The Innovation

Report

A Static Magnetic Field Improves Iron Metabolism and Prevents High-Fat-Diet/Streptozocin-Induced Diabetes

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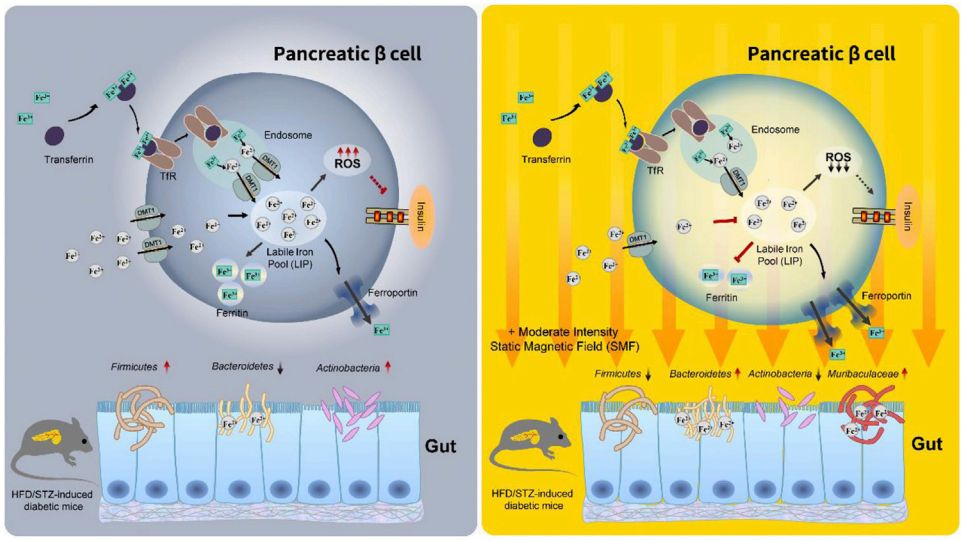
Received: October 22, 2020; Accepted: January 3, 2021; Published Online: January 6, 2021; https://doi.org/10.1016/j.xinn.2021.100077

GRAPHICAL ABSTRACT



PUBLIC SUMMARY

- A device made of permanent magnet could lower blood sugar level in type II diabetic mice
- The fatty liver, weight gain, and tissue injury were reduced
- Iron metabolism, pancreatic β cell function and gut microbiota were improved
- The magnetic field intensity, direction and distribution are important

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Received: October 22, 2020; Accepted: January 3, 2021; Published Online: January 6, 2021; https://doi.org/10.1016/j.xinn.2021.100077

Citation: Yu B., Liu J., Cheng J., et al. (2021). A Static Magnetic Field Improves Iron Metabolism and Prevents High-Fat-Diet/Streptozocin-Induced Diabetes. The Innovation **2(1)**, 100077.

Type 2 diabetes (T2D) is a metabolic disorder with high prevalence and severe complications that has recently been indicated to be treatable by a combined static magnetic field (SMF) and electric field. We systematically compared four types of SMFs and found that a downward SMF of ~100 mT could effectively reduce the development of hyperglycemia, fatty liver, weight gain, and tissue injury in high-fat-diet (HFD)/streptozocin-induced T2D mice, but not the upward SMF. The downward SMF markedly restored the Bacteroidetes population and reversed the iron complex outer membrane receptor gene reduction in the mice gut microbiota, and reduced iron deposition in the pancreas. SMF also reduced the labile iron and reactive oxygen species level in pancreatic Min6 cells *in vitro* and prevented palmitate-induced Min6 cell number reduction. Therefore, this simple SMF setting could partially prevent HFD-induced T2D development and ameliorate related symptoms, which could provide a low-cost and non-invasive physical method to prevent and/or treat T2D in the future.

KEYWORDS: type 2 diabetes; static magnetic field; ROS; iron metabolism; insulin

INTRODUCTION

Type 2 diabetes (T2D), the predominant type of diabetes, a metabolic disorder characterized by high blood sugar (hyperglycemia) and a series of complications, is becoming a major burden on the health care system and severely affects patient life quality. Diabetes symptoms often vary between individuals, depending on how much their blood sugar is elevated. For some T2D patients and people with prediabetes, they may not experience symptoms initially. Therefore, preventing the development of diabetes in a safe and effective way will be important for the people with T2D or prediabetes.

The magnetic field (MF) is a non-invasive physical tool, which has been indicated by a few studies to influence diabetes, but the results are controversial. There are some studies indicating that various MFs could have beneficial effects in treating or preventing diabetes. For example, exposure to a 45 mT static magnetic field (SMF) reduced numbness and pain on the foot of diabetic neuropathy patients.[1] Exposure to 10 Hz, 8 mT pulsed electromagnetic fields (PMFs) with complex modulation was found to have therapeutic efficacy, especially in the initial stages of diabetic polyneuropathy and in patients with diabetes mellitus for up to 10 years.[2] Moreover, exposure to 15 Hz, 1.6 mT PMF was found to have positive effects on diabetic peripheral neuropathy in streptozocin (STZ)-treated mice.[3] The blood glucose level of diabetic mice was reduced when treated with ~2.8–476.7 mT inhomogeneous SMF for 12 weeks.[4] It has even been reported in a case report that applying a 0.25 T magnet at auricular acupuncture points in a diabetic patient could help reduce the blood glucose level and improve eye conditions.[5,6] In addition, various MFs have also been shown to ameliorate diabetic wound healing.[7]

Recently, Carter et al. performed multiple mouse experiments to demonstrate that combined static magnetic and electric fields have obvious therapeutic effects on T2D, which provides promising evidence for developing it as a potential therapeutic physical method.[8] However, there are also some studies showing opposite or no effects of SMFs on the blood glucose level, which were performed on normal non-diabetic animals. For example, exposure to a 128 mT SMF induced a decrease in serum insulin secretion and an increase in the blood glucose level in normal rats.[9] Abbasi et al. found that a constant MF of 50 mT had no significant effect on weight gain or the blood glucose level in BALB/c mice.[10]

It has been clearly demonstrated that different MF parameters, including MF type, frequency, intensity, gradient, or even direction can directly affect their biological consequences.[11] There are different MF types, including SMFs and various dynamic MFs, such as pulsed MF, alternating MF, etc. Even for the simplest MF type, SMF, which has a fixed field direction and intensity over time, there are still multiple changeable parameters, including SMF intensity, gradient, direction, and spatial distribution, that could contribute to the differential bioeffects. For example, our group previously found that the spindle orientation in human cells can be affected by a 27 T ultra-high SMF, but not SMFs below 1 T.[12] We also found that a gradient ultra-high SMF could change the relative position of the cell nucleus inside a cell, while a non-gradient homogeneous SMF did not.[13] More interestingly, multiple studies, including ours, found that SMF direction can produce differential effects on mice and cells.[14–18]

In this study, we compared four different SMFs with different intensities and spatial distributions on high-fat-diet (HFD)/STZ-induced T2D mice. Our results showed that a moderate-intensity vertically downward SMF can effectively prevent the development of HFD/STZ-induced high blood glucose, weight gain, fatty liver, gut microbiota aberration, and tissue damage by regulating iron metabolism, increasing insulin secretion, and reducing oxidative stress in pancreatic β cells.

