An Acad Bras Cienc (2024) 96(1): e20220282 DOI 10.1590/0001-3765202320220282
Anais da Academia Brasileira de Ciências | *Annals of the Brazilian Academy of Sciences*
Printed ISSN 0001-3765 I Online ISSN 1678-2690
www.scielo.br/aabc | www.fb.com/aabcjournal

HEALTH SCIENCES

Neuromuscular electrical stimulation changes glucose, but not its variability in type 2 diabetes: a randomized clinical trial

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Abstract: Neuromuscular electrical stimulation (NMES) can be an alternative to conventional exercising. This randomized clinical trial evaluated the effect of NMES in type 2 diabetes patients. Twenty-eight individuals with type 2 diabetes were assigned to NMES (n=14) or NMES-placebo (n=14) applied to knee extensor muscles for 60 minutes. Glucose variability, microvascular function and endothelial function were evaluated through continuous glucose monitoring system, near infrared spectroscopy and flow-mediated dilatation, respectively. Glucose levels (mg/dl) decreased 2h (184 ± 11 *vs* 223 ±15), 3h (179 ± 12 *vs* 219 ±14) and 4h (177 ± 12 *vs* 212 ±12) after NMES, in comparison to NMES-placebo. No differences in glucose variability were found: coefficient of variation (%) at 0-6h (11.4±1.3 *vs* 11.4±1.2), 6-12h (9.8±1.0 *vs* 11.6±1.6), 12-18h (15.5±2.0 *vs* 11.4±2.1), 18-24h (12.8±2.3 *vs* 10.0±1.6); standard deviation (mg/dl) at 0-6h (21.6±2 *vs* 24.6±3.5), 6-12h (19.5±1.8 *vs* 20.3±2.8), 12-18h (29.9±3.5 *vs* 21.3±2.8),18-24h (22.8±4.1 *vs* 16.6±2.0) and mean amplitude of glycemic excursions (mg/dl) 54.9±25.0 *vs* 70.3±35.7. Endothelial and microvascular functions did not change. In conclusion, one acute NMES session was strong enough to trigger glucose reduction in individuals with type 2 DM, but it failed to induce any significant change in glucose variability, endothelial and microvascular functions.

Key words: Blood glucose, electrical stimulation therapy, glycemic control, physical therapy modalities.

INTRODUCTION

Exercising is one of the cornerstones of diabetes mellitus (DM) treatment, associated with greater metabolic control in type 2 diabetes patients (Umpierre et al. 2011). However, individuals with diabetes present reduced exercise tolerance. Barriers to exercise reported by patients include health issues and feeling that exercising is uncomfortable (Korkiakangas et al. 2009). In addition, deterioration of lean mass and muscle functions are also physiological parameters capable of predicting lower exercising capacity in diabetic patients (Guerrero et al. 2016).

Alternative exercise interventions, such as inspiratory muscle exercises, were capable of acutely reducing glucose levels (Schein et al. 2020) and after 8-week training (Pinto et al. 2020). Neuromuscular electrical stimulation (NMES) can also be an alternative method to conventional exercising, since it helps increase muscle strength, endurance, cardiorespiratory fitness, and quality of life (Gomes Neto et al. 2016). In individuals with diabetes, NMES application mitigated postsurgical muscle weakness and functional decline (Takino et al. 2022), reduced postprandial glucose levels (Miyamoto et al. 2015), increased insulin sensitivity (Joubert et

al. 2015) and glucose control (Crowe & Caulfield 2012). Moreover, a single bout of NMES has led to higher total body glucose uptake than voluntary ergometric exercising at identical effort levels (Hamada et al. 2004), and a 12-week intervention of lower limb NMES reduced the blood levels of glycated hemoglobin (HbA1c) in T2DM patients with post-stroke hemiplegia (Rubinowicz-Zasada et al. 2021).

Assessing glucose variability may add to the evaluation of glucose control (Danne et al. 2017). Glucose fluctuation amplitude, frequency and duration can be calculated based on data from continuous glucose monitoring (CGM) by reflecting intra- and inter day glycemic excursions, as well as acute events such as hypoglycemia and postprandial hyperglycemia (Monnier et al. 2018). Acute glucose variability has been linked to both microvascular and macrovascular complications, which can be influenced by exercising (Mitranun et al. 2014). A study has identified reduced glycemic variability after exercising interventions (Figueira et al. 2019).

