UNIVERSIDADE FEDERAL DO RIO GRANDE DO SUL
ESCOLA DE EDUCAÇÃO FÍSICA, FISIOTERAPIA E DANÇA
Programa de Pós-Graduação em Ciências do Movimento Humano
Tese de Doutorado

Matias Fröhlich

Neuromuscular Electrical Stimulation: Current Parameters and Reproducibility of Measures for Evaluation and Rehabilitation of Critically Ill Patients in Intensive Care Units

Estimulação Elétrica Neuromuscular: Parâmetros de Corrente e Reprodutibilidade de Medidas para Avaliação e Reabilitação de Pacientes Críticos em Unidades de Terapia Intensiva

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ESTIMULAÇÃO ELÉTRICA NEUROMUSCULAR: PARÂMETROS DE CORRENTE E REPRODUTIBILIDADE DE MEDIDAS PARA AVALIAÇÃO E REABILITAÇÃO DE PACIENTES CRÍTICOS EM UNIDADES DE TERAPIA INTENSIVA

RESUMO

A presente tese teve por objetivosdeterminar os parâmetros de corrente elétrica, a variabilidade da frequência cardíaca (VFC) e a confiabilidade de medidas da morfologia e da função muscularesem sujeitos saudáveis submetidos a protocolo de estimulação elétrica neuromuscular (EENM)em ambiente de unidade de tratamento intensivo (UTI) simulada. Ela está dividida em três capítulos que são descritos a seguir. **CAPÍTULO 1:** ESTIMULAÇÃO ELÉTRICA NEUROMUSCULAR: EFEITOS DE DIFERENTES DURAÇÕES DE PULSO SOBRE O DESCONFORTO E FADIGA EM SUJEITOS JOVENS SAUDÁVEIS.*Introdução*: O objetivo do presente estudo foi avaliar os efeitos de diferentes durações de pulso e de diferentes tempos de contração/relaxamento da EENM sobre a intensidade de corrente, produção de torque, desconforto e fadiga muscular. *Métodos*: 24 jovens saudáveis (12 ♂ e 12 ♀, 23,8±3,4 anos) foram submetidos a quatro protocolos de EENM (10 min de duração, duração de pulso = 1,2 e 2ms; tempos de contração/relaxamento = 5/25s e 10/50 s, respectivamente). Antes e após os protocolos de EENM, os sujeitos realizaram contrações voluntárias máximas isométricas (CVMIs) dos extensores de joelho, seguidas de um teste supramáximo de torque evocado (PS_{TE}) afim de avaliar a fadiga. A intensidade de corrente (IC), o torque evocado ($EENM_{TE}$) e o desconforto produzido (EVA) foram registrados no início e no final dos protocolos de EENM. *Resultados*: Menores níveis de IC foram necessários durante os protocolos com 2 ms de duração de pulso (efeito observado para duração do pulso: p=0,001). O desconforto relatado pelos sujeitos diminuiu durante os protocolos (efeito observado para momento: p<0,0001) e não foi diferente entre os quatro protocolos de EENM (efeito observado para: duração do pulso, p=0,203; tempos de contração/relaxamento, p=0,679). O PS_{TE} foi significativamente maior para os protocolos com duração de pulso de 2 ms (efeito observado para duração de pulso: p=0,044). *Conclusão*: Embora não tenha havido diferença entre a sobrecarga mecânica produzida durante os protocolos de EENM realizados na máxima IC tolerada, uma duração maior do pulso permite o uso de uma menor IC. **CAPÍTULO 2:** EFEITO DO TEMPO DE CONTRAÇÃO/RELAXAMENTO DA ESTIMULAÇÃO ELÉTRICA NEUROMUSCULAR SOBRE A VARIABILIDADE DA FREQUÊNCIA CARDÍACA EM SUJEITOS SAUDÁVEIS EM UTI SIMULADA.*Introdução*: O presente estudo teve como objetivo avaliar o efeito de dois protocolos de EENM sub-máximos, com diferentes tempos de contração/relaxamento, sobre a VFC. *Métodos*: 24 sujeitos saudáveis (12 ♂ e 12 ♀, 26,6±4,8 anos) foram submetidos a dois protocolos de EENM (20 minutos de duração) com tempos de contração/relaxamento de 5/25 s ($EENM_{5/25}$) e de 1/5 s ($EENM_{1/5}$). Um monitor cardíaco digital foi colocado na região do tórax e cada sujeito descansou por 10 minutos. A VFC foi avaliada por meio da análise de períodos de 5 min obtidos da segunda metade do repouso (10 min) e da segunda metade dos protocolos de EENM (20 min). Para a análise na VFC no domínio do tempo, calculou-se a média da frequência cardíaca (MFC) e a média dos intervalos RR (MRR). Para a análise no domínio de frequência, os componentes de baixa frequência (BF) e alta frequência (AF) do espectro de potência,seguidos de sua razão (BF/AF), foram calculados.

