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RESEARCH ARTICLE

Neuromuscular electrical stimulation increases serum brain-derived neurotrophic factor in humans

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Abstract

Brain-derived neurotrophic factor (BDNF) plays several important roles in nervous system function including neuronal growth and plasticity. The purpose of the present study was to clarify whether neuromuscular electrical stimulation (NMES) and voluntary exercise to the same integrated force as by the NMES-induced exercise would enhance serum BDNF. Eleven healthy male subjects completed three interventions (NMES, voluntary exercise, and resting interventions) for 20 min on different days. In the NMES intervention, NMES was applied to the quadriceps femoris muscles. The stimulus intensity of NMES was progressively increased to the highest tolerated intensity during the experiment. In the voluntary exercise intervention, subjects performed an isometric knee-extension task; in this intervention, the target torque was calculated in accordance with the integrated force of knee extension obtained during the NMES intervention. In the resting intervention, subjects relaxed in a sitting posture. We measured serum BDNF, blood lactate, heart rate, oxygen uptake, respiratory ratio, and blood pressure. Serum BDNF was increased in the NMES ($p = 0.003$) and voluntary exercise interventions ($p = 0.004$) after each intervention. At the post-timepoint, serum BDNF in the NMES intervention was highest among all interventions ($p = 0.038$) and significantly higher than in the voluntary exercise ($p = 0.036$) and resting ($p = 0.037$) interventions. Our results showed that NMES was more effective for enhancing serum BDNF than voluntary exercise at least when employing the same method and integrated force.

Keywords Brain-derived neurotrophic factor · Voluntary exercise · Blood lactate · Neuromuscular electrical stimulation

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Introduction

Brain-derived neurotrophic factor (BDNF) is a member of the neurotrophin family that plays several important roles in nervous system function including neuronal growth and plasticity (Kesslak et al. 1998; Knaepen et al. 2010; Yamada et al. 2002). Patients with depression, dementia, and Parkinson's disease have significantly lower circulating BDNF (Brunoni et al. 2008; Costa et al. 2015; Scalzo et al. 2010; Shimada et al. 2014). An enhancement of the circulating BDNF by dosing drug or exercise improved cognitive function and each symptom of depression and Parkinson's disease (Brunoni et al. 2008; Erickson et al. 2011; Ferris et al. 2007; Hirsch et al. 2018). Therefore, it is important to develop more effective and easily-applicable methods for the enhancement of BDNF.

A popular method for enhancing circulating BDNF is physical exercise. Acute or chronic physical exercise enhances peripheral BDNF (Erickson et al. 2011; Ferris et al. 2007; Winter et al. 2007). Especially, moderate-to-high intensity aerobic exercise contributes to the enhancement of peripheral BDNF as reported by human studies (for review see Huang et al. 2014). In addition, Schiffer et al. reported that lactate infusion using the lactate clamp method produced elevations in plasma BDNF (Schiffer et al. 2011). In this way, physical exercise at the intensity of producing lactate (above lactate threshold) might be required to enhance peripheral BDNF. However, exercise of such intensity cannot be easily performed by elderly people or patients.

To address this issue, we focused on neuromuscular electrical stimulation (NMES). NMES is commonly used in the clinical field for healthy adults and patients such as those with knee osteoarthritis as a strength training tool to induce neuromuscular adaptation, or a rehabilitation tool to preserve muscle mass and function (Bax et al. 2005; de Oliveira Melo et al. 2013; Langeard et al. 2017). Previous studies have shown that blood lactate was increased by applying NMES without voluntary muscle contraction (Hamada et al. 2004; Miyamoto et al. 2018). This phenomenon was attributed to a specific pattern of motor unit recruitment. When we performed voluntary muscle contraction, motor units were recruited in accordance with the size principle and activated slow-twitch fibers. In contrast, NMES non-selectively and/or randomly recruits motor units and might activate slow- and fast-twitch fibers (Maffiuletti et al. 2011). Because fast-twitch fibers are major physiological producers of lactate (Philp et al. 2005), blood lactate would be increased by applying NMES compared with voluntary exercise. To our knowledge, only one study reported that a 30-min session of NMES to both lower limbs enhanced peripheral BDNF accompanied by increment of blood lactate (Miyamoto et al. 2018). However, we considered that the finding of the previous study was insufficient to conclude that NMES contributes to enhancing serum BDNF because a voluntary exercise to the same total volume of work (i.e., integrated force) as by the NMES-induced exercise might enhance peripheral BDNF. That is, without using NMES, peripheral BDNF might be enhanced by voluntary exercise to the same integrated force as with NMES-induced exercise.

The purpose of present study was to clarify whether NMES and voluntary exercise to the same integrated force as by the NMES-induced exercise would enhance serum BDNF. In the present study, we administered NMES, voluntary exercise (i.e., isometric knee extension), and resting interventions on different days. We calculated the intensity and duration of the voluntary exercise by reference to the integrated force of knee extension during the application of NMES. In addition, because the amount of the change in serum BDNF might be different between NMES and voluntary exercise intervention, we measured heart rate, blood pressure, oxygen uptake, and the respiratory ratio throughout the experiment to investigate which factors would produce the different result between the NMES and voluntary exercise intervention.

