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Effect of the pulsed electromagnetic field on the release of inflammatory mediators from adipose-derived stem cells (ADSCs) in rats

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Abstract: Objective: The aim of this study was to verify if the exposure to the pulsed electromagnetic field (PEMF) influenced the release of proinflammatory cytokines from adipose-derived stem cells (ADSCs) of normal and overweight rats of various age and sex. Moreover, we compared body temperatures of normal-weight and overweight rats.
Methods: ADSCs of Wistar rats were isolated from the subcutaneous area in females and paratesticular region in males, cultured and exposed to PEMF (7 Hz, 30 mT). Concentrations of proinflammatory cytokines were determined in rat sera and supernatant from ADSCs cultures exposed and non-exposed to PEMF. Body temperature (BT) was measured twice a week, using an infrared and rectal thermometer.
Results: Irrespective of age and sex, animals maintained on low-fat (LF) diet had higher BT than those grown on high-fat (HF) diet. Exposure to PEMF reduced the release of TNF-α and enhanced the production of IL-6 in ADSCs cultures from female pups maintained on LF diet. In contrast, a decrease in IL-6 level was observed in PEMF-exposed ADSCs cultures from female pups grown on HF diet. A similar phenomenon, i.e. a post-exposure increase in IL-6 level was also observed in male pups fed with the LF diet. In the case of ADSCs cultures from adult rats maintained on an HF diet, either males or females, PEMF exposure contributed to a dramatic increase in TNF-α production.
Conclusion: Our findings suggest that PEMF exposure may affect the production of proinflammatory cytokines in ADSCs cultures. The intergroup differences in BT may result from the presence of an underlying inflammation in obese rats.

Key words: obesity, PEMF, ADSCs, proinflammatory cytokine, temperature.

Introduction

The effect of electromagnetic field (EMF) as a modulator of immune response has been recently a subject of many studies [1]. Previous research demonstrated that exposure to the pulsed electromagnetic field (PEMF) might affect proliferation, differentiation and viability of various cell types, as well as their metabolic and signal transduction pathways [2–6]. Extremely low-frequency electromagnetic fields (ELF-EMF) were shown to modulate the release of inflammatory mediators and keratinocyte proliferation [7]. According to Vincenzi *et al.*, the treatment of N9 microglial cell cultures with lipopolysaccharides and exposure to PEMF contributed to a decrease in concentrations of proinflammatory cytokines, such as tumor necrosis alpha factor (TNF-α), interleukin 6 (IL-6) and interleukin-1β (IL-1β), in cell culture medium [8]. Also, a study of adipose-derived stem cells (ADSCs) isolated from adipose tissue (AT) of male and female Wistar rats showed that exposure to PEMF modulated the synthesis of proinflammatory cytokines and adipokines by these cellular population [9].

Almost a half billion people worldwide are obese, and according to one hypothesis, the predisposition to overweight may correlate with metabolic activity and energy balance in homeothermy [10]. According to literature, normal body temperature (BT) of laboratory rats approximates 37.5–38.5/39.0°C. A decrease below those values may be a marker of immune response associated with an inflammatory process [11–13]. A drop off in BT below normal values was inter alia observed in adult female rats with experimentally-induced cystitis caused by *E.coli* strains [14].

The function of AT later in life is modulated by maternal nutritional status during fetal and immediate postnatal period; this phenomenon is referred to as metabolic programming [15]. Fat depots forming adipose tissue differ in terms of their structure and function [16].

Brown adipose tissue (BAT) is involved in thermogenesis acting via catecholamine signaling pathways. Homeostatic hormones, such as leptin and insulin, affect a release of uncoupling protein 1 (UCP-1) and the generation of thermal energy in brown adipocytes [17]. In turn, the primary function of adipocytes in white adipose tissue (WAT) is an accumulation of lipids and endocrine activity. Thus, an excess of WAT leads to obesity [18, 19]. In one study, rats maintained on high-fat (HF) diet showed an increase in UCP-1 level, but this effect was observed only in males. However, the authors of this study did not analyze changes in BT [20]. In contrast, Almeida *et al.* demonstrated that maternal HF diet contributed to an increase in UCP-1 and tyrosine hydroxylase (TH) contents in BAT from female, but not male pups [21]. Obesity was shown to cause disorders of BT in nonpregnant rats. Consumption of cafeteria diet contributed to a decrease (by up to 0.29°C) in BT of overweight female rats during the estrous cycle and pregnancy [22].

