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Is static magnetic field exposure a new model of metabolic alteration? Comparison with Zucker rats

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Abstract

Purpose: The aim of this study was to investigate if the metabolic alterations observed after static magnetic field (SMF) exposure participates in the development of a pre-diabetic state. A comparison study using the insulin resistant animal model, the Zucker rat and the SMF-exposed Wistar rat was carried out.

Materials and methods: Zucker rats were compared to Wistar rats either exposed to a 128 mT or 0 mT SMF (sham exposed) and analysed. This moderate-intensity SMF exposure of Wistar rats was performed for 1 h/day during 15 consecutive days.

Results: Wistar rats exposed to the SMF showed increased levels of carbohydrate and lipid metabolites (i.e., lactate, glycerol, cholesterol and phospholipids) compared to sham-exposed rats. Zucker rats displayed a normoglycemia associated with a high insulin level as opposed to Wistar rats which presented hyperglycemia and hypoinsulinemia after exposure to the SMF. During the glucose tolerance test, unexposed Zucker rats and Wistar rats exposed to the SMF exhibited a significantly higher hyperglycemia compared to sham-exposed Wistar rats suggesting an impairment of glucose clearance. In muscle, glycogen content was lower and phospholipids content was elevated for both unexposed Zucker rats and Wistar rats exposed to the SMF compared to Wistar rats sham control.

Conclusions: This study provides evidence that the metabolic alterations following exposure to a static magnetic field of moderate intensity could trigger the development of a pre-diabetic state.

Keywords: adaptive response, biochemistry, E-M fields

Introduction

According to their frequency, electric and magnetic fields (EMF) are classified into static, extremely low frequency, intermediate frequency and radiofrequency fields. Static magnetic fields (SMF) are characterised by a frequency of 0 Hertz (Hz) and a field which does not vary with time (Repacholi and Greenebaum 1999). SMF are naturally present everywhere as the earth is surrounded by fields that vary between 25 and 65 μT (Feychting 2005). Superimposed on the earth’s magnetic field are man-made static magnetic fields resulting in an increase of people exposed to SMF. Moreover, SMF are widely used in the treatment of musculoskeletal pain relief (Pilla 2006), refractory neuropathic pain (Weintraub and Cole 2004) and symptomatic diabetic neuropathy (Weintraub et al. 2003).


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Wistar rats were randomly divided into the following cycle, with free access to a standard diet and water. Male Wistar rats (n = 12) (Pasteur Institute, Tunis, Tunisia) and male Zucker rats (Janvier, Le Genest-Saint-Ilse, France) were housed in a temperature-controlled room at 25°C under a 12 h/12 h light/dark cycle, with free access to a standard diet and water. Wistar rats were randomly divided into the following groups: exposed rats (n = 6) to SMF (128 mT; 1h/day) for 15 consecutive days and sham-exposed control rats (n = 6) placed in the Lake Shore Electromagnetic processor (1h/day) for 15 consecutive days but not exposed to SMF (0 mT). Animals were cared for in compliance to the Tunisian code of practice for the “Care and Use of Animals for Scientific Purposes”. The experimental protocols were approved by the Faculty Ethics Committee. Faculte des Sciences de Bizerte, Tunisia.

Exposure system

We used an electromagnet (Model EM4-HVA, Lake Shore Cryotronic Inc., Westerville, OH, USA) charged by a magnet power supply (Model 647, Lake Shore Cryotronic Inc., Westerville, OH USA) containing an air gap of 11 cm (Figure 1). This apparatus incorporates water-cooled coils and precision yokes that assure precise cap alignment and excellent field stability and uniformity when high power is required to achieve the maximum field capability for the electromagnet. SMF intensity was measured and standardised over the total floor area of the Plexiglas cage at 128 mT. SMF uniformity in the active exposure volume was ±0.2% over 1 cm³. The experimental cage measured 20 × 10 × 20 cm. The two bobbins of the Lake Shore electromagnet were separated by a 12.1 cm gap. Exposed and sham control rats (n = 2/each time) were placed in the cage at the center of the uniform field area and exposed, or not, to 128 mT SMF. This intensity was chosen according to previous data of our laboratory which revealed that 128 mT was the minimal intensity for inducing alterations of physiological parameters (Abdelmelek et al. 2000, 2001, 2006, Chater et al. 2006).

