Obesity related adipokines release in rat adipose derived stem cell cultures influenced by pulsed electromagnetic field

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Abstract: Objective: The main goal of our studies was to investigate the effect exerted by pulsed electromagnetic filed (PEMF) on adipocytokines secretion in cell culture supernatants from rat adipose derived stem cells (ADSCs) grown on varied energy-rich diet. Offspring and adult animals were randomly selected for two types of experimental diets: low (LF) or high fat (HF) diet for 7 weeks. After the diet period, serum glucose level was measured, ADSCs were isolated from adipose tissues from different locations. ADSCs from all experimental groups were exposed to PEMF, supernatants collected and adipokines level was determined.

Results: HF diet feed in pups/adult animals elevated blood glucose level and increased the level of adiponectin (Apn) and leptin of both genders and age measured in serum. ADSCs cell cultures originated from female pups on LF diet and exposed to PEMF released large amounts of Apn. PEMF effect exerted on Apn release was also observed in ADSCs isolated from male pups HF diet. ADSCs from female pups on LF diet exposed to PEMF released smaller amounts of leptin in comparison to cell cultures without PEMF treatment. PEMF exposure of ADSCs cell cultures originated from female adults on LF diet decreased release of Apn, contrary adult male on LF diet ADSCs under PEMF treatment produced more leptin. PEMF treated male HF diet-originated ADSCs cultures released significantly more leptin than controls.

Conclusion: Our results suggest that PEMF exposure is responsible for metabolic physiological balance effects obtained in ADSCs cultures originating from adult animals on HF diet.

Key words: high/low fat diet, adipose tissue derived stem cells, adipokines, glicemia, PEMF.
Introduction

Obesity has become a worldwide society health problem associated with metabolic and chronic disorders. It concerns both male and female genders in most age groups [1, 2]. Adiposity is progressive, connected with adipocytokines such as adiponectin (Apn), by it decreased levels and the opposite effect is observed in leptin serum level. These metabolically active hormones in association with proinflammatory cytokines like TNFα and IL-6 are responsible for dysfunction of endothelium development and insulin resistance [3]. Apn plays an important role in metabolic balance through insulin-sensitizing, antidiabetic, antioxidant, anti-inflammatory, anti-atherogenic and vasoprotective effects [4, 5]. Leptin is engaged in obesity, insulin resistance, etiopathogenesis of cardiovascular diseases, inflammation, diabetes and plays an essential role in the long-term regulation/control of body weight [6, 7]. The western diet, rich in fat and sugar with combination of less activity results in excess weight and expression of genetic predispositions leading to type 2 diabetes mellitus (T2DM). This long-term developing disease with diagnosed hyperglycemia is caused by impaired insulin secretion, invalid insulin activity, defects of insulin receptors or post receptor insulin resistance [8]. Short term HF diet food highly increased plasma glucose and insulin level in adult male mice but didn’t affect the body mass and inflammation [9, 10]. Rodent studies based on maternal HF diet during pregnancy and lactation resulted in later life obesity and metabolic syndrome in offspring [11]. Introduction of HF diet in utero during pregnancy caused disturbances in appetite and energy balance during formation of the hypothalamic nervous system. Increased blood glucose and leptin level was noted in the offspring of both genders [12].

Low frequency pulsed electromagnetic field (PEMF) exposure of different cell types is known to exert biological effects such as cell proliferation/differentiation, cell death induction/inhibition, changes in gene expression and release of cytokines, growth factors and hormones [13]. It has been proved that pulsed electromagnetic field (PEMF) in a non-invasive way affected the proliferation and osteogenic differentiation into stem cells [14, 15].