Despite NMES improvement in glucose control, glucose variability was not previously evaluated. On the other hand, NMES effects on endothelial function present controversial results in the current literature. A single acute NMES session was capable of improving endothelial function in patients with heart failure (Tanaka et al. 2016), which was not observed by other study (Nicolodi et al. 2016). Moreover, near infrared spectroscopy (NIRS) was used to identify microvascular dysfunction in diabetic individuals, even before overt microangiopathy (Tagougui et al. 2015), but NMES effects were not explored.

The current study hypothesized that NMES can become a new method to help patients with diabetes to reduce glycemic levels and glucose variability, which, in its turn, could help improving patients' microvascular (evaluated through NIRS) and endothelial functions (evaluated though flow-mediated dilatation - FMD). The aim of this study was to investigate the effect of a single bout of NMES on glycemia, glucose variability, as well as microvascular and endothelial functions in type 2 diabetes individuals.

MATERIALS AND METHODS

Study design, setting and participants

The present randomized clinical trial was reported in compliance with the CONSORT Statement (Schulz et al. 2010). Individuals with type 2 diabetes were recruited from the endocrinology outpatient clinic at a tertiary teaching hospital in Brazil and through a website. All participants were at least 18 years old and presented HbA1c levels ranging from 7.5% to 10%. Exclusion criteria were: to be treated with insulin, pregnancy, varicose veins and having neuromusculoskeletal conditions capable of hindering the safe completion of the NMES interventions.

This study was in accordance with the Declaration of Helsinki and was approved by the Hospital de Clínicas de Porto Alegre Scientific and Research Ethics Committee (Certificate of Presentation for Ethical Appreciation 68437417.0.0000.5327) and registered at ClinicalTrials.gov (NCT03256747). Participants' selection, evaluation and intervention processes were performed from October 2018 to January 2020.

Data collection

The study protocol was conducted for four days, as follows:

- Day 1: Individuals were admitted to the laboratory at 08.00 AM, signed the informed consent and were subjected to baseline

evaluation comprising clinical examination, and blood collection for HbA1c and plasma glucose.

- Day 2: Individuals undergone autonomic neuropathy evaluation (Ewing's noninvasive cardiovascular reflex tests); the glucose sensor (CGM) was subcutaneously inserted.

- Day 3: Individuals were admitted to the laboratory at 08.00 AM, 30-min after eating a standardized breakfast meal (500 kcal; 60% carbohydrate, 30% fat, and 10% protein). They underwent allocated intervention (NMES or NMES-placebo); next, microvascular, and endothelial functions were evaluated.

- Day 4: Glucose sensor was removed.

Interventions

Neuromuscular electrical stimulation: NMES was applied with the aid of low frequency electrical stimulator *(Neurodyn II, IBRAMED)* attached to participants' knee extensor muscles for 60 minutes. NMES parameters comprised biphasic symmetric pulsed current stimuli, pulse frequency = 20Hz, pulse duration = 500μs, ON time = 10s, OFF time = 5s and rectangular wave. Self-adhesive hypoallergenic electrodes (130 x 75 mm) were bilaterally positioned at the motor point of the vastus lateralis and vastus medialis muscles (Gobbo et al. 2014). NMES intensity was individually adjusted at the maximum tolerated value in order to promote tetanic contraction of the knee extensor muscles, without leading to pain onset.

NMES – Placebo: duration, parameters and electrode positions were similar to the ones applied to the NMES group; however, current intensity was individually adjusted at sensorial level. Sensory stimulus was detectable by participants, but it was not enough to promote tetanic muscle contraction.

Outcome measurements

Interstitial glucose levels measured through CGM, 24h before (6-h timeframe) and 24h after (6-h timeframe) NMES application, were defined as the primary outcome. Secondary outcomes comprised (1) glucose variability, which was also evaluated through CGM: mean amplitude of glycemic excursions (MAGE), glucose coefficient of variation (CV%), and glucose standard deviation (SD); (2) microvascular function, which was evaluated through NIRS; and (3) flow-mediated dilation (FMD).

Laboratory analyses

HbA1c was analyzed through ion-exchange high-performance liquid chromatography *(HPLC, Merck-Hitachi L-9100 HbA1c analyzer; Merck, Darmstadt, Germany)*, whereas plasma glucose was analyzed based on the glucose oxidase method *(Sigma-Aldrich, St Louis, MO, USA).*

Autonomic neuropathy evaluation

Diabetes-associated cardiovascular autonomic neuropathy was assessed through five noninvasive cardiovascular reflex tests previously standardized (Neumann & Schmid 1997). Cardiovascular autonomic neuropathy was diagnosed in case of two or more, abnormal test results.