RESULTS

Exposure to a Downward Magnetic Field Ameliorated Hyperglycemia in T2D Mice

To investigate the effects of SMFs on T2D, four different SMF settings were used in addition to their "sham" control, which maximally mimicked SMF exposure conditions without real magnets (Figures 1A and 1B). We compared the vertically upward versus downward SMFs to examine the impact of different SMF directions (Figure 1A). In addition, we used magnetic plates with magnets inserted with alternating poles (AP-SMF), facing up, and compared two different intensities (Figure 1B). It should be noted that the 0.4 and 0.6 T AP-SMFs reflect only the approximate SMF intensity at the magnet surface. To get an accurate measurement of the SMF distribution at the mouse's location, we used a magnet analyzer to scan the horizontal planes at 1 cm above the magnetic plate for all four plates (Figures 1C and 1D),

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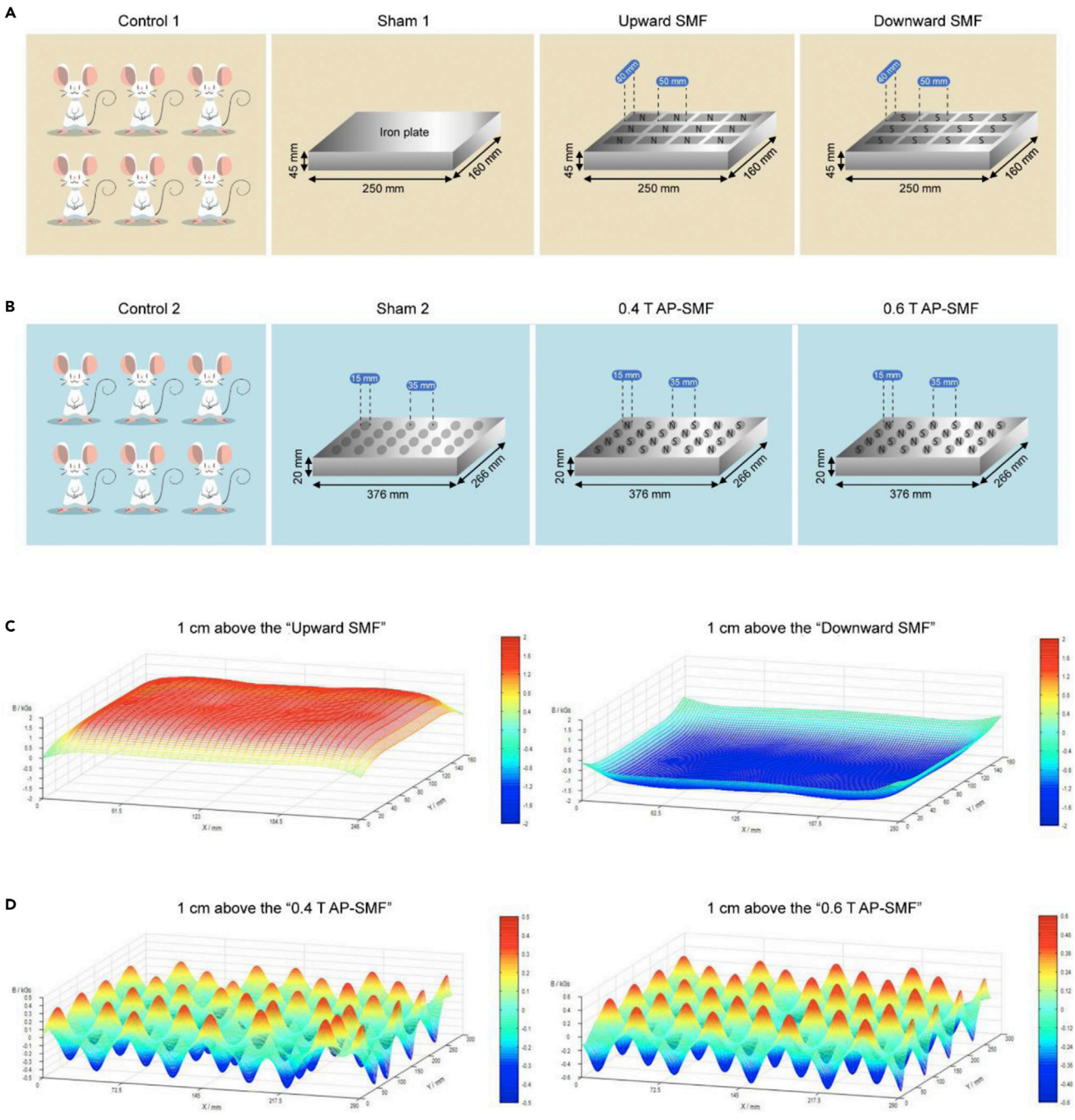

Figure 1. Four Different Magnetic Field Exposure Conditions (A) A diagram of the sham versus two magnetic plates that provide upward and downward static magnetic field (SMF). (B) A diagram of the sham versus alternating pole SMF (AP-SMF) plates. "0.4 T" and "0.6 T" mean that the magnetic field intensity of the magnet surface is around 0.4 or 0.6 T. Control mice were normally fed and not exposed to any sham or magnetic plates. Experimental mice were placed on top of the sham or magnetic plates. (C and D) Magnetic field distribution in the mouse exposure area, 1 cm above the magnetic plates. (C) Upward and downward SMF. (D) 0.4 and 0.6 T AP-SMF.

where the mouse's abdomen was located. At this horizontal plane, although the average SMF intensity of the upward and downward SMF is ~100 mT (Figure 1C), and the average peak intensities of the two AP-SMF plates are in similar ranges (~40–50 mT) (Figure 1D), the MF distributions, gradients, and magnetic fluxes are completely different.

HFD induces insulin resistance, but a robust β-cell response could prevent hyperglycemia. Therefore, STZ is frequently used to prevent β-cell compensation and used in combination with HFD to set up the T2D mouse model. Here we used HFD/STZ-induced T2D diabetic mice to investigate the effects and mechanisms of the four different SMF setups (Figure 2A). As expected, HFD alone resulted in gradually elevated fasting blood glucose (FBG) levels in a time-dependent manner, which reached approximately 10 mmol/L on week 11. Then, after STZ injection, the FBG levels reached ≥11.1 mmol/L, the standard for hyperglycemia (Figure 2B). Interestingly, our results showed that the FBG levels in only the downward SMF treatment group were significantly reduced compared with those of the sham control group, and not those of the other three magnetic conditions (Figure 2B). In fact, the upward SMF treatment even had increased FBG levels, which showed that the SMF direction is a key factor that contributes to the blood glucose regulation.

