



Safety of exposure to high static magnetic fields (2 T–12 T): a study on mice

Shenghang Wang^{1,2,3} · Jie Luo^{1,2,3} · Huanhuan Lv^{1,2,3} · Zhihao Zhang^{1,2,3} · Jiancheng Yang^{1,2,3} · Dandan Dong^{1,2,3} · Yanwen Fang⁴ · Lijiang Hu⁴ · Mengyu Liu⁴ · Zhongcai Liao⁴ · Jun Li⁴ · Zhicai Fang⁴ · Yunpeng Wei^{1,3} · Wei Han^{1,3} · Atik Badshah Shaikh^{1,3} · Dachuan Yin^{1,2,3} · Peng Shang^{1,3}

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Abstract

Objectives We aimed to evaluate the biological effects of high static magnetic field (HiSMF, 2–12 Tesla [T]) exposure on mice in a stable and effective breeding environment in the chamber of a superconducting magnet.

Methods C57BL/6 mice were bred in the geomagnetic field and HiSMF with different magnetic field strengths (2–4 T, 6–8 T, and 10–12 T) for 28 days. The body weight, blood indices, organ coefficients, and histomorphology of major organs were analyzed.

Results The results showed that the HiSMF had no significant effect on the body weight, organ coefficients, or histomorphology of major organs in mice. The HiSMF had no effect on most routine blood and biochemical indices, but the value of the mean corpuscular hemoglobin (MCH) was increased in the 2–4 T group compared with that of the other groups, and the uric acid level (UA) was decreased in the three HiSMF groups compared with that of the control group.

Conclusion The C57BL/6 mice were not affected when they were exposed to different HiSMF environments for 28 days.

Key Points

- No physiological problems were observed in mice with long-term whole-body exposure to HiSMF.

Keywords Magnetic fields · Mice, inbred C57BL · Safety management · Organ size · Staining and labeling

Abbreviations

ALB	Albumin
ALT	Alanine aminotransferase
BUN	Blood urea nitrogen
CHOL	Total serum cholesterol
CREA	Creatinine
CT	Computed tomography
GMF	Geomagnetic field
HGB	Hemoglobin

HiSMF	High static magnetic field
MCH	Mean corpuscular hemoglobin
MCV	Mean corpuscular volume
MRI	Magnetic resonance imaging
PDW	Platelet distribution width
PLT	Platelet count
RBC	Red blood cell count
RDW-CV	Coefficient variation of red blood cell volume distribution width
SMF	Static magnetic field
TBIL	Total bilirubin
TG	Triglyceride
UA	Uric acid
WBC	White blood cell count

✉ Peng Shang
shangpeng@nwpu.edu.cn

¹ Research & Development Institute of Northwestern Polytechnical University in Shenzhen, Shenzhen 518057, China

² School of Life Sciences, Northwestern Polytechnical University, Xi'an 710072, Shaanxi, China

³ Key Laboratory for Space Biosciences and Biotechnology, Northwestern Polytechnical University, Xi'an 710072, Shaanxi, China

⁴ Zhejiang Heye Health Technology Co., Ltd., Anji, Huzhou, China

Introduction

The earth is surrounded by a geomagnetic field (GMF). The magnetic flux density of the GMF is between 30 and 70 μT and changes with geographical position [1]. The GMF not

only protects organisms from sunlight radiation and solar wind but is also closely related to the living state of the organisms [2]. Artificial magnetic fields widely exist in our daily life, such as from various household appliances (telephone, TV) and magnetic resonance equipment for medical imaging and diagnosis. According to the direction and intensity of the magnetic field, the fields can be mainly divided into static (SMFs) and dynamic magnetic fields. Studying SMFs improves the understanding of the magnetic field's biological effect mechanism, without interference from electrical and thermal effects [3].

According to strength, SMFs have been divided into weak (< 1 mT), moderate (1 mT–1 T), and high SMFs (HiSMFs) (> 1 T). Because HiSMFs require special magnetic materials or superconducting technology, which means high design and maintenance costs, most of the existing literature on the biological effects of SMFs is with moderate magnetic fields [4, 5]. As an extreme environment, HiSMF experiments can better explain the biosafety and biological effects of SMFs. The International Commission for Non-Ionizing Radiation Protection (ICNIRP) declared in 2009 that magnetic fields are safe for occupational exposures as long as the time-weighted average intensity is not more than 200 mT, and the maximum exposure is not more than 2 T. Because there are no large blood vessels and organs, exposure of the extremities to 8 T or below is considered acceptable [6]. Chakeres et al found that short-term exposure to 1–8-T HiSMF had no significant effect on human vital signs, such as heart rate, respiratory rate, systolic and diastolic blood pressure, finger pulse oxygenation levels, and core body temperature [7].