Methods

Subjects

Eleven healthy male subjects (age, 21.9 ± 1.0 years; height, 173.7 ± 5.1 cm; weight, 68.5 ± 9.5 kg) with no regular participation in strength or endurance training were enrolled in this study. According to a detailed medical history questionnaire, all subjects were free of mental disorders and orthopedic or neurological disease affecting the lower limbs. Additionally, subjects aversive to needle injection were excluded. All subjects provided written informed consent for participation. There were no dropouts and all subjects successfully completed each experiment. The present study was performed in accordance with the Declaration of Helsinki (last modified in 2000) and approved by the Ethical Committee of Sapporo Medical University.

Experimental procedure

All subjects completed four separate experiments on different days. In the first experiment (Exp1), we measured the maximal voluntary contraction (MVC) of knee extension. The resultant MVC value was then used to calculate the percent MVC (% MVC) in other experiments. The remaining three experiments included three interventions: NMES, voluntary exercise, and resting. Each experimental

intervention was separated by an interval of at least 2 weeks. In the NMES intervention (Exp2), NMES was applied to the bilateral quadriceps femoris muscles. In the voluntary exercise intervention (Exp3), subjects performed an isometric knee-extension task. Both ankles were fixed via a strap that was attached to a load-cell (LUR-A-1KNSA1, Kyowa, Tokyo, Japan) and secured to the chair. Subjects extended their knees against the resistance of the fixed ankle repeatedly. In the resting intervention (Exp4), the subjects rested in a sitting posture. The experiments were completed in numerical sequence except for Exp4; Exp4 was completed before Exp2, between Exp2 and Exp3, or after Exp3. The allocation of Exp4 was randomized across subjects (Fig. 1).

We measured serum BDNF and blood lactate from blood samples. Blood samples were obtained pre-intervention (pre) and immediately after completion of the intervention (post). Heart rate, blood pressure, oxygen uptake and the respiratory ratio were measured throughout the experiment.

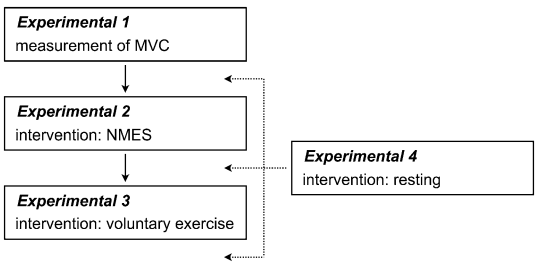

Fig. 1 The allocation of Exp1–4. The experiments were completed in numerical sequence except for Exp4; Exp4 was completed before Exp2, between Exp2 and Exp3, or after Exp3. The allocation of Exp4 was randomized across subjects

In addition, in the present study, we attempted to generate the same total volume of work in the NMES and voluntary exercise interventions.

Experimental environments

In the Exp1, subjects performed isometric MVC of the unilateral knee joint using an isokinetic dynamometer (BiodexTM, System 3, Sydney, USA). Subjects were required to sit with 90° knee flexion and 60° hip flexion. Isometric MVC was performed three times (5 s each) and measured from side-to-side. We set resting periods between MVC attempts for 3 min. The best performance value was recorded. Additionally, for practice of Exp2, NMES was applied to the bilateral quadriceps femoris muscles for 5 min.

In Exp2–4, subjects were prohibited from drinking alcohol on the day before each experiment and fasted breakfast on the day of each experiment. Subjects were also asked not to perform strength or endurance training for at least 48 h prior to an experiment. Subjects reported to the laboratory (24 °C ambient temperature) at 08:00 and rested in a seated position for 60 min. Experiments began at 09:00. After the start of each experiment, subjects rested for 30 min and subsequently proceeded to the intervention phase, which lasted 20 min. After the intervention phase, subjects rested for 10 min. The intervention environments are shown in Fig. 2. Subjects were seated on a comfortable chair with 90° knee flexion and 60° hip flexion. The trunk and both thighs were fixed into place using a strap or pad. A load-cell (LUR-A-1KNSA1, Kyowa, Tokyo, Japan) secured to a strap around the ankle was used to measure bilateral knee extensor force. Subjects were fitted with a facemask connected

