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Effects of verbal encouragement on force and electromyographic activations during handgrip exercise

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Abstract

Background: The aim of the present study was to examine the effects of verbal encouragement on isometric force and associated electromyographic (EMG) parameters during a handgrip task.

Methods: Twenty-three participants (12 women and 11 men) performed maximal voluntary isometric handgrip contractions following three conditions: (1) verbal encouragement (VE) condition: participants executed isometric contractions while being verbally encouraged. (2) non verbal encouragement (nVE) condition: the same starting and stopping signal but without encouragement. (nVE) condition: and non motivation (nCM) condition: self-initiated contractions without concentration and motivation. Start and stop of the contraction were self-initiated. The maximal voluntary force (MVF) and the maximal rate of force development (MRFD) were measured. Integrated EMG corresponding to MVF (iEMG_{MVF}) and to MRFD (iEMG_{MRFD}) were collected from Flexor Digitorum Superficialis (FDS) and Extensor Digitorum Communis (EDC) muscles.

Results: MVF was higher during VE compared with nVE (+11.7%; P<0.05) and nCM (+23.2%; P<0.05) conditions. Likewise, MRFD was significantly higher during VE, compared with nVE (+21.7%; P<0.05) and nCM (+55.4%; P<0.05) conditions. iEMG_{MVF} increased for FDS and EDC during VE, compared to nVE (+26.19%, +20.5%) and nCM conditions (+68.85%, +48.91%), respectively. iEMG_{MRFD} increased for FDS and EDC during VE, compared to nVE (+21.2%, +46.07%) and nCM conditions (+23.79%, +42.32%). Furthermore, the reproducibility of all these indices was higher with VE condition.

notes wheel have the state of t Conclusion: Taken together, force production (MVF and MRFD) and EMG data

Introduction

Many previous studies have demonstrated that muscular explosive force production during maximal voluntary contraction depends on histological, morphological and biomechanical factors, such as muscle typology 1, muscle size and architecture anatomic muscle cross-sectional area and volume 3, synchrony of activation onset 4 musculo-tendinous stiffnerss 5, recruitment of motor units and the modulation of their firing 6 and the mechanical properties of the myotendinous complex 7-9. Moreover, the kind of instruction given by the investigator is also known to influence the modulation of motor unit activity during explosive force production 10-13. Indeed, the effect of instruction has largely been studied in small muscle groups with a large cortical representation (somatotopic map), which are involved in precise voluntary movements. For example, Bemben et al. 14 and Sahaly et al. 13-15 studied the effect of instruction during isometric exercises on MVF, MRFD and EMG. MRFD was significantly higher when subjects were instructed to exert the most explosive force by concentrating on the fastest contraction without concern for achieving maximal force than when they were asked to exert muscle force with hard-and-fast instruction ^{13,15}. Explosive muscular force production expressed by MVF and MRFD is considered to be important in many sport-specific and functional daily tasks. MVF and MRFD are involved in the performance of sporting movements, such as jumping 16-18, and in providing dynamic joint stability and ligament protection 19. The rate of force development should also depend on nervous factors. It has been demonstrated that MRFD is lower during voluntary contractions than during electrical stimulations 20,21. Furthermore, since the pioneering study by Adrian and Bronk ²², it is well known that the gradation of muscle force is the result of an increasing firing rate and additional motor unit recruitment.

Among studies analyzing the instruction effect, the use of positive VE has been shown to improve motivation and motor performance in various activities, such as voluntary isometric contractions ²³⁻²⁵. VE represents a cognitive and motivational process, which is reflected in a large, distributed bilateral cerebro-cerebellar network 26. According to McNair et al. ²³, VE leads to suppression of the supraspinal inhibition, thus resulting in enhancement of muscle strength. Furthermore, Belanger and McComas 6 suggested that the activation of motor units may be inhibited when a maximal voluntary contraction is required due to a supraspinal drive acting on the motor units. In a recent functional Magnetic Resonance Imaging (fMRI) study, Belkhiria et al. 26 found that mean handgrip force measured during VE (29.26 kg; p< 0.05) was significantly higher relative to the non-verbal condition (26.97 kg; P = 0.004). The motivation induced by VE was maintained thanks to a closed-connectivity loop between cerebral and cerebellar cortex specifically through the red nucleus and striatal network 26. Furthermore, Generating a maximum muscle tension involves not only the recruitment of motor units, but also a modulation of their firing rates ⁶ by the brain networks, reinforced by the type of VE given to the participant during the contraction ²⁶. However, these VE works did not study at any time the effect of VE at specific and crucial force instants such as MVF, MRFD or associated EMG. Collectively, these findings indicated that VE is known to influence cerebral activity, and thus may lead one to suggest that VE could affect the neuromuscular activation (force and associated electromyography).

