UNIVERSITY OF SZEGED

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PhD Thesis

EFFECTS OF PHYSICAL ACTIVITY ON THE AGING PROCESS

- with non-invasive follow-up and invasive cross-sectional researches -

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1 Introduction:

Obesity is a growing problem in the world and the group of elderly people is one of the most vulnerable ones to this disease. Obesity is a risk factor for many internal diseases and musculoskeletal problems. The severity of obesity can be determined by the body mass index (BMI), the waist-hip ratio (WHR) and the body fat percentage (BF%). Previous studies revealed that physical activity and energy restriction are the most important factors in the prevention or management of obesity. In a 6-year follow-up study, a notable 15% difference was revealed in the walking speed of obese and normal body-type participants with the age of ≥65 years. The aging-assotiated loss of strength is slower than the loss of explosive power, this way daily movements, like standing up from a chair, slow down. Even a 3-month exercise programme can have positive effects on body composition and dynamics of movement. Human movements might be appropriately characterised by the dynamics of moves of the body centre of gravity (CG). Close connection can be assumed between the changes in body composition and the dynamic characteristics of movements. If the body weight (BW) of a person decreases but the skeletal muscle mass (SMM) is unchanged, the individual becomes stronger compared to the previous state, because the same muscle mass moves less weight. Several theories have emerged in relation to aging, such as apoptosis, sirtuin, free-radical, and mitochondrial theories, all of which summarize the processes of decline in old age. 40% of the human body mass is skeletal muscle, which also includes 60% of the total protein content. Skeletal muscle is necessary for

movements and body posture, and also important for metabolic functions like storage of energy in form of glycogen. 50% of muscle fibres is lost between the age of 50 and 80, thus muscle mass also decreases. Regular exercise increases muscle cross-section, fibre number, strength, endurance, mitochondrial function, and insulin sensitivity. Dynamics of mitochondrial function deteriorates and skeletal muscle miRNA profile changes during aging. Numerous functional and biochemical changes occur as a result of physical activity. Effects of short- and long-term endurance trainings change the levels of miRNAs, which play part in skeletal muscle regeneration, gene expression, and regulation of mitochondrial biogenesis. These processes can be observed not only in a healthy body. Persistent exercise and hypertrophic stimuli enhance the increase in miRNAs that target transcripts involved in inflammation, metabolism, muscle atrophy, and hypertrophy. Impairment of the miRNA profile and mitochondrial function caused by aging can also be reversed by regular exercise.

One of the targets of my research was to explore the beneficial effects of medium level intensive training on the body composition and on the dynamics of movements. I was concerned about the differences between the improvements of the younger and the elder groups after systematic physical activity. I also wanted to know whether there was any connection between the changes of body composition and the characteristics of daily movements after five months of training. My further aim was to gain insight into the biochemical nature of muscles of elderly people with different

lifestyles and to find out what could influence their aging, health and quality of life. I also wanted to detect the differences between the mitochondrial functions and miRNA levels of master athletes over sixty-five and their inactive contemporaries.

2 Materials and methods

2.1 Non-invasive follow-up research

Ninety-two physically inactive participants (56 females and 36 males) took part in the study, whose average age was 31.66 ± 19.27 years. They were divided into five groups based on their ages: second childhood (marked as G1; n = 14, mean age 11.5 ± 0.14 years); adolescence (marked as G2, n = 20, mean age 13.1 ± 0.25 years); mature age I (marked as G3, n = 22, mean age 26.55 ± 1.09 years); mature age II (marked as G4, n = 23, mean age 47.52 ± 1.48 years); ageing (marked as G5, n = 13, mean age 63.46 ± 1.23 years). Participants did recreational training periods of 60 minutes, repeated three times a week for five months at 80.36% ± 0.51% of the maximal heart rate. Polar Team System (Finland) was used for heart rate measurement, InBody230 Body Composition Analyzer (BIA; Biospace, Seoul, Korea) was used for body composition determination and Ariel Performance Analysis System version 12.3.0.2 (Ariel Dynamics Inc. USA) was used for motion analysis. Heart rate measurements were performed at random during the exercise programme, while body composition and movement analysis data were recorded at the beginning and end of the exercise programme. Daily movements were simulated in the motion analysis, with counter movement jump (CMJ) (stair climbing) and crouching (sitting, standing up). Data are expressed by mean and

standard error of mean (SEM), the differences of which were calculated by one-sample t-test and analysis of variance (ANOVA) ($p \leq 0.05$).

