Effect of GSM Electromagnetic Waves on the Activity, Morphology, and Structure of Skeletal Muscles

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***Abstract*—The use of cellular technology is overwhelming our lives these days. GSM waves - the basis of cellular technology - are high frequency, high energy electromagnetic waves that may pause as a threat to man. The current work studies the effect of such waves on two types of skeletal muscles, the slow and fast twitching muscles. The activity, morphology, and structure of the affected muscles are studied and analyzed against control. Our results shows that in both muscles, there were changes in the distribution of muscles proteins and in the percentage of MHC isoforms suggesting that the GSM antenna relay affects the plasticity of skeletal muscle fiber by transforming slow type to faster one.**

Keywords-GSM electromagnetic waves; skeletal muscles; proteins content; testosterone level; MHC isoforms.

I. INTRODUCTION

Striated muscle myofibrils are composed of repeating units called sarcomeres that are arranged in series. Sarcomeres in turn are composed of contractile filaments termed myofilaments that are of two major types, actin (thin filament) and myosin (thick filament), which interact together to generate force and contraction. These myofilaments are large polymers of noncovalently associated contractile proteins, actin and myosin, that comprise 70% of myofibrillar proteins in skeletal muscles [1]. The isomers of Myosin Heavy Chains (MHC) are often used to distinguish the types of skeletal muscle fibers: slow-twitch – or type I – muscle fibers, where MHC I isoform is abundantly expressed; and fast-twitch – or type II – muscle fibers (types IIa, IIb, and IId/x), where MHC IIa, IIb, IIx/d predominate respectively [2-4]. Slow-twitch fibers are adapted for continuous activity, and they are rich in myoglobin and oxidative enzymes. A typical example is the soleus muscle. Fast-twitch fibers are adapted for rapid activity, and they produce energy through glycolytic metabolism. A typical example is the *extensor digitorum longus* (*edl*) muscle [5].

A remarkable characteristic of striated muscles is plasticity. This term refers to the ability of these muscles to remodel and thus change their contractile and metabolic makeup, and – hence – their type from slow to fast, or vice versa, in response to specific environmental challenges, such as exercise, temperature, or gravitational loading, or internal challenges such as nutritional conditions as well as neuronal, mechanical, metabolic or hormonal stimuli [6]. This may be attributed to a reversible change in the muscle gene expression that leads to reversible structural and functional modifications [7].

One of the important challenges that have developed in the last decade and is thought to have an effect on health is the population exposure to electromagnetic waves, particularly the Global System of Mobile communication signals (GSM) signals**.** These signals are emitted from diverse sources particularly from cell phones and base station antennas. Also, they may come from industrial processes where workers in broadcasting, transport, and communication industries are highly exposed. They are also emitted from medical devices like electrosurgical devices and diagnosis equipment. Thus, concerns from the risk of GSM signals on health arise from long term exposure as well as from the cumulative effect of these waves. The major mechanism by which such waves can induce an effect on biological systems is the thermal mechanism by which the Electromagnetic Field (EMF) at high intensities can increase the tissue or body temperatures above the normal

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value. Non-thermal mechanisms are under wide investigation in recent studies [8-11].

Few studies have investigated the effect of electromagnetic waves on skeletal muscles. In fact, Radicheva *et al.* in 2002 has shown that a 2.45 GHz microwave field could possess a stimulating effect on muscle fiber activity, which is in part due to its specific non-thermal properties [12]. Moreover, our previous study has shown that one hour of exposure to electromagnetic field at 900 MHz modulated by human voice could have an effect on the excitation-contraction coupling mechanism of mammalian fast- twitch skeletal muscles [13]. However, no study to date has investigated the effect of electromagnetic waves emitted by GSM relay antenna on muscle composition. Consequently, this study is designed to investigate the effect of 25V/m of electromagnetic waves emitted by GSM relay antenna on animal body weight, muscle mass, proteins and water content, total RNA expression, serum testosterone level and myosin heavy chain isoforms expression in the two types of skeletal muscle fibers, slow and fast- twitches.

This paper studies the effect of GSM waves on skeletal muscles. A background of the study is given in Section I. The materials and methods used in the study are mentioned in Section II. The results are presented in Section III, and discussed in Section IV.