Although PEMF exposure induces a neuroprotective effect in patients with peripheral neuropathy in diabetes, usage of PEMF therapy in medicine remains controversial and needs further experiments to explain the cellular mechanism engaged in PEMF influenced activities [16].
Materials and Methods

Experimental animals

The use of animals in experimental research was approved by the First Local Ethics Commission of Jagiellonian University, Cracow, Poland (resolution no. 84/2014). Wistar rats [WistarKrf: (Wi) Wu] emanated from the Pharmacy Faculty at the UJCM in Krakow. Animals were acclimatized for 7 days before the research was started. Dams with offspring and adult animals were kept in an environmentally controlled room (20 ± 5°C, humidity 55 ± 10% and 12 h light-dark cycle with lights on from 07.00 h to 19.00 h). The intensity of light in experimental room was at 25 lux as a result of albinotic strain used. Animals were provided with enrichment in the cage in accordance with the guidelines of the 3R rule.

Diets

The rats were randomly divided into 8 groups and treated for 7 weeks: low fat diet group (LF), received a standard chow for rodents (Labofeed B Pasze Kcynia, Poland) and a high-fat group (HF) with higher content of fat (VERSELE-LAGA Opti Life Adult Active, Belgium). All experimental study groups based on different type of chow and also included age and gender characteristics. Standard diet contained the following: protein 25%, fat 8%, carbohydrates, ashes and minerals 67% (energy density was 2.75 kcal/g), HF diet with a higher fat content contained: protein 32%, fat 22%, carbohydrates, ashes and minerals 46%, (energy density 4.70 kcal/g).

Isolation and culture of ADSCs

Adipose tissue (AT) was obtained from animals from all 8 study groups under sterile conditions and placed in a sterilized culture dish under a laminar hood. AT was carefully collected according to a previously described methodology [17]. The specimens were washed with phosphate-buffered saline (PBS, Sigma-Aldrich, Germany) containing 1% penicillin/streptomycin solution (Sigma-Aldrich, Germany), homogenized and digested with type collagenase (1 mg/mL; Gibco by Life Technologies, USA) at 37°C for 1 h. Enzymatic activity 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. Subsequently, the reaction cocktail obtained from disintegrated tissues was filtered (filters with 100-μm pore diameter, Fisher Scientific, USA) and centrifuged at 300 × g for 10 min to obtain a high-density cell pellet. The pellet was suspended in DMEM supplemented with 10% FBS (Gibco by Life Technologies,
USA) and 1% penicillin/streptomycin solution (Sigma-Aldrich, Germany), placed in T75 flasks (Corning, Sigma-Aldrich, Germany) and incubated overnight at 37°C in a 5% CO₂ incubator with 90% humidity. After the 24-h incubation, non-attached cells and non-adherent erythrocytes were washed with antibiotic-supplemented PBS. Adherent cells (ADCSs) were suspended in DMEM with 10% FBS and 1% penicillin/streptomycin solution. The medium was changed every 72 h until the cells became confluent. After reaching 80–90% confluence, the cells were placed in trypsin-EDTA solution (0.25% weight/volume; Sigma-Aldrich, Germany), incubated at 37°C for 10–15 min, and dissociated by trituration. The trypsin reaction was blocked by addition of DMEM with 10% FBS and 1% penicillin/streptomycin solution, and the cell suspension was centrifuged at 416 × g for 10 min. ADSCs were suspended in DMEM medium supplemented with 10% FBS and 1% penicillin/streptomycin solution, counted with a hemocytometer, seeded on a 96-well plate (Corning, Sigma-Aldrich, Germany) in triplicate at a density of 0.25 × 10⁶ cells/ml per sample, and cultured at 37°C at humidified atmosphere of 5% CO₂.

PEMF exposure of ADSCs cell cultures

PEMF stimulation was started after 24 h of ADSCs cell culture duration. A generator was designed and manufactured by the Institute of Electron Technology (Cracow, Poland) with a frequency of 7 Hz at a flux density of 30 mT inside the cell culture incubator. A 96-well plate with ADSC culture was placed in the generator's pocket and exposed to PEMF for 4 h a day at 24-h intervals, during three consecutive days.

Euthanized animals

At the end of the experiment (21st day), animals from all study groups were sacrificed with an anaesthetic (Pentobarbital, Morbital, Pulawy) at a dose of 200 mg per kg body weight to collected blood sample and AT.