Next, we used intraperitoneal glucose tolerance test (IPGTT) and intraperitoneal insulin tolerance test (IPITT) to evaluate glucose tolerance and insulin sensitivity at 14–15 weeks of age. The mice were fasted for 12 h prior to

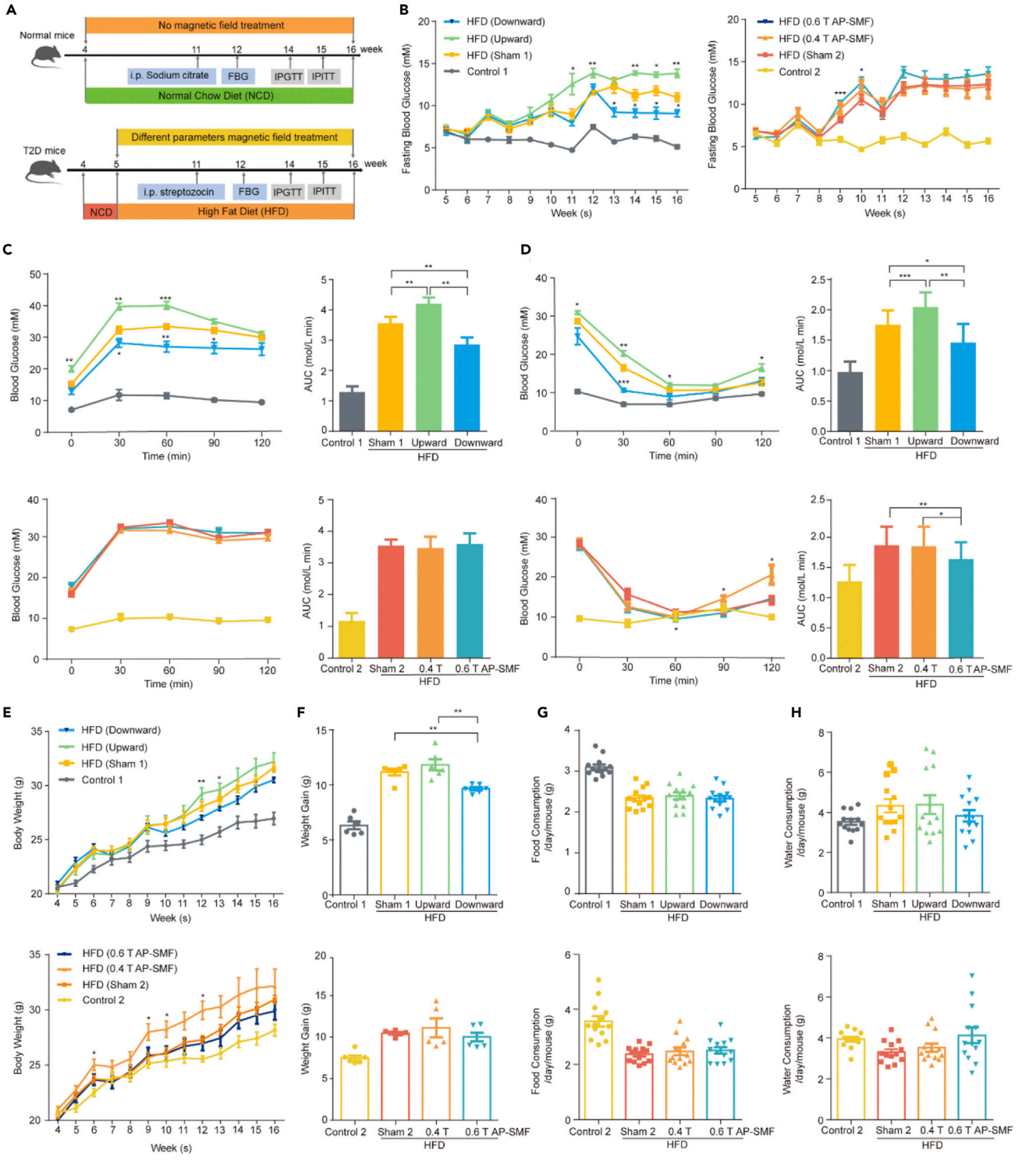

Figure 2. Exposure to a Downward Magnetic Field Ameliorated Hyperglycemia in T2D Mice (A) Experimental design. (B–D) Blood glucose level measurement in control group and T2D mice groups. (B) FBG (fasting blood glucose) level; (C) IPGTT (intraperitoneal glucose tolerance test); (D) IPITT (intraperitoneal insulin tolerance test). (E and F) Body weight (E) and body weight gain (F) in different magnetic field treatments. (G and H) Food (G) and water (H) consumption in control group and T2D mice groups. Values were expressed as means ± SEM (n = 5 or 6). *p < 0.05, **p < 0.01, and ***p < 0.001.

IPGTT assays. It is obvious that only the downward SMF treatment could reduce the blood glucose excursions in T2D mice, and not any of the other three magnetic conditions (Figure 2C). The downward SMF significantly lowered the area under the curve in IPGTT, showing a potent anti-diabetic effect. In contrast, the upward SMF significantly decreased the ability of glucose clearance compared with the sham control, suggesting an unfavorable effect on glucose-dependent insulin secretion (Figure 2C). For IPITT, it is obvious that the downward SMF could improve the insulin sensitivity of T2D mice (Figure 2D). Moreover, the body weight growth curves showed that the downward SMF-treated T2D mice had gained less weight than the other HFD groups (Figures 2E and 2F), while there was no food and water consumption difference among different HFD groups (Figures 2G and 2H).

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Since T2D could induce and aggravate mental disorders such as depression and anxiety,[19] we used the open field test (OFT) to measure the anxiety or exploration-related behavior of the mice (Figure S1). OFT results showed that the total traveled distance and the number of entries into the outer zone did not significantly differ among T2D mice, but the time spent in the center zone was increased ($p < 0.05$), which indicated a lower anxiety-like behavior and increased exploratory behavior in the downward SMF-treated mice compared with the upward SMF and the sham group (Figures S1A–S1G).

Therefore, by comparing four different SMF settings, our results show that only the moderate-intensity vertically downward SMF can effectively prevent the development of HFD/STZ-induced high blood glucose and weight gain, enhance exploratory activity, and decrease anxiety behavior in T2D mice.

Exposure to a Downward SMF Reduces Hypercholesterolemia and Lipid Accumulation in Liver

HFD usually leads to hypercholesterolemia and lipid accumulation in liver, which is one of the most prominent symptoms of HFD-fed mice. Our blood biochemistry analysis showed that, although the serum triglyceride, high-density lipoprotein cholesterol, and low-density lipoprotein cholesterol in T2D mice were not obviously affected by various MFs, the downward SMF and 0.4 T AP-SMF could reduce the total cholesterol level in diabetic mice (Figures S2A and S2B). Furthermore, routine blood assays indicated that the downward SMF treatment upregulated numbers of white blood cells, monocytes, and neutrophilic granulocytes in T2D mice (Figure S3).