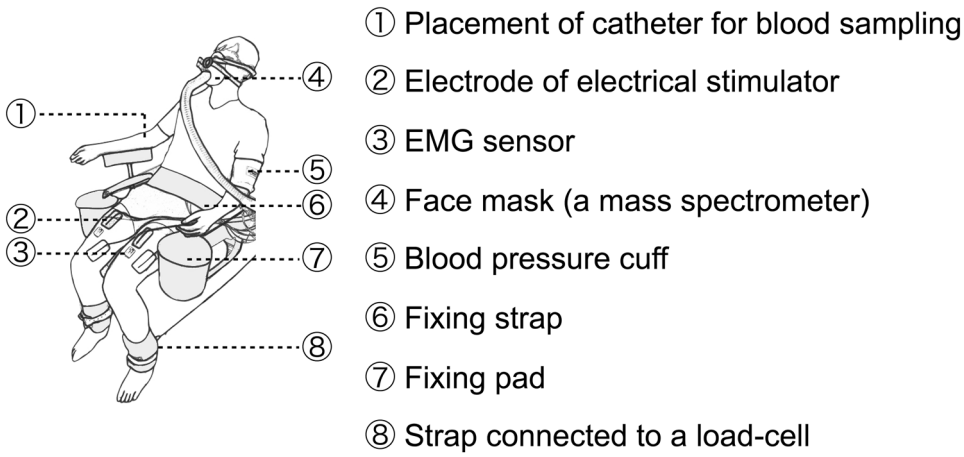

Fig. 2 Experimental environment of subjects. The trunk and both thighs were fixed into place using a strap or pad. A load-cell secured to a strap around the ankle was used to measure bilateral knee extensor force. Subjects were fitted with a facemask connected to a mass spectrometer. A catheter for blood sampling was placed into the antecubital or antebrachial vein of the right arm. Blood pressure and heart rate were monitored using an automated blood pressure cuff applied to the left upper arm. An EMG sensor was attached to the bilateral thighs to record NMES signal

to a mass spectrometer (ARCO-2000, Arco System, Chiba, Japan) to monitor the volume of oxygen consumed and that of carbon dioxide produced. A catheter for blood sampling was placed into the antecubital or antebrachial vein of the right arm. Blood pressure and heart rate were monitored using an automated blood pressure cuff applied to the left upper arm. To record the NMES signal, electromyographic (EMG) bipolar electrodes (Trigno Wireless EMG System, Delsys Incorporated, Boston, MA, USA) were placed on the bilateral thighs (i.e., rectus femoris muscle) according to the SENIAM recommendations (Hermens et al. 2000). Prior to electrode placement, the skin in the measurement area was cleaned with alcohol swabs. EMG signals were sampled at 1000 Hz using a Bio Amp device Power Lab System (Power Lab 16/35; AD Instruments, NSW, Australia), and data were stored on a computer. After that, the EMG signals were filtered (Butterworth band-pass at 50–500 Hz) using the Chart 7 software for Windows (Power Lab; AD Instruments, NSW, Australia). The filtered EMG signals were used to calculate the target torque for Exp3. The method of calculation of the target torque is shown in "Details of each intervention" [Voluntary exercise (Exp3)].

Details of each intervention

NMES (Exp2)

We used an electrical stimulator (Electrostimulator ES-520, Ito Chotanpa, Tokyo, Japan) to administer NMES. Stimulation was applied to the quadriceps femoris muscles of both lower limbs. Four rubber stimulation surface electrodes (9 × 5 cm) were placed over the motor points of the medial vastus and rectus femoris muscles and the proximal and distal points of the lateral vastus muscle. Before electrode placement, the skin surface was prepared by sanding and alcohol application. The stimulation protocol consisted of an interference wave at an amplitude-modulated frequency of 20 Hz (carrier frequency, 2000 Hz; pulse length, 0.05 msec) with a stimulus duration of 20 min and an on–off ratio of 4.5:4.5 s (rise time, 1.0 s and fall time, 0.5 s). The right and left lower limbs were stimulated alternately (i.e., when right lower limb was in the "on" phase, the left lower limb was in the "off" phase). The stimulus intensity was progressively increased to the highest tolerated intensity during the experiment. As a result, the stimulus intensity reached 31.3 (± 7.6) mA at the end of experiment.

Voluntary exercise (Exp3)

We devised the placement of the NMES electrodes and the part fixed on the body in a manner that the exercise induced by NMES was a simple movement (i.e., single joint movement). Given this, we expected subjects to be able to easily reproduce the movement with voluntary exercise. In the present study, NMES induced knee extension exercise (in essence, this exercise was isometric because the ankles were fixed). Therefore, in the voluntary exercise intervention, subjects were instructed to repeat the knee extension exercise isometrically.

The voluntary exercise had four phases: in the first phase, knee extension was increased to achieve the target torque (1.0 s); in the second phase, knee extension was sustained at the target torque (3.5 s); in the third phase, knee extension was decreased to resting (0.5 s); and the fourth phase was a resting phase (4.5 s). Subjects moved the right and left lower limbs alternately (e.g., when the right lower limb was exercised, the left lower limb was resting) and referred to a display readout of target torque located in front of them. The target torque was calculated individually for each subject in accordance with the value of knee-extension torque in Exp2 (Fig. 3): first, we selected torque waves stimulated by NMES in Exp2 based on the presence of NMES signal on thigh-EMG. Signal onset was determined when EMG signal value exceeded five standard deviations from the mean resting EMG value (the value 1 s before NMES stimulation). The identified torque waves were averaged and the target torque in Exp3 was restructured from the averaged torque wave into a single remodeling wave (exercise–resting ratio, 4.5:4.5 s [rise time, 1.0 s and fall time, 0.5 s]). Finally, the target torque wave was constructed by repeating the single remodeling wave. We aimed for the integrated force of knee extension in Exp3 to be congruent with the integrated force of knee extension in Exp2.

Resting (Exp4)

In the Exp4, the placement of rubber stimulation surface electrodes was similar to that in Exp2. However, we did not apply any electrical stimulation for subjects.