Adipokines level measurement

Adiponectin was measured in serum and ADSCs cell culture supernatants with ELISA (Mediagnost /Germany) in duplicates. Leptin in rat plasma and supernatants from ADSCs cell cultures were determined by ELISA kit (BioVendor/Czech Republic) in duplicates.
Blood glucose level measurement

Rat blood was collected on heparin with anti-glycololytic fluoride for determination of blood glucose level. Glucose concentration was evaluated with an enzymatic method using the Cobas 80 000 analyzer.

Statistical analysis

The results were presented as means (±) and their standard deviations (SD). Intergroup comparisons were conducted with Student's t-test. The differences were considered statistically significant at P < 0.05.

Results

Adipokines serum level in animals of both genders grown on LF or HF diet

Serum adiponectin level in female pups and in male pups was elevated in animals grown up on HF diet in comparison to animals grown on LF diet. LF diet fed male pups showed to release more Apn into serum than female pups grown on the same type of chow (Fig. 1). Opposite effect was found in serum of adult animals grown on LF diet (both females and males) — adiponectin level was higher than in adult animals fed with HF diet (Fig. 1). Serum leptin level measured in pups of both genders was higher in animals fed with HF diet. Exactly the same relationship occurred in adult rats, which grown on HF diet produced more leptin.

![Adipokines serum level in groups of offspring](image-url)

**Fig. 1.** The influence of LF/HF diet on sera adiponectin and leptin levels in offspring. Statistical significance of intergroup differences verified with Student’s t-test; differences significant at P < 0.05 marked with asterisks (*).
Fig. 2. The influence of LF/HF diet on sera adiponectin and leptin levels in adult animals. Statistical significance of intergroup differences verified with Student’s t-test; differences significant at P <0.05 marked with asterisks (*).

Fig. 3. The blood glycemia in pups. Statistical significance of intergroup differences verified with Student’s t-test; differences significant at P <0.05 marked with asterisks (*).

Fig. 4. The blood glycemia in adult animals. Statistical significance of intergroup differences verified with Student’s t-test; differences significant at P <0.05 marked with asterisks (*).
than LF fed animals, respectively (Fig. 1). The highest amount of leptin was obtained in adult males fed with HF diet, nearly four times more than adult females on the same diet (Fig. 2).

**Blood glucose level in animals of both genders grown on LF or HF diet**

Glucose level in rat pups of both genders fed with HF diet was much higher than glucose level measured in pups grown on LF diet (Fig. 3). Blood glucose level in adult animals fed with HF diet exceeded 200 mg/ml in males — characteristic for diabetes mellitus diagnostic criterion, and was slightly elevated in females fed with HF diet in comparison to LF diet glucose level, however not exceeding the value of 200 mg/ml (Fig. 4).

**Adipokines level in supernatants of ADSCs cell cultures isolated from pups exposed to PEMF**

ADSCs cell cultures originating from female pups grown on LF diet when treated with PEMF exposure released extremal amounts of Apn in comparison to ADSCs cell cultures without PEMF treatment. The PEMF exposure effect exerted on Apn release was also observed in ADSCs cell cultures isolated from HF diet fed animals, but it was not as spectacular as in case ADSCs isolated from LF diet grown animals (Fig. 5). ADSCs cell cultures originating from female pups grown on LF diet exposed to PEMF released smaller amounts of leptin in comparison to ADSCs cell cultures without PEMF treatment, whereas PEMF has not caused any effect on leptin level in supernatants from male ADSCs cell cultures (Fig. 5).

![Fig. 5. Adiponectin and leptin levels in supernatants of ADSCs cell cultures isolated from pups on LF diet exposed to PEMF. Statistical significance of intergroup differences verified with Student’s t-test; differences significant at P <0.05 marked with asterisks (*).](image-url)
Fig. 6. Adiponectin and leptin levels in supernatants of ADSCs cell cultures isolated from pups grown on HF diet exposed to PEMF. Statistical significance of intergroup differences verified with Student’s t-test; differences significant at P <0.05 marked with asterisks (*).

ADSCs cell cultures treated with PEMF originating from HF diet males and females produced less leptin to cell culture milieu than control ones (Fig. 6).