To directly assess the impact of different SMF conditions on tissues, we collected the mouse liver, pancreas, kidney, and retina for H&E stain. The hepatocytes were arranged in cords radiating around the central vein and showed apparent hepatic steatosis in liver sections of T2D mice. Tissue examination results showed that the downward SMF reduced the lipid accumulation in T2D mouse liver (Figures S2C and S2D). Conversely, the upward SMF exposure led to aggravating macrovesicular steatosis in hepatocytes (Figure S2C). In addition, the downward SMF-treated T2D mice also revealed amelioration in vacuolation, irregular shape, and/or even atrophy state in glomerulus and islet tissues (Figure S2C). Compared with the non-diabetic mice, T2D mice developed an irregular arrangement of cells in the inner and outer nuclear layer in retina tissues, whereas these were alleviated by downward SMF exposure (Figure S2C). Therefore, these results suggest that the downward SMF can ameliorate tissue injury in T2D mice.

The Downward SMF Effectively Restores Bacteroidetes and *f_Muribaculaceae* Populations in the Gut Microbiota Composition of T2D Mice

By comparing four different SMF settings, we found that only the moderate-intensity downward SMF could have significant beneficial effects on alleviating symptoms in the diabetic mice. Since gut microbiota metabolism was reported to play a critical role in insulin resistance and blood glucose regulation,[20,21] we set out to test whether the glucose regulation effect of the downward SMF in T2D mice was associated with the gut microbiota and found that the two SMFs with opposite directions differentially changed the relative abundances of some bacteria that may function in insulin and blood glucose regulation (Figure 3).

Feces samples were collected and analyzed at 16 weeks of age, when the mice had been treated with SMFs for 12 weeks. Interestingly, the downward SMF led to significant overall structural changes in the T2D mouse gut microbiota (Figures 3A and S4). Compared with the normal mice, T2D mice had increased Firmicutes, Proteobacteria, and Actinobacteria but reduced Bacteroidetes (Figures 3B and S5). It is interesting that the downward SMF effectively partly reversed the HFD/STZ-induced effects in these diabetic mice. For example, the downward SMF treatment increased the Bacteroidetes:Firmicutes ratio by 3.4-fold ($p < 0.01$) and decreased the population of Actinobacteria 5.3-fold ($p < 0.05$) (Figures 3C and S5). In contrast, the upward SMF had no significant effect at the phylum level (Figures 3B and S5). At the genus level, *f_Muribaculaceae* abundance was significantly increased in the downward SMF group (18.3 ± 5.9%; $p < 0.01$) but not in the upward SMF group (5.0 ± 2.1%) (Figures 3D and 3E). In addition, the downward SMF also stimulated the growth of another two genera of the Bacteroidetes, named *Alistipes* and *Odoribacter* ($p < 0.01$), but restored T2D-induced upregulation of *f_Lachnospiraceae* ($p < 0.05$), *Coriobacteriaceae*_UCG-002 ($p < 0.01$), and *Corynebacterium*_1 ($p < 0.01$) (Figure S6).

To detect specific bacteria that covaried with different SMF directions, linear discriminant analysis coupled with effect size was subsequently employed, which identified 38 operational taxonomic units in the three T2D mouse groups that were significantly different (Figures 3F and S7). Obviously, *g_Rikenellaceae*_RC9_gut_group, *g_Alistipes*, *g_Odoribacter*, and especially *f_Muribaculaceae*, which all belong to order Bacteroidales, were associated with the downward SMF. But *g_Corynebacterium*_1, classified to order Corynebacterium, and *g_Weissella*, classified to order Lactobacillales, were associated with the upward SMF.

Exposure to the Downward SMF Regains Iron Complex Outer Membrane Receptor Genes in the T2D Gut Microbiota

We speculated that the composition alterations in the gut microbiota could result in a microbial function change responsive to the upward and downward SMFs. Metagenomic sequencing was used to explore different KEGG pathways in the four groups. The community structure and abundance of gut microbiota among different samples derived from unigenes fit 16S rDNA analysis (Figure S8). Among the assigned 525 KEGG pathways, carbohydrate metabolism, global and overview maps, membrane transport, translation, nucleotide metabolism, cofactors, and vitamin metabolism accounted for the top six highest proportions (Figure 4A). As shown in Figures 4B and 4C, only the downward SMF had recovery effects on genes for butanoate metabolism and glycoside hydrolases families GH2 and GH3, related to carbohydrate metabolism in T2D mice (Figures 4B and 4C and Table S1).

Furthermore, among the top 30 enriched KEGG function terms, KOs associated with replication and repair (K04763, K03655, K00558, K02315) and membrane transport (K01992, K02014) were upregulated with exposure to the downward SMF but not the upward SMF (Figure 4D). Notably, K02014, annotated to the iron complex outer membrane receptor protein, was reduced sharply in T2D mice (about 2.7-fold), and the upward SMF treatment further lessened its abundance, but the downward SMF treatment restored its abundance by 2.1-fold. This gene was mainly derived from the phylum Bacteroidetes (Figure 4E). In addition to the unclassified genera, there were eight genera mainly harboring K02014, and T2D significantly changed their proportions (Figure 4F). Among them, Bacteroides accounted for the most variable abundance of this gene under SMFs with different directions (Figure 4F).

Collectively, our data show that different SMF directions can affect the content of iron complex outer membrane receptor genes in gut microbiota by regulating the microbiota redistribution *in vivo*, which may allow more iron in the diet to enter microbiota. In other words, it is possible that less iron in the diet will enter the mouse body in the downward SMF-treated mice. Consistently, Prussian blue staining assays were used to assess the iron content in mouse islets and showed that the iron storage in T2D mice was decreased by the downward MF treatment and increased by the upward MF, as indicated by blue-stained spots (Figure S9A).

The Downward SMF Alters Mouse Pancreatic β-cell Iron Metabolism *In Vitro*

Next, we used the saturated fatty acid palmitate (PA) to induce mouse pancreas Min6 cell lipotoxicity *in vitro* (Figure 5A), which mimics the HFD-induced pancreatic cell alteration. We placed the cell culture plates on the top of the magnets, where the cells were exposed to ~0.1–0.5 T SMFs (Figures 5B–5D). It is interesting that, although the total iron levels in Min6 cells were not affected, the ferrous (Fe^{2+}) levels were decreased by the 0.24 and 0.5 T downward SMFs, but not upward SMFs (Figures 5E–5H). This indicates that the conversion between Fe^{2+} and Fe^{3+} was affected.