Excessive proliferation of AT can cause adipocyte dysfunction and stimulate secretion of proinflammatory cytokines [23]. Inflammatory mediators, such as TNF-α and IL-6, are the main proinflammatory cytokines associated with the development of endothelial dysfunction in obesity and type 2 diabetes mellitus (T2DM) [24]. TNF-α plays a crucial role as a chemotactic and activating agent attracting neutrophils and monocytes to the site of inflammation [25]. Overweight is known to be associated with excessive secretion of TNF-α in adipose tissue. Rat's male offspring from mothers maintained on HF diet during pregnancy and lactation presented with elevated serum levels of TNF-α [26]. An increase in serum TNF-α was also observed in HF diet-fed rats with experimentally induced kidney damage [27]. One study demonstrated that the development of low-grade inflammation in mice with diet-induced obesity was associated with upregulation of IL-6 [28]. In another experiment, male mice maintained on two types of diet, HF and cafeteria feeding, presented with elevated serum levels of IL-6 [29].

The aim of this study was to verify if the exposure to PEMF influenced the release of proinflammatory cytokines from ADSCs of normal and overweight rats of various age and sex. Before harvesting the AT, we measured BT of the study animals.

Material and Methods

Animal care and preparation

Wistar rats were obtained from the Animal House of the Faculty of Pharmacy, Jagiellonian University Medical College. Following a 5-day quarantine, 64 animals of various sex and age were randomized to eight groups, maintained on low- (LF) and high-fat (HF) diet. The rats were kept in an experimental room with controlled air temperature (20 ± 5°C) and humidity (55 ± 10%), under a 12-hour light cycle (light on from 7:00 AM to 7:00 PM), with unlimited access to water and chow. Every effort was made to provide animal welfare in line with the principles of the 3Rs.

Dietary treatment

The study animals were maintained on two types of diet: regular low-fat diet (LF, Labofeed B, Pasze Kcynia) containing 25% protein, 8% fat and 67% carbohydrates, and obesity-inducing high-fat diet (HF, DIO, VERSELE-LAGA Opti Life Adult Active) with 32% protein, 22% fat and 40% carbohydrates.

Cell culture and PEMF exposure

Adipose-derived stem cells (ADSCs) were isolated using the method described by Safford *et al.* [30]. AT were obtained from both control and obese animals. The tissues were washed with phosphate-buffered saline (PBS, Sigma-Aldrich, Germany) containing

1% penicillin/streptomycin solution (Sigma-Aldrich, Germany), homogenized and digested with type 1 collagenase (1 mg/mL; Gibco by Life Technologies, USA) at 37°C for 1 hour. Enzymatic activity of the samples was neutralized with Dulbecco's modified eagle's medium (DMEM, Sigma-Aldrich, Germany) containing 10% fetal bovine serum (FBS, Gibco by Life Technologies, USA) and 1% penicillin/streptomycin solution. Then, the ADSCs were filtered (filters with a 100-µm pore diameter, Fisher Scientific, USA) and centrifuged at 300 g for 10 min. The cell pellets were suspended in DMEM supplemented with 10% FBS (Gibco by Life Technologies, USA) and 1% penicillin/streptomycin solution (Sigma-Aldrich, Germany), and left overnight in T75 flasks (Corning, Sigma-Aldrich, Germany) in a 5% CO_2 incubator set at 37°C and 90% humidity. After one day of culture, non-adherent cells were washed out with PBS containing 1% penicillin/streptomycin solution and resuspended in a fresh cell culture medium. Adherent cells were cultured until a 90% confluence was achieved, with cell culture medium changed every 72 hours. When the cells became confluent, they were treated with trypsin-EDTA solution (Gibco by Life Technologies, USA), followed by enzymatic neutralization. Then, the cells were centrifuged at 300 g for 10 min. Isolated ADSCs were counted with a hemocytometer and then cultured in triplicates onto 96-well plates, at the density of 0.25×10^6 cells/ ml. After a 24-hour incubation, the cells were exposed to PEMF (7 Hz, 30 mT, three exposures, each lasting 4 hours, with 24-hour intervals in between.

Euthanasia and tissue harvestings

On the 21st day of the experiment, animals from all groups were sacrificed by anesthetic overdose (Pentobarbital, Morbital, Puławy), to harvest adipose tissue specimens.

