Cellular and Molecular Biology
E-ISSN : 1165-158X / P-ISSN : 0145-5680
www.cellmolbiol.org
C M B Association Publisher

# Neuromuscular electrical stimulation effect on mRNA expression of skeletal muscle in elderly

Qingchun Jiang[1], Ruixue Wei[2], Leilei Gong[3], Ruiying Zhao[4*]

[1] Department of Nephrology, Rheumatology and Immunology, Qingdao Jiaozhou Central Hospital, 99, Yunxi Henan Road, Jiaozhou City, Qingdao 266300, China
[2] Sensory Control Division, Qingdao Jiaozhou Central Hospital, 99, Yunxi Henan Road, Jiaozhou City, Qingdao 266300, China
[3] Outpatient Department, The Eighth People's Hospital of Qingdao, 84 Fengshan Road, Licang District, Qingdao, 266100, China
[4] Department of Gynecology, Jiaozhou Peoples Hospital, Qingdao, Yunxi Henan Road, Jiaozhou City, Qingdao 266300, China

**ARTICLE INFO**

***Original paper***

***Article history:***
*Received: August 26, 2022*
*Accepted: September 21, 2022*
*Published: September 30, 2022*

***Keywords:***

*NMES, Elderly, Gene Expression, Skeletal Muscle*

**ABSTRACT**

Considering the increasing number of elderly in the world, this research aimed to investigate the effect of neuromuscular electrical stimulation (NMES) on changes in muscle mRNA abundance of a number of gene targets for improving the balance of the elderly. Twenty-six elderly undertook 30 minutes of quadriceps NMES (50 Hz, current at the limit of tolerance). Vastus lateralis muscle biopsies were obtained at rest immediately before and 24 hours after the intervention. The expression of 384 targeted mRNA transcripts was assessed by Real-time TaqMan PCR. A significant change in expression from baseline was determined using the ΔΔCT method with a false discovery rate (FDR) of <5%. The results showed that the biological functions of upregulated genes included muscle protein turnover, hypertrophy, inflammation, and muscle growth, while downregulated genes included mitochondrial and cell signaling functions. In general conclusion, it can be said that NMES can improve balance in the elderly. Therefore, considering the importance of balance in old people, it is suggested to use this method to improve the balance of the elderly.

**Doi:** http://dx.doi.org/10.14715/cmb/2022.68.10.10
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## Introduction

According to the announcement of the World Health Organization in 2020, one of the most significant changes we have faced in this century is the doubling of the elderly population, which is expected to continue to increase (1). It is predicted that in China, the elderly population will increase significantly in the coming years. In the next forty years, a quarter of the country's population will be elderly (2). Therefore, paying attention to the issues and needs of this era is considered a social necessity. With increasing age and reaching old age, there are changes in the functioning of various organs, such as the vestibular system, muscular-skeletal system, sensory-body system, visual system, etc., which affect multiple body functions, including a person's balance (3, 4). Balance describes the dynamics of the body to prevent falling and is necessary and unavoidable to perform most movement skills in daily life and is influenced by strength, reaction, and proprioception (5).

Systems theory is one of the most common and accepted theories regarding balance. Systems theory believes that maintaining balance and controlling posture in space results from interaction and function between different nervous, muscular, and skeletal systems (6). According to the systems theory, the central nervous system uses the information of visual, vestibular, and sensory-body systems. It is aware of the body's position and the center of gravity of the body in space (7). If needed, it activates the appropriate movement response in the form of pre-programmed movement patterns to deal with the body position (6). Neuromuscular electrical stimulation (NMES) may be an effective strategy for maintaining or improving muscle function and clinical studies have suggested that NMES increases muscle mass and strength in the elderly (8). This study investigated changes in the expression of muscle mRNA transcripts after NMES in the elderly.