2.2 Invasive cross-sectional research

Twenty-three participants took part in this research, 10 master athletes (mean age: 65 ± 5 years) and 13 sedentary subjects (mean age: 64.67 ± 2.08 years). Muscle biopsies were obtained from the vastus lateralis. Samples were stored at −80 °C until biochemical analysis. RNA, and miRNA, were isolated by miRNeasy Mini Kit (Qiagen #217004). miRNA expression analysis was performed on 4 samples from master athletes (68.75 ± 8.54 years old) and on 4 samples of sedentary subjects (70.25 ± 11.3 years old) gained by skeletal muscle biopsy samples with Agilent Human miRNA Microarray Release 14.0 8×15K resolution array (Agilent Technologies, USA), that distinguishes 887 human miRNAs. After hybridization, slides were washed at room temperature and scanned using an Agilent DNA microarray scanner. Raw data were extracted with the Agilent Feature Extraction Software 11.0., and TaqMan miRNA assays (Applied Biosystems, Foster City, CA) were used to quantify mature miRNA expression levels. The reverse transcription reactions were run on a PRISM 7900HT Fast Real-Time PCR System (Applied Biosystems) with miRNA specific reverse transcriptase primers. Twofold dilution series were performed for all target miRNAs to verify the linearity of the assay. Particular miRNAs were normalised to miR-423, and quantified using the cycle threshold method. cDNA was synthesised using a Tetro cDNA Synthesis kit (Bioline #BIO-65026 Luckenwalde, Germany). Based on the principle of the SybrGreen

detection method, EvaGreen® dye (Biotium, Hayward, CA, USA) was used to detect PCR products and target proteins (VEGF, SIRT1, FOXO1, MCU, IGF-1, PGC1α, MGF). Amplifications were performed in a Rotor-Gene 6000 thermal cycler (Corbett Life Science/Qiagen, London, UK). The validity of the signal was evaluated by melting analysis and agarose gel electrophoresis (human 28 S rRNA gene served as an endogenous control gene). Tissue homogenates of the muscle biopsy samples were generated with an Ultra Turrax® (IKA®-Werke) homogeniser using 10 vol of lysis buffer. Five to ten micrograms of protein were electrophoresed on 10-12% v/v polyacrylamide SDS-PAGE gels. Proteins were electrotransferred onto polyvinylidene difluoride (PVDF) membranes. The membranes were subsequently blocked in 0.5% BSA, and after blocking incubated with primary antibodies. Membranes were incubated with chemiluminescent substrate and protein bands in order to have them visualised on X-ray films. The bands were quantified by ImageJ software, and normalised to GAPDH, which served as an internal control.

Data gathered from the miRNA array validation and gene expression experiments were analysed with an unpaired Mann-Whitney U-test, and unpaired, two-tailed Student's t-test or χ_2 test were used for qPCR and Western blot variables, as appropriate. Data are presented as mean ± standard deviation ($p < 0.05$).

2.3 Results

2.4 Non-invasive follow-up research

The measurements of body composition showed several significant changes within and between the groups. (*Figure 1*)

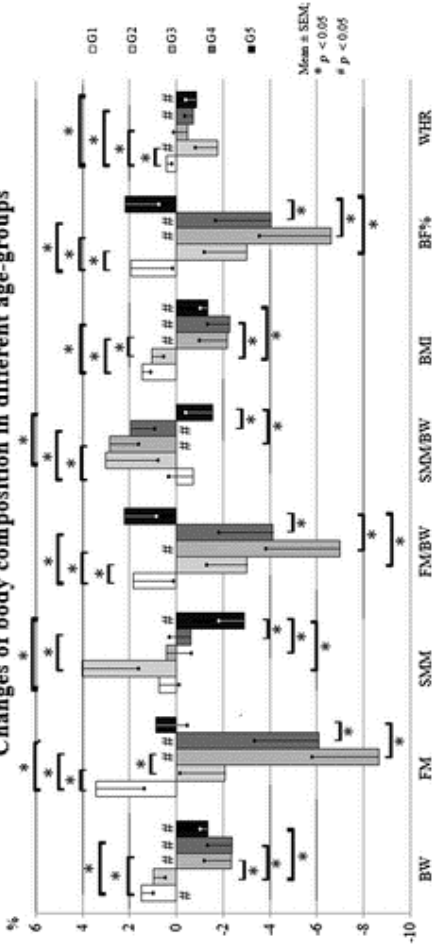

Figure 1: Changes of body composition in different age-groups

*(Values are means ± SEM. G1: second childhood; G2: adolescence; G3: mature age I; G4: mature age II; G5: ageing; BW: body weight; FM: fat mass; SMM: skeletal muscle mass; FM/BW: fat mass–body weight ratio; SMM/BW: muscle mass–body weight ratio; BMI: body mass index; BF%: body fat percentage; WHR: waist–hip ratio. *Significant differences between changes of groups; #Significant changes in groups; $p \leq 0.05$)*

The lifting of the position of the CG increased in G3, G5 ($p < 0,05$) during CMJ. The sinking of the position at pre-jump momentum was lower ($p < 0,05$) in all groups except G1. The measurement showed significant changes ($p < 0,05$) in the velocity of the CG because it decreased in G2, and increased in G4 and G5. However, improvements ($p < 0,05$) could also be seen in the acceleration of the CG in G3 and G5 during CMJ. Changes in the characteristics of CMJ showed significant differences between the groups. The lifting of CG increased ($p < 0,05$) in G3, G4, and G5 compared to G2; while the sinking of position was lower ($p < 0,05$) in G4 than in G2. The velocity of CG significantly improved ($p < 0,05$) in G3 in contrast with G2, similar to in G4 and G5 opposed to G1 and G2. The acceleration of CG increased ($p < 0,05$) in G3 compared to G2 and G5.