Magnetic resonance imaging (MRI) is the most important biomedical application of HiSMF. The magnetic field strength of most clinical devices is between 0.35 T and 3 T, some reaching 10 T or even higher, mainly dedicated to research [8–10]. Preliminary research on the biosafety and biological effects of MRI-based HiSMF has been reported [11–15]. There was no effect on embryo development in pregnant mice when they were exposed to 1.5-T HiSMF daily, but the exposure was accompanied by some developmental retardation responses on weight gain and eye-opening after birth [16]. HiSMF can cause human discomfort due to the influence of the Lorentz forces on the ionic current in the vestibule [17]. However, most MRI-based HiSMF research addresses short-term and discontinuous exposure conditions, which may mainly reveal the stress effect of the magnetic field on the organism. Meanwhile, due to the existence of a sequence acquisition which delivers stress during MRI imaging, the research results do not allow a conclusion on the effect of static field. The study of long-term continuous exposure aimed at detecting biological effects of HiSMFs without interference from stress and providing some theoretical bases and research ideas for future studies about the application of HiSMFs in biomedicine.

Methods

Construction of the HiSMF environment

Magnetic fields of 2–4 T, 6–8 T, and 10–12 T were provided by a non-refrigerant superconducting magnet (CRYOF12/150) with a 15-cm-diameter chamber (Fig. 1a). The maximum magnetic field strength was 12 T and decreased symmetrically from the middle to the outside (Fig. 1b).

Animals and treatments

In this study, 8-week-old male C57BL/6 mice were used. The mice were fed standard rodent diet ad libitum, maintained at 25 °C, and kept on a 12-h light/dark cycle. After adapting for a week, 48 mice were randomly assigned to one of following four groups: (1) the sham control group in which mice were kept inside the GMF environment, (2) the 2–4-T HiSMF group, (3) the 6–8 T HiSMF group, and (4) the 10–12-T HiSMF group. The three HiSMF groups were distinguished by the magnetic field strength and the active area of free movement for the mice (5 cm above the bottom of the cage) ($n = 12$) (Fig. 1e). The sham control group (hereafter called the control group) was fed in the same system without the HiSMF. The cages were removed from the magnet for cleaning every 3 days and to resupply the water and food. The experiment lasted for 28 days. On day 28, blood samples were collected via cardiac puncture under anesthesia. Subsequently, the mice were killed by cervical dislocation. The experiments were performed twice and data were pooled. All animal protocols used in this study were approved by the Lab Animal Ethics and Welfare Committee of Northwestern Polytechnical University.

Routine blood and biochemical analysis

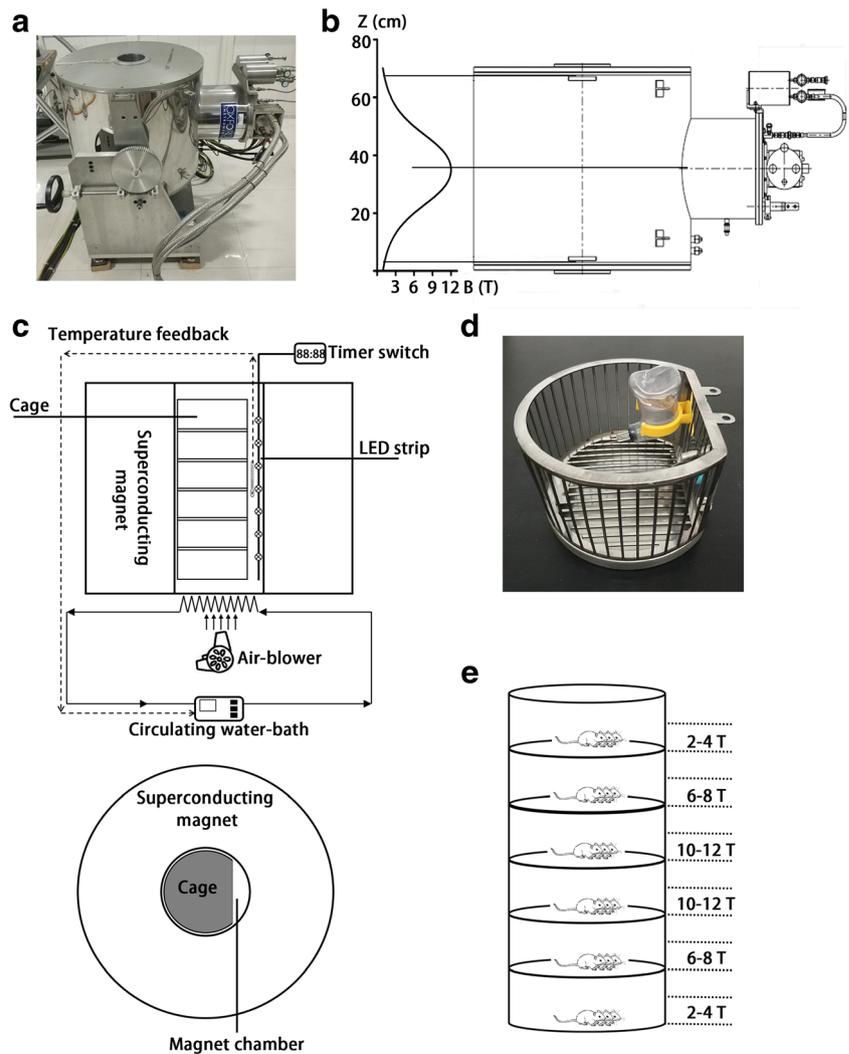
The blood samples were mixed with EDTA-K2 anticoagulant immediately after collection and subjected to routine blood analysis using a Sysmex xs-800i automated hematology analyzer (Sysmex TMC).