Adipokines level in supernatants from ADSCs cell cultures exposed to PEMF originating from adults

PEMF-exposed ADSCs cell cultures originating from female adults grown on LF diet released lower amounts of adiponectin in comparison to ADSCs cell cultures without PEMF treatment. The PEMF exposure effect exerted on adiponectin production was not observed in ADSCs cell cultures originating from female and male adults fed HF diet (Fig. 7). PEMF-exposed ADSCs cell cultures originating from adult males grown

Fig. 7. Adiponectin and leptin levels in ADSCs cell culture supernatants originating from adult animals exposed to PEMF. Statistical significance of intergroup differences verified with Student’s t-test; differences significant at P <0.05 marked with asterisks (*).
on LF diet produced more leptin than ADSCs cell cultures without PEMF treatment. PEMF-exposed ADSCs cell cultures originating from adult females grown on LF diet, produced less leptin than control ADSCs cell cultures (Fig. 7).

PEMF exposure of female HF diet grown ADSCs cell cultures caused slight decrease of leptin level in cell culture supernatants and significant leptin release in PEMF treated male HF diet grown ADSCs cell culture supernatants (Fig. 8).

**Fig. 8.** Adiponectin and leptin levels in ADSCs cell culture supernatants originating from adult animals fed with HF diet, exposed to PEMF. Statistical significance of intergroup differences verified with Student’s t-test; differences significant at P <0.05 marked with asterisks (*).

**Conclusion**

ADSCs cell cultures isolated from adult males fed with LF or HF diet when PEMF exposed, released more leptin than those not exposed to PEMF. PEMF treated ADSCs cell cultures from male pups caused significant increase of Apn. The metabolic effects exerted by PEMF treatment of ADSCs cell cultures could be used as a possible antidiabetic, metabolic balance- influencing therapy.

**Discussion**

Adipokines and obesity

Obesity is nowadays considered as one of the leading health problems resulting in increased rates of cancers, heart disease, stroke and diabetes. The suggested solution for this problem was to restrict calories, make wiser dietary choices and encourage physical activity [18]. Research derived from murine projects based on HF diet has shown that females have lower body weight (BW), body mass index (BMI) and also smaller body waist circumference (WC) than male animals. Similarly, significantly higher values of fasting glycaemia were noticed in males on the same diet in
comparison to female ones [19, 20]. Hyperglycemia as a metabolic disorder was observed in offspring of both genders from dams fed with HF diet during pregnancy and lactation period, which is consistent with our experimental results [21]. Some signal transduction hormones like adipokinins are released from adipocytes and directly related with pathogenesis of obesity. Low level of Apn is negatively correlated with inflammation markers in overweight animals when BMI increases over 30 [22]. The leptin target activity is localized in the hypothalamus which regulates food intake and impact on BW [23]. It has been proved that increased leptin level is associated with the growth of AT. It defines pathogenesis of obesity and resulting complications [24]. Our data revealed that HF diet led to higher leptin and glucose levels in adult animals of both genders while adiponectin amount was decreased in adults. Similarly to our research studies, in adult male rats and other animal models (rabbits) grown on HF diet, blood glycaemia was out of normal range [25, 26]. Experiments carried out on adult obese females have shown high leptin concentration in their serum results, that stay in agreement with results obtained by our group [27]. Masuyama et al. stated that in offspring mice of both genders fed with HF diet, blood glucose level and leptin release were elevated contrary to adiponectin value [28]. Other results indicated that in adult male rats plasma adipocytokines — Apn and leptin amount increased in animals grown on high fat rich diet. Differences in our results in terms of adiponectin level might result from later introduction of HF diet (24 post natal day) [29]. Some research studies presented correlation between HF diet effect and increase of glucose and leptin level in young male rats as well as in adults [30]. Our data are in agreement with Hou et al. who proved that animals of age 3 and 8 weeks after HF diet had impaired glycaemia. Rodent studies showed fasting glycaemia level in young male species on HF diet and decreased adiponectin level [31, 32].