II. MATERIALS AND METHODS

A. Experimental Design

All procedures in this study were performed in accordance with the stipulations of the Helsinki Declarations, and with the current Lebanese laws for animal experimentation. Twenty adult Sprague-Dawley male rats with an average weight of 190 ± 5 g were divided equally into 2 groups. One group was subjected for 6 weeks to whole continuous (24 hours/day) body exposure to EMW (900 MHz, E_{eff}= 25V/m). The other group was considered as control and maintained in the same environmental conditions under the turned off antenna. Both exposed and control animals were housed in a temperature-controlled room (22ºC) on a 12:12-h light-dark cycle. They were daily supplied with the same kind of food and water.

B. Dissection

After the exposure period, the rats were gently sacrificed and trunk blood was collected. Soleus and *edl* muscles were rapidly excised from the hind limbs of each rat. The muscles are weighted and preserved at - 80ºC for later analysis.

C. Total RNA extraction

Total RNA was extracted from muscle samples using **RiboZol™ RNA Extraction Reagent** from AMRESCO, according to the vender's instructions (American Research Products, 30175 Solon Industrial Parkway, Solon, OH 44139-9827 USA).

D. Serum testosterone level determination

Collected blood was centrifuged at 3500 rpm for 5 minutes. Serum of each rat was preserved at -20°C. Serum Testosterone levels in control and exposed groups were measured by Enzyme-Linked ImmunoSorbent Assay (ELISA) technique based on the principle of competitive binding, according to instructions supplied by the vender.

E. Proteins dosage

Protein dosage was performed according to Bradford Technique [14]. Pieces of frozen muscles were mechanically disrupted and spliced in 5 volumes of washing buffer containing 20 mM NaCl, 1 mM EGTA (pH 6.4), and 5 mM PO4. After 5 minutes of centrifugation at a high speed (12000 rpm), the supernatant is collected and the quantity of the protein is determined with the Bradford method (Bio-Rad, Hercules, CA), where the results were expressed as a ratio of milligrams of proteins to 100 milligrams of muscles. The pellet was then washed with 3 volumes of extraction buffer containing 5 mM EGTA, 1mM dithiothreitol (pH 8.5), and 100 mM sodium pyrophosphate, and incubated in cold overnight. The next day, the mixture was centrifuged at 12000 rpm for 10 minutes and the supernatant – which contained the protein myosin – was collected and the amount of myofibrillar proteins was determined. Small volumes (50µL) of the supernatant were diluted twice with glycerol and stored at -20°C for electrophoresis.

F. SDS-PAGE electrophoretic separation of Myosin Heavy Chain isoforms

To analyze the content of MHC I, MHC IIa, IIb, IId/x isoforms in the extracts, we used simple vertical migration of SDS-PAGE electrophoretic separation. The separating gel was prepared from 99.5% glycerol, 30% acrylamide, 0.6% bis acrylamide, 1.5 M Tris (pH 8.8), 1 M glycine, 10% SDS, 10% ammonium persulfate, and TEMED. The staking gel was prepared from 99.5% glycerol, 30% acrylamide, 0.6% bis acrylamide, 0.5 M Tris (pH 6.8), 10% SDS, 0.1 M EDTA (pH 7), 10% ammonium persulfate, and TEMED. For best quantification, 2-3 µg of myosin were loaded in each well. Electrophoresis was performed using a Cleavage, Scientific ltd, system. Gels were run at constant voltage (70V) for 24 h and then stained with silver reagent that allowed the detection of the MHC bands corresponding to I, IIa, IIb, and IId/x isoforms. The stained gels were scanned using a Canon digital imaging system and the density of bands was estimated using the UN-Scan-IT software [15].

G. Statistical analysis

All values are expressed as means ± SE for n observations. Data were analyzed by One-Way ANOVA (StatView; Alsyd, Meylan, France) statistical test. A level of $p< 0.05$ indicated statistical significance.