## Materials and Methods

### Subjects

The study's statistical population included all inactive male seniors (people who did not exercise regularly and at least twice a week). The research environment had elderly care and retirement centers to reach the subjects. After obtaining written consent, 26 old people from the target population were selected to participate in this research by the available sampling method. The criteria for entering the study included the age of 65 years and above, the absence of cognitive problems, the lack of brain and orthopedic injuries, and the ability to stand for at least one minute and walk a distance of 10 meters independently. The criteria for leaving the research were a lack of independence in daily activities, neuro-psychological disorders, and musculoskeletal disorders. People over 65 were selected as older adults based on the available backgrounds (9, 10).

### Experimental protocol

The elderly attended baseline assessments to perform spirometry, undergo body composition measures and complete NMES a minimum of one week before the first

* Corresponding author. Email: zhaoruiying2021@163.com

biopsy visit. Resting biopsies were performed on the vastus lateralis muscle using the micro-biopsy technique. The tissue was snap frozen in liquid nitrogen and stored for later analysis. After tissue acquisition, a light dressing was applied to the biopsy site, and NMES was performed. Twenty-four hours later, a second resting biopsy was performed at least 2.5 cm from the previous biopsy site, minimizing confounding changes in mRNA abundance due to tissue sampling.

**Basic assessments**

Body mass and height were measured using dual-energy X-ray absorptiometry (DEXA; Lunar Prodigy, GE, Buckinghamshire, UK) before assessing body composition. Fat-free mass index (FFMI) was calculated using the same equation, but body mass was replaced by total body fat-free mass + total body bone mineral mass (kg). Body mass index (BMI) was calculated as total body mass (kg)/height ($m^2$). Spirometry was performed using a portable spirometer according to British Thoracic Society guidelines.

**Muscle mRNA expression**

RNA was extracted from muscle samples using TRI Reagent (Applied Biosystems/Life Technologies, Paisley, UK) and reverse transcribed using SuperScript III (Life Technologies/Invitrogen) to synthesize complementary DNA. Complimentary DNA was loaded onto TaqMan 384-well custom Low-Density Array (LDA) microfluidic cards (Applied Biosystems/Life Technologies) and 40 cycles of automated polymerase chain reaction (PCR) were performed on a TaqMan 7900HT Real-Time PCR Instrument (Applied Biosystems, Paisley, UK). The abundance of mRNA was assessed using automated 384-well LDA cards.

**Data analysis**

Gene expression data were analyzed using the comparative CT method (ΔΔCT), which permits relative quantification of the target gene transcript against an internal control gene transcript (11). A house-keeping gene (hydroxymethylbilane synthase; HMBS) was selected with stable CT values across time points. Paired t-tests were used to identify significant changes in expression of the target gene relative to HMBS (ΔCT) from baseline to 24 hours. The False Discovery Rate (FDR) adjustment was applied to control for multiple comparisons using the R Statistical Package (R Version 3.0.0, 2013–04-03, The R Foundation for Statistical Computing). Expression values are presented as fold change from baseline ($2^{-\Delta\Delta CT}$), and significant within-group change was defined by an FDR <5%. Missing values occurred where gene expression was below the limit of detection after 40 cycles of PCR. A gene was excluded from the analysis if there were more than two missing data points. Between-group differences in physiological variables were tested by t-test, Mann-Whitney U-test (ordinal data), or Pearson $X^2$ test (categorical data).

**Results**

The average age of the studied elderly was 69.08 ± 2.84 years, the average weight was 67.66 ± 9.59 kg, and the average height was 1.62 ± 0.07 meters. According to the results, gene transcripts were significantly changed in amount (FDR <5% compared to baseline, Figure 1). The within-group variation in response to NMES is illustrated in Figure 2. Mean fold change values for all targets measured are listed in Table 1. Genes with physiological roles related to anti-inflammatory action, protein breakdown, cell cycle regulation, and antioxidant action, respectively (CHI3L1, CTSL1, CDK2, and SOD2), were upregulated following NMES. Other upregulated transcripts DYSF, MYC, FOS, TIMP1, and RUNX1 have physiological roles relating to anti-wasting, growth, regeneration, repair, and muscle hypertrophy, respectively. Downregulated transcripts CCNG2, ATP2B2, and RASGRP3 influence cell cycle/signaling regulation. RASGRP3 also has a physiological role in cancer, as does MN1, with both of these transcripts