Temperature measurements

BT of rats from all the study groups was measured twice a week. To minimize stress and pain, BT of rat pups was measured with an infrared thermometer (Anima, Vivari), whereas the measurements in adults were taken with a rectal thermometer (Anima, Vivari).

ELISA tests

Concentrations of cytokines, TNF-α and IL-6, in serum and ADSCs cultures were measured using ELISA with commercially available kits purchased from Diaclone (SAS, France), strictly following the manufacturer's instructions.

Statistical analysis

All results are presented as arithmetic means ± their standard deviations (SD). Intergroup comparisons were carried out with Student t-test, with the threshold of statistical significance set at $p<0.05$. Statistically significant differences were designated with asterisks.

Results

Female pups maintained on LF diet had significantly higher BT than female pups grown on HF diet. The same phenomenon was also observed in the case of male pups. BT of rat pups turned out to be lower than in adult rats, but this difference might be associated with the fact that the measurements in these two age groups were taken with different types of thermometer, infrared and rectal one, respectively (Fig. 1 and 2).

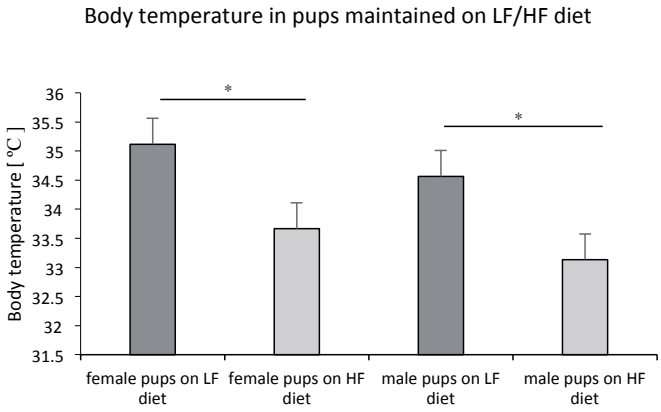

Fig. 1. Effect of HF/LF diet on body temperature in female and male rat pups. The results are presented as mean (+SD), the statistical significance of intergroup differences verified with Student t-test, * $p<0.05$.

BT of female adult rats maintained on LF diet was significantly higher than the temperature of adult females kept on HF diet. Also, male adult rats maintained on LF diet presented with significantly higher BT than the males receiving HF diet (Fig. 2).

Serum concentrations of cytokines, TNF-α and IL-6, in female pups and adult females maintained on HF diet were significantly higher than in their counterparts grown on LF diet (Fig. 3).

Both female and male pups received the same type of diet (HF or LF) as was given to their mothers during pregnancy.

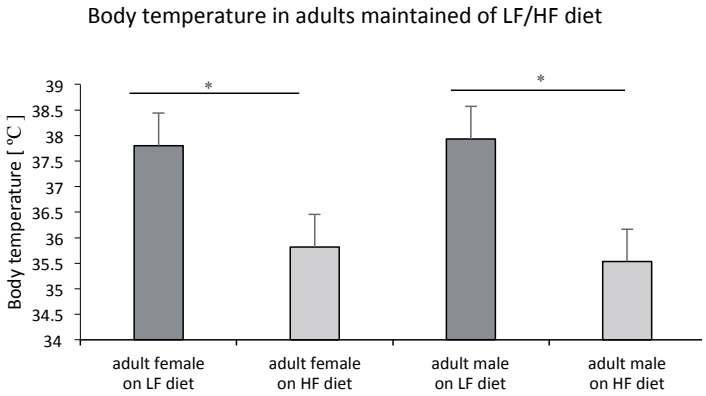

Fig. 2. Effect of HF/LF diet on body temperature in female and male adult rats. The results are presented as mean (+SD), the statistical significance of intergroup differences verified by Student t-test, * $p < 0.05$.

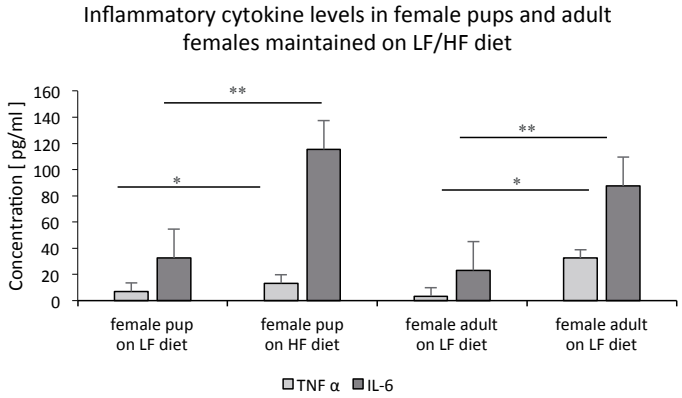

Fig. 3. Serum concentrations of TNF-α and IL-6 in female pups and adult females maintained on LF and HF diet, as determined by ELISA. The results are presented as mean (+SD), the statistical significance of intergroup differences verified by Student t-test, * $p < 0.05$, ** $p < 0.001$.

