

## EFFECTS OF SUB-ACUTE EXPOSURE TO MAGNETIC FIELD ON SYNTHESIS OF PLASMA CORTICOSTERONE AND LIVER METALLOTHIONEIN LEVELS IN FEMALE RATS

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### ABSTRACT:

**Objective:** To investigate the bio-effects of MF exposure on synthesis of plasma corticosterone and liver metallothionein (MT) concentrations in female rats

**Design:** Female rats were exposed to 128 mT 1hour/day for 10 consecutive days. Trunk blood of decapitated rats was collected and used for determination of corticosterone concentration. Quantification of MT was performed by using <sup>109</sup>Cd. Whole liver were homogenized in 1 ml of a 0.25 M sucrose solution. Surgical Adrenalectomy (ADX) and sham-ADX were performed via dorsal approach under ether anesthesia.

**Setting:** Female Wistar rats were housed in a cage, with free access to food and water (Faculté des Sciences de Bizerte, Tunisia). Rats were cared for under the Tunisian Code of Practice for the Care and Use of Animals for Scientific purpose and the Experimental Protocols were approved by the Ethics Committee.

**Subjects:** Treated and control groups (n=12) weighing 100-150g at the time of experiments were housed in the same condition three weeks before the beginning of the experiments.

**Main outcome measures:** Counting radioactivity will be used as analysis marker of bio-effects of magnetic field.

**Results:** Sub-Acute exposure to magnetic field the exposition of rats 1hour/day for 10 consecutive days to MF of 128 mT induced a significant increase (+104%, p<0.05) of plasma corticosterone concentrations showing a stress-state. Interestingly, MF induced an increase of metallothionein levels (+122%, p<0.05) in liver compared to controls. By contrast, levels of MT in adrenalectomised rats remained unchanged following MF exposure.

**Conclusions:** The results presented above show, for the first time, that sub-acute exposure to MF stimulates plasma corticosterone and MT activities in female rats. Indeed, we have noted an apparent lack of MT response to MF exposure in adrenalectomized rats, indicating that probably biosynthesis of MT was induced by stress.

**KEYWORDS:** Magnetic field, metallothionein, corticosterone, adrenalectomy rat.

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## INTRODUCTION

Over the past few years, considerable attention has been given to the potential bio-effects of magnetic field (MF). Epidemiological studies have suggested that MF may increase the risk of various types of cancer, including leukemia, brain and breast tumours.<sup>1-4</sup> The characteristic biological effects of MF appear to be functional changes in the central nervous system, endocrine and immune systems<sup>5</sup>.

Recent investigations revealing an increase in corticosterone concentration in rats exposed to MF, and the efficiency of the hypophysis-hypothalamic system changed, as indicated by an increase in the cortisol level was also observed<sup>6-8</sup>. By contrast, conflicting data reported no significant differences in corticosterone levels between exposed and sham-exposed rats<sup>6,9</sup> although recent studies have shown that various stressor can induce metallothionein (MT) synthesis in animal tissues<sup>10</sup>. Interestingly, the induction of MT synthesis by exposure to MF has not been reported. MTs are low molecular weight proteins (6-7 kDa), with a high affinity for soft trace metals and with characteristic amino-acid composition and sequence<sup>11,12</sup>. MTs were firstly detected in equine kidney cortex<sup>13</sup> and subsequently purified and characterized by Kagi et al<sup>14</sup>. Moreover, MT has been proposed to play an important role in essential-metal homeostasis, such as zinc and copper<sup>15</sup>. Induction of MT is thought to be an important adaptive mechanism which protects against the toxicity of heavy metals such as cadmium<sup>16</sup>, acts as free radical scavenger protecting against oxidative damage<sup>17</sup>, and protects against toxicity of alkylating anti-cancer drugs and other electrophiles<sup>18</sup>. Their gene expression and biosynthesis are regulated by heavy metals such as cadmium, zinc and copper as well as by endogenous factors such as cytokines and steroid hormones<sup>19-22</sup>. Specific steroid hormones shown to regulate MT are glucocorticoid<sup>23</sup>, progesterone<sup>24</sup> and catecholamines<sup>25</sup>.

The aim of the present study is to investigate the bio-effects of MF exposure on synthesis of plasma corticosterone and liver metallothionein concentrations in female rats.

## MATERIALS AND METHODS

### *Animals and surgery*

Female Wistar rats (Pasteur Institute, Tunisia) weighing 100-150g at the time of experiments were housed at 25°C in a cage under a 12-12 h light/dark cycle, with free access to food and water. Treated rats (n=12) were exposed to MF (128 mT; 1h/day) for 10 consecutive days.

Control and treated animals were sacrificed under light anesthesia (halothane 2.5%, in air). Rats were Cared for under the Tunisia Code of Practice for The Care And Use of Animals for Scientific Purposes and the Experimental Protocols were Approved by The Ethics Committee (Faculté des Sciences de Bizerte, Tunisia).

