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The effects of different durations of exposure to hypomagnetic field on the number of active mitochondria and ROS levels in the mouse hippocampus

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ABSTRACT

Reactive oxygen species (ROS) are one of the potential molecules in response to a hypomagnetic field (HMF), and exposure to an HMF for eight weeks led to an increase in ROS levels in the whole hippocampus area in mice. ROS are mainly derived from the byproducts of mitochondrial metabolism. However, previous *in vivo* studies mostly focus on the influence of one time point of HMF exposure on the mouse hippocampus and lack comparative studies on the effects of different durations of HMF exposure on the mouse hippocampus. Here, we investigated the effects of different durations of HMF on the number of active mitochondria and ROS levels in mouse hippocampus. Compared with the geomagnetic field (GMF) group, we found that the number of active mitochondria in the hippocampus was significantly reduced during the sixth week of HMF exposure, whereas the number of active mitochondria was significantly reduced and the ROS levels was significantly increased during the eighth week of HMF exposure. The number of active mitochondria gradually decreased and ROS levels gradually increased in both GMF and HMF groups with prolonged exposure time. In addition, the expression level of the PGC-1α gene in the hippocampus, the main regulator of mitochondrial biogenesis, decreased significantly in the eighth week of HMF exposure. These results reveal that the changes in active mitochondria number and ROS levels were dependent on the durations of HMF exposure, and prolonged exposure to HMF exacerbates these changes.

1. Introduction

The elimination of geomagnetic field (GMF), also called hypomagnetic field (HMF), is one of the risks for astronauts during deep-space exploration or workers in magnetically shielded rooms on Earth; also happened during geomagnetic reversals [1–6]. HMF exposure caused many adverse biological effects on cellular and individual levels [7–9]. Intracellular reactive oxygen species (ROS) are considered as one of the critical factors in response to changes in the external magnetic field [10,11]. ROS are an array of derivatives of molecular oxygen in cells and enable cells to rapidly respond to changing environmental factors and stress [12,13]. Many cellular studies have shown that HMF exposure affects ROS levels and exerts multiple effects on cell growth, proliferation, and survival [10,14–19]. Only a few studies have addressed the effects of HMF exposure on the levels of ROS in the hippocampus and negatively on mice's learning and memory capacity [9,20]. For example, we found that HMF exposure for eight weeks led to increased levels of ROS in the dentate gyrus and cornu ammonis regions of the hippocampus accompanied by significant changes in the expression levels of redox-related genes in mice [20]. ROS function as signaling molecules that affect various cellular processes, while excessive ROS may cause oxidative stress and damage cellular structures [12,21,22]. These results suggest that the mechanisms of many biological effects of HMF exposure may be closely related to intracellular ROS levels [11]. Mitochondria are considered one of the major sources of ROS, which are actively involved in regulating the ROS signaling pathway and cellular redox. The number of active mitochondria (mitochondria number) per cell directly influences ROS production [23]. *In vivo*, evidence demonstrates that long-term HMF exposure causes decreases in the citric acid level and the number of subsarcolemmal mitochondria in mouse skeletal muscles [17]. Therefore, mitochondria may be one of the targets for cellular responses to the HMF environment, which are closely associated with ROS levels and oxidative stress [24,25]. The exposure-time window is very important which corresponds to the period where the effects would plausibly occur, and that could substantially differ from the comparison group [26].

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However, previous *in vivo* studies mostly focus on the influence of one time point of HMF exposure on mouse hippocampus and lack comparative studies on the effects of different exposure durations of HMF on the number of mitochondria and ROS levels in mouse hippocampus. To decipher the above issues, we quantitatively measured active mitochondrial number and ROS levels by flow cytometry in adult mice exposed to GMF or HMF for four, six, and eight weeks. Because thyroid hormones are a major factor controlling metabolic and respiratory rates in mammalian cells, closely associated with oxidative stress in cells [27–29], the serum thyroxine (T4) levels of mice were measured using the ELISA method after exposed to GMF or HMF for 6 and 8 weeks. Mitochondrial content is regulated by the dynamic balance between mitophagy and biogenesis. PINK1 (PTEN-induced kinase 1), LC3 (activated form of the microtubule-associated protein 1 light chain 3), Parkin (E3 ubiquitin ligase), and PGC-1α (peroxisome proliferator-activated receptor gamma (PPARγ) coactivator 1 alpha) are key proteins involved in mitophagy and biogenesis. PINK1-Parkin-mediated mitophagy is a key mechanism of mitochondrial quality control. PINK1 is recruited to damaged mitochondria to phosphorylate Parkin and thus activate Parkin; the latter links LC3 to initiate mitophagy [30]. PGC-1α is a key indicator in mitochondrial biogenesis and regulates the expression of mitochondrial antioxidant genes, therefore PGC-1α may regulate intracellular redox homeostasis and inflammatory responses [31,32]. After finding the exposure-time window, we further determined the effect of HMF exposure on the expression levels of key genes encoding PINK1, LC3, Parkin, and PGC-1α involved in mitophagy and biogenesis by quantitative real-time polymerase chain reaction (qRT-PCR) method. Exploring these questions will contribute to understanding the cellular mechanisms underlying the effects of HMF on cognitive abilities in animals and are useful for assessing the space exploration risks of humans.