Resultados: A MFC aumentou, enquanto, concomitantemente, a MRR reduziu (efeito observado para momento: p=0,049 e p<0,0001, respectivamente). Houve um aumento do componente de BF durante ambos os protocolos (efeito observado para momento: p=0,001), mas o aumento do componente de BF foi maior durante a $EENM_{5/25}$ (tempos de contração/relaxamento: p<0,0001). Concomitantemente, o componente de AF apresentou diminuição tanto para a $EENM_{5/25}$ quanto para a $EENM_{1/5}$, mas observou-se maior decréscimo para a $EENM_{5/25}$ (efeito observado: momento, p=0,001; tempos de contração/relaxamento, p<0,0001). Consequentemente, a razão BF/AF aumentou tanto em $NMES_{5/25}$ quanto em $EENM_{1/5}$, mas um aumento maior foi encontrado para $EENM_{5/25}$ (efeito observado: momento, p=0,008; tempos de contração/relaxamento, p=0,001). *Conclusão*: O aumento do tempo de contração/relaxamento da EENM de 1/5 s para 5/25 s aumentou a razão BF/AF, sugerindo um aumento na atividade simpática em indivíduos saudáveis. **CAPÍTULO 3:** CONFIABILIDADE DE MEDIDAS DA MORFOLOGIA MUSCULAR E DA FUNÇÃO MUSCULAR EM UM AMBIENTE DE UTI SIMULADA.*Introdução*: O objetivo do presente estudo foi o de avaliar a confiabilidade intra-avaliador, inter-avaliador e inter-analisador de medidas da morfologia do músculo quadríceps,obtidas por meio de ultrassonografia,e das medidas de força voluntária e evocada dos extensores de joelho em uma UTI simulada. *Métodos*: 32 sujeitos saudáveis (16 ♂ e 16 ♀; 26,6±4,9 anos) foram submetidos a doisdias de avaliações,separados por uma semana. Em um dos dias, os participantes foram avaliados por um único avaliador, que repetiu a avaliação no dia de testes seguinte, juntamente com outros dois avaliadores. A morfologia muscular foi avaliada por meio de ultrassonografia para obtenção da área de secção transversa do músculo reto femoral (RF_{AST}), espessura muscular do quadríceps femoral (Q_{EM}), reto femoral (RF_{EM}), vasto intermédio (VI_{EM}), vasto medial (VM_{EM}) e vasto lateral (VL_{EM}), o comprimento das fibras musculares do vasto lateral (VL_{CF}) e seu respectivo ângulo de penação (VL_{AP}). Em seguida, a força foi avaliada por meio de contrações voluntárias máximas isométricas (CVMIs) e pela força evocada (FE). Para a determinação da confiabilidade, foram calculados o coeficiente de correlação intra-classe (CCI), erro padrão de medida (EPM) e mínima mudança detectável (MMD). *Resultados*: As comparações intra-avaliador mostraram um alto valor de CCI (>0,90). Nas comparações inter-avaliadores, os parâmetros Q_{MT}, VL_{MT}, VM_{MT}, RF_{MT} e VI_{MT} apresentaram CCIs elevados (0,87 a 0,96), enquanto os parâmetros VL_{AP} e VL_{CF} apresentaram um CCI moderadamente elevados (0,66 e 0,69, respectivamente). Para as comparações entre os analisadores das medidas de RF_{AST}, o ICC foi alto (0,98). Para as comparações intra-avaliador das avaliações de força, os CCIs foram altos tanto para CVMI quanto para FE (0,91 e 0,94, respectivamente). Nas comparações inter-avaliadores das medidas de força, os CCIs foram menores do que aqueles para as comparações intra-avaliador, mas ainda considerados altos tanto para a CVMI quanto para a FE (0,89 e 0,86, respectivamente). *Conclusão*: As medidas ultrassonográficas são precisas na avaliação da morfologia muscular, quando realizadas pelo mesmo avaliador em diferentes momentos. Foi alta a confiabilidade inter-avaliadores e inter-analistas encontrada para a RF_{AST} e medidas de espessura muscular quando realizadas por diferentes avaliadores e analistas. A confiabilidade inter-avaliadormoderadamente alta encontrada para VL_{AP} e VL_{CF} sugere que esses parâmetros são avaliador-dependentes. A confiabilidade das medidas de força foi alta tanto para a CVMI quanto para a FE.