Serum concentrations of TNF-α and IL-6 in male pups and adult males grown on HF diet were significantly higher than in respective groups of male rats maintained on LF diet (Fig. 4).

While the exposure to PEMF contributed to a significant decrease in the release of TNF-α from ADSCs obtained from female pups grown up on LF diet, the amount of TNF-α synthesized by PEMF-exposed ADSCs from female pups maintained on HF diet was significantly higher than in non-exposed ADSCs from the same group of animals. Conversely, the exposure to PEFM resulted in a significant increase in the amount of IL-6 secreted by ADSCs from female pups maintained on LF diet, but PEMF-treated ADSCs from female pups grown on HF

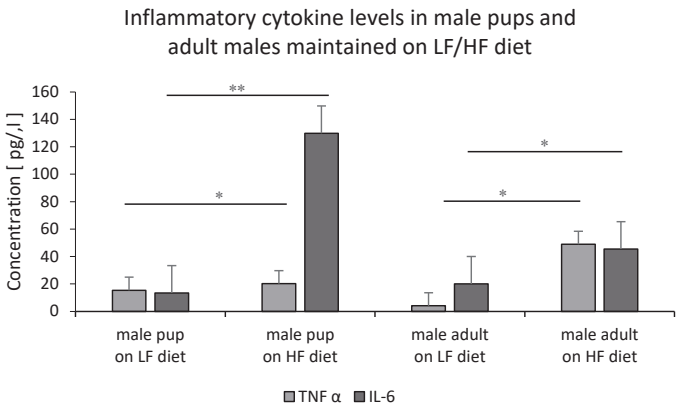

Fig. 4. Serum concentrations of TNF-α and IL-6 in male pups and adult males maintained on LF and HF diet, as determined by ELISA. The results are presented as mean (+SD), the statistical significance of intergroup differences verified by Student t-test, * $p < 0.05$, ** $p < 0.001$.

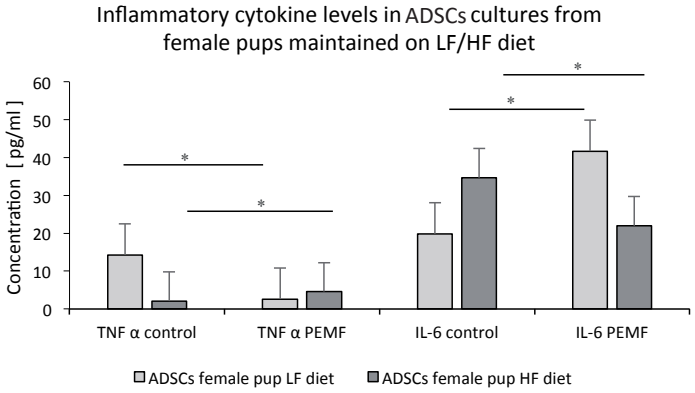

Fig. 5. Concentrations of TNF-α and IL-6 in supernatants from adipose-derived stem cell (ADSCs) cultures from female pups maintained on LF and HF diet, as determined by ELISA. The results for control cultures and cultures treated with the pulsed electromagnetic field (PEMF) are expressed as mean (+SD), statistical significance of intergroup differences verified by Student t-test, * $p < 0.05$.

diet produced significantly lesser amounts of this cytokine than the non-treated cells (Fig. 5).

PEMF-exposed ADSCs from adult females grown on either LF or HF diet produced significantly larger amounts of TNF-α than respective non-exposed ADSCs cultures. While the exposure to PEMF contributed to a significant decrease in the amount of IL-6 synthesized by ADSCs from adult females maintained on LF diet, no significant differences were found in the concentrations of this cytokine in PEMF-treated and non-treated ADSCs cultures from females grown on HF diet (Fig. 6).