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III. RESULTS

A. Effect of GSM waves exposure on Body mass

As shown in Fig. 1, all animals steadily gained weight and there was no difference observed between the control and exposed animals after 6 weeks of GSM waves exposure (Control: 283 ± 8 g; Exposed: 295 ± 7 g, n=10)

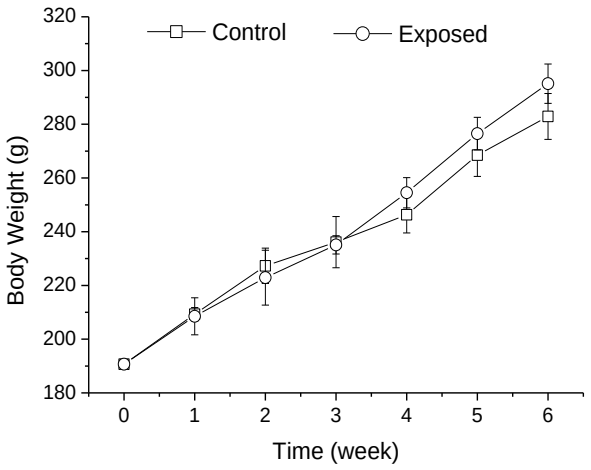

Fig. 1. Effect of GSM electromagnetic waves exposition on Body mass. Each value displays mean ±SE.

B. Effect of GSM waves exposure on muscles mass

Although body weight was not affected by the exposure, six weeks of exposure resulted in a significant decrease in *edl* mass by 16% (Control; 133.56 ± 3.69 mg; Exposed: 112.19 ± 2.57 mg, n=20, p<0.05). However, no significant effect was observed in soleus muscle mass (Control; 120.06 ± 2.89 mg; Exposed: 117.49 ± 3.11 mg, n=20) (Fig. 2).

Such a decrease in muscle mass observed in *edl* muscle could be related to modification in water content and/or in the proteins content. Consequently the water content and the soluble and myofibrillar proteins content were estimated.

C. Effect of GSM waves exposure on muscles water content

In control group, water content expressed as percentage is estimated to 37.5 ± 0.7% in soleus muscles and 26.9 ± 0.6% in *edl* muscles. After 6 weeks of continuous electromagnetic waves exposure, and although no significant effect was observed in soleus muscle mass, an increase by 17% in percentage of water content was observed (43.9 ± 1.2%, n=12, p<0.05). However, no significant effect was observed in *edl* muscles (28.1 ± 0.6%, n=12) (Fig. 3)

D. Effect of GSM waves exposure on proteins content

In soleus control muscles, soluble and myofibrillar proteins content were 2.71±0.13 and 3.42 ±0.26 mg/g of muscle, respectively. The six weeks of continuous electromagnetic waves exposure induced an increase by 23% of soluble proteins (3.34±0.16 mg/g, n=24, p<0.05); however, a decrease by 32% of myofibrillar proteins was observed (2.33±0.29 mg/g, n=24, p<0.05) (Fig. 4).

In *edl* control muscles, soluble and myofibrillar proteins content were 3.56±0.18 and 3.83 ±0.11 mg/g of muscle, respectively. Six weeks of continuous GSM waves exposure induced a decrease by 28% and 24% of soluble and myofibrillar proteins, respectively (Soluble proteins content: 2.78±0.19 mg/g; Myofibrillar proteins content: 2.91±0.22 mg/g of muscle, n=24, p<0.05) (see Fig. 4).

These modifications in proteins content in both soleus and *edl* muscles should be correlated to total RNA level expression.