Palavras-chaves: Estimulação elétrica neuromuscular, fadiga muscular, variabilidade da frequência cardíaca, reprodutibilidade.

NEUROMUSCULAR ELECTRICAL STIMULATION: CURRENT PARAMETERS AND MEASURES REPRODUCIBILITY FOR EVALUATION AND REHABILITATION OF CRITICALLY ILL PATIENTS IN INTENSIVE CARE UNITS.

ABSTRACT

The aim of the present thesis was to determine electrical current parameters, heart rate variability (HRV), and reliability of measures of muscular morphology and function in healthy subjects submitted to a neuromuscular electrical stimulation (NMES) protocol in a simulated intensive care unit (ICU). It is divided into three chapters, which are described below. **CHAPTER 1:** NEUROMUSCULAR ELECTRICAL STIMULATION: EFFECTS OF DIFFERENT PULSE DURATIONS ON DISCOMFORT AND FATIGUE IN HEALTHY YOUNG SUBJECTS.*Introduction*: The purpose of the present study was to evaluate the effects of different NMES pulse durations and of different NMES contraction/relaxation (ON/OFF) times on current intensity, torque production, discomfort and muscle fatigue. *Methods*: 24 healthy (12 ♂ and 12 ♀, 23.8±3.4 years) were submitted to four 10-min duration NMES protocols with distinct electric current parameters (pulse duration = 1.2 and 2 ms; ON/OFF times = 5s/25s and 10s/50s). Subjects performed maximum voluntary isometric contractions (MVICs) followed by a supramaximal evoked torque test (SP_{ET}), before and after the NMES protocol for fatigue evaluation.The current intensity (CI) used, torque ($NMES_{ET}$) and discomfort produced (by the Visual Analogue Scale - VAS) were registered at the beginning and at the end of the NMES protocols. *Results*: Lower CIs were necessary during longer pulse duration (2 ms) NMES protocols (observed effect for pulse-duration: p=0.001). Discomfort reported was no different between NMES protocols (observed effect: pulse-duration, p=0.203; ON/OFF times, p=0.679). $NMES_{ET}$ (observed effect moment: p<0.0001) and $\Delta NMES_{ET}$ (observed effect: pulse-duration, p=0.261; ON/OFF times, p=0.303) were similar between NMES protocols. No difference was found on ΔMVIC between NMES protocols (observed effect: pulse-duration, p=0.261: ON/OFF times, p=0.303). SP_{ET} was significantly greater for 2 ms pulse duration NMES protocols (observed effect for pulse-duration: p=0.044). *Conclusion*: Although there was no difference between the mechanical overload produced during NMES protocols performed at maximal tolerated CI, a longer pulse duration allows for the use of a lower CI. **CHAPTER 2:** EFFECT OF NEUROMUSCULAR ELECTRICAL STIMULATION CONTRACTION/RELAXATION TIMES ON HEART RATE VARIABILITY IN HEALTHY SUBJECTS: A SIMULATED ICU SETUP. *Introduction*: The present study aimed to evaluate the effect of two submaximal NMES protocols with contraction/relaxation times (ON/OFF times) of 5/25 s and 1/5 s, respectively, over the heart rate variability (HRV). *Methods*: 24 healthy (12 ♂ and 12 ♀, 26.6±4.8 years) were submitted to two 20-minutes NMES protocols with ON/OFF time of 5/25 s ($NMES_{5/25}$) and 1/5 s ($NMES_{1/5}$). A digital heart rate monitor was placed in the chest region and each subject rested for 10 minutes. HRV was assessed and recorded throughout the NMES protocols, and analyzed through 5-min periods obtained at the second half of the 10-min rest and at the second half of 20-min NMES protocols. For HRV time domain analysis, mean heart rate (MHR) and mean RR intervals (MRR) were calculated. For HRV frequency domain analysis, low frequency (LF) and high frequency (HF) power spectralcomponents, followed by their ratio (LF/HF), were calculated. *Results*: MHR was increased (moment: $p=0.049$), while concomitantly, MRR was reduced (moment: $p<0.0001$) during the NMES protocolos. There was an increase in LF power component during both