The increase in labile iron could be from iron import, storage, and/or efflux. IRP2 (iron-dependent protein 2) plays a central role in the regulation of intracellular iron metabolism by binding to IREs (iron-responsive elements)

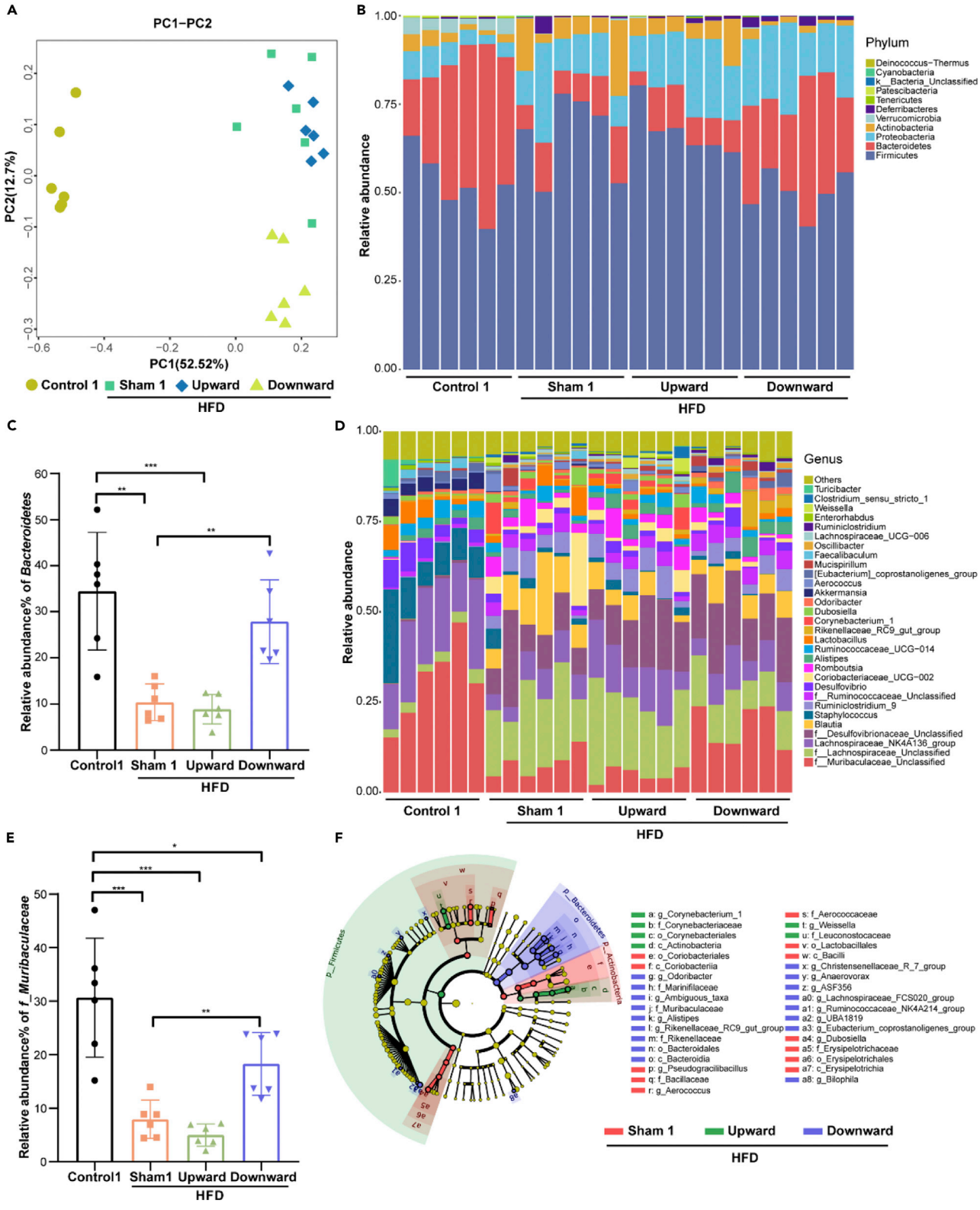

Figure 3. The Downward Magnetic Field Treatment Effectively Restores Phylum Bacteroidetes and Genus *f_Muribaculaceae* Populations in the Gut Microbiota Composition of T2D Mice (A–E) (A) Principle coordinates analysis. (B) The taxonomic composition distribution at the phylum level. (C) The relative abundance of phylum Bacteroidetes. (D) The taxonomic composition distribution at the genus level. (E) The relative abundance of genus *f_Muribaculaceae*. (F) Cladogram generated from linear discriminant analysis (LDA) coupled with effect size exhibits Bacteroidetes covarying with the downward SMF and Firmicutes covarying with the upward SMF. Taxa enriched in microbiota from sham 1 (red), upward SMF (green), or downward SMF (blue) are indicated with a positive LDA score (taxa with LDA score >2 and significance of $\alpha < 0.05$ determined by Wilcoxon signed-rank test). *p < 0.05, **p < 0.01, and ***p < 0.001.