The blood samples were collected and centrifuged at $10,000\times g$ for 10 min to obtain serum. The serum biochemical indices were analyzed using an Au-400 automated biochemical analyzer (Olympus).

Organ coefficient analysis

The organs, including the heart, liver, spleen, lung, kidney, testicle, and tibia (right), were collected from the sacrificed mice after the cardiac perfusion was performed with normal saline solution. Organ coefficients were calculated as the ratio between organ weight and total body weight.

Fig. 1 Experimental HiSMF environment device. **a** Non-refrigerant superconducting magnets (CRYOF12/150, OXFORD). Arrowheads, magnet chamber. **b** Magnetic field distribution in the superconducting magnets. **c** Schematic diagram of the mouse feeding system in the HiSMF. **d** The 304 stainless steel mouse cage. **e** The arrangement of the mice cages (three mice per cage) and the corresponding magnetic field strength of the main active area



Histomorphology analysis

The heart, liver, spleen, lung, kidney, and testicle were fixed in 4% paraformaldehyde for 2 days. Then, the organs were embedded with paraffin and sectioned in 5- μ m-thick slices via a semiautomated rotary microtome (Leica Biosystems RM2245). The histological changes in the sections were detected by histomorphology with hematoxylin and eosin staining (H&E; Beyotime Biotechnology) according to the standard procedures. The histological data were obtained by double-blind trial.

Statistics

All statistical analyses were performed using GraphPad Prism statistical software for Windows (version 5, GraphPad Software, Inc). The differences between the control group and the three HiSMF groups were revealed by using an ordinary one-way ANOVA. The normal distribution was tested by

the Kolmogorov-Smirnov test with $p > 0.05$, and equal variances were tested by Bartlett’s test with $p > 0.05$ for the requirements of the ANOVA test. The results are expressed as the mean \pm standard deviation. For all statistical tests, $p < 0.05$ was considered to indicate statistical differences.

Results

Construction of the animal feeding environment in the HiSMF

Due to the limited magnetic field space, traditional animal feeding devices could not be used in our superconducting magnet. For the experiments in this study, 304 stainless steel was used as the material for the mouse cages because of its sturdy and nonmagnetic properties. The cage size was determined to be 15 cm in inner diameter and 8 cm in height, and a chassis with a height of 1 cm was placed for easy replacement