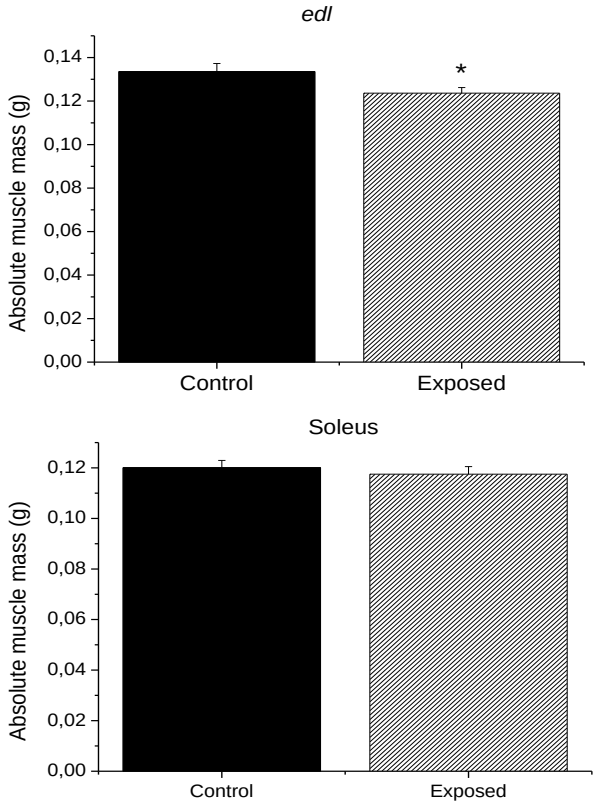

Fig. 2. Effect of GSM waves exposure on muscles mass of the *edl* and soleus muscles. Each histogram displays mean ±SE. * p < 0.05

E. Effect of GSM waves exposure on total RNA

In control condition, the total RNA values were 755.21± 16.72 µg/µl and 548.32± 14.98µg/µl in *edl* and soleus muscles, respectively. After 6 weeks of GSM exposure, a decrease by 29% and 25% were shown in both *edl* and soleus muscles, respectively (n=10; p<0.05).

This decrease in the amount of total RNA expression could be related to the modification in the serum testosterone level.

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F. Effect of GSM waves exposure on serum testosterone level

In control condition, the Enzyme-Linked ImmunoSorbent Assay showed that serum testosterone level was 82.12 ± 4.39 ng/ml. Six weeks of GSM waves exposure induced a decrease by 50% in serum testosterone level (40.91 ± 5.71 ng/ml, n=10, $p<0.05$).

G. Effect of GSM waves exposure on Myosin Heavy Chain Isoforms expression

Separation and analysis of MHC isoforms by SDS-PAGE allowed the estimation of the density of the bands corresponding to each of the MHC isoforms (MHC I, II_a, II_b, and II_x) using the UN-Scan-IT software. These isoforms are differentially expressed in the different muscle fiber types. In control conditions, the *edl* muscle expresses 34.6 ± 0.2 % of MHC IIx and 66.4±2.8 % of MHC IIb, while the soleus muscle expresses 5.1 ± 1.7% of MHC IIa and 94.6 ± 1.5% of MHC I.

The 6 weeks of GSM waves exposure induced, in *edl* muscle, a significant increase by 63% in the expression of MHC IIx isoforms (56.3±4.2%) and a significant decrease by 33% in MHC IIb isoforms expression (44.9±3.4%, n=24, $p<0.05$).

Moreover, in soleus muscle, the exposure induced a significant increase in the expression of MHC IIa isoforms (16.1 ± 1.7%) with a significant decrease in the expression of MHC I isoforms (84.3±1.5%, n=24, $p<0.05$). (Fig. 5)

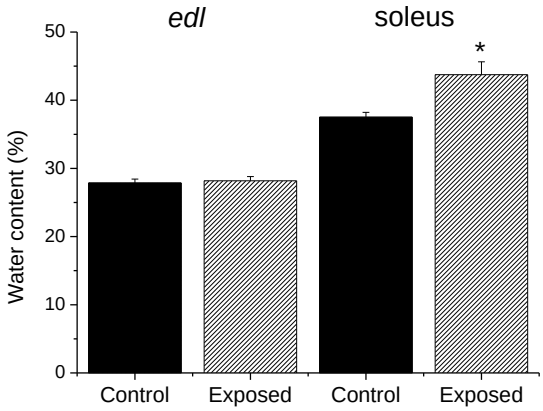

Fig. 3. Effect of GSM electromagnetic waves exposition on muscles water content of the *edl* and soleus muscles. Each histogram displays mean ±SE. * $p < 0.05$