From a methodological perspective, one also has to consider the relative advantages and limits of previous cited studies. Whereas verbal encouragement previous studies revealed neuromuscular increases and yielded a high specificity, their efficiency may be limited by the selection of only large time intervals and the lack of time accuracy, and

reproducibility. Independent of methodological differences, interpretation of the current literature thus faces the standing that VE effect is present in several neuromuscular activation methods but absent in the specific and crucial force and iEMG instants. In order to provide a precise identification of VE effect, this study aimed to: i) determine how VE could influence force parameters during high accuracy and crucial force time intervals such as MVF and MRFD; ii) determine whether the EMG activity might be substantially responsive to VE effect; iii) assess how the reproducibility of MVF, MRFD and EMG could depend on VE. To address these aims, we compared MVF, MRFD and EMG across three diverse handgrip conditions: VE, nVE and nCM. It was hypothesized that VE condition, acting as a motivational factor, would result in higher MVF and MRFD parameters. This increase would be explained by neuromuscular activation expressed by iEMG activity of solicited muscles.

Materials and methods

Participants

Twenty-three active young adults (12 active women and 11 active men, aged 25.1 ± 5.9 years ranging from 18 to 32 years) participated in this experiment. All of them were right-handed and volunteers. They had to strongly concentrate on the movement to be performed of pressing a handgrip as hard as possible without making any sudden movements. For the assessment of inclusion and exclusion criteria, a questionnaire was used to examine the participant's health condition. None of them practiced intense or isometric activities of upper or lower limbs (e.g., body building, climbing, weightlifting...). They have no neuromuscular or cardio-respiratory disease. Female participants were not pregnant or lactating. Participants have not a history of alcohol or recreational drug abuse within 12 months prior to the study. Participants were

asked to respond to the questionnaire before the beginning of the experiment under supervision of a study nurse to ensure accurate understanding of the questions. All questions were answered with "yes" or "no," including space for additional comments.

All participants were healthy and had no known neuromuscular disorders at the time of the study. They have given their informed consent for participation in this research study. The study was approved by the Institutional Review Board, and was conducted according to the guidelines of the Declaration of Helsinki.

Protocol

The protocol began with a familiarization and warm-up phase with the handgrip device. Participants were lying on their backs with connected forearm EMG electrodes and with headphones placed on their ears. They were asked to repeat the following sequence five times: mental preparation, then motor execution by squeezing a handgrip as hard as possible, and afterwards rest. In this paper, we only focus on the execution period of the three following randomized conditions:

- i) Verbal Encouragement (VE) condition: participants achieved a preparation period of 6.6 seconds and then they received a starting and stopping contraction signal (Go/Stop). During the contraction period of 4.4 seconds, participants were verbally encouraged by a recorded human voice repeating firmly, "Go Go Go..." 20 times to encourage them to squeeze the handgrip as hard as possible. Finally, a long rest period of 44 seconds allowed participants to recover effort. The verbal encouragement was recorded by WavePad Audio Editing Software.
- ii) Non-verbal encouragement (nVE) condition: It includes exactly the same steps of VE condition but without verbal encouragement during contraction movement.

Non concentration and non motivation (nCM) condition: the start and stop of contractions were self-initiated by the participants themselves. They heard a beep every 55 seconds (five times) to remind them to squeeze the handgrip as hard as possible during 4.4 seconds. The participants had to prepare and to squeeze the handgrip when they felt ready without motivation and concentration.

This protocol was tested in a precedent fMRI study (14) and the used experimental periods were multiples of 2.2 seconds, which is a time repetition parameter needed in fMRI measures. Both studies used the same paradigm with different hypotheses and they are completely independent.