Intraperitoneal glucose tolerance test (IPGTT)

Two days before being sacrificed, rats underwent an intraperitoneal glucose tolerance test (IPGTT), as previously described (Metz et al. 2005). Briefly, after 4 h of fasting, a glucose solution (2 g/kg body weight) was administered intraperitoneally (i.p.). Blood was collected 0, 20, 40, 60 and 90 min after i.p. glucose administration for consequent measurements of glucose and insulin plasma levels.

Biochemical analysis

Zucker rats and Wistar rats, exposed or sham exposed, were sacrificed by decapitation while in a post prandial state. Blood samples were immediately centrifuged and plasma aliquots were frozen and stored at −80°C until further use. Plasma glucose concentration was measured using the enzymatic...
method (Sigma 510, St-Quentin Fallavier, France), triglyceride and glycerol content were quantified by the Serum Triglycerides Determination Kit (Sigma TR0100, St-Quentin Fallavier, France). Insulin concentration was determined by radioimmunoassay following manufacturer’s instructions (SRI-13K, Labodia, Paris, France). We used a colorimetric enzymatic test for cholesterol analysis (CHOD-PAP, Biomagrheb 20111, Ariana, Tunisia). For lactate assay, a 50 μl blood sample aliquot was immediately mixed with 200 μl of ice-cold 7% perchloric acid and centrifuged at 1500 g for 10 min at +4°C. The supernatant was analysed enzymatically for lactate content according to the method of Gutmann and Wahlefeld (Gutmann and Wahlefeld 1974). Phospholipids were analysed according to the method developed by Shibuya et al. (1967). All reagents used were obtained from Sigma (St-Quentin Fallavier, France).

**Tissue sampling**

Immediately after sacrificing each rat, the soleus (SOL; oxidative muscle) and the extensor digitorum longus (EDL; glycolytic muscle) of the hindlimb were removed, frozen in liquid nitrogen and stored at −80°C until use for various enzymatic activity measurements. Quadriceps and liver biopsies were carried out in order to quantify tissular glycogen, phospholipids, triglycerides and glycerol levels.

**Enzymatic activities**

Citrate synthase (CS) activity was measured at 412 nm and 30°C for 2.5 min as suggested by Srere (1969). Also, 3-hydroxyacyl-coenzyme A-dehydrogenase (HADH) and lactate dehydrogenase (LDH) activities were measured at 340 nm during 10 min and 2.5 min, respectively. Results are expressed in micromoles per minute per g of tissue weight (μmol/min/g).

**Muscle and liver glycogen contents.** Muscle and liver glycogen contents were measured on portions of quadriceps and liver using the procedure described by Lo et al. (1970). Briefly, liver and muscle were

![Figure 1. Model EM4-HVA Electromagnet dimensions (Front view) (A) and magnetic field propagation (B). B (T) = Magnetic induction.](image-url)
boiled in 30% potassium hydroxide (KOH) saturated with Na$_2$SO$_4$ for 30 min to become soluble, and glycogen was then precipitated from the solution by addition of a 1.2 volume of 95% ethanol. Samples were centrifuged for 30 minutes at 840 g and pellets were resuspended in H$_2$O. Assays were conducted on aliquots in triplicate against appropriate blanks at 490 nm. Results were determined from a standard curve generated at the same time and expressed in mg glycogen/g tissue.$^1$

Data presentation and statistical analysis. Data were reported as the mean ± standard error of the mean (SEM). Statistical significance of the differences between mean values was assessed by Student’s $t$-test. Differences within groups for the IPGTT values were assessed by analysis of variance (ANOVA) method followed by Bonferroni post hoc tests. The level of significance was set at $p < 0.05$.

Results

**Metabolic parameters**

Basal metabolic parameters related to carbohydrate and lipid metabolism are reported in Table I. In post prandial state, we observed an increase in glycemia for SMF-exposed Wistar rats and a normoglycemia in unexposed Zucker rats compared to sham-exposed Wistar rats. However, insulin concentration showed a marked difference between hyperinsulinemic Zucker rats and SMF-exposed Wistar rats which on the contrary are hypoinsulinemic. Unexposed Zucker rats and Wistar rats exposed to SMF displayed a significant increase of plasma lactate levels compared to sham-exposed Wistar rats ($p < 0.01$). Additionally, both unexposed Zucker and Wistar SMF-exposed rats presented enhanced plasmatic concentrations of glycerol, cholesterol ($p < 0.01$) and phospholipids ($p < 0.01$) as opposed to triglycerides (TG) levels which were significantly increased only in the Zucker rat group whereas the SMF-exposed Wistar rat group showed no alteration in TG levels compared to sham-exposed animals.