Examining the dynamic features of crouching, groups G4 and G5 achieved significant improvements ($p < 0,05$) in movement velocity and acceleration by the end of the training programme. Evaluating the differences between the results achieved by the groups, the change in the CG deviation was greater ($p < 0,05$) in group G4 than in group G1. The velocity of crouch significantly increased in G4 compared to G1, G2 and G3 ($p < 0,05$), similar to G5 opposed to G1. Improvements ($p < 0,05$) were also observed in the acceleration of CG in G4 in contrast with G1 and G3, as well as in G5 opposed to G1.

Changes in the dynamic of movements and body composition showed several correlations. The improvement of CMJ elevation was positively influenced by the decrease in BW, fat mass (FM), and BMI, as well as the increase in muscle mass-body weight ratio

(SMM/BW) in group G3. The rate of pre-jump gain was increased by the improvement in SMM/BW in G4 and by the decrease in BW, BMI, and WHR in G5. In case of crouching, the velocity of movement was increased by the decreased BW, BMI, and WHR in group G5, while acceleration was positively affected only by the decreased WHR in the same group.

2.5 Invasive cross-sectional research

The microarray analysis revealed that 21 of the 887 miRNA sequences were lower in master athletes than in control muscles. Four miRNAs were selected based on the greatest difference in the miRNA array for further q-PCR analysis. This revealed that only miR-7 was expressed more ($p < 0.05$) in the muscles from controls than in those from master athletes. Then from the remained muscle samples, key mitochondrial mRNA and protein contents were measured. SIRT1 ($p < 0.01$) and FOXO1 ($p < 0.05$) mRNA levels were higher in master athletes than in control groups, while the SIRT3 and SOD2 proteins ($p < 0.01$; *Figure 11*) from the muscle samples of master athletes were higher than in the control subjects.

3 Discussion

Various studies proved the beneficial effects of physical activity on body composition: it can decrease the BW, FM, BMI, BF% and increase the SMM. Regular exercise not only has a beneficial effect on the musculoskeletal system, but also on the whole body, and reduces the risk factor for diabetes, hypertension, heart disease, dyslipidemia, cerebrovascular disease and

metabolic syndrome, which play an important role in old age. The level of miR-7 increases with aging, and its expression can be increased by the sarcopenia-associated inflammations. The expression of miR-7 also increases in airways of patients suffering from chronic obstructive pulmonary disease, in peripheral blood mononuclear cells of HIV patients, and in muscles of facioscapulohumeral muscular dystrophy patients. Expression of epidermal growth factor receptor (EGFR) is reduced (via degradation of its mRNA) by the elevated miR-7 regulation in aged cells,and it can also interact with the EGFR dependent mRNA signalling pathway points (MAPK/ERK, CaMKII, Rho- GTPase, PI3K, Akt and mTOR), which are essential to wound healing in striated muscle. Due to the diminished level of miR-7 the expression of EGFR mRNA increases, and the functionality of aging fibroblasts is restored. Effects of physical activity may reduce the expression of inflammatory markers, thus the occurrence of systemic inflammation in muscle. This anti-inflammatory effect can lead to the lower miR-7 levels in master athletes than coeval sedentary people. miR-7 also has a significant part in lipogenesis, by taking part in cross-talks between peroxisome proliferator-activated receptor (PPAR), sterol regulatory element-binding proteins (SREBP), and liver X receptors signalling pathway. Regular physical activity needs higher level of energy supply, that can be affected by the intensified lipid metabolism mediated by the increased miR-7 regulation. The SIRT3 enzyme also regulates lipid metabolism, by the deacetylation of acyl-CoA dehydrogenase and medium-chain acyl-CoA dehydrogenase. Previous studies showed that the ATP-synthase F-complex can be deacetylated by SIRT3, thus

SIRT3 regulates ATP production directly. The age related decline of SIRT3 level is a generally accepted fact, explaining the decline of ATP production during aging. Results of related studies proved that the level of SIRT3 is higher in master athletes who had lifelong training than in samples of sedentary people. Physical activity affects the function of mitochondria in older age, which is helped by the fact that SIRT3 activates SOD2, thereby the antioxidant activity improves and the level of ROS decreases in mitochondria. During the research we stated that the mRNA levels of SIRT1 and FOXO1 were higher in the muscles of master athletes than in sedentary subjects. FOXO 1 takes part in mitochondrial metabolism, glycolytic and lipolytic flux. When FOXO1 becomes deacetylated by SIRT3, the expression of FOXO1 target genes, like SOD2, increases. The positive effects elicited by these enzymes and proteins can be observed in the skeletal muscles of people who exercise throughout their lives, with their energy exchange and quality of life deteriorating to a lesser extent with age.