Force data acquisition

The isometric contraction (duration 4.4 seconds) was measured by a pressure captor (hand dynamometer TSD121B-MRI, Biopac Systems Inc., Santa Barbara, CA) connected to the Biopac system MP150 (Biopac MP150, Systems Inc., Santa Barbara, CA). The handgrip device was held by the right hand of the participant. The transducer was connected to an amplifier DA100C (Biopac MP150, System Inc., Santa Barbara, CA), whose output was directed to the AcqKnowledge software (Version 4.2, System Inc., Santa Barbara, CA). This amplifier recorded the data on the hard drive of a personal computer. The sampling frequency of the force signal was digitized at 1000

EMG data acquisition

Simultaneously with force data measurements, electromyographic signals were digitized and recorded on the same computer. The flexor digitorum superficialis (FDS) and extensor digitorum communis (EDC) muscles of the right forearm were identified by palpating the skin when participants flexed and extended their fingers. The overlying

skin was shaved and rubbed with an alcohol wipe to remove dead cells, dirt or skin oils. Bipolar electrodes (ADD208, 8-mm recording diameter, System Inc., Santa Barbara, CA) were firmly attached on the skin surface overlying each of the two muscles. A reference electrode was placed on the skin overlying the lateral epicondyle near the elbow joint of the right arm. Surface EMG signals were recorded using a sampling rate of 1,000 Hz. The EMG signal was amplified (gain range at 2000) and filtered using a band-pass filter (125 Hz).

Force and EMG data processing

MVF was defined as the highest peak force recorded during one trial. MRFD was equal to the steepest slope calculated in a 20-ms time window ¹³. The value of MRFD was expressed in newtons per second and in relative units, i.e. as e percentage of MVF per second [MRFD%=(MRFD/MFV)*100] ¹³. EMG data for both muscles were rectified and integrated (iEMG) during the specific phase and divided by the duration of the phase corresponding to average EMG. The value of iEMG corresponding to MVF (iEMG_{MVF}) was computed by integrating EMG during a 128-ms window previously to MVF. The value of iEMG corresponding to MRFD (iEMG_{MRFD}) was computed by integrating EMG during a 128-ms window around MRFD (64 ms before and 64 ms after MRFD) ¹⁵. Five trials have been executed in each condition. For each condition, the mean of the three best trials of MVF and MRFD were averaged to analyze the data.

Statistical analyses

Statistical analyses were carried out using Statistica 10.0 (StatSoft, Maisons-Alfort, France) and data are presented as mean (± SD). The normality was tested using the Shapiro-Wilk's test for all dependent variables. One-way ANOVA with repeated measure and Bonferroni post-hoc test were used to examine the effect of instruction on

measured variables. Relative changes (%) in dependent variables are expressed with 95% confidence interval (95% CI). The standardized differences or Cohen effect sizes (ES, 95 % CI) of differences in measured variables between the conditions were calculated using the pooled standard deviation ²⁷. Threshold values for Cohen ES statistics were 0–<0.20 (trivial), 0.2–<0.50 (small), 0.50–<0.80 (medium), ≥0.80 (large) ²⁸. Person Correlation Coefficient was used to correlate MFV with MRFD. The intraparticipant coefficient of variation in each condition (CV%) of the three best trials of MVF and MRFD and the associated EMG_{MVF} and EMG_{MRFD} activities were examined using two-way ANOVA with repeated measures and a Bonferroni post-hoc test. All significance thresholds were set at P < 0.05.

Results

MVF and MRFD

The mean (\pm SD) values of MVF, MRFD and MRFD % for each condition were presented in Table 1. A significant main effect of instruction was found for all force data (Table 1). The Bonferroni Post-hoc test showed that the values of MVF, MRFD, and MRFD% were significantly higher with VE compared with nVE and nCM. There were significant correlations between MVF and MRFD for each instruction $0.82 \le r \le 0.84$ (P < 0.001) for nCM, nVE and VE, respectively. There were no significant differences between instructions for both CV% MVF and CV% MRFD. However, CV% MRFD was significantly higher than CV% MVF.

EMG activity

The mean (± SD) values of iEMG for MVF and MRFD were presented in Table 2. A significant main effect of instruction was found for EMG data (Table 2). The value of

iEMG_{MVF} was significantly higher in VE than in nVE and nCM for all participants for FDS and EDC. Similar significant results were also found for iEMG_{MRFD}.