EMF influence

Electromagnetic field (EMF) interaction has been evidenced to be therapeutically effective and useful in a wide range of its parameters as well as medical implications. There are many research studies which confirm the hypoglycaemic activity of magnetic field exposure but still frequency and exposure time vary in different projects [33, 34]. Studies carried by Sieroń et al. which marked glucose by 3H uptake in organs and tissues of rats exposed to an extremely low electromagnetic field (ELEMF, 10 Hz, 1.8–3.8 mT), showed significantly higher glucose uptake in the liver, kidney, heart muscle, cartilage, connective tissue, skin and tendon. They hypothesised that ELEMF can facilitate glucose transport through the cell membrane [35]. Diabetic male mice when exposed to EMF (25 Hz, 250 μT) for 45 minutes for 2 weeks revealed lower blood glucose comparative to metformin and insulin application [33]. Research of Sakurai et al. run on hamster-derived insulin secreting cells (TIH-T15) with ELEMF
(60 Hz, 5 mT) has found increased concentration of intracellular insulin in the cell lines [36]. Öcal et al. investigated changes in blood glucose level in healthy and diabetic rats caused by alternating magnetic field (5 mT and 8 mT). They found out that alternative magnetic field exposure resulted in a decrease of blood glucose level in healthy and diabetic rats [37].

Endocrine system is sensitive to PEMF influence and possibly responds with changes of hormone production in the course of experimental animal research [38, 39]. Analysis of Holstein cow’s milk productivity in the interaction of the vertical field and the horizontal magnetic field showed rise in insulin-like growth factor 1 (IGF-1) and milk production [40]. Influence of extremely low electromagnetic field on gender hormones is partially reversible [41]. Leoci et al. observed that application of EMF in the course Benign Prostatic Hyperplasia (BPH) in canine model led to a reduction a gland volume. Furthermore it did not negatively affect testosterone level and quality of the semen [42]. High rate of EMF (2450 MHz) in the prenatal period in female rats showed postnatal growth restriction and delayed maturation. In addition, in the brain and ovarian tissue EMF resulted in high oxidative stress index [43]. Currently, magnetotherapy is an easy and direct method used to treat a variety of illness and pathologies [44]. Studies with a low frequency electromagnetic field showed to affect different physiological processes in vitro and in vivo experiments, thus we chose the frequency of 7 Hz with a flux density of 30 mT [45]. Apn is a hormone from the fat tissue with anti-inflammatory effect in obesity, in contrast to the majority of adipokines [46, 47]. Apn lower values are associated with abundance of abdominal fat [48]. Unfortunately, there is little evidence when analyzing PEMF effect on metabolic hormones in rat ADSCs in experimental models of obesity. Therefore, we could not compare our findings with the results by other authors. In ADSCs cultures obtained from female pups grown on LF diet we proved strong PEMF effect with higher Apn level contrary to values of leptin. In turn, ADSCs cultures originating from HF diet grown animals showed the PEMF influence on higher serum leptin level in supernatants from ADSCs female pups. ADSCs from male offspring on the same diet presented elevated supernatant leptin level but adiponectin value was lower under PEMF exposure. Application of ELEMF from the 6th day of pregnancy across 21 days of lactation in male offspring did not affect the spermatogenesis and fertility [49].

Our PEMF treatment of ADSCs from adult females on LF diet presented lower Apn and leptin values but in males leptin scores were higher as compared to controls. Our PEMF application showed that ADSCs originating from obese adult male rats improved Apn on higher level, in turn ADSCs from females showed low leptin level after exposure. Research of Trent et al. presented activity growth and weight loss in obese adult knockout male mice in electromagnetic field of 0.5 Tesla (T) conditions. Other research studies dealing with human embryonic stem cells which
were underwent static magnetic fields exposure showed upregulation of genes encoding insulin factors [18]. Cell line HIT-T15 under ELEMF demonstrated limited insulin secretion in response to increased glucose level [50]. Contrary, in the same conditions, HIT-T15 cells treated with electromagnetic field at a frequency of 60 Hz, 5 mT without glucose therapy, responded with an increase in intracellular insulin concentration [51].

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Conflict of interest

None declared.

References