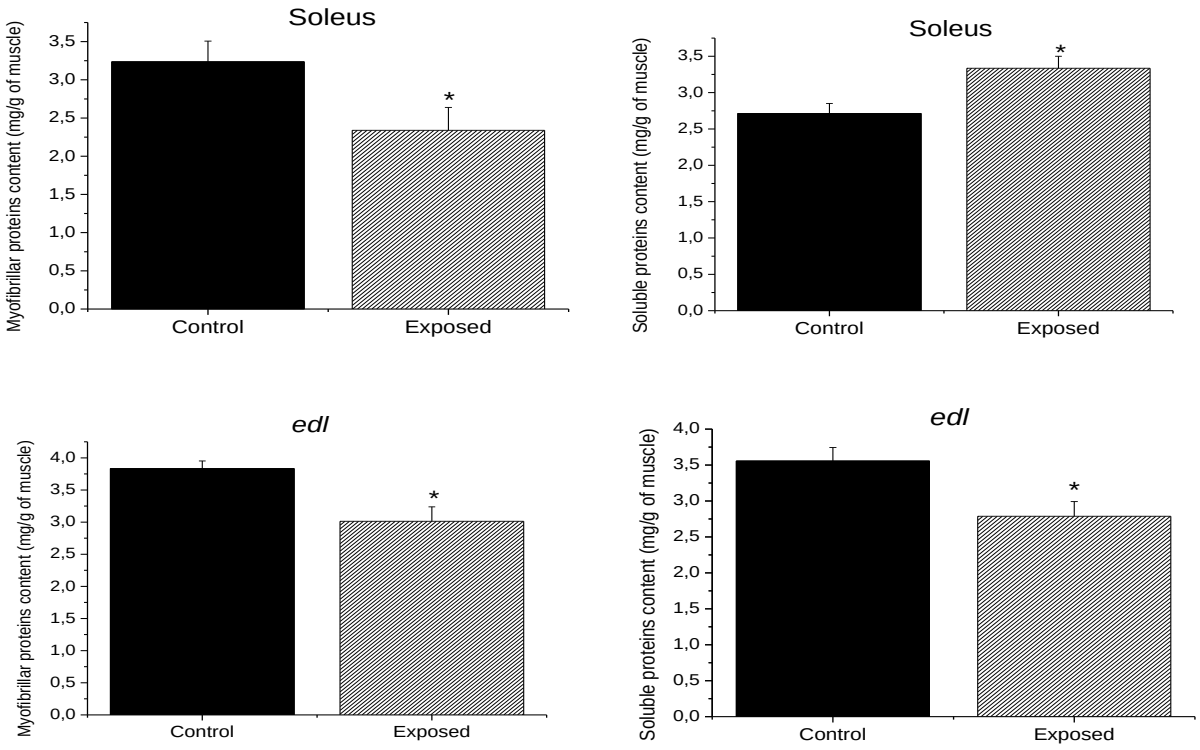

Fig. 4. Effect of GSM waves exposure on Myofibillar proteins content and soluble proteins content on both soleus and *edl* muscles. Each histogram displays mean ±SE. * $p < 0.05$

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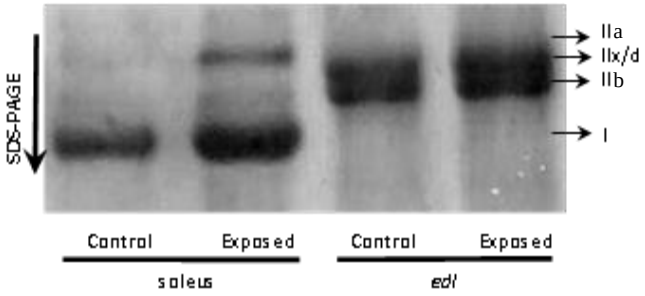

Fig. 5. Separation and analysis of MHC isoforms by SDS-PAGE in soleus and edl muscles in control rats and in rats exposed to 6 weeks of GSM waves. This figure show the bands corresponding to each of the MHC isoforms (MHC I, IIa, IIb, and IIx/d)

IV. DISCUSSION

Skeletal muscle is the most abundant tissue in animals representing up to 50% of body mass in some athletic species such as dogs and horses. Muscle fibers are composed of myofibrils arranged in parallel which constitute the major compartment in muscle cells, comprising from 73.2% of muscle fiber volume in horses to 83.3% of muscle fiber volume in goats. Also, total myofibrillar volume is directly proportional to muscle mass with a scaling factor of 0.98 [16].

This study aims to examine the effect of electromagnetic waves emitted from GSM antenna relay, at 900 MHz frequency, on body weight, muscle mass, protein content and the expression of the isoforms of the myosin heavy chain (MHC) in 2 types of muscle fiber types. One is slow oxidative fiber and the other is fast glycolytic fiber.