The mean (± SD) values of coefficient of variation of iEMG for MVF and MRFD were presented in Table 3. The CV% of EMG_{MRFD} was significantly higher than CV% of EMG_{MVF} only during nCM for EDC muscle. CV% of EMG_{MVF} and EMG_{MRFD} for both FDS and EDC muscles were significantly higher with VE.

Standardized differences between instructions were presented in table 4. Taken together, these results indicated supplementary significant differences between the three instructions. For all tested parameters, the verbal encouragement effect was higher compared to both nVE and nCM.

Discussion

This study investigated for the first time the verbal encouragement effect during handgrip exercise by the combination of force production and EMG analysis. In contrast to previous instruction studies ^{13-15,23}, we pursued a different approach and found some novelties. Addressing many aims, the present results confirmed our hypothesis and indicated that: i) verbal encouragement increased significantly MVF and MRFD; (ii) iEMG activity was substantially related to VE; iii) VE had positive and significant effect on EMG reproducibility; iiii) VE would decrease intra-subject MRFD variability.

The previous researches reported VE increases only during large time intervals such as during aerobic Wingate test ²⁹, VO2 max and blood lactate concentration during a treadmill test ³⁰, muscular endurance ³¹, elbow flexors during an isometric muscle action ²³, knee extension ²⁴, and triceps surae flexion ²⁵. An advantage result of the present

study was the concurrent dependence of both MVF and MRFD on VE, as suggested by the comparison to nVE and nCM conditions (Table 4). This VE effect on MRFD was observed on the different indices of rate of force development: maximal slope in absolute values and relative MRFD. Increases in both absolute and relative values of rate of force development with VE condition implied a decrease in the time to develop a specific level of force output. The positive correlation between MVF and MRFD for each instruction confirmed that MRFD expressed in absolute values depends on maximal strength ³², in addition to shortening velocity, skeletal muscle compliance and muscle activation. Moreover, it was confirmed that the rapid force production is closely related to central parameters ³³. More specifically, RFD can be strongly affected by both the supraspinal drive and presynaptic inhibition ³³. Gandevia ³⁴ suggested that diverse spinal and supraspinal mechanisms influence the supraspinal drive and/or motor units firing rate. In support of previous studies ³⁵⁻³⁸, whatever the instruction, coefficients of variation and consequently intra-subjects variabilities were significantly higher in MRFD than MVF.

To the best of our knowledge, this study is the first to evaluate VE effect on neuromuscular activation during explosive voluntary contractions. Maximal contraction data showed greater iEMG in FDS and EDC muscles during VE condition when compared with nVE and nCM conditions (Tables 2, 4). Current literature suggested that this increase might be partly related to voluntary drive to human skeletal muscles through changes in the spinal and peripheral transmission of the neural drive ³⁹. The increased iEMGs are synonymous with increases in specific neural drive, including increases in motor unit recruitment and/or firing frequency ^{40,41} driven by the spinal and/or supraspinal centre ⁴²⁻⁴⁴. Our results indicated that the abilities of the central nerve

system to activate and recruit motor units of the FDS and EDC muscles, including early recruitment and greater motor unit synchronisation, were significantly increased during maximal voluntary contractions associated to VE. According to McNair et al. ²³, VE may lead to a disengagement of the supraspinal inhibition, thus resulting in an enhancement of muscle strength. Moreover, our results corroborate with Lieber's explanation ⁴⁵ stating that the greater the number of motor units recruited through a stimulus, the greater the resultant muscle generated will also be. The iEMG data explain the highest performances of both MVF and MRFD but do not explain the higher increase of MRFD compared to MVF.

Moreover, the important electric activity of EDC during VE condition revealed a significant effect of the instruction on antagonist muscle during isometric contraction. One possible explanation for this result is that, in humans, the forearm extensor offers static stability of the elbow wrist joint. For example, the role of the extensor carpi ulnaris (similar to EDC) is to control or resist wrist flexion ⁴⁶. The extensor muscles also act to generate large and dynamic torque through a high rate of afferent or efferent activation. The primary functional role of the extensor muscle is to open the hand, preparing for manual manipulations during both finest control and power grip ⁴⁷. It has been demonstrated that the amplitude of EDC-EMG was much higher than the amplitude of FDS-EMG ⁴⁸.