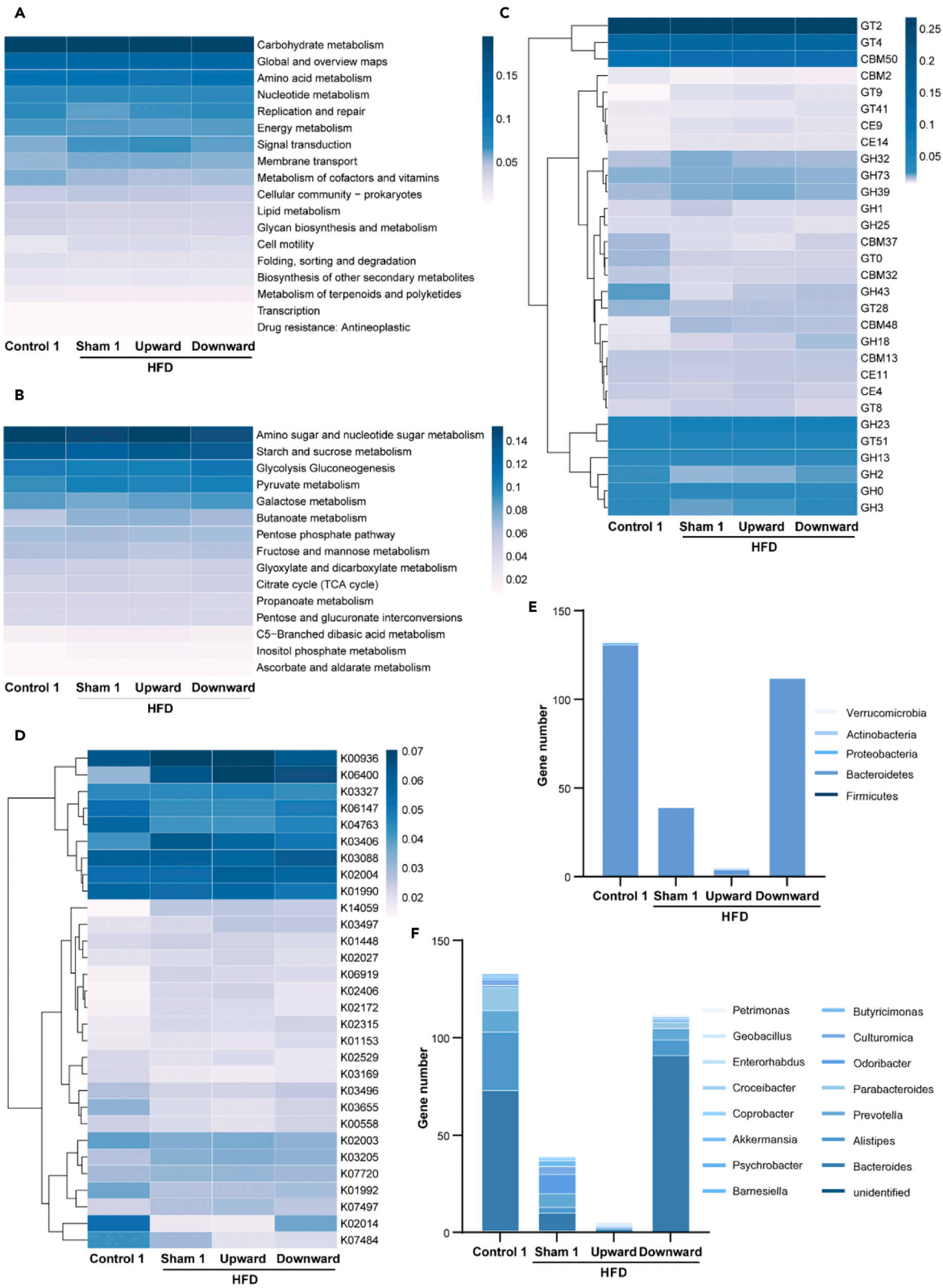

Figure 4. Exposure to the Downward SMF Regains Iron Complex Outer Membrane Receptor Genes in the T2D Gut Microbiota (A–C) Heatmaps of the relative abundance of various metabolic pathways (A), the carbohydrate metabolism pathway (B), and carbohydrate-active enzymes (C). (D) Heatmap of the top 30 enriched KEGG function terms showing different enrichments among the four groups. (E and F) Comparisons of the gene numbers of the outer membrane receptor protein mapped to various phyla (E) and genera (F).

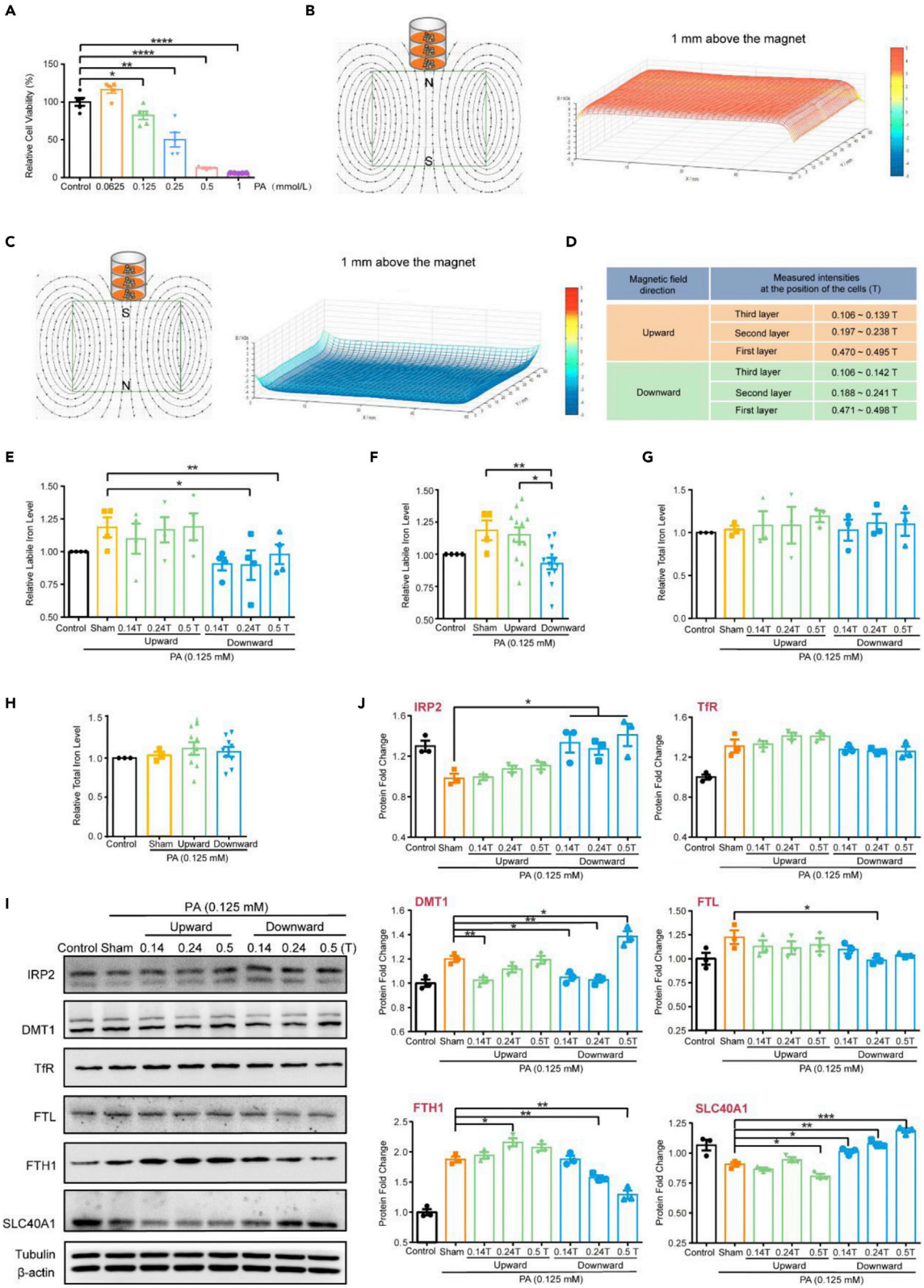

Figure 5. The Downward SMF Alters Mouse Pancreatic β-Cell Iron Metabolism *in vitro* (A–E) (A) Cell viability assay. (B and C) Diagrams show examples of upward magnetic field (B) and downward magnetic field (C) treatments, in which the SMF directions are in parallel with and opposite to that of gravity, respectively. The arrows indicate the direction of SMF from the north (N) to south (S) pole. (D) Measured intensity at the position of cells. (E) Relative labile iron content was determined by flow cytometry analysis. (F) Relative labile iron level treated by different magnetic field direction in Min6 cells. (G and H) (G) Relative total iron level was determined. (H) Relative labile iron level treated by different directions of magnetic field in Min6 cells. (I and J) Protein expression levels of TfR, DMT1, FTH1, FTL, SLC40A1, and IRP2 in PA-treated Min6 cells by different SMFs. (I) Western blot. (J) Protein fold change. Values are expressed as means ± SEM. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, and ****$p < 0.0001$.

present in the untranslated region of mRNAs encoding iron-related proteins. Its level is often correlated with labile iron (Fe^{2+}) level. Western blot results showed that IRP2 expression was slightly increased in the downward SMF-treated Min6 cells, indicating lower levels of labile iron (Figure 5I). Meanwhile, the iron intake proteins TfR and DMT1 and intracellular iron storage proteins FTH1 and FTL were all downregulated (Figure 5J). In contrast, the iron export protein SLC40A1 was upregulated. These results indicate that the downward SMF can reduce iron uptake and storage and increase iron export in pancreatic cells.