Glucose variability

Individuals were admitted in the laboratory at 04:00 p.m., ~30 hours before the NMES or NMES-placebo intervention, for CGM sensor placement *(Enlite Glucose Sensor, Medtronic MiniMed Inc., Northridge, CA, USA)*, and were monitored for four days, with glucose data obtained every 5 min. Glucose variability analyses comprised SD, CV% and MAGE (Hill et al. 2011). These indices, except for MAGE, were calculated within a 6-h timeframe (Fofonka et al. 2018, Schein et al. 2020). MAGE index was calculated 24h after

intervention based on differences between peak and nadir points higher than 1SD (Hill et al. 2011).

Blood pressure and heart rate

Blood pressure and heart rate were measured with noninvasive oscillometric device (Dinamap, Critkon, USA) during interventions. Measurements were performed every 5 minutes at rest, NMES or NMES-placebo application.

Microvascular function

Microvascular function was evaluated based on changes in oxy[hemoglobin+myoglobin] ([OHb]) through NIRS *(Oxymon, Artinis Medical Systems, Zetten, The Netherlands)* right after the interventions (NMES or NMES-placebo) (Mason Mcclatchey et al. 2017). NIRS generates light at 764 nm and 856 nm. The three-optode set was in the holder; inter-optode distance was 45 mm (tissue penetration depth was 22.5 mm). The holder was positioned on the distal portion of the vastus lateralis muscle, as well as on the belly of the lateral gastrocnemius muscle, and fixed with adhesive stickers to stop the optode from moving during evaluation. Differential pathlength factor values were 5.0 (vastus lateralis muscle) and 5.51 (gastrocnemius muscle); sampling frequency equal to 10Hz was used for NIRS data collection purposes. NIRS data were averaged to find values at 1-s intervals. After patients were left to rest for 3 minutes (basal data acquisition), the cuff was inflated at 50mmHg to suprasystolic levels for 5 minutes, in order to obstruct blood flow to leg muscles and to measure the maximal O_2 extraction capacity by skeletal myocytes. Next, it was deflated in order to measure reperfusion indices (Mclay et al. 2016): (1) desaturation rate during cuff occlusion (slope 1), which can be considered the indirect measure of skeletal muscle metabolic rate; (2) reperfusion rate, which was quantified as mean upslope after cuff release (slope 2); (3) reactive hyperemia, which was evaluated based on the area under the curve (AUC) calculated according to the cuff release time of 3-minutes after its occlusion; and (4) the lowest OHb value recorded during ischemia, which measures the magnitude of the ischemic insult (the stimulus to vasodilate); (5) recovery time (s), time interval between cuff release and the time when initial OHb values were reached (Rosenberry et al. 2018).

Endothelial function

Noninvasive measurements of endothelial function were taken through two-dimensional ultrasonography *(HD7XE: Phillips, USA)* based on the FMD technique (Thijssen et al. 2019). Individuals were advised to refrain from drinking caffeine and from smoking for at least 4 h before the examination. Arterial occlusion cuff was inflated to enable arterial occlusion in the distal hand. Next, it was deflated to allow vasodilation in response to reactive hyperemia in distal and proximal vascular beds. The FMD method comprises ultrasound arterial imaging taking under two conditions, namely: at rest (baseline) and during reactive hyperemia (after 5-minute arterial occlusion). FMD was herein expressed as relative variation in brachial diameter at hyperemic stage and defined as [(post-hyperemic diameter – baseline diameter) / baseline diameter] x 100.

Randomization and allocation concealment

Patients were randomly allocated to the NMES or NMES-placebo group at 1:1 ratio. The randomization sequence (7 blocks) was generated in the web randomization.com software and concealed in opaque numbered (1 to 28) envelopes, which were opened in numerical order just before the interventions had begun.

Statistical analyses and sample size

Variables were expressed as means ± SD or SE, medians and interquartile ranges or number and percentage. Data normality was assessed through Shapiro-Wilk test. The effect of both NMES or NMES-placebo on glucose and glucose variability were analyzed through generalized estimating equations (GEE), which were followed by Bonferroni's post-hoc test. Endothelial function parameters were compared based on covariance analysis (ANCOVA). Microvascular evaluation parameters were compared through Student's t test or Mann-Whitney test. Statistical significance was set at $p < 0.05$. All data were analyzed in Statistical Package for Social Sciences (SPSS- version 18.0) and GraphPad Prism software (version 8).

Sample size was estimated in 28 individuals, which enabled 10% dropout. This sample size allowed detecting 34 mg/dl difference in glucose levels between NMES and NMES-placebo interventions at standard deviation of 30 mg/dl, based on preliminary results obtained in our laboratory (Corrêa et al. 2015). Statistical power of 80% and 5% type I error were established. WinPepi software was used to calculate sample size.