Explosive strength during a fast contraction is determined not only by muscle contraction speed-related characteristics but also by neural factors, as classically inferred from the rate of EMG rise (i.e., the EMG counterpart of RFD), especially in the earlier time intervals of the contraction (i.e., < 100 ms) ^{49,50}. In support of this notion, previous studies proposed that iEMG changes during muscle contraction offer a

combined measure of the number of active fibres and their frequency of excitation ^{51,52}. These observations suggested that EMG data in response to VE might partly be due to the facilitation of the recruitment of large, fast motor units, which are expected to be recruited only at higher forces, increasing motor units firing rates, and a greater degree of motor units synchronization. Consequently, the current results corroborated with various pathological investigations ^{53,54} reporting that a high degree of motivation is an important factor to obtain higher EMG activity. These studies provided encouraging verbal feedback to help focus the patient's attention and increase motivation. Thus, our results associated to physical and sports activities as well as pathological studies suggest that the ability to produce higher EMG activity associated to force parameters depends on motivational factors (e.g. verbal encouragement) as well as the increase of muscle activation at the onset of the contraction.

The results of the present study highlight the global reproducibility during isometric exercise associated to VE. The coefficient of variation of EMG was higher in MRFD compared with MVF (Table 3) as observed in the previous studies⁵⁵.

From neuronal side, in a previous fMRI handgrip study ²⁶, verbal encouragement reinforced the activity of brain regions (e.g. primary motor cortex, dorsolateral prefrontal cortex, orbitofrontal cortex, superior temporal gyrus and lobule VI of cerebellum). Specifically, the anterior part of the right cerebellum lobule VI was activated by motor execution, while its posterior part was activated by VE. More specifically, VE increased constituted a closed connectivity between cerebral and cerebellar through the red nucleus and striatal network.

Notwithstanding, this study has some limitations. The main one was the restriction of our EMG analysis only to the motor execution stage. The EMG treatment focused only

on high EMG magnitudes and not on low magnitudes proper to the preparation phase. Further study is needed to develop EMG descriptors during preparation stage in order to understand the relationship between motor preparation and execution tasks during verbal encouragement for both agonist and antagonist muscles. It would be interesting to demonstrate how the complementarities of these force indices could allow to better understand temporally the underlying of neurophysiological and muscular mechanisms associated with cerebral activity. Based on the literature and on the somatotopic organization of the sensorimotor and motor cortex, it would be interesting to study the relationship between the neuromuscular parameters FMV, MRFD and EMG and the corticospinal neuronal transmission from motor cortex to the forearm muscles. This relationship could be measured by using electroencephalography based on the analysis of the cortico-muscular coherence of the beta bands during an isometric force contraction task.

Conclusion

Taking into account the VE condition on MVF, MRFD and EMG, the present study might provide the potential motivational basis of how the neuromuscular system and less intra-participant variability in MVF compared to MRFD and the decrease of intra-participant variability in MRFD. It seems reasonable to assume that VE improves the reproducibility of the different measured parameters. The constant encouragements effects associated to motivational system might provide the necessary process here. The good agreement between anatomical motivational and motor areas and their respective functional roles provide strong evidence for their mutual involvement in specific neural networks during VE. Finally, these results imply that it is important for researchers investigating the improvement of neuromuscular parameters and human motor

performance to focus on verbal encouragement, particularly for the exploration of explosive force production.

Titles of tables

Table 1: Force and rate of force development for each condition

Table 2: EMG activity for each condition for FDS and EDC

Table 3: Coefficient of variation of EMG_{MVF} and EMG_{MRFD} for each condition

Table 4: Standardized differences between instructions and percent chances that the true differences were higher/similar/lower

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Table 1: Force and rate of force development for each condition

						The state of the s
	nCM	nVE	VE	F(2,44)	η^2	P
MVF (kg)	26.74 ± 12.36	$28.98 \pm 12.45 \#$	32.28 ± 13.76 \$\dd{\}	44.65	0.67	< 0.001
MRFD (kg.s ⁻¹)	120.36 ± 75.09	$147.33 \pm 82.99 \#$	182.14 ± 110.65 \\$\\$\\$	28.04	0.56	< 0.001
MRFD% (.s ⁻¹)	439.77 ± 148.36	$495.41 \pm 144.56*$	542.32 ± 174.16 §	11.12	0.36	< 0.001
CV% MVF	6.61 ± 5.7	6.2 ± 3.25	5.74 ± 3.26	0.33	0.01	0.71
CV% MRFD	13.8 ± 7.1 A	$10.56 \pm 6.1 \text{A}$	10.5 ± 5.75 A	2.16	0.08	0.122