Our experimental results showed that the percentage of mitochondria active cells in the hippocampus of HMF-exposed mice decreased significantly in the sixth week of magnetic field exposure, while ROS levels were significantly increased until the eighth week of HMF exposure. However, HMF exposure for four weeks did not affect the number of mitochondria active cells and ROS levels. Interestingly, the results of intra-group comparisons showed that the number of active mitochondria gradually decreased and the ROS levels increased with the exposure durations within the GMF/HMF groups. For the GMF control group, the changes can be considered as the effect of the increasing age of animals. The changes in the HMF group suggest that the HMF exposure plus the increasing age have combined effects on the number of active mitochondria and ROS levels in the hippocampus. The results of qRT-PCR clearly showed that the expression level of PGC-1α was significantly reduced in the eighth week of HMF exposure, whereas the expression levels of other key genes PINK 1, LC3, and Parkin were unchanged. This result suggests that HMF exposure inhibits mitochondrial biogenesis.

2. Material and methods

2.1. Animals and magnetic fields exposure

Seven-week-old adult male mice (C57BL/6 J) were obtained from Beijing HFK Bioscience (Beijing, China) and housed in the experimental coils to acclimate to the exposure environments for one week before the magnetic field exposure, with a constant temperature and relative humidity (21 ± 1 °C; 60 ± 1%). Since the results of previous studies have shown that HMF exposure had a significant inhibitory effect on hippocampal cognitive behavior in 8-week-old male mice [9,20], in order to further investigate the mechanism of these effects, 8-week-old male mice were also selected for different durations of magnetic fields exposure in this study. Animals were randomly assigned to the GMF-exposed and the HMF-exposed groups, and the experimenter was blinded to treatment groups of the animals until the experiment was complete. The body weight and food intake of mice were recorded weekly during exposure to GMF or HMF (n = 12 per group). The serum of mice was collected for thyroxine assay using the ELISA method after exposed to HMF or GMF for 6 and 8 weeks (n = 4 per group). For flow cytometry analysis, three or four animals in each group were included, the qRT-PCR assay involved a minimum of three animals and each repeated three times. The study was approved by the Institutional Animal Care and Use Committee at the Institute of Geology and Geophysics, Chinese Academy of Science.

The experimental HMF/GMF environments were simulated using a double-wrapped coils system. This system has two sets of coils powered by the same power supply, and each coil contains two matched sets of windings to allow operation in active or sham mode. In active mode, currents in paired windings were parallel, generating a magnetic field that countered the surrounding geomagnetic field to produce an HMF. In sham mode, currents ran antiparallel, resulting in no measurable external field. Then, the surrounding GMF remained, with similar ohmic heating and magneto-mechanical effects as in active mode [33,34]. The magnetic induction intensity of the HMF was about 32 ± 7 nT (mean ± SEM) during the experimental duration. The magnetic induction intensity for GMF control was 55,574 ± 12 nT (Supplementary Fig. 1). The intensity of the ambient magnetic field at 50 Hz power frequency inside the GMF and HMF cages was about 1.2 nT and 0.5 nT, respectively. Body weights of mice were not significantly different between GMF- and HMF-exposed groups.

2.2. Cell staining and flow cytometry analysis

Mice were quickly euthanized by cervical dislocation after HMF and GMF exposures. The hippocampus samples of mice, including the dentate gyrus and cornu ammonis areas, were quickly isolated from brain tissues under a stereomicroscope and soaked in frozen HBSS solutions. The anatomical method of the hippocampal tissue was described in the Supplementary Materials according to the experimental procedures modified from Sultan [35]. Dissociated hippocampal cells were obtained through mechanical mincing and trypsin digestion, as described in reference [36]. The quality of single-cell suspension was evaluated by trypan blue staining and microscopic observation according to the references [37,38]. In order to check the percentage of viable cells, one sample from each group was randomly selected and stained with trypan blue staining (Supplementary Fig. 2). Samples with a high average viability of 86% (ranging from 83.3% to 88.1%) are qualified for subsequent flow cytometry detection.

The flow cytometry method was used to simultaneously labeled active mitochondria and ROS with MitoTracker® Deep Red and CellROX™ Green probes in this study. MitoTracker® Deep Red Reagent is a far red-fluorescent dye that labels active mitochondria in live cells [39,40]. CellROX™ Green Reagent is a novel fluorogenic probe for measuring oxidative stress in live cells. Cell staining was performed as per the manufacturer's instructions (Thermo Fisher). All flow cytometry experiments were performed on the BD LSRFortessa™ Cell Analyzer (BD Biosciences, USA) in the Institute of Biophysics, Chinese Academy of Sciences. Data were analyzed with FlowJo software (Tree Star Inc., Ashland, OR, USA). The percentage of positive cells and the fluorescence intensity of all samples were both collected, with negative and positive controls for the unstained gate and the FMO gate (Supplementary Fig. 3). The calculation of mean fluorescent data was calculated by arithmetic mean. The concentration and incubation time for two probes were as follows: MitoTracker® Deep Red (200 nmol l−1, 37 °C, 30 min), and CellROX™ Green Reagent (5 μmol l−1, 37 °C, 30 min). The whole experimental procedure can be completed within 8 h after magnetic field exposure, which greatly guarantees the reliability and stability of the experiment.