**Intraperitoneal glucose tolerance test responses (IPGTT)**

To investigate whole body glucose metabolism, the intraperitoneal glucose tolerance test (IPGTT) was performed on fasted animals two days prior to their sacrifice (Figure 2A). Intraperitoneal administration of glucose resulted in an increase of plasma glucose and insulin concentrations in all groups. ANOVA analysis showed that overall, unexposed Zucker rats and Wistar rats exposed to SMF had higher glucose levels than sham-exposed animals. Therefore, the period of hyperglycemia was longer for SMF-exposed Wistar and Zucker rats than in sham-exposed rats suggesting an impairment of glucose clearance. These higher glucose levels detected in SMF-exposed Wistar rats were accompanied by

Table I. Basal metabolic parameters in sham exposed (C), Static Magnetic Field exposed (SMF), and Zucker (Z) rats.

<table>
<thead>
<tr>
<th></th>
<th>C</th>
<th>SMF</th>
<th>Z</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glycemia (mg/dl)</td>
<td>166 ± 4</td>
<td>206 ± 6*</td>
<td>152 ± 8</td>
</tr>
<tr>
<td>Insulin (ng/ml)</td>
<td>4.7 ± 0.1</td>
<td>1.7 ± 0.5*</td>
<td>11.3 ± 0.6*</td>
</tr>
<tr>
<td>Lactate (mM)</td>
<td>1.4 ± 0.1</td>
<td>3.2 ± 0.4**</td>
<td>2.8 ± 0.2**</td>
</tr>
<tr>
<td>Triglycerides (mg/dl)</td>
<td>66 ± 11</td>
<td>47 ± 9</td>
<td>116 ± 15*</td>
</tr>
<tr>
<td>Glycerol (mg/dl)</td>
<td>14 ± 3</td>
<td>23 ± 5*</td>
<td>44 ± 16**</td>
</tr>
<tr>
<td>Cholesterol (g/l)</td>
<td>1.0 ± 0.1</td>
<td>1.3 ± 0.1**</td>
<td>1.8 ± 0.1**</td>
</tr>
<tr>
<td>Phospholipids (mg/ ml)</td>
<td>1.0 ± 0.1</td>
<td>1.6 ± 0.1**</td>
<td>2.06 ± 0.6**</td>
</tr>
</tbody>
</table>

Data represent the means ± SEM of six animals per group. *$p < 0.05$; **$p < 0.01$ significantly different from sham exposed (C).

Figure 2. (A) Glucose response to an IPGTT in sham exposed (C), Zucker (Z) and SMF-exposed rats (SMF). (B) Insulin response to an IPGTT in sham exposed (C) and SMF-exposed rats (SMF). (C) Insulin response to an IPGTT in sham exposed (C) and Zucker (Z). Error bars indicate the standard error of the mean (SEM) for $n=4–6$ independent experiments. *$p < 0.05$ vs. C, **$p < 0.01$ vs. C.
Data represent the means ± SEM of six animals per groups. *p < 0.05; **p < 0.01 significantly different from sham exposed rats (C).

Table II. Muscular and hepatic parameters in sham exposed (C), Static Magnetic Field exposed (SMF), and Zucker (Z) rats.
SMF exposure at 128 mT. Navakatikyan et al. (1994) had previously measured serum insulin levels after daily 23 h exposure to magnetic fields of 50 Hz at 10, 50, and 250 mT for 11 days. Serum insulin levels were decreased for medium and high-flux magnetic densities when catecholamine levels were increased. Moreover, it is important to note that hyperglycemia could also be due to alterations in other hormones implicated in glucose homeostasis since Gorczynska and Wegrzynowicz (1991) found an increase in glucagon, cortisol, thyroid hormones and growth hormone levels after magnetic field exposure suggesting a diabetic-like state.

Regarding the level of blood lactate, we noticed a strong hyperlactatemia in both unexposed Zucker rats and Wistar SMF-exposed rats. Previous studies have found a relation between hyperlactatemia and lactate exchange alterations in the etiology of insulin resistance (Vettor et al. 1997, Lombardi et al. 1999). These alterations were due to both impaired lactate metabolism (Vettor et al. 2000, Miller et al. 2002) and impaired lactate exchange in skeletal muscle (Py et al. 2001, 2002). Thus, the hyperglycemia observed after SMF exposure, could be explained by a reduced glucose uptake due to high lactate levels. The tissues mainly responsible for glucose uptake are skeletal muscles and liver, thus we aimed to evaluate glucose storage in these tissues. Muscular glycogen was reduced in both unexposed Zucker rats and Wistar SMF-exposed rats, whereas a decrease in hepatic glycogen was only observed in Wistar rats following SMF exposure, in accordance with previous findings (Chater et al. 2006). This reduction could be caused either by a decrease in glucose uptake and insulin level or an increase glycogenolysis due to epinephrine (Abdelmelek et al. 2006).