The Downward SMF Reduces Oxidative Damage in Palmitate-Induced Pancreatic β Cells

Iron homeostasis has emerged as a key modulator of glucose and lipid metabolism, and is involved in oxygen binding, transport, and metabolism by activating key enzymes in the processes of cell growth, proliferation, and differentiation.[22] Due to less expression of antioxidant enzymes, the pancreatic islets are particularly susceptible to oxidative damage caused by excess iron, which results in β-cell apoptosis, insulin secretion reduction, and insulin resistance.[23] To elucidate the mechanisms of the SMF effects on the pancreas, we performed cellular assays on PA-treated Min6 cells. Although the cell apoptosis and protein expression levels in apoptosis pathways were only slightly changed by various SMF treatments (Figures 6B–6E and S9B), the PA-induced intracellular reactive oxygen species (ROS) elevation and cell number reduction can be remarkably prevented by the downward SMF ($p < 0.05$, Figure 6A). Furthermore, the downward SMF exposure resulted in a slightly larger size of pancreas islets and more insulin secretion compared with the other HFD mice as shown by insulin immunohistochemical staining (Figures 6F–6H). These results suggest that the downward SMF treatment has a protective effect on the pancreas against HFD-induced pancreatic cell dysfunction.

DISCUSSION

There have been several studies reporting that SMFs of different directions can lead to differential bioeffects.[14,17,18,24–26] For example, the levels of copper and zinc in different organs could be changed differentially by SMFs of different orientations.[17,18] In this study, we systematically investigated the biological effects of different SMF directions, distributions, and intensities on T2D mice. We discovered that the downward SMF of hundreds of milliteslas could partially prevent the development of HFD-induced diabetes. The downward SMF-treated T2D mice have reduced blood cholesterol and glucose levels, as well as reduced body weight gain and lipid accumulation in liver. These beneficial effects are correlated with improved gut microbiota, reduced labile iron, and reduced ROS levels in pancreatic cells, which have protective effects on the pancreas and increase insulin secretion.

Our results show that MFs of different directions affected gut microbiota composition differentially, which may explain the beneficial effects of the downward SMF in relieving T2D symptoms. Generally, the increased ratio of Bacteroidetes:Firmicutes is a common signature seen in lean and healthy individuals, and is usually reduced in obesity and obesity-induced T2D.[27–29] We found that the downward SMF could significantly increase this ratio. Moreover, Muribaculaceae, also called family S24-7 or Bacteroidaceae S24-7 group, decreased significantly in HFD-fed mice, which is consistent with other reports using high-calorie diets.[30,31] Our results show that downward SMF treatment could effectively restore *f_Muribaculaceae*, which might lead to a hyperglycemia-ameliorating effect. In addition, HFD could cause gut inner mucus layer barrier deficiency and further leave the host confronting a high bacteria load and insulin resistance.[32,33] We found that the downward SMF-treated T2D mice have restored *f_Muribaculaceae* and *g_Odoribacter*, which are correlated with improved inner mucus layer barrier function,[34–38] which may result in improved inner mucus layer barrier in T2D mice. Furthermore, the microbiota is involved in iron availability regulation as verified by both *in vitro* and animal assays.[39,40] Therefore, the increased iron complex outer membrane receptor genes corresponding to the upregulated Bacteroidetes population on exposure to the downward SMF here might be used for iron storage or scavenging from the diet, thereby limiting the iron availability in mice, like other reported gut microorganisms.

Iron is known as an essential nutrient obtained mainly from dietary sources and is involved in the processes of cell growth, proliferation, and differentiation as a cofactor of several enzymes and as a major component of oxygen transporters.[22] Iron homeostasis has emerged as an essential modulator, not only for glucose and lipid metabolism, but also for gut microbiome composition.[22,41] In fact, there are multiple correlations between iron overload disorder and T2D. For example, excessive iron stores can increase the risk of developing T2D among patients with hemochromatosis.[42] In another two prospective cohort studies, moderately increased body iron stores at baseline were found to be significantly related to T2D incidence in both men[43] and women.[44] In addition, a meta-analysis of five studies showed higher intake of heme iron can lead to T2D.[45] Iron metabolism is tightly controlled by a set of iron-dependent proteins and divided into the processes of iron intake, utilization, storage, and efflux. TfR and DMT1 mediate the intake of transferrin-bound iron and non-transferrin-bound iron; intracellular iron can be stored in ferritin for reducing oxidative stress from excess labile iron; and iron is exclusively exported by SLC40A1.[46] In our study, we found that downward SMF downregulated TfR, FTL1, and FTH1 and upregulated SLC40A1, which are consistent with the decreased labile iron in pancreatic Min6 cells, which makes them less prone to oxidative stress. In addition, we suspect that the magnetic susceptibility difference of Fe^{2+} and Fe^{3+} may affect their responses in an MF, which could contribute to the changed conversion between Fe^{2+} and Fe^{3+} in cells.[47]

It has been shown that pancreatic β cells are exquisitely sensitive to ROS formation due to their inadequate antioxidative defense,[48,49] which may contribute to their impaired function and viability in diabetes. There have been multiple studies indicating that moderate-intensity MFs can reduce ROS levels,[50] including the recently published study that used a combination of SMF and electric field.[8] Our results show that the lipid-induced ROS elevation can be reversed by SMFs, which likely contributes to the protective effects of SMFs on pancreatic β cells.

It should be mentioned that although HFD/STZ is a very commonly used way to induce T2D mice, we found that these mice are still sensitive to insulin, as shown in Figure 2D. In fact, Srinivasan et al. also report that HFD/STZ-treated rats are sensitive to glucose-lowering effects of insulin sensitizing (pioglitazone) as well as insulinotropic (glipizide) agents.[51] Our results show that the downward SMF could increase the glucose tolerance and insulin sensitivity in HFD/STZ-induced diabetic mice. Using more types of diabetic animal models will be the next step in unraveling the potential therapeutic effects of SMFs.