RESULTS

From October 2018 to March 2020, 31 individuals were randomized, and 28 were included in data analysis (NMES=14 and NMES-placebo=14) (figure 1).

Table I shows participants' characteristics. They were predominantly women, age ranged from 42 to 83 years, and body mass index ranged from 21.9 to 44.6 Kg/m^2. No differences in fasting plasma glucose, HbA1c and diabetes

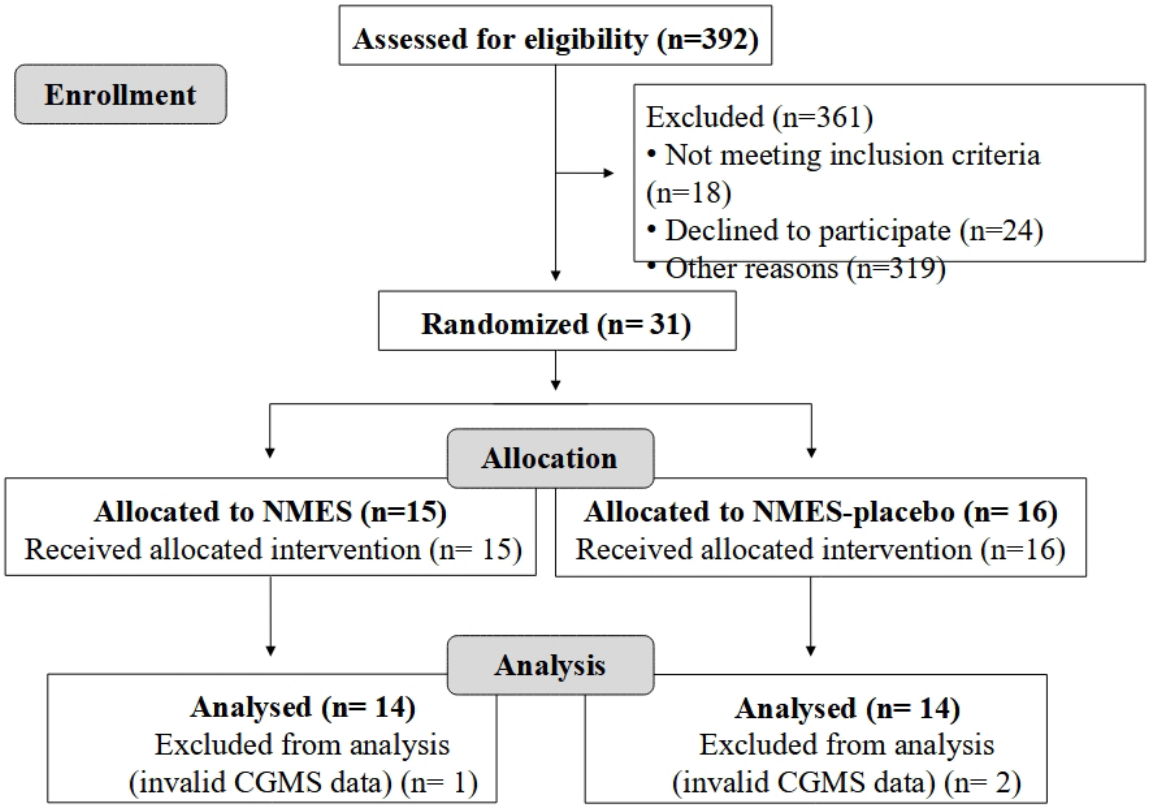

Figure 1. Flow diagram of patient's selection.

duration were found between groups. Less than half (35%) of patients reported practice mild exercising (walking or cycling 30 to 45 minutes, twice a week).

Figure 2 shows interstitial glucose levels 60 min before breakfast, 30 min during breakfast, 60 min during the intervention, and 300 min (6h) after the intervention. Glucose levels decreased 2h (184 ± 11 mg/dl *vs* 223 ±15 mg/dl; p=0.034), 3h (179 ± 12 mg/dl *vs* 219 ±14 mg/dl; p=0.031) and 4h (177 ± 12 mg/dl *vs* 212 ±12 mg/dl; p=0.035) after the intervention (NMES), in comparison to that of NMES-placebo. Glucose AUC did not differ between NMES and NMES-placebo from 0 to 6h after interventions (67328 ± 4109 mg/dl x h *vs* 74933 ± 4105mg/dl x h; p= 0.058).