2.3. qRT-PCR analyses

Hippocampus samples in mice after HMF and GMF exposures were

Table 1
Primers used for real-time PCR analysis.

Gene Name	Primer	Sequence	GenBank Accession No.	Product Size (bp)
GAPDH	Forward	5′-TCATTGACCTCAACTACATGGT-3′	OX439033	363
	Reverse	5′-CTAAGCAGTTGGTGGTGCAG-3′		
PINK1	Forward	5′-GAGGAGCAGACTCCCAGTTC-3′	NM_026880	150
	Reverse	5′-CCAGGGACAGCCATCTGAGT-3′		
MAP1LC3A	Forward	5′-GATAGCCTTGGAGTCGGTGG-3′	NM_001290769	127
	Reverse	5′-CCGGGGATCAGCCTCTGTAG-3′		
PRKN	Forward	5′-GACAAGGACACGTCGGTAGC-3′	NM_001317726	101
	Reverse	5′-CCAGATGACAGAGGAAGATGA CTGA-3′		
PPARGC1A	Forward	5′-TTTGCATCCAGAGCATGGC-3′	NM_001402987	114
	Reverse	5′-CCAGAGCAGCACACTGGTT-3′		

Note. MAP1LC3A encoding LC3. *PRKN* encoding Parkin. *PPARGC1A* encoding PGC-1α.

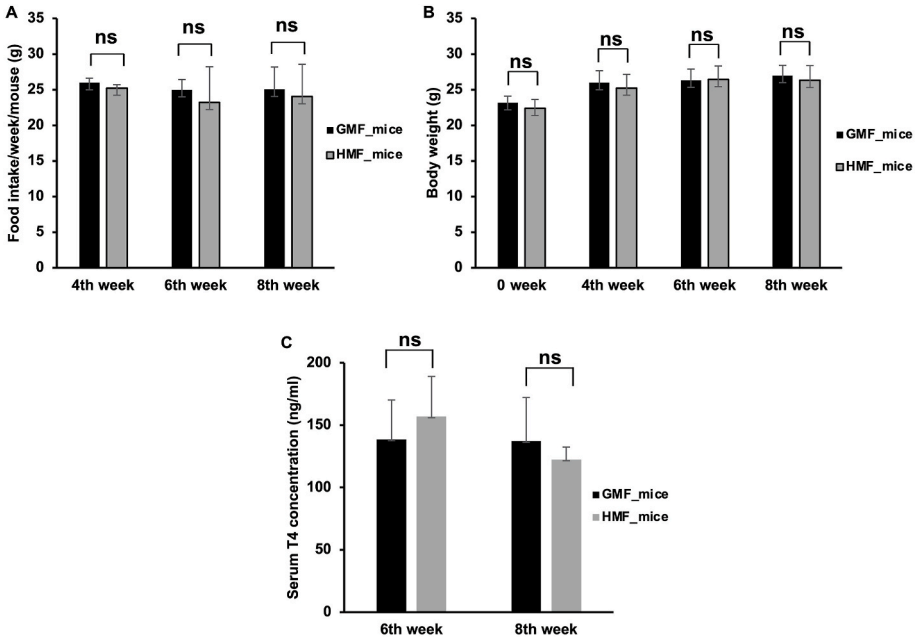


Fig. 1. Average weekly food intake (A), weekly body weight (B) and serum thyroxine (T4) concentration (C) of mice exposed to geomagnetic field (GMF) or hypomagnetic field (HMF) for 4, 6 and 8 weeks. Values are presented as mean ± SD. n = 12 per group for food intake and body weight measurements. n = 4 per group for thyroxine measurement. "ns" indicates not significant ($p > 0.05$).

quickly isolated and frozen at −80 °C. Total RNA was extracted using TRIzol (Invitrogen, Thermo Fisher Scientific, US) according to the manufacturer's instructions. RNA quality was determined using the NanoDrop 2000 spectrophotometer (Thermo Fisher Scientific, US). About 1 μg total RNA was reverse transcribed using the RT2 First-Strand Kit (Qiagen, US). Based on sequences retrieved from GenBank (Table 1), specific primers were designed by balancing for melting temperatures, and primer dimers were avoided where possible. The housekeeping gene GAPDH was used as an internal reference gene because the transcripts for GAPDH were not influenced by magnetic fields [41]. The amplification efficiency of all primers ranged from 95 to 102%. The primers used for qRT-PCR were listed in Table 1. RT-PCR was performed with the QuantStudio Real-Time PCR system (Applied Biosystems, Thermo Fisher Scientific, US). $2^{-\Delta\Delta CT}$ was used to calculate the relative expression level of target genes by normalization to GAPDH.

2.4. Statistical analyses

Statistical analyses were carried out with GraphPad Prism software (La Jolla, CA, USA). Unpaired t-tests were performed to test the significant difference between the GMF group and the HMF group. All data are presented as mean ± standard error of the mean (SEM). The P values < 0.05 were considered significant. One-way analysis of variance (ANOVA) analysis was used to test the statistical significance between multiple groups of data.