Measurements

A 5-mL volume of blood was taken from the antecubital or antebrachial vein to quantify blood lactate and serum BDNF. Blood samples were obtained pre- and post-timepoints. Blood lactate was measured immediately after blood-drawing using the Lactate Pro assay (Arkray, Inc., Japan). The detection limit of this assay was 0.5 pg/mL Thereafter, blood was kept for 90 min to allow for clotting and was centrifuged at 1500 relative centrifugal force for 10 min at room temperature to obtain serum. The supernatant was decanted and stored at − 80 °C until analysis. Serum BDNF was measured using a BDNF Emax Immunoassay Kit (Promega, Madison, WI, USA) according to manufacturer specifications. The detection limit of the assay was 15.6 pg/mL. Serum dilution (1/100) with sample buffer supplied by the kit was required

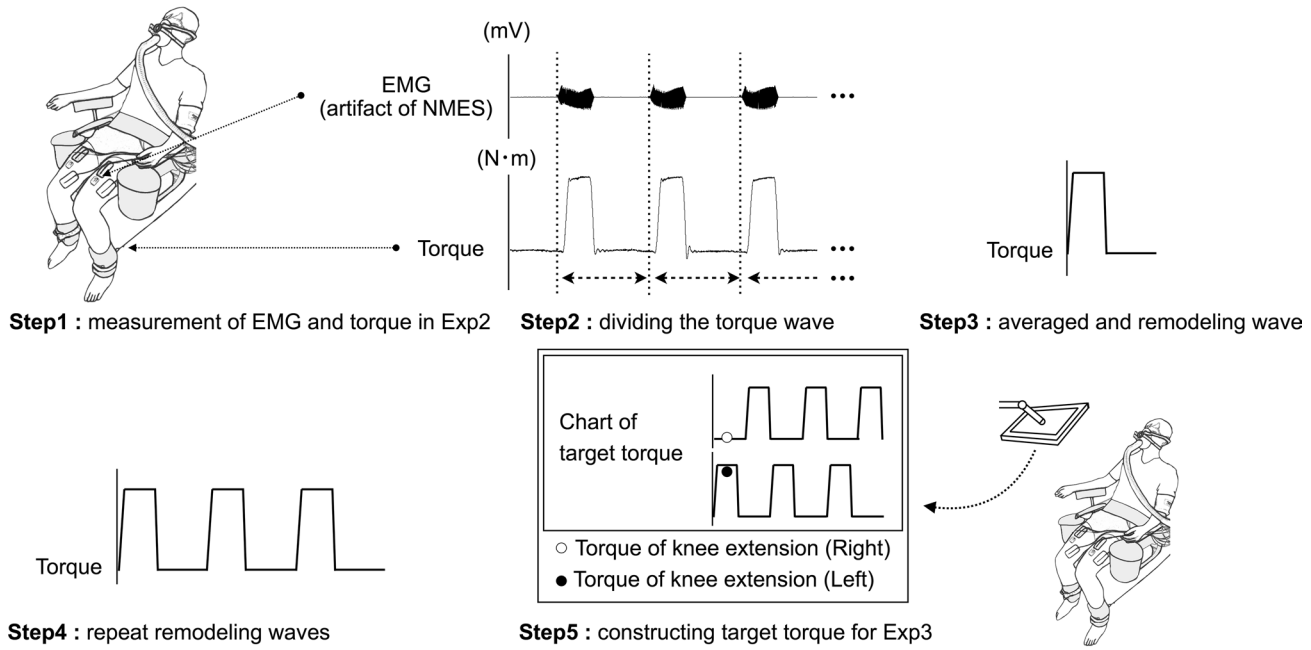

Fig. 3 The method of calculating and constructing the target torque in Exp3. Step 1, we measured EMG and knee-extension torque in Exp2. Step 2, we selected torque waves stimulated by NMES in Exp2 based on the presence of NMES signal on thigh-EMG. Signal onset was determined when the EMG signal value exceeded five standard deviations from the mean resting EMG value (the value 1 s before NMES stimulation). Step 3, the identified torque waves were averaged. The target torque in Exp3 was restructured from the averaged torque wave into a single remodeling wave with the same torque production (exercise–resting ratio, 4.5:4.5 s [rise time, 1.0 s and fall time, 0.5 s]). Step 4, a target torque wave was constructed by repeating the single remodeling wave. Step 5, the target torque wave was used in Exp3. We aimed for the integrated force of knee extension in Exp3 to be congruent with the integrated force of knee extension in Exp2

to obtain adequate concentration to measure. The assay was read using a TECAN Infinite M1000 pro microplate reader (Tecan Japan, Kanagawa, Japan) at 450 nm. The intra-assay coefficient of variation for serum BDNF was 5.7%. All samples were assayed in duplicate.

Oxygen uptake and respiratory ratio were determined breath-by-breath. Respiratory variables were continuously analyzed every 30 s using a mass spectrometer (ARCO-2000, Arco System, Chiba, Japan). Blood pressure and heart rate were measured using an automated blood pressure monitor (Tango; SunTech Medical Instruments, Morrisville, NC, USA) at 2-min intervals throughout the experiment. We calculated mean blood pressure with a formula as follows: mean blood pressure = diastolic blood pressure + 1/3 [systolic blood pressure − diastolic blood pressure]. Torque and integrated force of knee extension were measured using the load-cell (LUR-A-1KNSA1, Kyowa, Tokyo, Japan). Oxygen uptake, respiratory ratio, heart rate, and knee-extension torque were recorded using a Bio Amp device Power Lab System (Power Lab 16/35; AD Instruments, NSW, Australia) at a sampling rate 1000 Hz and data were stored on a computer for further analysis using Chart 7 software for Windows (Power Lab; AD Instruments, NSW, Australia). Integrated force was calculated by integrating the difference between the baseline and resultant torque values. The baseline torque was determined by averaging the torque for 1 s during the resting phase of the knee-extension task or NMES task.