Glycemic variability evaluated through mean glucose, SD, and CV is shown in Table II. Before interventions the mean glucose was approximately 18 md/dl and 30 md/dl higher in the NMES group at 6-12h and 18-24h in in relation to 0-6h, respectively. After interventions mean glucose was approximately 35 mg/dl higher in the NMES-placebo group at 6-12h and at 18-24h in relation to 0-6h, and was higher at 0-6h after *vs* before interventions in the NMES-placebo group. Mean glucose was 10 mg/dl higher at 6-12h in the NMES group in relation to 0-6h after

Table I. Type 2 diabetes features of the included participants.

	NMES-Placebo (n=14)	NMES (n=14)	p
Male sex	8 (57)	4 (28)	0.127
Age (*years)*	63 ± 10	62 ± 10	0.836
BMI (kg/m^2)	30.8 ± 6.3	28.7 ± 3.7	0.242
HbA1c (%)	7.8 ± 0.5	7.8 ± 0.8	1.0
Fasting plasma glucose *(mg/dL)*	183 ± 40	172 ± 38	0.474
Diabetes duration (*years)*	10.3 (6.8 - 13.8)	12.2 (8.3 - 16.0)	0.430
SBP (mmHg)	120 ± 15	115 ± 21	0.896
DBP (mmHg)	73 ± 9	68 ± 8	0.133
HR (bpm)	70 ± 12	72 ± 14	0.816
Family history of diabetes	10 (71)	12 (86)	0.357
Autonomic neuropathy	6 (43)	6 (43)	1.0
Hypertension	11 (79)	12 (86)	0.622
Dyslipidemia	8 (57)	10 (71)	0.430
Smoker	1 (7)	1 (7)	1.0
Regular exercise	6 (43)	4 (29)	0.430
Medications			
Metformin	9 (64)	8 (57)	0.699
Sulfonylureas	10 (71)	9 (64)	0.686
ACE inhibitors	6 (43)	6 (43)	1.0
Statins	8 (57)	8 (57)	1.0

BMI: body mass index; HbA1c: glycated hemoglobin; SBP: systolic blood pressure; DBP: diastolic blood pressure; HR: heart rate. Continuous variables are expressed as mean ± standard deviation or median [interquartile range (p25-p75)]. Categorical variables are expressed as n (%). Comparisons were tested through Pearson's χ2 test, Student's t test or Mann–Whitney test.

interventions. After interventions, mean glucose at 6-12h was approximately 20 mg/dl higher in NMES compared to NMES-placebo. Both SD and CV at 18-24h were reduced within NMES-placebo group before *vs* after interventions. SD at 6-12h and at 12-18h was approximately 8 mg/dl lower after *vs* before interventions in the NMES group. CV at 6-12h was 5% lower after *vs* before interventions, and at 12-18h was 5% higher after *vs* before interventions in the NMES group. Analysis based on diary records registered every 6h was performed to separate the effects of meals and NMES intervention on glucose variations. There were no differences in macronutrient intake between groups 24h after interventions (Supplementary Material - Table SI). MAGE also did not show difference between NMES and NMES-placebo groups (54.9 ± 25.0 mg/dl *vs* 70.3 ± 35.7 mg/dl, p= 0.201).

Microvascular and endothelial functions were evaluated right after, and 15 minutes after the interventions, respectively (Table III). Microvascular function evaluated through NIRS, in both muscles, vastus lateralis and gastrocnemius, did not show differences in desaturation (slope 1) and reperfusion rates (slope 2), reactive hyperemia AUC, magnitude of ischemic insult (lowest OHb) and recovery time between NMES and NMES-placebo groups. Endothelial function evaluated through FMD did not show difference in absolute brachial artery diameter at baseline and at reactive hyperemia, neither between groups.

Systolic arterial pressure (p=0.057) and heart rate (p=0.193) did not show differences between groups in all moments (basal or intervention's time) (Supplementary Material - Figure S1).

DISCUSSION

To the best of our knowledge, this is the first randomized clinical trial focused on assessing the effect of acute NMES on glucose levels and glucose variability in patients with type 2 diabetes. Acute 60-minute session of low frequency NMES with maximal tolerable intensity was capable of reducing glucose levels, but it did not reduce glucose variability or improved microvascular and/or endothelial function in comparison to placebo intensity.