NMES$_{5/25}$ and NMES$_{1/5}$ (observed effect for moment: $p=0.001$) with a greater increase on LF (ON/OFF times: $p<0.0001$). Concomitantly, the HF component showed a higher decrease for NMES$_{5/25}$ (observed effect: moment, $p=0.001$; ON/OFF times, $p<0.0001$). LF/HF increased for both NMES$_{5/25}$ and NMES$_{1/5}$, but a much larger increase was found for NMES$_{5/25}$ (observed effect: moment, $p=0.008$; ON/OFF times, $p=0.001$). *Conclusion*: The increase in NMES contraction/relaxation times from 1s/5s to 5s/25s largely increased LF/HF ratio, suggesting an increase on sympathetic activity in healthy subjects. **CHAPTER 3:**RELIABILITY OF ULTRASSOUND ACQUIRED MUSCLE MORPHOLOGY AND MUSCLE FUNCTION IN A SIMULATED ICU SETTING.*Introduction*: The goal of the present study was to evaluate the intra-rater, inter-rater and inter-analyst reliability of ultrasonography muscle morphology measurements, and of voluntary and evoked knee extensor torque measurements in a simulated ICU. *Methods*: 32 healthy participants (16 ♂ and 16 ♀; 26.6±4.9 years)were submitted to two batteries of evaluations, performed on two separate days, separated by a week. In one of the testing days, subjects were evaluated by a single experienced rater, who repeated the evaluation on the next testing day along with two other raters. Muscle morphology was evaluated by means of ultrasonography in order to obtain the rectus femoris muscle cross-sectional area (RF$_{CSA}$), the muscle thickness of the quadriceps femoris (Q$_{MT}$), rectus femoris (RF$_{MT}$), vastus intermedius (VI$_{MT}$), vastus medialis (VM$_{MT}$), and vastus lateralis (VL$_{MT}$), the fascicle length of vastus lateralis muscle fibers (VL$_{FL}$) and its respective pennation angle (VL$_{PA}$). Then, the maximum torque produced by means of both maximal voluntary isometric contractions (MVICs) and by supramaximal doublet-pulse evoked force (EF) were evaluated. *Results*: Intra-rater comparisons showed a high intra-class correlation coefficient (ICC) value (>0.90). In the inter-rater comparisons, the parameters Q$_{MT}$, VL$_{MT}$, VM$_{MT}$, RF$_{MT}$ and VI$_{MT}$ showed high ICCs (0.87 to 0.96), while the VL$_{PA}$ and VL$_{FL}$ parameters presented moderately high ICCs (0.66 and 0.69, respectively). For inter-analyst comparisons of RF$_{CSA}$ measurements, ICC was high (0.98). For force evaluations, intra-rater comparisons ICCs were high for both MVIC and EF (0.91 and 0.94, respectively). In the inter-rater comparisons of force measures, ICCs were high for both MVIC and EF (0.89 and 0.86, respectively). *Conclusion*: Ultrassound measurements are accurate in the muscle morphology evaluation, when performed by the same rater at different moments. Inter-rater and inter-analyst reliability was high for RF$_{CSA}$ and muscle thickness measures when performed both by different evaluators and analysts. The moderately high reliability found for VL$_{AP}$ and VL$_{FL}$ parameters in the inter-rater comparisons suggests that these parameters are evaluator-dependent. The reliability of force measures was high for both MVIC and EF.

Key-words: Neuromuscular electrical stimulation, fatigue, heart rate variability, reliability.

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LIST OF ABBREVIATIONS AND ACRONYMS

Chapter 1:

CI: Maximal tolerated current intensity (mA).

CI_{END}: CI values recorded during the last NMES contraction (END).

CI_{PRE}: CI values recorded before the beginning (PRE) of each NMES protocol.

ET: Evoked torque (Nm).

MVC: Maximal voluntary contraction (Nm).

$MVIC_{POST}$: MVIC immediately after the end of the 10-min NMES protocol (POST).

$MVIC_{PRE}$: The highest peak torque value recorded during the three contractions at baseline (PRE).

MVICs: Maximum voluntary isometric contractions (Nm).