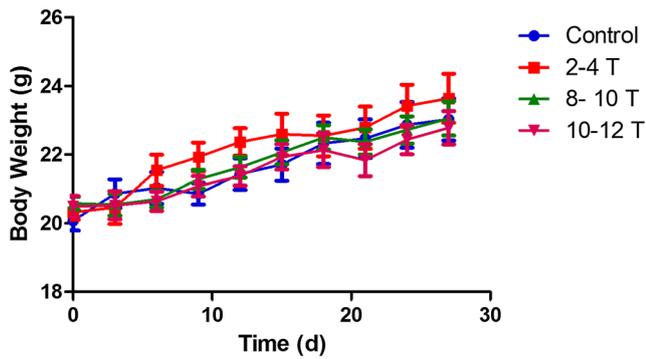


Fig. 2 Effect of the HiSMF on the weight of mice. The 28-day HiSMF exposure had no significant effect on body weight in mice. The mice were weighed every 3 days. Control group: mice were raised in normal GMF for 28 days; 2–4 T, 6–8 T, and 10–12 T groups; mice were raised in a HiSMF with a magnetic field strength of 2–4 T, 6–8 T, and 10–12 T, respectively ($n = 12$)

of the bedding (three mice per cage). To maintain air circulation in the relatively closed HiSMF chamber, the side of the animal cage could not be completely sealed (Fig. 1d). The animal cages were inserted into the superconducting magnet chamber (Fig. 1e).

A LED strip (12 V, 1 A) was attached in the superconducting magnet chamber and used as the light source for a 12-h light/dark cycle.

Due to the low temperature during the process of generating a HiSMF with a superconducting magnet, the cavity was relatively closed. A circulating water bath was used as a heating device. The heated air was blown into the magnet cavity by an air blower with a pipe to ensure that the air circulation and temperature were maintained at 25 °C.

The construction and application of these devices can provide a stable and effective animal feeding environment for long-term HiSMF exposure. The control group was fed in the same system but without HiSMF.

Effects of the HiSMF on the body weight of mice

The differences in body weight between the experimental groups are shown in Fig. 2. There were no significant differences in body weight between any two groups after 28 days of treatment. The body weight of each group showed an upward trend during the experiment. Interestingly, the body weight of the three HiSMF groups showed stagnation on the third day and gradually recovered later on.

Effects of the HiSMF on the blood in mice

Routine blood and biochemical analyses are important tests to evaluate the indicators that reflect the health of the body. In this study, the white blood cell count (WBC), red blood cell count (RBC), mean corpuscular volume (MCV) coefficient, variation of red blood cell volume distribution width (RDW-CV), hemoglobin (HGB), mean corpuscular hemoglobin (MCH), platelet count (PLT), and platelet distribution width (PDW) were used to reflect the effects of the HiSMF on routine blood parameters (Table 1). The WBC was used to evaluate the effect of the HiSMF on white blood cells (leukocyte), and we did not find a significant difference in WBC between the different groups ($p > 0.05$). For red blood cell-related indicators, the data showed that there were no significant changes in RBC, MCV, or RDW-CV in the three HiSMF groups compared with that in the control group ($p > 0.05$). The changes in hemoglobin-related indicators were evaluated by the HGB and MCH. The data showed that HiSMF exposure had no significant effect on HGB ($p > 0.05$), but the MCH in the 2–4 T group was significantly increased compared with that in the control group ($p < 0.05$). Interestingly, the MCH in the 10–12 T group was significantly decreased compared with that in the 2–4 T group ($p < 0.01$). Compared to that in the control

Table 1 Effect of HiSMF exposure on routine blood parameters in mice

Blood parameter	Control	HiSMF		
		2–4 T	6–8 T	10–12 T
WBC ($10^9/L$)	2.528 ± 0.8223	4.135 ± 2.800	2.813 ± 1.312	2.852 ± 0.9628
RBC ($10^{12}/L$)	9.240 ± 0.2062	9.355 ± 0.2908	9.033 ± 0.3427	9.750 ± 0.4664
MCV (fL)	52.34 ± 1.050	52.50 ± 1.988	52.83 ± 1.691	53.38 ± 1.053
RDW-CV%	18.34 ± 0.7021	18.30 ± 0.6132	18.88 ± 1.068	19.40 ± 0.9338
HGB (g/L)	133.2 ± 3.564	137.7 ± 3.670	134.3 ± 5.538	139.3 ± 5.279
MCH (pg)	14.42 ± 0.1304	14.80 ± 0.2280*	14.48 ± 0.2994	14.28 ± 0.1835 ^{###}
PLT ($10^9/L$)	849.6 ± 89.78	883.3 ± 96.60	919.3 ± 163.8	854.3 ± 241.6
PDW (fL)	6.580 ± 0.1924	6.683 ± 0.7574	6.883 ± 0.5742	6.917 ± 0.2858