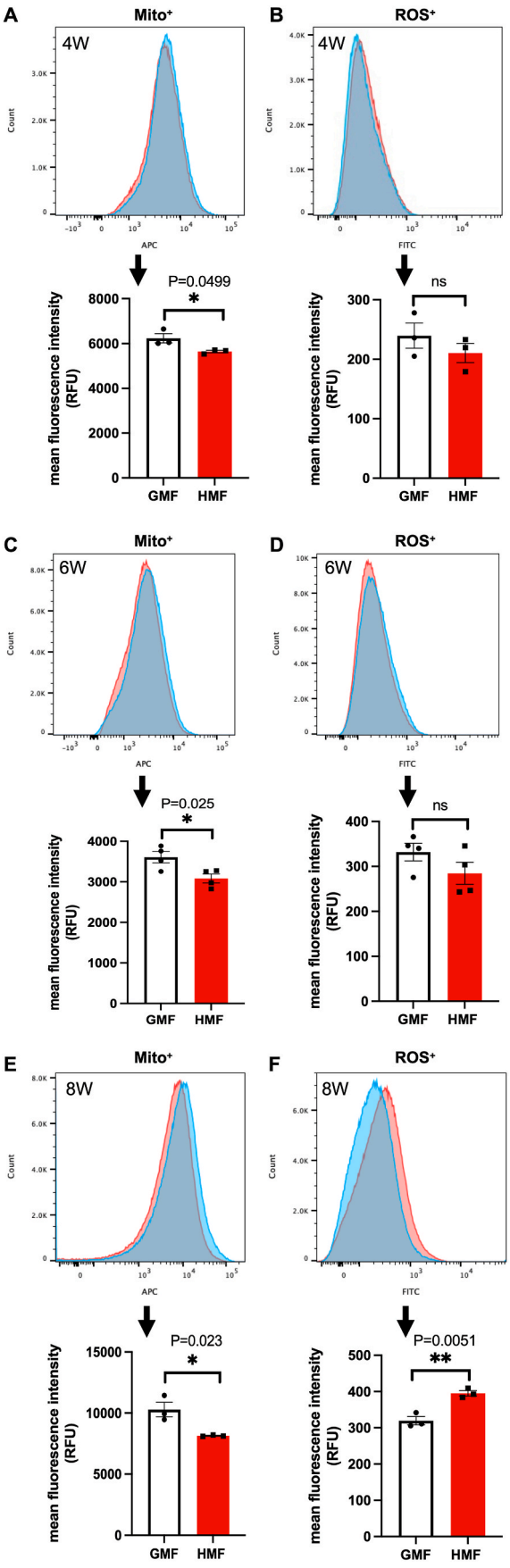
3. Results

3.1. The effects of HMF exposure on the weekly food intake, body weight and serum thyroxine (T4) levels in mice

There were no significant differences in food intake and body weight of mice between the GMF and HMF groups when exposed to GMF and HMF for 4, 6, and 8 weeks. The weekly food intake of mice ranges from 23–26 g and the mice gained weight gradually each week (Fig. 1A and B). The serum T4 concentration of mice did not differ significantly between the GMF and HMF groups after 6 and 8 weeks of exposure, as shown in Fig. 1C.

3.2. The effect of HMF exposure on the number of active mitochondria and ROS levels in hippocampal cells

The mean fluorescence intensity (MFI) represents the average levels of active mitochondria and ROS in the cell population in the experiment



(caption on next column)

Fig. 2. Representative flow cytometry histograms of positive cells stained with allophycocyanin (APC-Mito$^+$) and fluorescein isothiocyanate (FITC-ROS$^+$), and mean fluorescence intensity of active mitochondria (Mito$^+$) (A, C, E) and reactive oxygen species (ROS$^+$) (B, D, F) in hippocampal cells exposed to GMF or HMF for 4, 6 and 8 weeks. n = 3–4 mice/group. Data are presented as means ± SEM with data points superimposed in the histograms. Statistical significance was calculated by unpaired t-tests. **P < 0.01, *P < 0.05, ns means no significance.

(Fig. 2). The results showed that the number of mitochondria in the hippocampus was significantly decreased in the HMF-exposed mice in the sixth week of exposure (Fig. 2C–D), with a weak significance in the fourth week (p = 0.0499) (Fig. 2A–B). There was no difference in reactive oxygen species levels in the fourth and sixth week of exposure (Fig. 2B–and D). However, in the eighth week of HMF exposure, the number of active mitochondria in the hippocampal cells of mice was significantly reduced (Fig. 2E), accompanied by a significant increase in ROS levels in hippocampal cells (Fig. 2F). The uneven baselines of mean fluorescence intensity among different exposure weeks are due to different experimental batches.