Investigation of enzymatic activity in SMF-exposed rats seems to indicate a shift from oxidative to glycolytic metabolism consistent with previously published studies (Abdelmelek et al. 2006, Chater et al. 2006). It is important to consider that SMF exposure preferably affects glycolytic muscles and favours lactate production. However, unexposed Zucker rats presented an increased muscular oxidative capacity as previously found (Pujol et al. 1993, Dourmashkin et al. 2005).

Since glucose metabolism strongly interacts with lipid metabolism, lipid parameters were also measured. Not counting triglyceride levels which remained unchanged after SMF exposure, a large increase in glycerol, cholesterol and phospholipids levels was noticed in both groups (unexposed Zucker rats and Wistar SMF-exposed rats) compared to sham-exposed Wistar animals. An excess level of circulating lipid is often associated with cardiovascular diseases and participates in the dysregulation of glucose metabolism (Boden and Shulman 2002, Savage et al. 2007).

**Conclusion**

We propose a block diagram (Table III) which reveals the main similarities between Wistar rats exposed to static magnetic fields and unexposed Zucker rats. These data suggest that the metabolic alterations observed in Wistar rats following SMF exposure were similar in many ways to those obtained in Zucker rats. Our study provides evidence that a 128 mT static magnetic field exposure might favour the development of a prediabetic state or at least the emergency of some characteristics found in type 1 and type 2 diabetes. Thus, it seems that in addition to lifestyle and
Table III. Comparison between metabolic alterations observed in Static Magnetic Field exposed (SMF) and Zucker (Z) rats.

<table>
<thead>
<tr>
<th></th>
<th>SMF</th>
<th>Z</th>
</tr>
</thead>
<tbody>
<tr>
<td>Systemic: Glucose</td>
<td>↑</td>
<td>=</td>
</tr>
<tr>
<td>Insulin</td>
<td>↑</td>
<td>↑</td>
</tr>
<tr>
<td>Lactate</td>
<td>↑</td>
<td>↑</td>
</tr>
<tr>
<td>Triglycerides</td>
<td>=</td>
<td>↑</td>
</tr>
<tr>
<td>Glycerol</td>
<td>↑</td>
<td>↑</td>
</tr>
<tr>
<td>Cholesterol</td>
<td>↑</td>
<td>↑</td>
</tr>
<tr>
<td>Phospholipids</td>
<td>↑</td>
<td>↑</td>
</tr>
<tr>
<td>Liver: Glycogen</td>
<td>=</td>
<td>=</td>
</tr>
<tr>
<td>Phospholipids</td>
<td>↑</td>
<td>↑</td>
</tr>
<tr>
<td>Muscle: Glycogen</td>
<td>↑</td>
<td>↑</td>
</tr>
<tr>
<td>SOL</td>
<td>=</td>
<td>↑</td>
</tr>
<tr>
<td>CS activity</td>
<td>=</td>
<td>↑</td>
</tr>
<tr>
<td>EDL</td>
<td>=</td>
<td>↑</td>
</tr>
<tr>
<td>LDH activity</td>
<td>↑</td>
<td>↑</td>
</tr>
<tr>
<td>EDL</td>
<td>=</td>
<td>↑</td>
</tr>
<tr>
<td>SOL</td>
<td>=</td>
<td>↑</td>
</tr>
<tr>
<td>HADH activity</td>
<td>=</td>
<td>=</td>
</tr>
<tr>
<td>EDL</td>
<td>=</td>
<td>=</td>
</tr>
</tbody>
</table>

↑: Increase; ↓: Decrease; =: No effect; CS activity: Citrate synthase activity; LDH activity: Lactate dehydrogenase activity; HADH activity: 3-hydroxyacyl-coenzyme A-dehydrogenase activity; SOL: soleus oxidative muscle; EDL: Extensor digitorum longus glycolytic muscle.

genetic predisposition, experimental magnetic exposure at moderate intensity fields may be another factor promoting metabolic disorders. These results warrant further investigations to understand the mechanism and signalling pathways involved in these alterations.

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References