Values are means ± SD. WBC, white blood cell count; RBC, red blood cell count; MCV, mean corpuscular volume; RDW-CV, coefficient variation of red blood cell volume distribution width; HGB, hemoglobin; MCH, mean corpuscular hemoglobin; PLT, platelet count; PDW, platelet distribution width. * $p < 0.05$ vs. control, ^{###} $p < 0.01$ vs. 2–4 T ($n = 12$)

Table 2 Effect of HiSMF exposure on blood biochemical indices in mice

Biochemical parameter	Control	HiSMF		
		2–4 T	6–8 T	10–12 T
ALT (U/L)	26.63 ± 5.039	22.83 ± 4.364	28.87 ± 6.446	20.12 ± 2.805
ALB (g/L)	19.87 ± 1.917	19.55 ± 1.435	19.42 ± 0.8841	19.50 ± 0.7616
TBIL (μmol/L)	0.9333 ± 0.2805	0.8833 ± 0.2639	1.050 ± 0.2168	0.8500 ± 0.2588
CHOL (mmol/L)	2.468 ± 0.3388	2.425 ± 0.3871	2.520 ± 0.3256	2.163 ± 0.1916
TG (mmol/L)	0.9250 ± 0.2887	0.7467 ± 0.4252	1.130 ± 0.4320	0.7967 ± 0.1174
BUN (mmol/L)	11.43 ± 0.9606	11.22 ± 1.144	11.18 ± 1.981	13.43 ± 3.058
CREA (μmol/L)	21.57 ± 3.254	20.77 ± 2.270	21.33 ± 3.210	23.05 ± 3.114
UA (μmol/L)	237.8 ± 20.47	192.0 ± 21.02**	194.1 ± 22.45**	191.5 ± 19.14**

Values are means ± SD. ALT, alanine aminotransferase; ALB, albumin; TBIL, total bilirubin; CHOL, total serum cholesterol; TG, triglyceride; BUN, blood urea nitrogen; CREA, creatinine; UA, uric acid. ** $p < 0.01$ vs. control ($n = 12$)

group, the PLT and PDW in the HiSMF groups showed no significant changes ($p > 0.05$).

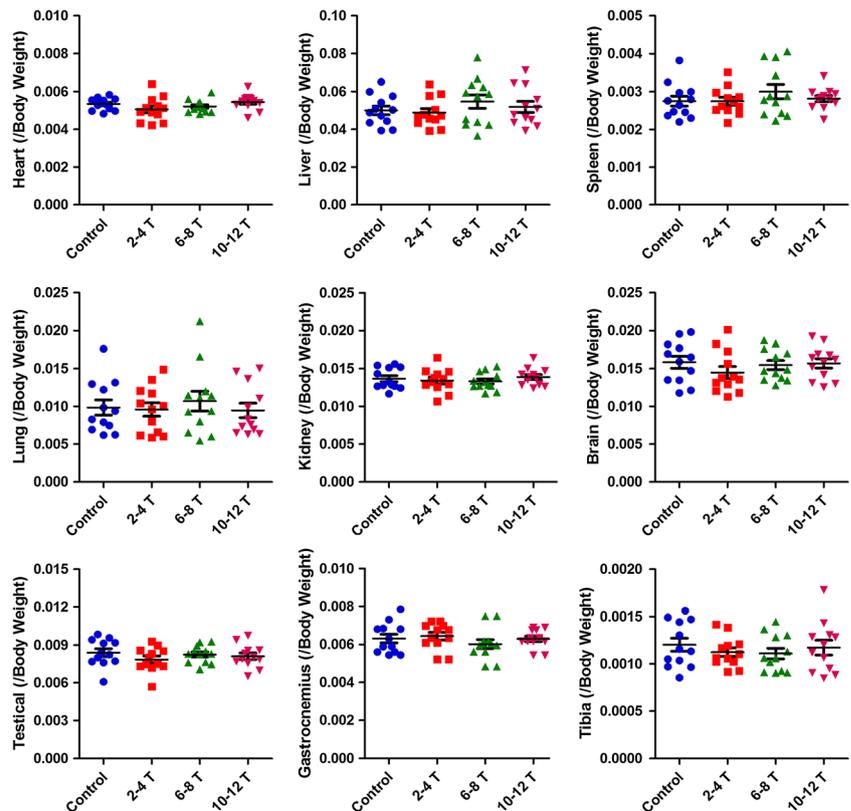
The functions of many systems in the body such as the liver, kidney, and lipid metabolism can be reflected by the serum biochemical analysis. Changes in alanine aminotransferase (ALT), albumin (ALB), total bilirubin (TBIL), total serum cholesterol (CHOL), triglyceride (TG), blood urea nitrogen (BUN), creatinine (CREA), and uric acid (UA) were evaluated in this study (Table 2). The data showed that there were no significant changes in ALT, ALB, TBIL, CHOL, TG, BUN, or CREA between the three HiSMF groups and the

control group ($p > 0.05$). The UA in the three HiSMF groups was significantly decreased compared with that of the control group ($p < 0.01$).

