Biological effects of strong static magnetic fields on insulin-secreting cells

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Abstract. The magnetic flux density of MRI for clinical diagnosis has been increasing. However, there remains very little biological data regarding the effect of strong static magnetic fields (SMFs) on human health. To evaluate the biological effects of strong SMFs, we cultured INS-1 cells under exposure to sham and SMF conditions for 1 or 2 h, and analyzed insulin secretion, mRNA expression, cell proliferation and cell number. Exposure to SMF with a high magnetic field gradient for 1 h significantly increased insulin secretion and insulin 1 mRNA expression. Exposure to SMF did not affect cell proliferation and cell number. Our results suggested that MRI systems with a higher magnetic flux density might not cause cell proliferative or functional damages on insulin-secreting cells.

1. Introduction

Magnetic resonance imaging (MRI) has been introduced to many hospitals as a non-invasive diagnostic tool. A strong static magnetic field (SMF) is one of the key components of MRI. Although, most MRI systems used in hospitals at present are made up of a SMF at a magnetic flux density of 1.5 or 3 \text{T} in Japan, MRI systems with a much higher magnetic flux density have been under development [1, 2]. During MRI diagnosis, patients are introduced into superconducting magnet and exposed to strong SMF, therefore, it is important to evaluate the biological effects of exposure to a strong SMF.

Various experimental studies to evaluate the effects of SMF have been performed over the last 30-40 years using laboratory animals [3 for review]. In contrast, there have been few studies on the effects of SMF at the cellular level [4 for review]. Meanwhile, information on the biological effects of strong SMF is still lacking.

We have been studying the effects of magnetic fields on insulin-secreting cells [5-8]. Insulin-secreting cells are appropriate for evaluating the effects of SMF on biological systems, because insulin secretion is affected by the behavior of potassium and calcium ions and their channels, which are considered to be a candidate for interaction with SMF [9].

In this study, we cultured INS-1 cells under exposure to sham and SMF conditions, and analyzed insulin secretion, mRNA expression, cell proliferation and cell number to evaluate the effects of exposure to strong SMF on insulin-secreting cells.
2. Materials and Methods

2.1. SMF exposure system
The horizontal exposure system for the strong SMF has been described previously [10]. Briefly, the SMF exposure system consists of a magnet with a bore 150 mm in diameter and 1000 mm long (JMTD-10T150M; Japan Superconductor Technology, Kobe, Japan), a DC power supply (Model 622; Lake Shore Cryotronics, Westerville, OH), cryocooler unit for the magnet (RDK-408D and CSA-71A; Japan Superconductor Technology, Kobe, Japan), a condition control unit (BNA-680S, Espec Engineering, Osaka, Japan), which supplies 5% CO₂ and 95% humidified air for the incubator, and a thermocontroller unit (CL-80R, Taitec, Koshigaya, Japan), which supplies warm water to regulate the temperature of the incubation space. The conditions of magnetic flux density and magnetic field gradient at the exposure positions are summarized in Table 1. For the sham-exposure system, we utilized a unit of the same bore shape, but without the magnetic field generator, which is covered by a high-nickel-content soft alloy (Permalloy C, JIS C 2531, Nakano Permalloy, Tokyo, Japan); the SMF in the sham exposure space was less than 5 μT.

Table 1. The magnetic flux density and magnetic field gradient at the center of the exposure positions.

| Exposure position | Magnetic flux density (T) | Magnetic field gradient (T/m) | Field-gradient product (T²/m) |
|-------------------|---------------------------|------------------------------|------------------------------|
| 3 T               | 3                         | 27                           | 81                           |
| 6 T               | 6                         | 42                           | 251                          |
| 10 T              | 10                        | 0                            | 0                            |

2.2. Insulin secretion
INS-1 cells (donated by Dr. Christopher B. Newgard, Duke University Medical Center) were seeded with 0.5 mL of culture medium on 4-well-MultiDishes (Nalge Nunc No. 176740; Thermo Fisher Scientific Inc., Waltham, MA) at a density of 7.5 × 10⁴ cells/cm², and cultured in RPMI 1640 supplemented with 10% fetal bovine serum (FBS), 10 mM 2-[4-(2-hydroxyethyl)-1-piperaziny]ethanesulfonic acid (HEPES), 1 mM sodium pyruvate, 50 μM 2-mercaptoethanol, 100 U/mL of penicillin, and 100 μg/mL of streptomycin at 37 °C with 95% humidified air and 5% CO₂ for 24 h. After cells were washed once with fresh medium, the culture medium was changed to another 0.5 mL/well of fresh medium. After cells were exposed to SMF for 1 or 2 h, aliquot of culture medium was collected and insulin concentration was measured by enzyme-linked immunosorbent assay (ELISA) kit (Sibayagi, Gunma, Japan). Insulin secretion was standardized to the cell number, which was counted by a cell and particle counter (COULTER Z1; Beckmann Coulter Inc., Fullerton, CA).