Verbal encouragement (VE), non verbal encouragement (nVE), non concentration and motivation (nCM)

^{* (}p < 0.05), # (p < 0.01), \S (p < 0.001) significantly higher compared with nCM

^{‡ (}p < 0.001) significantly higher compared with nVE

 $[\]Psi$ (p < 0.001) significantly higher compared with VE

^{‡ (}p < 0.001) significantly higher compared with VE

A (p < 0.001) significantly higher compared with CV% MVF

Table 2: EMG activity for each condition for FDS and EDC

		nCM	nVE	VE	F(2,44)	η^2	P
	FDS	30.41 ± 10.36	39.70 ± 15 §	$48.87 \pm$	27.645	0.56	< 0.001
EMC(uv)				17.93§‡		- 1	
$iEMG_{MVF}(\mu v)$	EDC	27.86 ± 8.63	32.78 ± 9.04 §	$39.15 \pm$	23.554	0.52	< 0.001
				12.81§‡	0	5	
	EDG	20.00 + 0.00	24.05 + 9.276	41.00	W1 022	0.66	< 0.001
'ENG ()	FDS	28.88 ± 8.80	34.05 ± 8.27 §	41.08 ±	41.933	0.66	< 0.001
$iEMG_{MRFD}(\mu v)$				10.93§‡			0.004
	EDC	29.87 ± 5.64	34.7 ± 7.38 §	42.03 ± 7.42 §‡	66.735	0.75	< 0.001

Verbal encouragement (VE), non verbal encouragement (nVE), non concentration and motivation (nCM)

^{§ (}p < 0.001) significantly higher compared with nCM

 $[\]ddagger$ (p < 0.001) significantly higher compared with nVE

Table 3: Coefficient of variation (CV%) of EMG_{MVF} and EMG_{MRFD} for each condition.

	CV%	nCM	nVE	VE	F(2.44)	η^2	P
EDC	EMG_{MVF}	19.88 ± 9.44	$12.39 \pm 12.52*$	11.77 ± 8.01*	4.826	0.18	0.013
FDS	EMG MRFD	23.06 ± 13.34	$14.23 \pm 9.35*$	$15.17 \pm 13.83*$	4.337	0.16	0.019
					6		
EDC	EMG_{MVF}	16.18 ± 7.27	$11.11 \pm 5.73 \#$	$8.40\pm4.67\S$	12.817	0.37	< 0.001
	EMG MRFD	$22.87 \pm 7.79 \text{ A}$	11.83 ± 8.82 §	10.43 ± 10.43 §	17.871	0.45	< 0.001

Verbal encouragement (VE), non verbal encouragement (nVE), non concentration and motivation (nCM)

A (p < 0.05) significantly higher compared with CV% MVF

Table 4: Standardized differences between instructions and rating higher/similar/lower

Variable	nVE compared with n	CM	VE compared with nCM		VE compared with nVE	
v al lable		V				
	ES (95% CI)	Rating	ES (95% CI)	Rating	ES (95% CI)	Rating
MVF	0.17 (0.13 to 0.21)	Trivial	0.42 (0.31 to 0.53)	Small	0.25 (0.16 to 0.35)	Small
MRFD (kg.s ⁻¹)	0.29 (0.18 to 0.40)	Small	0.67 (0.48 to 0.86)	Medium	0.38 (0.22 to 0.54)	Small
$MRFD\%(.s^{-1})$	0.35 (0.11 to 0.58)	Small	0.64 (0.31 to 0.98)	Medium	0.30 (0.05 to 0.54)	Small
$iEMG_{MVF}$	0.85 (0.49 to 1.22)	Large	1.79 (1.27 to 2.30)	Large	0.94 (0.63 to 1.24)	Large
iEMG _{MRFD}	0.79 (0.51 to 1.08)	Medium	1.94 (1.63 to 2.25)	Large	1.15 (0.87 to 1.43)	Large

^{* (}p < 0.05), # (p < 0.01), § (p < 0.001) significantly lower compared with nCM

 $[\]ddagger$ (p < 0.001) significantly higher compared with nVE

 $[\]Psi$ (p < 0.001) significantly higher compared with VE