Statistical analyses

All statistical analyses were conducted using SPSS version 22 (IBM, Corp, Armonk, New York, USA). Results are reported as the mean ± standard deviation. Changes in serum BDNF, oxygen uptake, respiratory ratio, heart rate, and blood pressure in each intervention (Exp2–4) between before and after the intervention (pre and post) were analyzed using a two-way repeated analysis of variance (ANOVA). When a significant interaction effect (time × intervention) was identified followed by a simple main effect, Bonferroni's post-hoc tests or paired t-tests were conducted. In addition, Pearson's correlation coefficient was used to test the associations between the degree of change in serum BDNF and blood lactate or mean blood pressure to explore the cause of the change in serum BDNF. Knee-extension torque was analyzed using a paired t-test. Effect sizes are expressed as partial eta-squared (partial η^2). Differences were considered to be statistically significant when $p < 0.05$.

Results

Serum BDNF

Serum BDNF at the pre-timepoint were 13164.0 ± 4579.4 pg/mL in the NMES intervention, 12843.3 ± 4099.1 pg/mL in the voluntary exercise intervention, and 12009.9 ± 4422.0 pg/mL in the resting intervention. Serum BDNF at the post-timepoint were 18625.6 ± 4173.5 pg/mL in the NMES intervention, 15103.0 ± 4177.9 pg/mL in the voluntary exercise intervention, and 13618.8 ± 4558.6 pg/mL in the resting intervention.

A two-way repeated ANOVA revealed a significant interaction ($F_{2,20}$ = 3.870, p = 0.038, partial η^2 = 0.279), main effect of time ($F_{1, 10}$ = 28.980, p < 0.001, partial η^2 = 0.743) and main effect of intervention ($F_{2,20}$ = 3.711, p = 0.043, partial η^2 = 0.271). A significant simple main effect of time in the NMES ($F_{1, 10}$ = 15.062, p = 0.003, partial η^2 = 0.601) and voluntary exercise interventions ($F_{1, 10}$ = 13.820, p = 0.004, partial η^2 = 0.580), and a significant simple main effect of interventions at the post-timepoint ($F_{2,9}$ = 4.807, p = 0.038, partial η^2 = 0.517) were observed. Post-hoc tests revealed that serum BDNF at the post-timepoint was significantly higher than that at the pre-timepoint in the NMES intervention (p = 0.003) and in the voluntary exercise intervention (p = 0.004) (Fig. 4). Additionally, at the post-timepoint, serum BDNF in the NMES intervention was significantly higher than serum BDNF in the voluntary exercise (p = 0.036) and in the resting (p = 0.037) interventions.

There were no significant associations between the degree of change in serum BDNF and blood lactate in the NMES intervention (r = 0.424, p = 0.194) and voluntary exercise intervention (r = − 0.009, p = 0.979). On the other hand, there was significant association between the degree of change in serum BDNF and mean blood pressure in the NMES intervention (r = 0.612, p = 0.045), but not significant association in the voluntary exercise intervention (r = 0.411, p = 0.209) (Fig. 5).

Blood lactate

Blood lactate at the pre-timepoint were 1.06 ± 0.29 mmol/L in the NMES intervention, 1.01 ± 0.18 mmol/L in the voluntary exercise intervention, and 1.16 ± 0.35 mmol/L in the resting intervention. Blood lactate at the post-timepoint were 1.55 ± 0.50 mmol/L in the NMES intervention, 0.95 ± 0.20 mmol/L in the voluntary exercise intervention, and 1.14 ± 0.31 mmol/L in the resting intervention.

There was significant interaction ($F_{2,20}$ = 12.079, p < 0.001, partial η^2 = 0.547) and main effects of time ($F_{1, 10}$ = 8.624, p = 0.015, partial η^2 = 0.463) and intervention ($F_{2,20}$ = 6.949, p = 0.005, partial η^2 = 0.410). A significant simple main effect of time in the NMES ($F_{1, 10}$ = 13.053, p = 0.005, partial η^2 = 0.566), and a significant simple main effect of interventions at the post-timepoint ($F_{2, 9}$ = 8.286, p = 0.009, partial η^2 = 0.648) were observed. Post-hoc tests revealed that, in the NMES intervention, blood lactate values at the post-timepoint was significantly higher than that at the pre-timepoint (p = 0.005). Additionally, at the post-timepoint, blood lactate in the NMES intervention was significantly higher than blood lactate in the