It is very interesting that the recently published work by Carter et al. found that T2D can be treated by electric and magnetic fields.[8] They combined a 3 mT SMF with an electric field, which is a much more complicated device than ours. Here in our study, we compared four different MF settings with variable MF intensities and distributions. Surprisingly, we found that an MF provided by an array of simple magnets, which have obvious advantages of low cost, could prevent the development of T2D. While both Carter's and our work provided very useful information for developing future devices for clinical application, the experimental settings and mechanisms are different. Carter et al. found that the combined weaker MF (3 mT) with an electric field could ameliorate insulin tolerance and glucose intolerance. We found that stronger MF (~100 mT) alone with a downward direction could improve pancreas function by regulating iron metabolism, ROS production, and gut microbiota, which partially prevent the development of HFD-induced T2D. However, it should be mentioned that, from a physical point of view, it is impossible to expect any difference in the interaction of the upward and downward SMFs with the iron complex, or its related genes. The observed difference in the effects of the upward and downward SMFs on gut microbiota and pancreatic cells can only arise due to underlying biophysical processes, which needs further investigations. The molecular mechanism may involve quantum biology intervention that includes ROS production changes through

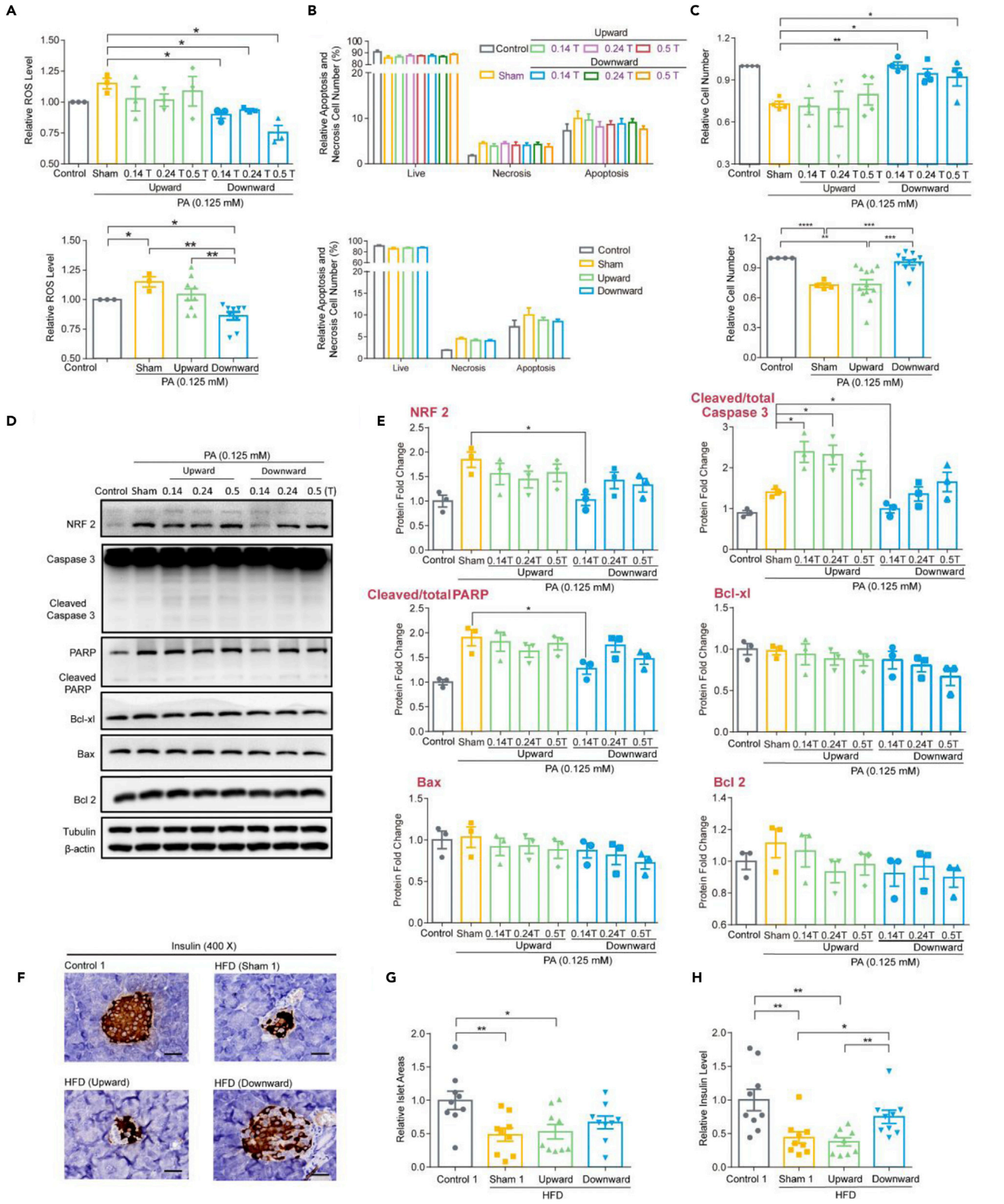

Figure 6. The Downward SMF Reduces Oxidative Damage in Palmitate-Induced Pancreatic β Cells (A–E) (A) ROS assay. (B) Cell apoptosis assay. (C) Cell number. (D and E) (D) The representative results of Western blot analysis of the NRF 2, total Caspase-3, cleaved Caspase-3, PARP, cleaved PARP, Bax, Bcl-xl, and Bcl 2 proteins in Min6 cells. (E) Protein fold change. (F) Histological images of the pancreas islets in group 1 mice. Immunohistochemical staining (in brown) for insulin is shown. Scale bars, 50 μm. (G and H) (G) Levels of relative islet area and (H) relative insulin levels in group 1 mice. Values are expressed as means ± SEM. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, and ****$p < 0.0001$.

the radical pair mechanism, free radical and SMF interaction. Consequently, labile Fe is attenuated by ROS and they collectively induced the other cellular effects.

In conclusion, we have provided the first evidence that a downward moderate-intensity SMF alone could have beneficial effects for prevention of HFD/STZ-induced diabetes in mice. This SMF can affect both gut microbiota and pancreas to regulate iron metabolism and improve cell oxidative stress and viability of pancreatic cells. This simple SMF setting could partially prevent HFD-induced T2D development and ameliorate related symptoms, which could provide a low-cost and non-invasive physical method to prevent and/or treat T2D in the future.

MATERIALS AND METHODS

See the supplemental information for details.

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ACKNOWLEDGMENTS

This work was supported by the National Key R&D Program of China (grant 2016YFA0400900), the Chinese Academy of Sciences Hefei Institutes of Physical Science Director's Fund (YZJJ201704), and the Chinese National Natural Science Foundations (grant

31800051). We would like to thank Heye Health Technology for providing the magnetic plates for the alternating magnetic field in Figure 1B. A portion of this work was supported by the High Magnetic Field Laboratory of Anhui Province. The authors have a patent pending related to this work.

AUTHOR CONTRIBUTIONS

X.Z. contributed to study design, project administration, methodology, data analysis and manuscript writing; B.Y., and J.L. contributed to methodology, performing experiments, data analysis and manuscript writing; J.C., L.Z., C.S., X.T., Y. F., and Y.L. performed experiments and data analysis. All authors have approved the final version of the manuscript.

DECLARATION OF INTERESTS

All authors declare no competing interests.

SUPPLEMENTAL INFORMATION

Supplemental Information can be found online at https://doi.org/10.1016/j.xinn.2021.100077.

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