Fig. 3 displayed the representative dot plot representing four quadrant images. Q1 represents the mitochondrial active cells with low ROS levels (Mito$^+$ ROS$^-$ cells). Q2 represents the mitochondrial active cells with high ROS levels (Mito$^+$ ROS$^+$ cells). Q3 represents mitochondrial inactive cells with high ROS levels, but they are very rare. The percentage of mitochondria active cells (Mito$^+$ cells) (Q1+Q2) in the hippocampus subjected to GMF exposure for 8 weeks (GMF_8W) was 89.7%, while the percentage of Mito$^+$ cells in the hippocampus subjected to HMF exposure for 8 weeks (HMF_8W) was 82.4%. However, the percentage of Q2 cells in the hippocampus increased from 7.73% to 17.0% when compared the GMF group with the HMF group (Fig. 3E and F). Fig. 4 showed the histogram of the percentage of Q1 cells or Q2 cells in the hippocampus when exposed to GMF and HMF for 4, 6, and 8 weeks. There was no significant difference in the percentage of Q1 cells or Q2 cells between the GMF and HMF groups for 4 weeks of exposure (Fig. 4A and B). The percentage of Q2 cells in the hippocampus significantly decreased in the HMF-exposed mice, while there was no significant difference in the percentage of Q1 cells between the GMF and HMF groups in the sixth week of exposure (Fig. 4C–and D). The percentage of Q2 cells in the hippocampus significantly increased in the HMF-exposed mice, while there was no significant difference in the percentage of Q1 cells in the eighth week of exposure (Fig. 4E–and F).

Intra-group comparison can assess the effects of different durations of HMF exposure on the active mitochondria and ROS levels in the hippocampus (Fig. 5). The percentage of Mito$^+$ cells decreased with prolonged exposure time in the HMF group. However, the percentage of cells containing the ROS (ROS$^+$ cells) in the HMF group showed an increasing trend with prolonged exposure time, especially a sharp increase from the sixth week to the eighth week of exposure. Similar trends in the percentage of Mito$^+$ cells or ROS$^+$ cells were also observed in the GMF group. Compared to the GMF group, the percentage of Mito$^+$ cells showed a significant decrease since the sixth week of HMF exposure, and then the percentage of ROS$^+$ cells began to increase significantly in the eighth week of HMF exposure.

3.3. The effect of 8-week HMF exposure on the expression levels of key genes related to the mitophagy and biogenesis of mitochondria

The expression levels of PINK1, LC3, and Parkin genes in the hippocampus had no significant difference between the HMF and GMF groups for 8 weeks of exposure. While the expression level of PGC-1α in the HMF group significantly decreased compared to the GMF group (Fig. 6).