Effects of the HiSMF on the organ coefficients in mice

The organ coefficient is one of the most common indicators of toxicological experiments. Increases in the organ coefficient are always accompanied by organ congestion, edema, or hypertrophy; in most instances, a decreased organ coefficient indicates organ atrophy and other degenerative changes. Our

Fig. 3 Effects of the HiSMF on the organ coefficients in mice. The heart, liver, spleen, lung, kidney, brain, testicle, gastrocnemius, and dry weight of the tibia were weighed, and their organ coefficients were calculated ($n = 12$)



experimental data showed that the organ coefficient of the heart, liver, spleen, lung, kidney, brain, testicle, gastrocnemius, and dry weight of the tibia (right) did not change significantly after HiSMF exposure ($p > 0.05$) (Fig. 3).

Effects of the HiSMF on the organ's histomorphology in mice

Histomorphology observation is a simple and effective method for the clinical detection of tissue lesions. In this study, the heart, liver, spleen, lung, kidney, brain, duodenum, testicle, and proximal femur were used to evaluate the influence of the magnetic field on the histomorphology of major organs in mice by H&E staining (Figs. 4 and 5). According to its physiological structure, the kidney was divided into two parts: the renal cortex and the renal medulla.

No significant histomorphological changes were observed in the heart, liver, spleen, lung, kidney, brain, duodenum, testicle, and proximal femur after HiSMF exposure. Meanwhile, no tissue lesions, such as fibrosis, edema, necrosis, and inflammatory, appeared in the structure or morphology of the organs.

Discussion

In this study, we aimed to measure the effect of continuous and prolonged HiSMF on mice, eliminating any confusing additional stress with an adapted environment for the animals. We concluded that there were no safety issues related to mice exposure to HiSMF.

The biosafety of SMFs has been widely debated. In experiments where weak magnetic fields were used as the research conditions, the subtle differences in magnetic fields often led researchers to completely different conclusions. HiSMF is increasingly used with the development of superconducting technology. Although some previous animal studies have examined the biological effects of HiSMFs, the animals were restricted in freedom or the exposure time was not continuous [9, 16, 18, 19]. These results could not rule out the interference of stress. A study based on long-term continuous exposure can better evaluate the safety and biological effects of HiSMFs.

A stable and effective HiSMF animal feeding environment is necessary to obtain reliable data. Our environmental control device provided the same temperature, humidity, and ventilation as in traditional animal experiments. Having a special

Fig. 4 Effects of the HiSMF on the histomorphology of the heart, liver, spleen, lung, brain, and duodenum in mice. The histological characteristics of the heart, liver, spleen, lung, brain, and duodenum in the three HiSMF groups (2–4 T, 6–8 T, and 10–12 T groups) did not show any changes or lesion structures, such as inflammation, necrosis, and fibrosis, compared with those in the control group. Scale bar, 100 μm