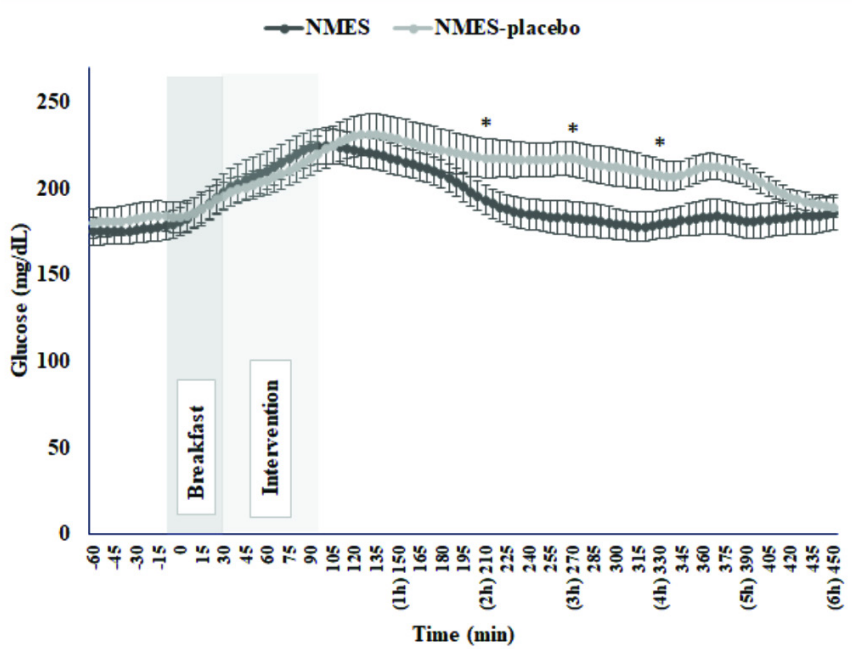

Figure 2. Interstitial glucose (continuous glucose monitoring, sampled every 5min) at baseline (fasting, -60min), before (breakfast 0-30min), during (30-90min) and after intervention (90-450min of recovery) (panel A). Data were presented as means and SE; *p<0.05 NMES *vs* NMES-placebo.

The present study adopted an alternative exercising method, and found interstitial glucose reduction 2, 3 and 4 hours after NMES; there was no detectable change after NMES-placebo application. Miyamoto et al. (2012) have also reported lower glucose levels after acute NMES session (30 minutes) at 1, 1.5 and 2 hours after meal intake in non-randomized crossover trial conducted with 11 patients with type 2 diabetes (Miyamoto et al. 2012). In addition, Jabbour et al. (2015) in a crossover trial have observed that glucose decrease 120 minutes after acute NMES session (60 minutes) was higher than that observed for the control session (Jabbour et al. 2015). Moreover, NMES increase energy expenditure and whole-body carbohydrate oxidation (Chen et al. 2021). An acute aerobic exercise session reduces glucose levels in individuals with diabetes in a way related to increased plasma membrane content of GLUT4 and glucose uptake (Ferrari et al. 2019), and since glucose reduction was only observed in the NMES group, it is possible that this effect is associated with glucose uptake induced by muscle contraction.

We evaluated short-term glucose variability by CGM to assess the effect of acute NMES on glucose control, since glucose fluctuations, including hypoglycemia or hyperglycemia, can be evaluated (Azhar et al. 2020). Lower glucose levels were observed after exercise performed in the fasting state or after lunch (Eshghi et al. 2017), and a single bout of exercise reduced the prevalence of hyperglycemia over the subsequent 24-h period (Van Dijk et al. 2013). In this way, we intent to evaluate if acute NMES could induce better glycemic control, however, no differences in glycemic variability were found between groups.

Although a few individuals reported practice mild exercise, it is unlikely that some adaptation to physical exercise affect the musculoskeletal response and, consequently, the effect of the study intervention, because the individuals are equally distributed in both groups. Previously, non-conventional analysis applied to glucose variability by our research group had shown reduced glucose variability after a single aerobic + resistance exercising session (Figueira et al. 2013), whereas another study focused on applying

Table II. Glucose variability responses before and after interventions (NMES or NMES-placebo).