It is never too late to start an active lifestyle. The body is able to adapt to regular exercise even in old age, which is reflected not only in the improvement of body composition and in its biochemical functions, but also in the dynamic marks of movement. As an effect of a 5-month training programme the body composition was normalized, and the characteristics of dynamic in CMJ and crouch improved in the groups over the age of 26. This can be explained by the fact, that while school-aged children have physical education lessons every day, which means regular physical activity for them, this regular activity is missing from the habits of elderly people. During CMJ, the range of motion of the CG

increased at both pre-jump gain and jump, as well as the acceleration of motion in G3. In G4, the sinking of CG at pre-jump gain and the velocity of CG increased during CMJ, as well as the velocity and acceleration of CG at crouch. However, the most stable improvement occurred in the oldest age group (mean age 63 years), where there was an improvement in all the examined dynamic characteristics of CMJ. In crouching the velocity and the acceleration of the movement of the CG also increased in this group. The positive effects of regular exercise showed correlations between changes in the dynamic of movements and body composition. The improvement in liftingt of CG during CMJ was positively influenced by the decrease in BW, FM, and BMI, as well as the increase in SMM/BW in the 21-35 age group. The lower CG of the pre-jump gain was aided by the increase in SMM/BW in participants aged 35–60 years and by the decrease in BW, BMI, and WHR in the elderly. In case of crouching, the velocity of movement was increased by the decreased BW, BMI and WHR in those over 60 years of age, while the acceleration was only positively affected by the decrease in WHR. Why the elderly were able to achieve better results in the dynamics of movement without an accertion in muscle mass, may be explained by the fact that the primary increase in strength in inactive people is due to the improved motor unit synchronization, i.e. neuromuscular coordination. Correlation calculations showed several connections between the changes of movement dynamics and the body composition, but by non-significant differences these relationships were not clear.

To get an accurate picture of the effects of training in the elderly, a larger scale research would be

needed. The study should be conducted to different age-groups, but with a larger number of participants and with more categories per age-groups (for example: athlete, recreational athlete and inactive individuals). A recreational training programme ought to be defined in addition to the usual activities supplemented by a weight-maintaining diet. The research shall concentrate on body composition, movement dynamics, biochemical, and neuromuscular parameters in the groups. Body composition determination should be supplemented by skeletal muscle mass - fat mass ratio (SMM/FM) and waist circumference (WC). Movement analysis shall measure the location of maximal velocity and of maximal acceleration in the motion, the average velocity and acceleration of movement. Similar biochemical parameters should also be considered and the neuromuscular changes should be researched in the muscles of the lower limb by EMG.

The most promising results are detected in the working-age and in near-retirement age-groups, as an outcome of recreational training and regular sport. Decreased miR-7 level in muscles by lifelong physical activity could lead to repression of sarcopenia-associated inflammation and to better fat metabolism. Increased SIRT3 level could aid more effective fat metabolism, production of ATP, and antioxidant function by SOD2 in striated muscles of physically active people. Lifelong regular sport may soothe the age-related decline in the antioxidant system and in the energy metabolism of muscle tissue. The positive effects of regular exercise are caused by even low-intensity training, but in elderly people, these alone are not enough to significantly improve body composition and reduce the risk of obesity,

although they can change the dynamics of movement. Based on these, it can be stated that by ensuring systematic physical activity muscle function and regeneration improve, the state of health consolidates, activity, work ability and ease of movements can remain lasting in old age. Related publications:

I.: **Szablics P, Orbán K, Szabó S, Dvorák M, Ungvári M, Béres S, Molnár AH, Pintér Z, Kupai K, Pósa A, Varga C.** *Effects of aerobic workout on the changes in the characteristics of dynamics of the center of gravity in different age categories.* Physiol Int. 2019 Jun 1;106(2):140-150. doi: 10.1556/2060.106.2019.13.

II.: **Koltai E, Bori Z, Osvath P, Ihasz F, Peter S, Toth G, Degens H, Rittweger J, Boldogh I, Radak Z.** *Master athletes have higher miR-7, SIRT3 and SOD2 expression in skeletal muscle than age-matched sedentary controls.* Redox Biol. 2018 Oct;19:46-51. doi: 10.1016/j.redox.2018.07.022. Epub 2018 Aug 7.

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