2.3. RNA extraction, cDNA synthesis, and PCR reaction
Cells were seeded on 4-well-MultiDishes at a density of 1.5 × 10⁵ cells/cm², cultured, and exposed to SMF as described in section 2.2. After exposure to SMF, cells were scraped and total RNA was isolated using an RNeasy® Mini kit (QIAGEN GmbH, Hilden, Germany) according to the manufacturer's instructions. cDNA synthesis was performed using random hexamers as primers and ExScript™ RTase (TaKaRa Bio, Siga, Japan) according to the manufacturer's instructions. Semi-quantitative PCR was performed using a SYBER® Premix Ex Taq™ kit (TaKaRa Bio) and a Smart Cycler® II System (Cepheid, Sunnyvale, CA) according to the manufacturers' instructions. The cycle conditions were as follows; after holding at 95 °C for 10 s, 45 cycles of denaturation at 95 °C for 5 s and primer annealing at 60 °C for 20 s. The primers used in this study are summarized in Table 2. β-Actin and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) expression were used to normalize the amount of template cDNA in the semi-quantitative PCR reaction.
Table 2. Sequences of primers used in real-time reverse transcription polymerase chain reaction.

| Gene    | Sequence 1 | Sequence 2 | Gene bank accession |
|---------|------------|------------|---------------------|
| insulin 1 | Forward: 5'-AAGCGTGGCATTGTGGATCA-3' | Reverse: 5'-GGAGTGGTGAGCTCAGTTGCAGTA-3' | NM_019129 |
| insulin 2 | Forward: 5'-TCAAACAGCACCTTTGTGGTTCTC-3' | Reverse: 5'-ACCTCCAGTGCCAAGGTCTGA-3' | NM_019130 |
| β-actin  | Forward: 5'-TGACAGGATGCAAGGAGA-3' | Reverse: 5'-GCTGGAAGGTGAGACAGTGAG-3' | NM_007393 |
| GAPDH   | Forward: 5'-GGCACAGTCAAGGCTGAGAATG-3' | Reverse: 5'-ATGGTGGTGAAAGACGCGCAGTA-3' | NM_017008 |

2.4. **WST-1 cell proliferation assay and Cell number**
Cells were seeded, cultured, and exposed to SMF as described in section 2.2. After SMF exposure, 50 μL of premixed WST-1 (TaKaRa Bio) was added to each well. All test plates were incubated for an additional 4 h in a conventional incubator. The optical density was then measured using a microplate reader (Sunrise™ Basic; Tecan, Zurich, Switzerland), at 450 nm. Cell number analysis was performed as described in section 2.2 excluding the measurement of insulin concentration in the culture medium.

2.5. **Statistical analysis**
Statistical comparisons were performed by analysis of variance and, when appropriate, using the Bonferroni correction method. All results are presented as the mean ± SE, and studies were repeated in at least three independent experiments.

3. **Results**

3.1. **Insulin secretion**
Figure 1 represents the insulin secretion during exposures to SMF for 1 or 2 h at 3, 6, or 10 T position. Exposure to SMF for 1 h at the 6 T position increased insulin secretion significantly. At the 3 T position, insulin secretion was slightly, but not significantly, increased by exposure for 1 or 2 h. At the 10 T position, exposure to SMF did not affect insulin secretion.

![Figure 1](image1.png)

**Figure 1.** Alterations of insulin secretion levels during exposure to static magnetic field (SMF). INS-1 cells were exposed to SMF at the 3, 6, or 10 T position for 1 or 2 h. Data are represented as the mean ± SE from three independent experiments. * P < 0.05.

3.2. **Expression of insulin mRNA**
We performed semi-quantitative RT-PCR using β-actin and GAPDH as house keeping genes. Only the data standardized to β-actin are shown in figure 2; no differences were observed between the results standardized to β-actin vs. GAPDH (data not shown). Similar to insulin secretion, a significant increase in insulin 1 (ins1) mRNA expression was detected after SMF exposure for 1 h at the 6 T position. A slight but not significant increase in insulin 2 (ins2) mRNA expression was observed after SMF exposure for 1 h at the 6 T position.