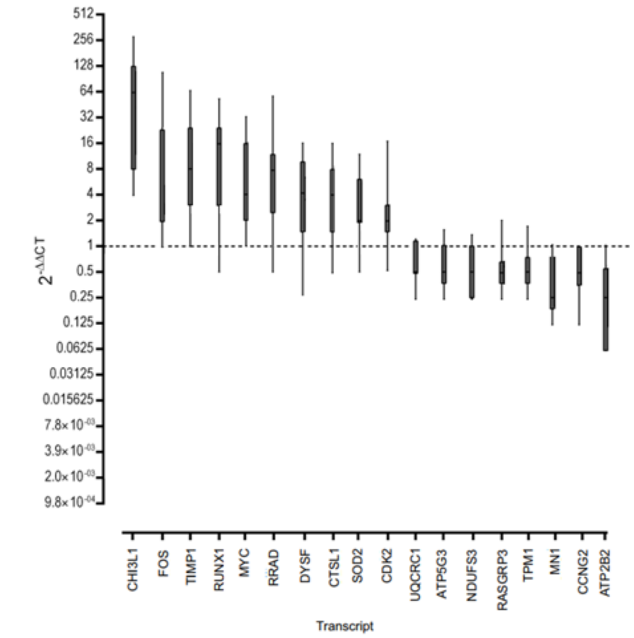


**Figure 1.** The expression of evaluated genes following transcutaneous neuromuscular electrical stimulation (NMES) with a false discovery rate (FDR) <5%; Data are expressed as fold change from baseline. Boxes denote median and interquartile range, whiskers are range.

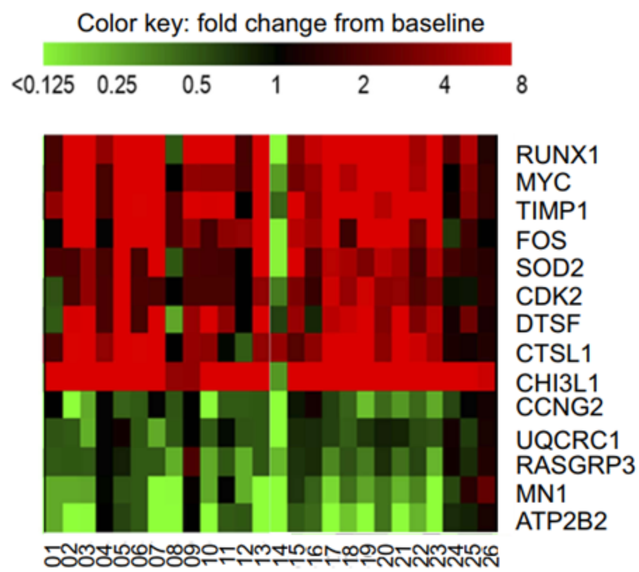


**Figure 2.** Heat map depicting individual variation in response to transcutaneous neuromuscular electrical stimulation (NMES) for the 14 transcripts that were significantly influenced.

**Table 1.** List of gene transcripts in expression following a bout of NMES.

| Gene name | Gene Full Name | Fold Change | FDR% |
|---|---|---|---|
| cathepsin L | | 5.3 | 0.029 |
| cyclin-dependent kinase 2 | | 3.1 | 0.41 |
| dysferlin | | 6.3 | 0.035 |
| superoxide dismutase 2 | | 3.5 | 0.028 |
| TIMP metallopeptidase inhibitor 1 | | 16.4 | 0.023 |
| FBJ murine osteosarcoma viral oncogene homolog | | 17.3 | 0.012 |
| v-myc avian myelocytomatosis viral oncogene homolog | | 10.2 | 0.12 |
| RUNX1 | runt-related transcription factor 1 | 15.8 | 0.006 |
| CHI3L1 | chitinase 3-like 1 | 84.6 | <0.001 |
| UQCRC1 | ubiquinol-cytochrome c reductase core protein I | 0.6 | 0.026 |
| ATP2B2 | ATPase, $Ca^{++}$ transporting, plasma membrane 2 | 0.5 | 0.025 |
| RASGRP3 | RAS guanyl releasing protein 3 | 0.7 | 0.026 |
| CCNG2 | cyclin G2 | 0.7 | 0.026 |
| MN1 | meningioma (disrupted in balanced translocation) 1 | 0.5 | 0.025 |