NME_{CHARGE}: Neuromuscular efficiency calculated by means of estimated current charge (current intensity*pulse duration) to maximal NMES evoked torque values obtained from the FIRST and LAST contraction of each protocol.

NME_{CI}: Neuromuscular efficiency calculated by means of relative values of CI to maximal NMES evoked torque values, obtained from the FIRST and LAST contraction of each protocol.

NMES: Neuromuscular electrical stimulation.

$NMES_{ET}$: Knee extensor NMES evoked torque (Nm).

ON/OFF time: NMES contraction/relaxation time (s).

$ON/OFF_{10/50}$: Contraction/relaxation time of 10/50 s.

$ON/OFF_{5/25}$: Contraction/relaxation time of 5/25 s.

PD: Pulse duration (ms).

$PD_{1.2}$: Pulse durations of 1.2 ms.

PD_2: Pulse durations of 2 ms.

SP_{POST}: Single-pulse evoked torque (Nm) determined by supramaximal stimulus applied immediately after the NMES protocol (POST).

SP_{PRE}: Single-pulse evoked torque (Nm) determined by the torque produced by three supramaximal stimulus applied at rest (PRE).

VAS: Visual analogue scale (cm).

ΔCI: Relative change from CI_{PRE} to CI_{POST} (%).

ΔMVIC: Relative reduction of MVIC torque from $\mathrm{MVIC}_{\mathrm{PRE}}$ to $\mathrm{MVIC}_{\mathrm{POST}}$.

ΔNME: Relative change of neuromuscular efficiency obtained from the FIRST and LAST contractions (%).

$\Delta\mathrm{NMES}_{\mathrm{ET}}$: Relative reduction of the $\mathrm{NMES}_{\mathrm{ET}}$ from the FIRST to LAST contraction (%).

$\Delta\mathrm{SP}_{\mathrm{ET}}$: Relative reduction of single-pulse evoked torque from $\mathrm{SP}_{\mathrm{PRE}}$ to $\mathrm{SP}_{\mathrm{POST}}$.

ΔVAS: Relative change of VAS from PRE to END (%).

Chapter 2:

$\mathrm{CI}_{\mathrm{END}}$: Current intensity values recorded at the NMES protocols (END – mA).

$\mathrm{CI}_{\mathrm{PRE}}$: Current intensity values determined before NMES protocol (PRE - mA).

ET: Evoked torque by electrical stimulation in the form of doublet-pulses.

$\mathrm{ET}_{\mathrm{POST}}$: Peak value of the doublet-pulse applied immediately after the NMES protocols (POST - Nm).

$\mathrm{ET}_{\mathrm{PRE}}$: Mean of the three doublet-pulses obtained before (PRE – Nm).

HF: High frequency component (n.u.).

$\mathrm{HF}_{\mathrm{NMES}}$: HF drawn from 5 min within the second half of the NMES protocols (n.u.).

$\mathrm{HF}_{\mathrm{REST}}$: drawn from the second half of the REST period (n.u.).

HRV: Heart rate variability.

ICU: Intensive care unit.

LF/HF: Low frequency to high frequency component ratio.

$\mathrm{LF/HF}_{\mathrm{NMES}}$: LF/HF drawn from 5 min within the second half of the NMES protocols.

$\mathrm{LF/HF}_{\mathrm{REST}}$: LF/HF ratio drawn from the second half of the REST period.

LF: Low frequency component (n.u.).

$\mathrm{LF}_{\mathrm{MNES}}$: LF drawn from 5 min within the second half of the NMES protocols (n.u.).

$\mathrm{LF}_{\mathrm{REST}}$: LF drawn from the second half of the REST period (n.u.)

MHR: mean heart rate (- bpm / min).

$\mathrm{MHR}_{\mathrm{NMES}}$: MHR drawn from 5 min within the second half of the NMES protocols (beats/min).

$\mathrm{MHR}_{\mathrm{REST}}$: MHR drawn from the second half of the REST period (beats/min).

MRR: Mean RR interval (ms).

MRR_{NMES}: MRR drawn from 5 min within the second half of the NMES protocols (ms).

MRR_{REST}: MRR drawn from the second half of the REST period (ms).

MVIC: Maximal voluntary contractions (Nm).

$MVIC_{POST}$: Knee extension MVIC evaluated immediately after the end of the NMES protocol (Nm).