Surgical Adrenalectomy (ADX) and sham-ADX were performed via dorsal approach under ether anesthesia. Sham operated-rats were exposed to MF (128 mT, 1h/day). This diet is known to increase the life span and general well-being of ADX rats and particularly to improve their cardiovascular state<sup>26,27</sup>. The completeness of ADX was controlled by visual examination at the autopsy and assay of plasma corticosterone.

### *Exposure system*

Lake Shore Electromagnets (Lake Shore Cryotronic, Inc, Westerville Ohio, USA) are compact electromagnets suited for many applications such as magnetic resonance demonstrations. For the present experiment, we used an air gap of 15 cm. Water-cooled coils provide an excellent field for stability and uniformity when high power is required to achieve the maximum field capability for the electromagnet. We have an accurate pole alignment by precise construction of the air gap adjustment mechanism<sup>28</sup>.

### *Determination of MT*

The determination of MT was performed according to the technique described by Eaton<sup>29</sup>. Quantification of MT was performed by using <sup>109</sup>Cd. Whole liver were homogenized in 1 ml of a 0.25 M sucrose solution. The homogenate were centrifuged at 10.000 g for 10 min at 4°C, the supernatant was stored at -80°C for analysis of MT protein.

Two hundred microliters of <sup>109</sup>Cd solution (2 µg/ml) was mixed with 200 µl of sample (heat-denatured supernatant) and allowed to incubate at room temperature for 10 min. Then 100 µl of a 2% bovine hemoglobin solution was

added to the tubes, mixed and heated in a 100°C boiling water bath for 2 min. Then the tubes were placed on ice for several minutes, and centrifuged at 10,000g for 2 min in a microfuge and another 100 µl aliquot of 2% hemoglobin was added. The heating, cooling and centrifugation are repeated once again. A 500 µl aliquot of the supernatant fraction should be carefully removed. Lastly, aliquot of 500 µl of the supernatant were recovered carefully and transferred in clean tubes for counting their radioactivity.

### Corticosterone assay

Trunk blood of decapitated rats was collected in plastic conical centrifuge tubes containing EDTA (1 mg/ml of blood). After centrifugation at 3000g for 15 min at 4°C, the plasma was collected. Corticosterone was extracted by ethanol and then assayed as previously described by Murphy et al<sup>30</sup>.

### Statistical analysis

Data were analyzed using Stat View 512+ software (Abacus Concept, Inc). The results were expressed as means ± SEM and comparison of two means was made using Student's *t*-test.

## RESULTS

### Effects of magnetic field on plasma corticosterone concentrations

As shown in Figure 1, the sub-acute exposure of rats to MF did not alter relative

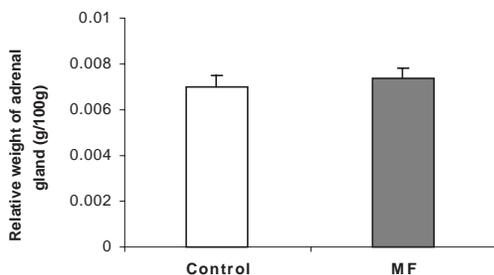


Fig. 1: Effect of exposure to magnetic field on adrenal gland weight of female rats.

Rats exposure to MF 128 mT (1h/ day) for 10 days [Each value is the mean ± SE of 12 determinations.  $P > 0.05$  compared to control]

weight of adrenal gland ( $0.007 \pm 0.0004$  g/100g vs  $0.0068 \pm 0.0006$  g/100g,  $p > 0.05$ ). However, exposure to MF increased the plasma corticosterone levels ( $25.35 \pm 1.24$  ng/100ml vs  $12.38 \pm 1.71$  ng/100ml,  $p < 0.05$ ) (Figure 2).

### Effects of magnetic field on metallothionein synthesis

Exposure to MF did not affected relative liver weight compared to control rats ( $4.13 \pm 0.29$ g/100g vs  $3.91 \pm 0.29$ g/100g,  $p > 0.05$ ) (Figure 3). Our data showed a significant increase in MT levels after sub-acute exposure of rats to MF (1h/day, 128 mT) for 10 consecutive days ( $2.94 \pm 0.53$ µg/g vs  $1.32 \pm 0.41$ µg/g,  $p < 0.05$ ). By contrast, the levels of metallothionein did not show any significant change ( $1.38 \pm 0.03$ µg/g vs  $1.54 \pm 0.05$ µg/g,  $p > 0.05$ )

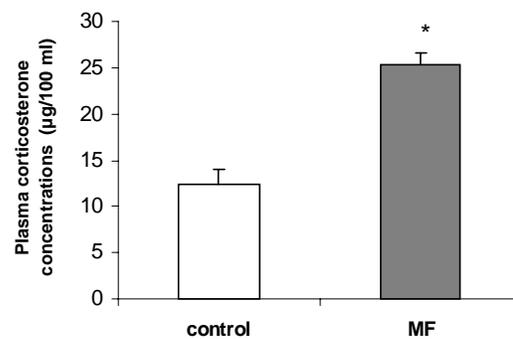


Fig 2: The effect of sub-acute exposure to magnetic field on plasma corticosterone concentrations in female rats.