Many questions were raised about the possibility that exposure to electromagnetic fields emitted by mobile phones or their base stations could affect the health of users. If there is a health impact, there will be a global impact because the number of active cell phones is estimated to reach 7.3 billion by 2014. For this, the World Health Organization (WHO) established the International EMF Project in 1996 to assess the science, and recommended research to fill gaps in the knowledge of risks arising from exposure to electromagnetic fields on health [7].

Human skeletal muscle is a highly heterogeneous tissue, able to adapt to environmental challenges to which it is subjected. This process is governed by a set of mechanical, hormonal and nutritional signals [17-19]. Phenotypic plasticity of muscle tissue allows it to be modified in order to meet the specific demands faced by an animal during its life [13]. This critical property leads to a conversion of muscle fibers from slow to fast or vice versa [2, 20]. A wide range of contractile properties are mainly attributed to the diversity of the isoforms of MHC, which can exist in different muscle fibers. Four MHC isoforms (I, IIa, IIx and IIb), each encoded by a separate gene can be expressed in adult skeletal muscle. The intrinsic differences in the properties of the ATPase of MJHC isoforms led to the classification of fiber muscle as slow or fast fibers [18]. Generally, the fast-type genes appear to be expressed at birth, while the slow-type genes are expressed in response to changes in activity during development [17].

Actually, only two studies have examined the effect of electromagnetic waves on the skeletal muscle. The first study was conducted in vivo and showed that the field of microwave of 2.45 GHz has a stimulating effect on the activity of the muscle fibers, which is in part due to its non-thermal specific properties [12]. The second study was conducted in vitro, and showed that one hour of exposure to 900 MHz of electromagnetic field modulated by the human voice could have an effect on the mechanism of excitation-contraction coupling of skeletal muscle fast-twitch [13].

The results of our present work show that electromagnetic waves do not affect the body weight of rat males. In addition, the exposure period results in a decrease in absolute edl muscle mass, while that of soleus is kept unchanged. The myofibrillar protein content is directly proportional to muscle mass. A decrease in the mass of the edl was translated by decrease of both myofibrillar protein content and soluble protein. The increase in soluble protein content in soleus muscle was compensated by the decrease of myofibrillar proteins content resulting in the maintenance of muscle mass of soleus. This can be explained by the compensatory mechanisms at the translational and post-translational levels as suggested by Nikolova et al. [22]. In addition, synthetic modification in myofibrillar and soluble proteins in edl and soleus may be the consequence of the changes of level of transcription of genes encoding these proteins or due to perturbation in the stability of their corresponding mRNAs [20]. Therefore, the quantification of the mRNA encoding the soluble and myofibrillar and measurement of protein half-life is necessary to detect the level at which the change has led to modify the level of myofibrillar proteins and other proteins expressed in these two types of skeletal muscles. Our results provide new information by showing a decrease in the total RNA values in both edl and soleus muscles after 6 weeks of exposure to GSM.

On the other hand, the effect of electromagnetic waves is specific and can be attributed to a modification in the activity of different hormones. In 2010, Meo et al. [23] showed that exposure to radiation emitted by mobile phone for 60 minutes / day for a period of 3 months significantly decreased the level of serum testosterone in albino rats Wistar. In 2008, Al-Akhras showed that exposure for 6 weeks in a sinusoidal electromagnetic field of 50 Hz resulted in a significant reduction in levels of gonadotropins (FSH and LH) in female rats [24]. These results are similar to our results, where we showed a decrease in the level of testosterone after 6 weeks of exposure to GSM waves. This decrease can explain the decrease in total RNA values in both muscles since it is known that testosterone binds with androgen receptors inside the nucleus of different target cells and turn on the synthesis of mRNA which are then translated into specified proteins. Thus, the decrease of the

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level of testosterone may explain the decrease of the RNA levels.

The change in the percentage of MHC isoforms was also detected. The results show an increase of the MHC IIa isoform, but a decrease in the MCH I isoform in the soleus muscle. In the edl muscle, a significant increase in MHC IIx and a significant decrease of MHC IIb isoforms were observed. These results suggest that the GSM antenna relay affects the plasticity of skeletal muscle fiber by transforming slow type to faster one.

Acknowledgments

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