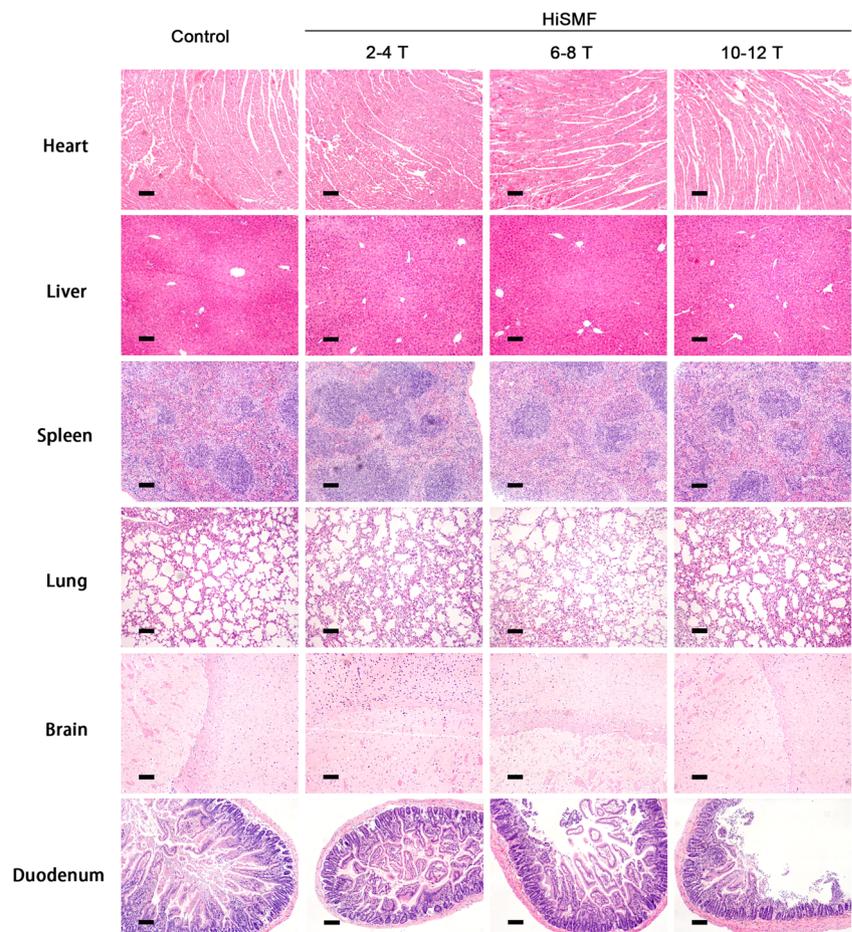
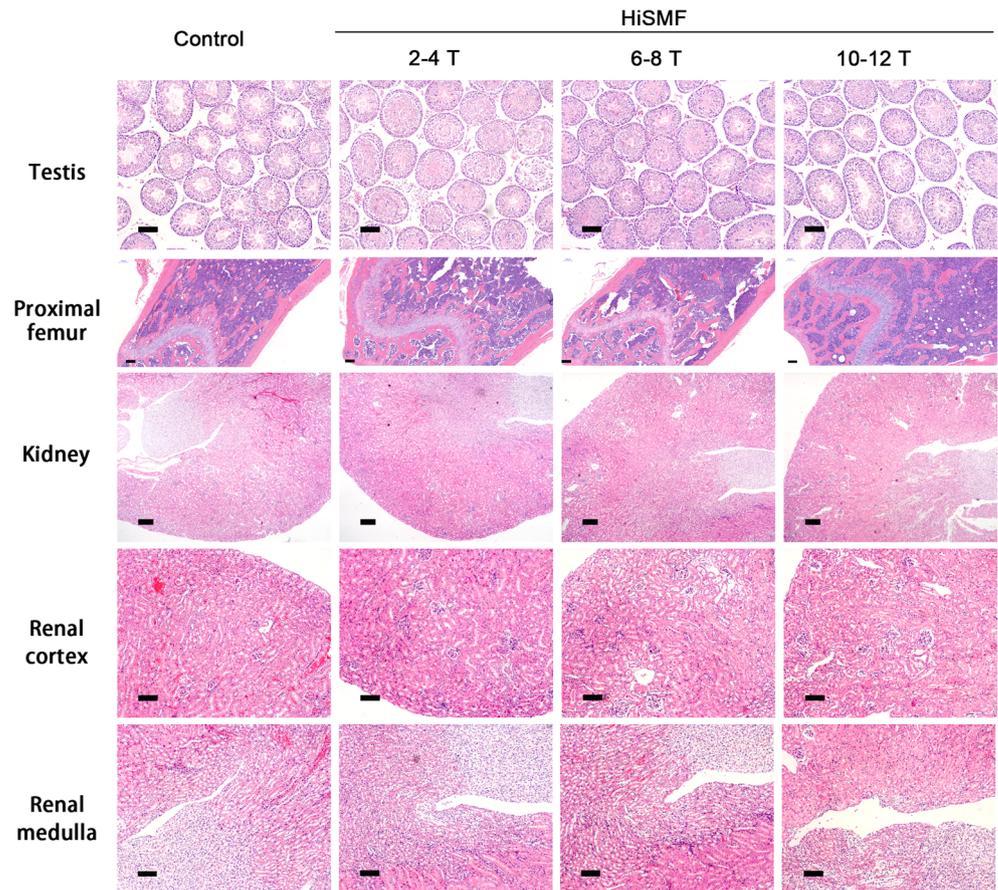


Fig. 5 Effects of the HiSMF on the histomorphology of the testicle, femur (proximal), and kidney in mice. The histological characteristics of the testicle, femur (proximal), and kidney in the three HiSMF groups (2–4 T, 6–8 T, and 10–12 T groups) did not show any changes and lesion structures, such as inflammation, necrosis, and fibrosis, compared with those in the control group. The testicle, femur (proximal), renal cortex, and renal medulla: scale bar, 100 μm ; the kidney: scale bar, 200 μm



cage with similar space to usual mice cage avoided stress due to space restrictions for their activity [20].