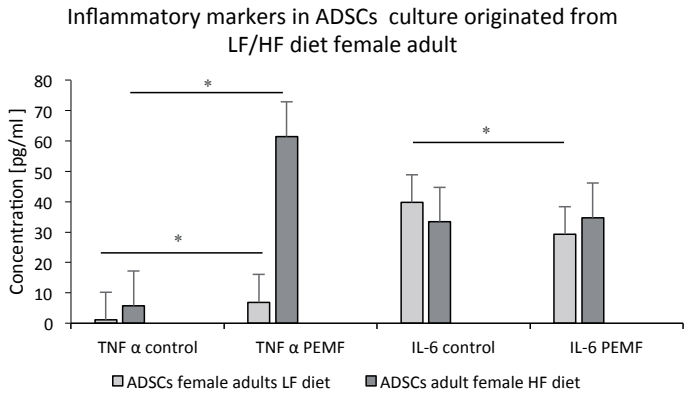

Fig. 6. Concentrations of TNF-α and IL-6 in supernatants from adipose-derived stem cell (ADSCs) cultures from adult females maintained on LF and HF diet, as determined by ELISA. The results for control cultures and cultures treated with the pulsed electromagnetic field (PEMF) are expressed as mean (+SD), statistical significance of intergroup differences verified by Student t-test, * $p < 0.05$.

Irrespective of the diet type, exposure to PEMF contributed to a significant increase in the concentration of TNF-α in the supernatants of ADSCs cultures from male pups. While IL-6 concentration in PEMF-exposed ADSCs cultures from male pups maintained on LF diet was significantly lower than in non-exposed cells, an opposite effect, i.e. post-exposure increase in IL-6 level was observed in ADSCs from male pups fed with HF diet (Fig. 7).

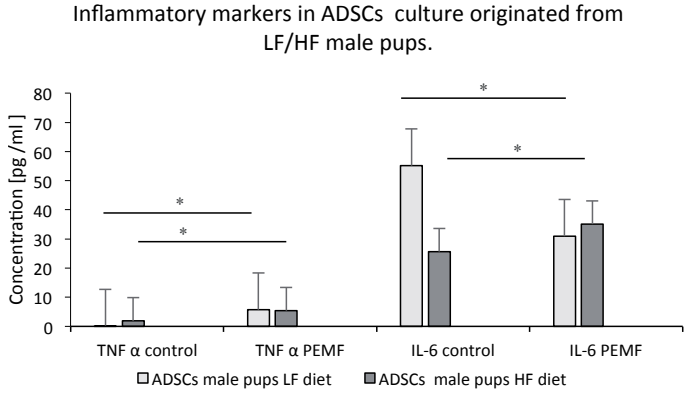

Fig. 7. Concentrations of TNF-α and IL-6 in supernatants from adipose-derived stem cell (ADSCs) cultures from male pups maintained on LF and HF diet, as determined by ELISA. The results for control cultures and cultures treated with the pulsed electromagnetic field (PEMF) are expressed as mean (+SD), statistical significance of intergroup differences verified by Student t-test, * $p < 0.05$.

Following the exposure to PEMF, ADSCs from adult male rats maintained on HF diet, but not from animals kept on LF diet, produced significantly more TNF-α than non-treated cells. Irrespective of the diet type, the exposure to PEMF contributed to a significant increase in the production of IL-6 by ADSCs (Fig. 8).

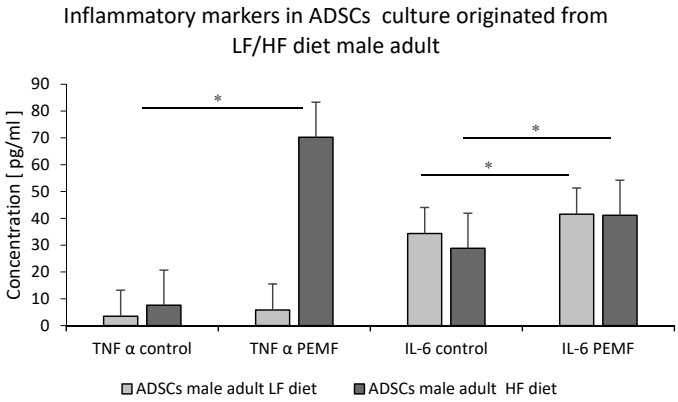

Fig. 8. Concentrations of TNF-α and IL-6 in supernatants from adipose-derived stem cell (ADSCs) cultures from adult males maintained on LF and HF diet, as determined by ELISA. The results for control cultures and cultures treated with the pulsed electromagnetic field (PEMF) are expressed as mean (+SD), statistical significance of intergroup differences verified by Student t-test, * $p < 0.05$.

