用三轴亥姆霍兹线圈系统模拟的亚磁环境，长期（一个月）曝露处理成年雄性C57BL/6小鼠，并从行为、组织、细胞、分子水平研究其对小鼠运动能力的影响. 相比于地磁组对照，亚磁组小鼠的耐力显著下降. 并且，其骨骼肌中柠檬酸水平和肌膜下线粒体数量的下降，以及骨骼肌线粒体形态的变化，表明亚磁场诱导了肌肉线粒体功能抑制，并可能导致其与耐力密切相关的能量代谢的下降. 我们的研究结果为线粒体直接响应亚磁场提供了体内证据，并且提示线粒体相关指标可能用于亚磁场效应的风险评估和干预药物的开发.

关键词　地磁场，亚磁场，运动能力，骨骼肌，能量代谢，线粒体

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