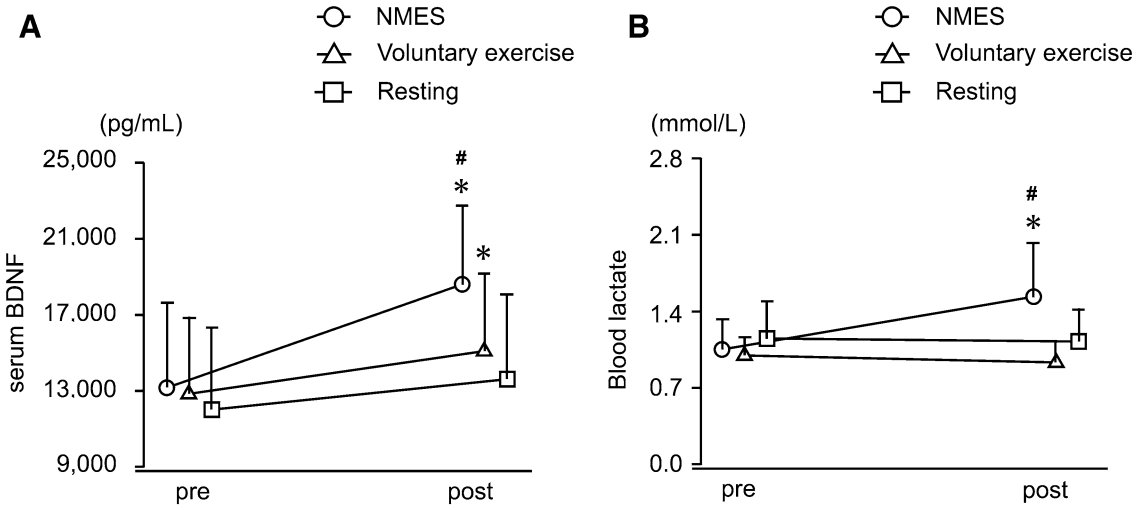

Fig. 4 Change in serum BDNF (**a**) and blood lactate (**b**) after each intervention. A two-way repeated ANOVA revealed a significant interaction in each parameter (serum BDNF: $F_{2,20}$ = 3.870, p = 0.038, partial η^2 = 0.279; blood lactate: $F_{2,20}$ = 12.079, p < 0.001, partial η^2 = 0.547). *Mean significant difference between the pre- and post-timepoints, at p < 0.05. #Mean serum BDNF in the NMES intervention was significantly higher than the voluntary exercise and resting intervention at the post-timepoint, at p < 0.05

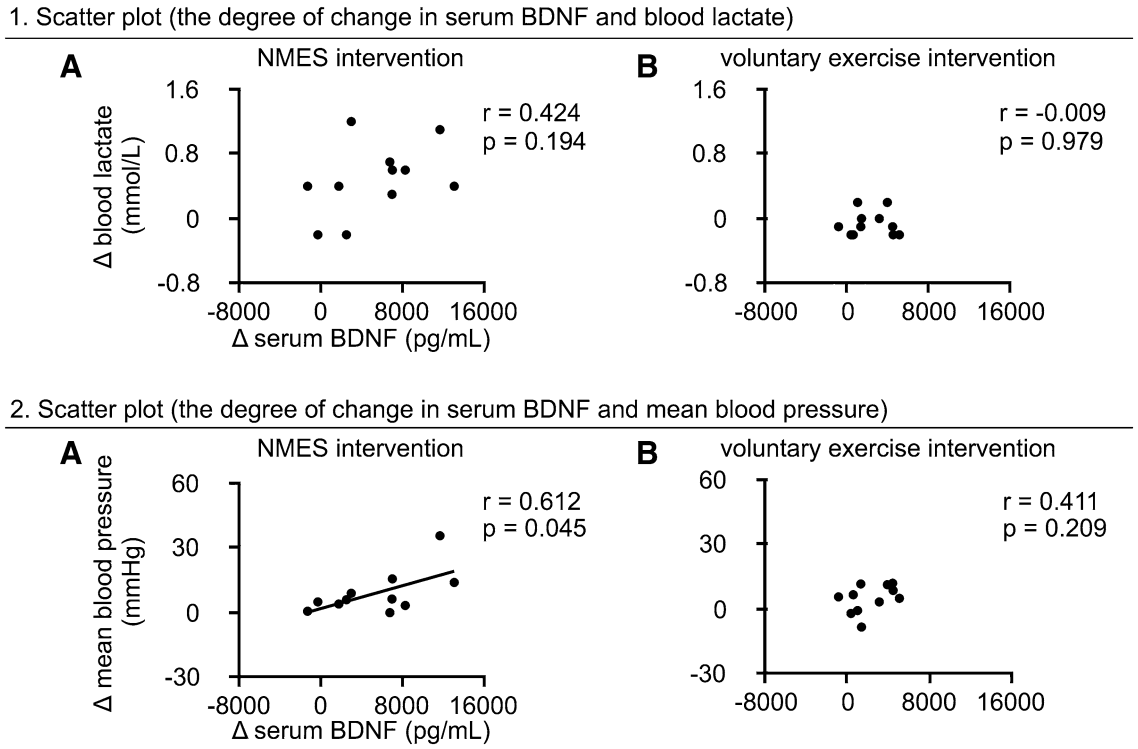

Fig. 5 Above: the scatter plot of the degree of change in serum BDNF and blood lactate in the NMES (**a**) and voluntary exercise intervention (**b**). Below: the scatter plot of the degree of change in serum BDNF and mean blood pressure in the NMES (**a**) and voluntary exercise intervention (**b**)

voluntary exercise ($p = 0.006$) and in the resting intervention ($p = 0.012$) (Fig. 4).