False discovery rate (FDR) <5% is the threshold for significance. Between group differences in fold change all P>0.05

downregulated after both interventions. Another transcript downregulated following NMES is UQCRC1 which codes for a mitochondrial sub-unit.

## Discussion

This study aimed to investigate the effect of the neuromuscular electrical stimulation (NMES) on improving the balance of the elderly. NMES was performed at the highest tolerable intensity to maximize muscle fiber functions. A limitation of this study is that muscle tension development during NMES was not measured but is known to typically be below 15% of maximal voluntary isometric force generation. In explaining the findings related to the improvement of the balance of NMES, it can be said that electrical stimulation of the brain can cause changes in neural plasticity, which is likely to be related to changes in functional connections in the human brain (12). This issue causes cerebral blood flow to be distributed in the stimulated area, and more blood flow will flow in that area. Hemoglobin will increase in the area where communication is strengthened (13). This issue causes better performance compared to the external stimulus; therefore, the person's balance also increases following these interactions. Also, direct electrical stimulation of the brain in the cerebellar region can affect the membrane potential of glial cells and, as a result, the balance of neurotransmitters (14). This change is similar to what is observed physiologically in astrocytes during the activation of neurons (15).

Research by Bondi *et al*. (16) investigated the short-term effect of NMES on static balance performance in elderly with chronic stroke and healthy people. The results showed that 20-minute stimulation with 1.5 mA improves static balance. Hardwick and Celnik (17) conducted a study to investigate the effect of electrical stimulation of the cerebellum on the motor learning rate of the elderly. The research results showed that fifteen minutes of electrical stimulation of the cerebellum significantly reduces the error rate and improves motor learning in the chain task.

On the other hand, Steiner *et al*. (18), in a study aimed at investigating the effect of NMES on balance and posture control, showed that electrical stimulation of the cerebellum does not affect balance and posture control. Also, Flöel *et al*. (19) reported in a study that electrical stimulation in the temporoparietal region does not significantly affect the learning rate of older people compared to the control group. The duration of stimulation in both mentioned studies was done only during one session, which is probably the reason for the lack of significant effect of electrical stimulation. However, in the present research, the number of stimulation sessions was considered to be three.

The results of the present research can be justified by systems theory. According to this theory, maintaining balance and controlling posture in space results from interaction and function between different nervous, muscular, and skeletal systems. According to the systems theory, the central nervous system (CNS) uses the information of visual, vestibular, and sensory-body systems and is aware of the position of the body and the center of gravity of the body in space and, if needed, provides the appropriate movement response in the form of movement patterns, which activates pre-programmed to deal with body position (20). According to this theory, NMES in the cerebellum can affect balance by activating the main areas of the brain that are involved in balance.

In general conclusion, it can be said that NMES can improve balance in the elderly. Therefore, considering the importance of balance in the elderly, it is suggested to use this method to improve the balance of the elderly. Among the limitations of the current research were the relatively small number of subjects and the use of the male gender alone, which is suggested to be observed in future research. It is also recommended to use other tests related to balance, such as Biodex, balance meter, etc., which are performed with advanced tools and devices, and evaluate the effectiveness of this protocol.

**Acknowledgement**
The authors are thankful to the higher authorities for the facilities provided.

**Authors' contribution**
This study was done by the authors named in this article, and the authors accept all liabilities resulting from claims which relate to this article and its contents.

**Conflicts of interest**
There are no conflicts of interest.

**Funding**
No funding received for this study.

**Availability of data and materials**
The data used to support the findings of this study are available from the corresponding author upon request.

**Statements and Declarations**
The author declares that no conflict of interest is associated with this study.

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