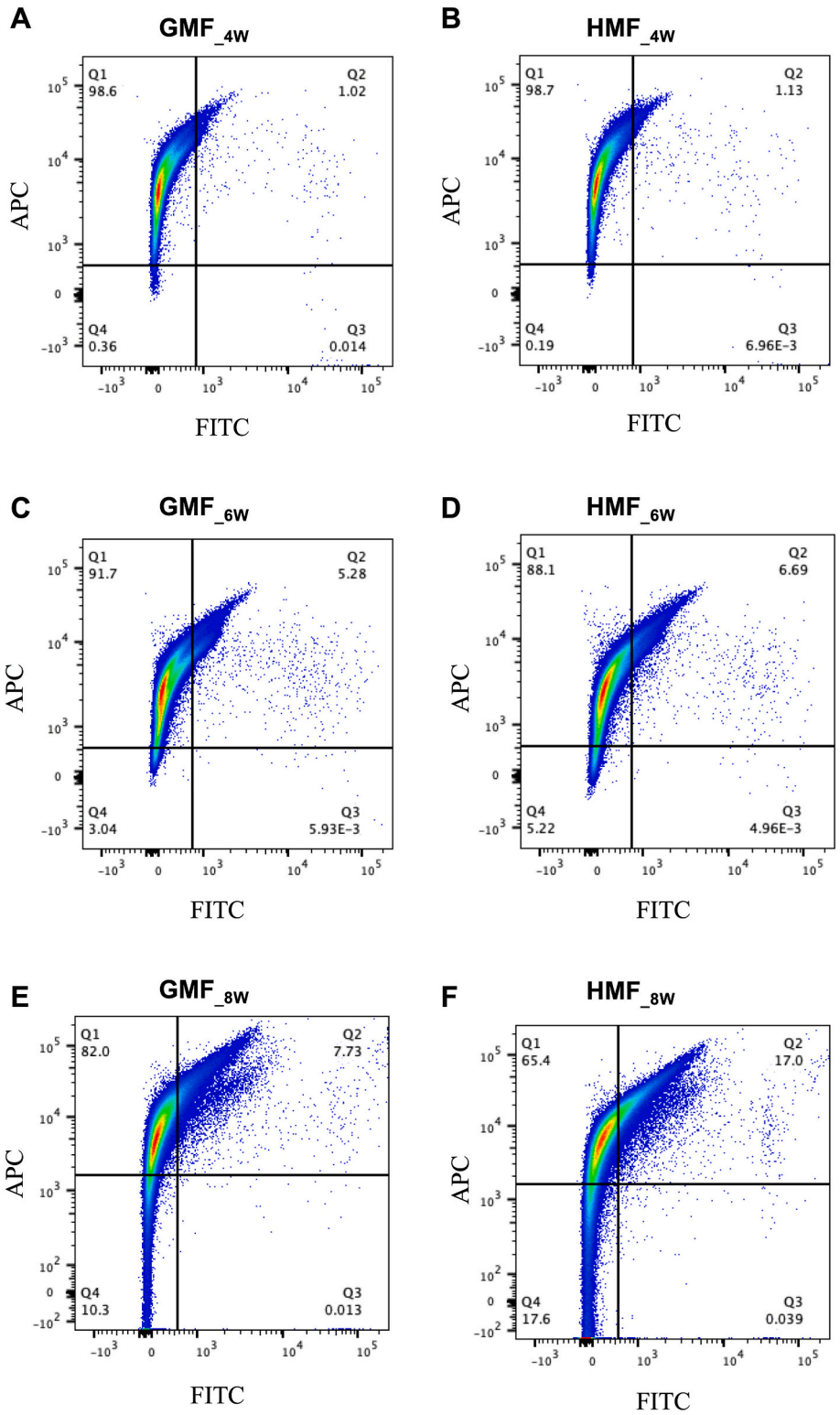


Fig. 3. Dot plot representing four quadrant images by flow cytometric analysis. Q1: mitochondria active cells with low ROS levels ($Mito^{+}$ ROS^{-} cells), Q2: mitochondria active cells with high ROS levels ($Mito^{+}$ ROS^{+} cells), Q3: mitochondria inactive cells with high ROS levels ($Mito^{-}$ ROS^{+}), and Q4: mitochondria inactive cells with low ROS levels ($Mito^{-}$ ROS^{-}).

4. Discussion

Here, we measured the changes in the number of active mitochondria and ROS levels in the hippocampus of mice continuously exposed to GMF or HMF for 4, 6, and 8 weeks. We found that since the sixth week of exposure, the number of active mitochondria in the hippocampus of GMF-exposed mice was significantly different from that in the HMF group (Fig. 2C and E). In the eighth week of HMF exposure, the ROS levels in the hippocampus of HMF-exposed mice significantly increased compared with that of GMF-exposed mice (Fig. 2F). Why was there a sudden increase in ROS levels in the eighth week of HMF exposure? ROS are mainly formed in the mitochondrial respiratory chain. The