3.3. WST-1 cell proliferation assay and cell number
Insulin-secreting cells store insulin for rapid release after glucose stimulation. Therefore, insulin is released to culture medium when insulin-secreting cells die. If it occurs during exposure to SMFs, observed increase in insulin secretion (figure 1) might be due to death of insulin-secreting cells. To estimate this possibility, we assessed the cell viability by WST-1 cell proliferation and cell number (table 3). Since the WST-1 cell proliferation assay only evaluated the cellular mitochondrial activity, we performed both analyses. No effects of exposure to SMF were observed under any exposure condition, using both the WST-1 cell proliferation assay and cell number. These results suggested that the increase in insulin secretion during exposure to SMFs were not due to insulin-secreting cell death.

Table 3. The effects of static magnetic field (SMF) on cell proliferation and cell number.

| Exposure position | Cell proliferation (% of sham exposure) | Cell number ($\times 10^5$ cells/cm$^2$) |
|-------------------|----------------------------------------|----------------------------------------|
|                   | 1 h                                    | 2 h                                    | 1 h                                    | 2 h                                    |
| 3 T               | 104.5 ± 4.7                            | 100.4 ± 1.4                            | 3.28 ± 0.06                            | 3.28 ± 0.07                            |
| 6 T               | 103.8 ± 2.3                            | 101.0 ± 1.1                            | 3.10 ± 0.04                            | 3.15 ± 0.10                            |
| 10 T              | 101.4 ± 1.2                            | 99.3 ± 1.8                             | 3.23 ± 0.03                            | 3.21 ± 0.12                            |
| Sham              | (100)                                  | (100)                                  | 3.25 ± 0.05                            | 3.28 ± 0.04                            |

4. Discussion
In this experiment, we evaluated the effect of a strong SMF on insulin-secreting cells by measuring insulin secretion, mRNA expression and cell viability. Exposure to SMF at 6 T position for 1 h significantly increased insulin secretion and ins1 mRNA expression (figures 1 and 2).

We have previously reported that magnetic field gradient and/or the product of magnetic field gradient and magnetic flux density played a critical role in the effects on biological systems [11, 12]. In this study, we also detected the effects of magnetic field gradient and/or the product of magnetic field gradient and magnetic flux density was the highest, and significant effects on insulin secretion were observed at this position. At the 3 T position, where the magnetic field gradient and magnetic force was modest, a slight increase in insulin secretion was observed; indicating the relationship between magnetic field gradient and/or the product of magnetic field gradient and magnetic flux density and the increase in insulin secretion. The magnetic field gradient and/or the product of magnetic flux density and magnetic field gradient affected the diamagnetic components of cells [13] and the distribution of dissolved oxygen in the culture medium [14]. These phenomena are thought to be possible mechanisms of the effects of SMF.

Regarding the effects on insulin secretion, ins1 mRNA expression was increased by exposure to SMF at the 6 T position for 1 h. The transcription of insulin mRNA has been reported to be regulated by increases in intracellular calcium concentrations, and by secreted insulin itself [15, 16]. Therefore, exposure to SMF might increase intracellular calcium concentration, which, in turn, increases insulin secretion and insulin mRNA, or exposure to SMF might directly increase insulin secretion resulting in increased insulin mRNA expression.

Evaluation of metabolic activity of insulin-secreting cells is possible by combination of WST-1 cell proliferation assay and cell count [17]. From our results of the WST-1 cell proliferation assay and cell count (table 3), exposure to SMF did not affect the metabolic activities of INS-1 cells. Although a reduction of metabolic activity in the human leukemic cell line HL-60 following 72 h exposure to SMF at 1 T has been reported [18], the time period of exposure and the cell line used in the current study were different from this report. This could explain why we did not detect the effects of SMF on metabolic activities in this study.

In conclusion, MRI systems with higher magnetic flux density are currently under development, despite the fact that the effects of exposure to strong SMF on biological systems have not been well clarified. Our results demonstrated that exposure to magnetic fields with high magnetic flux density and magnetic field gradient did not cause any damage to insulin-secreting cells, and suggest that MRI systems with higher magnetic flux density might not cause cell proliferative or functional damages on cells.

Acknowledgments
This study was supported in part by a Grant-in-Aid from the Magnetic Health Science Foundation and a Grant for Hirosaki University Institutional Research.

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