Rats exposure to MF 128 mT (1h/ day) for 10 days [Each value is the mean ± SE of 12 determinations. \*  $p < 0.05$  compared to control]

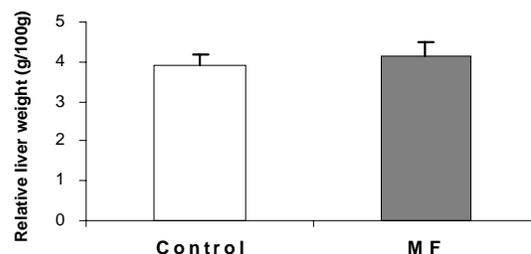


Fig 3: The effect of sub-acute exposure to magnetic field on relative liver weight in female rats.

Rats exposure to MF 128 mT (1h/ day) for 10 days [Each value is the mean ± SE of 12 determinations.  $P > 0.05$  compared to control]

in adrenalectomized rats following sub-acute exposure to MF (Table 1).

Table-1  
Effects of exposure to magnetic field on  
MT concentration of the liver in female rats

	Control	MF	ADX	ADX + MF
Hepatic MT (µg/g)	1.32 ± 0.18	2.94 ± 0.63*	1.54 ± 0.05	1.38 ± 0.03

Rats exposure to MF 128 mT (1h/ day) for 10 days. [Each value is the mean ± SE of 12 determinations,

\*P<0.05 compared to control

MF: magnetic field, ADX: adrenalectomized.

MT: metallothionein

## DISCUSSION

Our findings suggest that sub-acute exposure to magnetic field (MF) stimulates plasma corticosterone and metallothionein (MT) activities in female rats. Interestingly, we have noted an apparent lack of MT response to MF exposure in adrenalectomized rats, indicating that probably biosynthesis of MT was induced by stress.

Many reports have shown that electromagnetic field induced functional changes in the central nervous, endocrine and immune systems<sup>5</sup>. The present study, demonstrated that MF induced an increase in plasma corticosterone concentrations. In accordance with our results,<sup>31</sup> reported that exposure to low frequency magnetic field (2 Gauss) induces a significant increase in the level of corticosterone in blood plasma. By contrast, previous studies dealing with the bioeffect of MF on corticosterone point to the absence of biochemical effect<sup>9, 32</sup>. Moreover,<sup>7</sup> it has been shown that MF exposure induced a change in the efficiency of the hypophysis-hypothalamic, as indicated by an increase in the cortisol level leading to a temporarily diabetic-like response in rats.<sup>7</sup> In MF rats, we observed an increase of corticosterone concentration following sub-acute exposure to MF. These results suggest that MF acts as stressor in female rats, raises the question. The evidence that MF acts as a stressor raises the question of how corticosterone regulates MF response in rats.

Our data showed that sub-acute exposure to MF was associated with a high level of MT

compared to control rats, although recent studies have shown that various stresses can induce MT synthesis in animal tissues<sup>10, 33</sup>. The induction of MT synthesis by exposure to MF has not been reported. Likewise, Satoh et al<sup>33</sup> reported that the combination of CCl<sub>4</sub> injection and MF exposure induced elevation of the hepatic MT content exceeding the one induced by cadmium treatment alone. These results indicated that exposure to MF induced MT synthesis in mice liver and enhances the hepatic MT synthesis induced by CCl<sub>4</sub> administration. Moreover, MT also plays a role in the response to stresses such as cold, heat, exercise and injection of chemicals<sup>34</sup>. The stress-related increase of MT synthesis seems to be mediated by glucocorticoid hormones<sup>23, 35</sup>. The data in the present study indicated that sub-acute exposure to MF induces an elevation of hepatic MT. Our results, showed that MF can be regarded as a stressor, which stimulates the endogenous release of hepatic MT and that only are consistent with previously report in the literature<sup>23, 33</sup>. This is in keeping with results obtained in studies with various stressors, suggesting that this agent causes an increase of MT<sup>10</sup>. Yet, these findings support our studies, in adrenalectomized rats, which find a lack of the MT response and a low level of corticosterone. This lack of MT response is primarily ascribed to the low level of corticosterone associated to the adrenal hypoactivity. Besides its MT stimulation, MF caused an increase of adrenal activity. It is possible that corticosterone pathway is also involved in MT biosynthesis. Interaction between, corticosterone and MT is probably only one of several pathways involved in MF defense mechanism.

In conclusion, the results presented above show, for the first time, that sub-acute exposure to MF stimulates plasma corticosterone and MT activities in female rats. Indeed, we have noted an apparent lack of MT response to MF exposure in adrenalectomized rats, indicating that probably biosynthesis of MT was induced by stress. The mechanism underlying the change of corticosterone and MT after MF remains to be investigated.

## ACKNOWLEDGMENTS

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