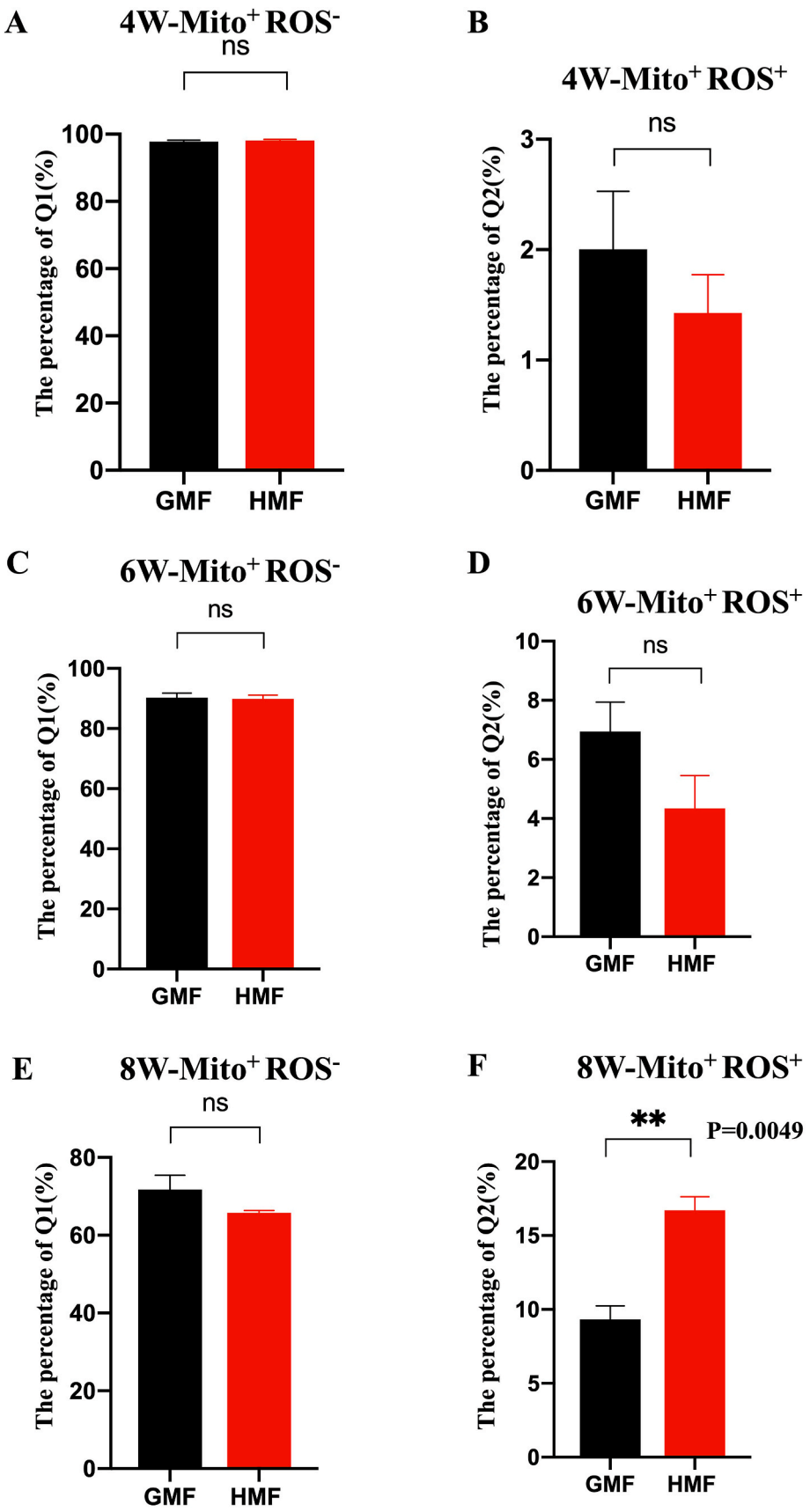


Fig. 4. Comparison results of the percentage of Q1 (A, C, E) and the percentage of Q2 (B, D, F) in hippocampus of mice between the GMF and HMF groups when exposed to GMF and HMF for 4, 6, and 8 weeks. Data were presented as mean ± SEM. Statistical significance was calculated by unpaired t-tests. **P < 0.01, ns means no significance.

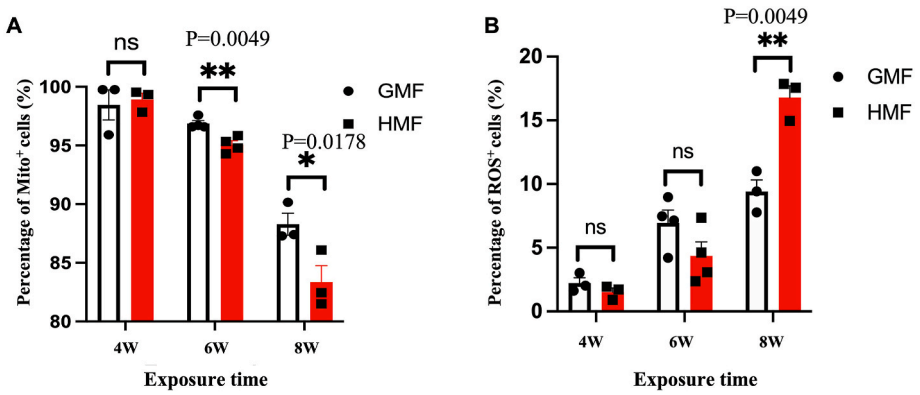


Fig. 5. Comparison results of the percentage of Mito $^{+}$ cells (A) and ROS^{+} cells (B) in hippocampus of mice when exposed to GMF and HMF for 4, 6, and 8 weeks. Data were presented as mean ± SEM with data points superimposed in the histograms. Statistical significance was calculated by unpaired t-tests. **P < 0.01, *P < 0.05, ns means no significance.

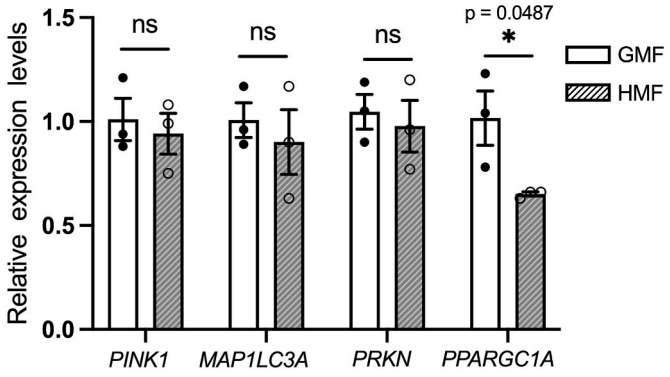


Fig. 6. Histograms with one standard error bar and each data point superimposed showing the results of relative expression levels of PINK1, LC3, Parkin, and PGC-1α genes in mouse hippocampal cells when exposed to hypomagnetic field (HMF) for eight weeks. The expression of these genes was determined by qRT-PCR analysis. The expression level of the geomagnetic field (GMF) group was set to 1. *MAP1LC3A* encoding LC3. *PRKN* encoding Parkin. *PPARGC1A* encoding PGC-1α. Statistical significance was calculated by unpaired t-tests. *P < 0.05, ns means no significance.

intracellular ROS are maintained at a constant low level by the balance between ROS generation and antioxidant defense systems [12,23]. We speculated that the decrease in the number of active mitochondria may have caused an increase in ROS production, which in turn could stimulate the antioxidant systems to eliminate the excess ROS. Hence, the ROS levels did not show a significant increase at 4 and 6 weeks of exposure and even presented a slight decrease (Fig. 4A–D and Fig. 5B). However, the balance was completely disrupted during the 8 weeks of HMF exposure. That is because 8-week HMF exposure induced an increase in ROS production and a decrease in the expression of key antioxidant genes, which resulted in a significant increase in ROS levels in the HMF group, as shown in our previous report [20]. Excessive ROS have toxic effects on nerve cells and may damage macromolecules within cells, leading to oxidative stress and ultimately affect cell function [42–44]. Mitochondrial dysfunction and oxidative stress are largely involved in aging, cancer, and neurodegenerative diseases [45,46].