In this study, we found that HiSMF had no effect on the body weight of mice after HiSMF exposure for 28 days. On the third day, the body weights of the three HiSMF groups showed significant stagnation, which is justified since the sudden exposure to HiSMF may cause physiological stress in mice. As the mice adapted to the HiSMF environment, their body weights gradually recovered.

Routine blood tests are widely used to study the biological effects of magnetic fields. Trbovich's group found that continuous exposure to a 16-mT magnetic field for 28 days had no effect on hemoglobin and red blood cells in rats, while intermittent exposure to a 128-mT magnetic field led to a decrease in the WBC and number of lymphocytes in the blood [21, 22]. Cardiovascular responses to an 8-T HiSMF were within the normal physiological range in human and animal studies [23]. In our study, after 28 days of HiSMF exposure, most of the indices related to blood cells, such as WBC, RBC, MCV, and PLT, did not show significant changes. The MCH was only significantly increased in the 2–4 T group compared with that in the control group ($p < 0.05$). This may be due to the threshold influence of HiSMF on the MCH. Many studies have shown that the

biological effects of magnetic fields are related to the magnetic direction [22, 24, 25]. In this study, there was a significant difference in WBC and MCV between the mice in two different cages (facing up and down) in the 2–4 T group. However, this phenomenon did not occur in the 6–8 T and 10–12 T groups, and its mechanism needs further research.

Blood biochemical analyses inform about systemic function and metabolism. There were no significant changes of any parameter excepting UA after HiSMF exposure ($p > 0.05$). This suggested that long-term whole-body HiSMF exposure did not affect the function of the liver, kidneys, or lipid metabolism in mice. Meanwhile, compared with that of the control group, there were no significant differences in serum ALB, CHOL, and TG values in the HiSMF group, which also reflected that the HiSMF had no effect on the thyroid function and nutrient uptake in mice. Interestingly, serum UA levels were significantly decreased in the three HiSMF groups compared with that of the control group ($p < 0.01$). Conversely to an increase in UA level, a decrease is not a disease symptom.

There was no difference in organ characteristics between the control group and the three HiSMF groups. The heart, brain, liver, spleen, kidney, lung, testicle, and femur of the HiSMF

exposure groups showed no obvious histomorphological changes or pathological damage as compared with the control group. This means that HiSMF exposure only has limited influence on the mice health. Previous studies on the safety of HiSMF mainly focused on the effects on the nervous system. A study showed that MRI could affect some patients' transient senses, such as the perception of magnet phosphenes, a metallic taste, or vertigo-like sensations [26]. In animal experiments, HiMSFs induced vestibular stimulation in mice [27]. A few studies have reported alterations in eye-hand coordination, visual contrast sensitivity, and visual and auditory working memory after MRI [28]. However, most of these results were based on a study of short-term HiSMF exposure. We did not find any anomalous behavior or brain tissue changes in this study. Therefore, the subtle changes in the nervous system are most likely due to the stress response to the magnetic field environment.

There are some limitations in this study. First, caused by the limitations of the feeding system, the data of animal diet and excretion could not be available during the course of this study. Second, since high magnetic fields interfere with video acquisition, the animal's behavioral characteristics could not be observed in real time. Third, due to the difference in strength and gradient, the magnetic force was different in the three HiSMF groups.

Because of the rapid development of MRI in clinical situations, the scientific community needs to acquire a deeper understanding of the magnetic field's effects on biological and physical characteristics, and finally patient safety. Our data showed that long-term exposure to HiSMFs had no significant effect on the physiological indices in mice. This provides an encouraging evaluation of safety concerning HiSMF.

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Compliance with ethical standards

Guarantor The scientific guarantor of this publication is Peng Shang, PhD.

Conflict of interest The authors of this manuscript declare no relationships with any companies whose products or services may be related to the subject matter of the article.

Statistics and biometry One of the authors has significant statistical expertise.

Ethical approval Institutional Review Board approval was obtained.

Methodology

- Prospective
- Experimental
- Performed at one institution

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