Heart rate

There was significant interaction ($F_{2,20} = 4.867$, $p = 0.019$, partial $\eta^2 = 0.327$) and main effects of time ($F_{1,10} = 57.406$, $p < 0.001$, partial $\eta^2 = 0.852$). A main effect of intervention was not observed ($F_{2,20} = 1.038$, $p = 0.373$, partial $\eta^2 = 0.094$). A significant simple main effect of time in the NMES ($F_{1,10} = 23.742$, $p = 0.001$, partial $\eta^2 = 0.704$) and voluntary exercise intervention ($F_{1,10} = 12.704$, $p = 0.005$, partial $\eta^2 = 0.560$) were observed. Post-hoc tests revealed that, heart rate at the post-timepoint was significantly higher than that at the pre-timepoint in the NMES intervention ($p = 0.001$) and in the voluntary exercise intervention ($p = 0.005$) (Table 1).

Oxygen uptake

There was significant interaction ($F_{2,20} = 10.993$, $p = 0.001$, partial $\eta^2 = 0.524$) and main effects of time ($F_{1,10} = 13.273$, $p = 0.005$, partial $\eta^2 = 0.570$) and intervention ($F_{2,20} = 3.594$, $p = 0.046$, partial $\eta^2 = 0.264$). A significant simple main effect of time in the NMES ($F_{1,10} = 20.475$, $p = 0.001$, partial

Table 1 The change in other parameters except serum BDNF and blood lactate

	Intervention	Pre	Post
Heart rate (beat/min)	NMES	55 ± 7	68 ± 11*
	Voluntary	59 ± 9	65 ± 11*
	Resting	58 ± 10	61 ± 11
Oxygen uptake (METs)	NMES	0.99 ± 0.17	1.41 ± 0.29*
	Voluntary	1.08 ± 0.20	1.21 ± 0.28
	Resting	1.07 ± 0.17	0.99 ± 0.14
Respiratory ratio	NMES	0.88 ± 0.06	0.96 ± 0.09*
	voluntary	0.93 ± 0.12	0.91 ± 0.07
	resting	0.91 ± 0.05	0.89 ± 0.05
Blood pressure			
1. Systolic (mmHg)	NMES	114 ± 7	124 ± 13
	Voluntary	112 ± 10	117 ± 8
	Resting	109 ± 6	112 ± 9
2. Diastolic (mmHg)	NMES	71 ± 10	80 ± 10
	Voluntary	69 ± 11	74 ± 8
	Resting	66 ± 13	71 ± 14
3. Mean (mmHg)	NMES	86 ± 7	95 ± 10
	Voluntary	83 ± 8	88 ± 4
	Resting	81 ± 9	84 ± 10

*Significant difference compared with pre in each group, at $p < 0.05$

$\eta^2 = 0.672$), and a significant simple main effect of interventions at the post-timepoint ($F_{2,9} = 12.328$, $p = 0.003$, partial $\eta^2 = 0.733$) were observed. Post-hoc tests revealed that, in the NMES intervention, metabolic equivalents (METs) values at the post-timepoint was significantly higher than that at the pre-timepoint ($p = 0.001$). Additionally, at the post-timepoint, METs in the NMES intervention were significantly higher than METs in the resting intervention ($p = 0.003$), and METs in the voluntary intervention were significantly higher than METs in the resting intervention ($p = 0.028$) (Table 1).

Respiratory ratio

There was significant interaction ($F_{2,20} = 4.957$, $p = 0.018$, partial $\eta^2 = 0.331$). Main effect of time and intervention was not observed (main effect of time: $F_{2,20} = 0.819$, $p = 0.387$, partial $\eta^2 = 0.076$; main effect of intervention: $F_{2,20} = 0.200$, $p = 0.821$, partial $\eta^2 = 0.020$). A significant simple main effect of time in the NMES ($F_{1,10} = 13.721$, $p = 0.003$, partial $\eta^2 = 0.595$) was observed. Post-hoc tests revealed that, the respiratory ratio at the post-timepoint was significantly higher than that at the pre-timepoint in the NMES intervention ($p = 0.003$) (Table 1).

Systolic blood pressure

Significant main effects of time and intervention were observed (main effect of time: $F_{1,10} = 7.002$, $p = 0.024$, partial $\eta^2 = 0.412$; main effect of intervention: $F_{2,20} = 5.638$, $p = 0.011$, partial $\eta^2 = 0.361$). There was no significant interaction ($F_{2,20} = 2.977$, $p = 0.074$, partial $\eta^2 = 0.229$) (Table 1).

Diastolic blood pressure

A significant main effect of time was observed ($F_{1,10} = 12.655$, $p = 0.005$, partial $\eta^2 = 0.559$). There was no significant interaction ($F_{2,20} = 0.764$, $p = 0.479$, partial $\eta^2 = 0.071$) and main effect ($F_{2,20} = 1.945$, $p = 0.169$, partial $\eta^2 = 0.163$) (Table 1).

Mean blood pressure

Significant main effects of time and intervention were observed (main effect of time: $F_{1,10} = 15.640$, $p = 0.003$, partial $\eta^2 = 0.610$; main effect of intervention: $F_{2,20} = 4.156$, $p = 0.031$, partial $\eta^2 = 0.294$). There was no significant interaction ($F_{2,20} = 1.562$, $p = 0.234$, partial $\eta^2 = 0.135$) (Table 1).