	NMES – placebo (n=14)				NMES (n=14)			
Before interventions	**0-6h**	**6-12h**	**12-18h**	**18-24h**	**0-6h**	**6-12h**	**12-18h**	**18-24h**
Mean glucose (mg/dL)	182 ± 15	190 ± 10	184 ± 14	192 ± 16	174 ± 11	192 ± 12$^{\#}$	204 ± 11	203 ± 11$^{\#}$
CV (%)	11.9 ± 1.8	11.4 ± 1.3	9.5 ± 1.0	15.6 ± 3.0	13.4 ± 2.0	14.8 ± 1.9	10.7 ± 1.5	10.7 ± 1.5
SD (mg/dL)	20.7 ± 2.8	20.9 ± 2.0	17.0 ± 1.9	29.2 ± 5.3	21.9 ± 2.8	27.3 ± 3.0	21.8 ± 2.4	21.8 ± 3.5
After interventions	0-6h	6-12h	12-18h	18-24h	0-6h	6-12h	12-18h	18-24h
Mean glucose (mg/dL)	213 ± 12 $^{¥}$	178 ± 7$^{\#}$	187 ± 14	178 ± 11$^{\#}$	192 ± 12	205 ± 11$^{\#*}$	200 ± 13	184 ± 12
CV (%)	11.4 ± 1.3	11.6 ± 1.6	11.4 ± 2.1	10.0 ± 1.^{6}¥	11.4 ± 1.2	9.8 ± 1.0$^{¥}$	15.5 ± 2.0$^{¥}$	12.8 ± 2.3
SD (mg/dL)	24.6 ± 3.5	20.3 ± 2.8	21.3 ± 4.2	16.6 ± 2.^{0}¥	21.6 ± 2.1	19.5 ± 1.8$^{¥}$	29.9 ± 3.5$^{¥}$	22.8 ± 4.1

Data (CGM) are presented as estimated mean ± SE calculated through GEE analysis; CV: coefficient of variation; SD: standard deviation. P interaction (group*day*time) to mean glucose, CV and SD were p=0.009, p=0.030 and p=0.034, respectively. $^{\#}$p<0.05 within group between 0-6h *vs* other 6h time frames in the same day (before or after interventions); $^{¥}$p<0.05 within group between before *vs* after interventions; * p<0.001 between NMES *vs* NMES-placebo.

conventional analysis to evaluate glucose variability has found that CV and SD decreased 12h and 18h after the inspiratory muscle exercise, although no difference was observed for MAGE (Schein et al. 2020). NMES was not capable of changing glucose variability. We carefully checked the number of macronutrients ingested by participants 24 hours after the interventions within 6h timeframes, which were evaluated in equal periods of glycemic variability, and no difference in the number of macronutrients was identified between groups. In addition, all participants were taking antidiabetic agents before the interventions had begun. Thus, it is possible to suggest two hypotheses: first, NMES was not capable of reducing glucose variability due to insufficient stimulus intensity, unlike what was observed for conventional aerobic or resistance + aerobic exercising (Figueira et al. 2013); second, since participants presented maximal CV of 15%, and unstable glucose levels were defined as CV ≥36% (Monnier et al. 2017), glucose level stability do not allow changes to take place.

Microvascular and endothelial function did not show differences between groups. Microvascular function evaluated based on post occlusive reactive hyperemia can show changes at microcirculatory level (Horiuchi & Okita 2020). The current study did not find differences in desaturation rate (slope 1) and in minimum OHb value among groups during the ischemic stage (occlusion). Since slope 1 was considered the indirect muscle oxidative metabolism parameter, the current results have suggested that muscle metabolic rate during the ischemic stage was not

Table III. Microvascular and endothelial responses after the intervention (NMES) or placebo (NMES-placebo).

Microvascular responses						
	Vastus lateralis			**Gastrocnemius**		
	NMES-placebo (n=10)	**NMES (n=12)**	**p**	**NMES-placebo (n=12)**	**NMES (n=12)**	**p**
OHb Slope 1(µmol/100ml)	-0.55 ± 0.2	-0.50 ± 0.1	0.429	-0.044 ± 0.02	-0.043 ± 0.012	0.871
OHb Slope2 (µmol/100ml)	1.1 (1.0 - 1.2)	0.9 (0.7 - 1.5)	0.453	0.85 ± 0.4	0.89 ± 0.3	0.778
Total AUC (mg/dL.h)	583.9 (304.2 - 780.9)	741.0 (555.6 - 1275.5)	0.184	768.4 ± 380.7	756.0 ± 153.1	0.917
Lowest OHb (µmol/100ml)	-16.9 ± 3.1	-17.1 ± 5.0	0.907	7.5 ± 2.4	6.7 ± 2.5	0.786
Time of recovery (s)	20 (16 – 29)	25 (16.5 – 91)	0.384	18.5 ± 8.9	15.6 ± 6.9	0.564
Endothelial responses						
	Brachial Artery					
	NMES-placebo (n=14)	NMES (n=14)	p			
Baseline diameter (mm)	0.305 ± 0.01	0.314 ± 0.020	0.686			
Peak diameter (mm)	0.334 ± 0.004	0.322 ± 0.004	0.061			
FMD% (%)	7.65 ± 1.5	4.34 ± 1.5	0.132			