Different from 8-week exposure window in this study, the *in vitro* experiment showed that the intracellular ROS levels changed significantly after 12 or 24 h of HMF exposure compared with the GMF group [14]. These results suggested that the exposure-time window of HMF on the number of active mitochondria was earlier than the ROS levels in the hippocampus. A decrease in the number of active mitochondria implies an increase in ROS production in cells [47,48]. Moreover, the result of a significant increase in ROS levels during the 8th week of HMF exposure was largely consistent with our previous immunofluorescence quantification in brain slices [20]. It indicates the feasibility of flow cytometry for quantitative determination of specific cell populations and ROS levels. These results demonstrated that mitochondria are most likely the primary targeted organelles when animal cells are exposed to the HMF environment, which is consistent with the results of NASA report on the effects of deep space environment on animals, including humans [49,50].

In the study, it was observed that there was a gradual decrease in the percentage of Mito^{+} cells and an increase in the percentage of ROS^{+} cells with the duration of the magnetic field exposure in both the GMF and HMF groups (Fig. 5). The changing trend of the intra-group might not be directly related to the increasing age of the animals, but rather influenced by other factors such as mental disorders. Animals suffering from mental disorders, like anxiety and depression, often experience some degree of oxidative stress or disruption of ROS homeostasis. However, it is currently unclear whether oxidative stress is the cause or consequence of mental disorders [51–53]. We previously found that exposure to an 8-week HMF resulted in significant anxiety-like behavior in mice, accompanied by high levels of ROS in the hippocampus [20,34]. Therefore, it is possible that the effects of the exposure duration of HMFs on ROS levels and active mitochondrial number may be related to anxiety-like behavior. However, this needs to be verified in future studies. Furthermore, the food intake, body weight, and serum T4 of mice showed no significant differences between the GMF and HMF groups (Fig. 1), indicating that HMF exposure did not affect the growth and basal metabolism of the mice. The changing trend of the intra-group of HMF is more rapid than that of GMF, especially in the 8th week of magnetic field exposure, which suggests that HMF exposure aggravates these trends. In addition, we previously found that 8-week HMF affected the hippocampal neurogenesis, and inhibited the proliferation and differentiation of hippocampal neural stem cells in mice, resulting in reduced numbers of newborn neurons and abnormal development [9]. It is still unclear whether the changing trend in the intra-group is related to the effect of HMF on the consolidation of brain function (hippocampal neurogenesis) in mice.

In this study, the results of qRT-PCR clearly demonstrated that HMF exposure significantly reduced the expression level of PGC-1 α and had no influence on the expression levels of PINK 1, LC3, and Parkin in the hippocampus of mice. Previous studies showed that PGC-1α is highly upregulated in skeletal muscles after cold exposure or exercise which leads to the increase of mitochondrial biogenesis and maintenance to meet energy demands [54], while the expression level/activity of PGC-1α and PGC-1α-dependent metabolic pathways are down-regulated in neurological and developmental disorders [55]. In this study, the low expression levels of PGC-1α indicated a decrease in mitochondrial

biogenesis, which may lead to the abnormal expression of antioxidant genes and increased ROS levels, even neuroinflammatory responses, as reported in our earlier studies [20,34]. Therefore, we believe that the low PGC-1α level in the HMF group may contribute to the reduced mitochondrial number and the higher ROS level shown in this study.

Hippocampal area cells are heterogeneous, including both neuronal and non-neuronal cells. The non-neuron/neuron ratio in the mouse hippocampus is approximately 1:1 [56,57]. However, due to the lack of suitable membrane-specific antibodies to simultaneously label the different types of cells, active mitochondria and ROS in hippocampus, this study firstly focuses on the number of active mitochondria and ROS levels of the whole hippocampus under different durations of HMF exposure, aiming to search the exposure time window of the changes of mitochondria number and ROS levels and the time sequence in which they showed significant changes. In the future, we will try to use viral tool to label neuronal and non-neuronal cells combined with specific antibodies to co-quantify the effects of HMF on the number of mitochondria or ROS levels in neurons, oligodendrocytes, astrocytes, and microglia.

5. Conclusion

This study revealed that prolonged exposure to HMF leads to a decrease in active mitochondrial number and an increase in ROS levels, associated with a decrease in mitochondrial biogenesis. These changes are dependent on the duration of the magnetic field exposure. The exposure time window for active mitochondria number was significantly earlier than the ROS levels in the hippocampus. Following these results, maintaining the homeostasis of mitochondria should be an essential strategy for humans against the adverse effects of HMF exposure.

Data availability statement

Data will be made available on request.

CRediT authorship contribution statement

Lanxiang Tian: Writing – review & editing, Writing – original draft, Supervision, Project administration, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Jie Ren:** Writing – review & editing, Writing – original draft, Methodology, Investigation, Formal analysis. **Yukai Luo:** Writing – review & editing, Formal analysis, Data curation.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bbrep.2024.101696.

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