Knee-extension torque and integrated force

The mean knee-extension torque value was $3.79 \pm 2.85\%$ MVC in the NMES intervention and $4.02 \pm 3.08\%$ MVC in the voluntary exercise intervention; these values were not significantly different ($t_{10} = 0.180$, $p = 0.861$). The mean integrated force was 13253.7 ± 10563.6 N s in the NMES intervention and 13664.4 ± 10548.5 N s in the voluntary exercise intervention; these values were also not significantly different ($t_{10} = 0.092$, $p = 0.928$). However, the standard deviations of the mean knee-extension torque and the mean integrated force were large because the stimulus intensity of NMES and the torque induced by NMES were different among subjects. Based on this result, we calculated the relative value of the results of these measurements in the voluntary exercise intervention in NMES intervention for each subject as a further analysis. We found that the relative value of knee-extension torque was 1.00 ± 0.24 and the relative value of integrated force was 1.00 ± 0.25.

Discussion

We investigated whether peripheral BDNF would be differentially enhanced by NMES and by voluntary exercise when using the same method and integrated force in both interventions. Serum BDNF was increased in the NMES and voluntary exercise interventions. However, serum BDNF at the post-timepoint in the NMES intervention was highest among all interventions and significantly higher than in the voluntary exercise and resting interventions. Integrated force was not significantly different between the NMES and voluntary exercise interventions. From these results, it was suggested that NMES was a more effective method for enhancing serum BDNF than voluntary exercise at least when employing the same method and integrated force.

BDNF plays several important roles in nervous system function including neuronal growth and plasticity (Kesslak et al. 1998; Knaepen et al. 2010; Yamada et al. 2002). Exercise is a frequently used and well-examined method to enhance peripheral BDNF in the clinical field (Huang et al. 2014). However, it was considered that exercise at the intensity of producing lactate might be required to enhance peripheral BDNF, and it may not be possible to use exercise of such intensity with elderly people or patients. In the present study, we revealed that NMES could enhance serum BDNF without being accompanied by high physical load (i.e., on average, the heart rate, METs and respiratory ratio in the NMES intervention reached 68 bpm, 1.41 METs and 0.96, respectively). Therefore, our results suggested that NMES might be an effective method of enhancing peripheral BDNF for people who have difficulty engaging in voluntary exercise or cannot exercise at high intensity.

In the present study, NMES was more effective for enhancing serum BDNF than voluntary exercise. As a cause of this difference between NMES and voluntary exercise, several possibilities may be considered. First, blood lactate might contribute to this difference. The increment of blood

lactate was considered to be a key factor for the enhancement of peripheral BDNF (Ferris et al. 2007; Schiffer et al. 2011). In the present study, we observed that blood lactate increased significantly only in the NMES intervention, although the association between the degree of change in serum BDNF and blood lactate in the NMES intervention was not significant ($r = 0.424$, $p = 0.194$). The increment of blood lactate induced by NMES might have contributed to the enhancement of BDNF to some extent in the present study. Second, increased sympathetic nerve activity in the NEMS intervention might influence an increase in the serum BDNF. Previous human studies reported that transcutaneous nerve stimulation enhanced sympathetic nerve activity through increased skin sensation and muscle contraction (Moreau et al. 1995; Wong and Jette 1984). In addition, animal study revealed an increase in sympathetic nerve activity led to increase in a production of BDNF in the adrenal medulla through the sympathetic-adrenal-medullary axis (Kondo et al. 2013). In these lines, the degree of change in mean blood pressure, an index of sympathetic nerve activity (Nobrega et al. 2014), was significantly and moderately correlated with serum BDNF in the NEMS intervention (Fig. 5). In the voluntary exercise, there were little changes in mean blood pressure and serum BDNF and therefore we could not find significant relationship between the changes in mean blood pressure and serum BDNF (Fig. 5). Further study was needed to clarify the relationship between sympathetic nerve activity and the serum BDNF in humans such as measuring the other blood components (e.g., cortisol and catecholamines).

Limitations

The present study had several limitations. First, our study had a limited sample size. Additionally, untrained individuals were recruited in the present study. We could not reveal whether NMES would enhance serum BDNF in trained individuals. Therefore, future study should recruit large samples and focus on the training history of subjects. Second, we did not measure the number of platelets. BDNF is mostly stored in platelets and was released by platelets for clotting (Fujimura et al. 2002). In the present study, we recruited healthy people, thus, it was expected that there was not remarkable individual difference of the number of platelet. However, if we set inclusion criteria based on the number of platelets, the variance of the result of serum BDNF might be more reduced. Third, serum BDNF enhanced in the voluntary exercise intervention not accompanied with the change of blood lactate. Previous study showed physical exercise at the intensity of producing lactate might be required to enhance peripheral BDNF (Huang et al. 2014). Therefore, although it was possible that there was an existence of another mechanism to enhance peripheral BDNF not depending on the change of blood lactate, it could not be clarified in the present study.

Conclusion

The present study showed that NMES enhanced serum BDNF. Additionally, NMES was more effective at enhancing serum BDNF than voluntary exercise using the same method and integrated force as in the exercise induced by NMES. NMES might be a new method of enhancing peripheral BDNF for people who have difficulty engaging in voluntary exercise or cannot exercise at high intensity.

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