Discussion

Non-shivering thermogenesis in mammals is associated with the activity of BAT, and is responsible for the maintenance of BT, especially during inflammatory processes [31]. BAT differs morphologically and functionally from WAT as it contains small intracellular lipid droplets, a greater number of mitochondria, synthesizes UCP-1 and shows enhanced metabolic activity [32]. Some published evidence suggests that the activity of BAT may be limited in obese humans [33]. In our present study, male and female rat pups grown on a standard LF diet had similar BT, 35.5°C ± 0.1 and 35.6°C ± 0.9, respectively [34]. Our findings are consistent with the results published by Tsushima *et al.* who demonstrated that HF diet had an effect on BT in adult male rats, which was lower than in the controls [35]. Also in another study, in which BT was measured twice a day, male adult rats fed with HF diet presented with lower body temperatures than the controls [36]. We observed that male and female rats maintained on an HF diet, either pups or adults, had increased piloerection (not shown) which may indicate disturbances at the BAT function level.

The results of our study are consistent with the observations made by De Almeida *et al.* according to whom male offspring from mothers grown on HF

diet during lactation period (with light and dark cycle kept) had lower BT then the controls maintained on LF diet [37]. However, another study conducted in mice produced contradictory findings, since 4-week-old animals that were grown on HF diet presented with higher BT than their counterparts maintained on LF diet; this effect was observed during the day and was followed by a decrease in early-night thermogenesis [38]. Finally, some authors did not find significant diet-related differences in rectal temperatures of adult male rats [39].

Maternal HF diet is known to promote the signs of early obesity, excessive proliferation of white adipocytes and enhanced accumulation of BAT in the offspring [40]. Male and female offspring from dams fed with HF diet during mating, gestation and lactation were overweight and showed greater body adiposity. However, some sex-specific differences were observed in the offspring's response to HF diet, as only males presented with hyperleptinemia and had higher energy expenditures [41]. Maternal HF diet was also shown to contribute to elevated plasma levels of TNF-α and IL-β in the offspring [42]. Our findings are consistent with the results of an *in vitro* study conducted by Tinkow *et al.*, in which the levels of IL-6 in adult female rats maintained on HF diet were significantly higher than in animals grown on a standard LF diet. Also, serum concentrations of IL-6 in male adult mice and rats grown on HF diet were shown to be higher than in control groups fed with LF diet [43, 44]. Likewise in our study, Díaz-Rúa *et al.* demonstrated that HF diet had an effect on serum TNF-α level in adult male rats [45]. Another study, conducted in overweight rodents, showed that elevated concentration of TNF-α was a marker of underlying inflammation [46]. An increase in serum TNF-α level was also previously observed in young male mice grown on HF diet [47]. Elevated levels of TNF-α and IL-6 were also found in visceral adipose tissue (VAT) harvested from male offspring grown on HF diet. Interestingly, however, the level of TNF-α in subcutaneous adipose tissue (SAT) from female pups maintained on the HF diet was similar as in the controls [48].

PEMF treatment is a non-invasive method to deliver electric and magnetic fields to tissues especially those affected by various pathological processes. Published evidence from clinical studies in humans and animal experiments suggests that PEMF treatment may produce beneficial effects in bone and wound healing, inflammation, treatment of post-operative pain and edema [49]. *In vitro* studies demonstrated that PEMF exerts an anti-inflammatory effect in cell culture models [50, 51].

Following the exposure to PEMF, the cells of the nucleus pulposus from adult male rats released less IL-1β and TNF-α to cell culture medium [52]. In another study, low-frequency PEMF treatment (2.5 ± 0.3 mT, 75 Hz, 1.3 ms pulse duration) maintained at low levels the production of proinflammatory cytokines (IL-1β, TNF-α and IL-6) in mononuclear cells obtained from adult male rats [53]. To the best of our knowledge, none of the previous studies except those conducted by our group [9, 54], have analyzed the effect of PEMF on ADSCs in an animal model for obesity. Our

findings suggest that the exposure to PEMF may alter the profile of biomarkers synthesized *in vitro* by undifferentiated ADSCs and that this effect may depend on animal age, sex and the type of diet.

Conflict of interest

None declared.

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