NMES: neuromuscular electrical stimulation; Total AUC: area under the curve of 3 minutes post occlusion reactive hyperemia; OHb; Oxyhemoglobin + myoglobin; FMD: flow-mediated dilation. Variables are expressed as mean ± standard deviation or median [interquartile range (p25-p75)]. Microvascular responses were tested by Student t test or Mann–Whitney test. Peak diameter and FMD% were evaluated at the following basal values and were compared by ANCOVA. Baseline diameter data were compared by Student's test t.

different among intervention groups (Horiuchi & Okita 2020). Similarly, no differences between interventions were found during the reperfusion (slope 2) and hyperemic stages (post-occlusion). Slope 2 represented the reoxygenation rate and AUC was considered the longest response to shear stress, i.e., persistent vasodilation (Soares & Murias 2018). Thus, NMES was not capable of improving arterial inflow and/or vasodilation at microvasculature after the intervention. The current results disagree with results from a previous study, according to which, NMES was capable of inducing vasodilatation in the lower limbs (Huang et al. 2019). Microvascular function was assessed through NIRS in both studies; however, Huang's study (Huang et al. 2019) has included healthy individuals and did not use post-occlusive reactive hyperemia, whereas the current study included patients with diabetes and observed similar changes in NMES and NMES-placebo groups during and after occlusive reactive hyperemia. The adopted technique and selected populations likely explain differences observed between studies. To the best of our knowledge, the literature in this field lacks studies about microvascular effects induced by NMES in the diabetic population evaluated through NMES, based on the post-occlusive reactive hyperemia technique.

Endothelial function was evaluated through FMD and did not show brachial artery dilatation or FMD after NMES or NMES-placebo application, suggesting that acute NMES at pulse frequency equal to 20Hz did not produce enough stimuli to modulate or change the vascular endothelial function of patients with diabetes. Tanaka et al (2016), using pulse frequency equal to 50Hz, had previously shown that a single NMES session was capable of enhancing vascular endothelial function in patients with acute myocardial infarction (Tanaka et al. 2016). However, Nicolodi et al. (2016), using pulse frequency equal to 20Hz, did not find changes in endothelial function after a single NMES application session in patients with heart failure (Nicolodi et al. 2016). In addition to different participants' conditions, NMES pulse frequency and endothelial function evaluation method can explain differences observed between studies. Tonometry assessed by Endo-PAT 2000 was the method used in Tanaka's study (Tanaka et al. 2016), whereas the current study, and the one conducted by Nicolodi et al (Nicolodi et al. 2016), have assessed endothelial function through ultrasonography. Therefore, it is possible that identifying improvements in induced endothelial NMES demands 50HZ pulse frequency to cause tetanic contraction of type II muscle fibers.

Some limitations of the present study comprises the small sample size; lack of nutrient intake standardization, although patients were instructed to maintain their usual diet; antidiabetic agents used by participants were taken at different times of the day, which may have affected glycemic variability. There is low sample homogeneity in the parameters age and BMI, but in both groups, minimizing the risk of bias. Furthermore CGMS method assesses interstitial, not plasma glucose.

Accordingly, one acute NMES session was capable of inducing glucose reduction in individuals with type 2 diabetes, but it failed to induce improvements in glucose variability, and in endothelial and microvascular functions. Glucose reduction through exercising is a cornerstone in diabetes treatment, so the current results encourage further long-term studies to be carried out with NMES-training diabetic populations.

Acknowledgments

This study was supported by Fundo Institucional de Apoio à Pesquisa do Hospital de Clínicas de Porto Alegre (FIPE), grant number 20170272 and Coordenação de Aperfeiçoamento de Pessoal de Nível Superior, Brazil

(CAPES), Finance Code 001. The experiments comply with the current laws of the country in which they were performed. The authors have no conflict of interest to declare. The datasets generated and analyzed during the current study are not publicly available, but are available from the corresponding author.

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SUPPLEMENTRY MATERIAL

Figure S1.
Table SI.

How to cite
MACEDO ACP, BOCK PM, SAFFI MAL, MADALOSSO MM, DAL LAGO P, CASALI KR & SHAAN BD. 2023. Neuromuscular electrical stimulation changes glucose, but not its variability in type 2 diabetes: a randomized clinical trial. An Acad Bras Cienc 96: e20220282. DOI 10.1590/0001-3765202320220282.

Manuscript received on March 25, 2022;
accepted for publication on June 9, 2023

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