$MVIC_{PRE}$: Highest torque value recorded during the three MVICs (Nm).

MVICs: Maximal voluntary isometric contractions (Nm).

NMES: Neuromuscular electrical stimulation.

$NMES_{1/5}$: NMES protocol with contractions of 1 s duration were produced (accompanied by 1 s of intensity increase ramp and 1 s of relaxation ramp) followed by a 5 s interval.

$NMES_{5/25}$: NMES protocol with contractions lasting 5 s (accompanied by 2 s of intensity increase ramp and 1 s of relaxation ramp) followed by 25 s intervals.

$NMES_{ABS}$: Mean absolute NMES protocol torque (Nm).

$NMES_{REL}$: Mean relative NMES protocol torque to MVIC torque (%).

ON/OFF: Relaxation/contraction time (s).

VAS: Visual Analogue Scale (cm).

VAS_{END}: Discomfort generated by the current applied through a visual analogue scale at the end of NMES protocols (cm).

VAS_{PRE}: Discomfort generated by the current applied through a visual analogue scale after the determination of current intensity before NMES protocols (cm).

ΔET: Relative reduction of ET from ET_{PRE} to ET_{POST} (%).

ΔMVIC: Relative reduction of MVIC torque (%).

Chapter3:

CIs: Intra-class correlation coefficients confidence intervals.

EF: Evoked force by means of supramaximal electrical stimulus (kgf).

ICC: Intraclass correlation coefficients.

ICUs: Intensive care units.

MDC: Minimal detectable change.

MVIC: Maximum voluntary isometric contraction (kgf).

NMES: Neuromuscular electrical stimulation.

Q_{MT}: quadriceps femoris muscle thickness (cm).

RF_{CSA}: Rectus femoris muscle cross-sectional area (cm^2).

RF_{MT}: Rectus femoris muscle thickness (cm).

SEM: Standard error of measurements.

VI_{MT}: Vastus intermedius muscle thickness (cm).

VL_{FL}: Vastus lateralis muscle fibers fascicle length (cm).

VL_{MT}: Vastus lateralis muscle thickness (cm).

VL_{PA}: Vastus lateralis muscle pennation angle (°).

VM_{MT}: Vastus medialis muscle thickness (cm).

SUMÁRIO

APRESENTAÇÃO

A perda de massa muscular leva a uma importante perda de funcionalidade no paciente adulto crítico em unidade de tratamento intensivo (UTI), aumentando o risco do desenvolvimento de outras comorbidades. Esta perda de funcionalidade aumenta o tempo de permanência e de reabilitação do paciente junto ao sistema de saúde, acarretando um aumento do custo para o paciente, para sua família e para o sistema público de saúde.

A estimulação elétrica neuromuscular (EENM) é uma técnica não-invasiva, de fácil utilização e baixo custo, que tem demonstrado efeitos significativos de melhora da estrutura e função neuromusculares. A melhora do sistema de movimento aumenta a funcionalidade, conduzindo os pacientes a uma condição de saúde mais rapidamente e reduzindo os custos para o paciente, sua família e sistema de saúde.

Entretanto, a diversidade de equipamentos e tipos e parâmetros de corrente elétrica utilizados na EENM aplicada em protocolos de intervenção, além da inexistência de valores normativos de parâmetros neuromuscularespara sujeitos saudáveis e da baixa qualidade metodológica dos estudos existentes impossibilitam se chegar a uma conclusão embasada em evidências científicas sobre a utilização da EENM na prática clínica em UTIs.

Portanto, os objetivos do presente estudo foram (1) testar uma série de parâmetros de EENM na estrutura e função neuromusculares e na função sistêmica em sujeitos adultos saudáveis a fim de melhor definir um protocolo de avaliação e outro de intervenção com pacientes adultos críticos internados em UTIs, e (2) avaliar a confiabilidade das medidas de avaliação a serem utilizadas com esses mesmos pacientes a partir da avaliação da repetibilidade das medidas com um mesmo avaliador em dias diferentes e a avaliação da reprodutibilidade inter-avaliadores dessas mesmas medidas em um mesmo dia. Por fim, também procuramos (3) estabelecer dados normativos em sujeitos adultos saudáveis a fim de definir a condição de saúde neuromuscular e sistêmica a ser atingida pelos pacientes adultos críticos durante o programa de reabilitação neuromuscular a ser utilizado na UTI do Hospital de Clínicas de Porto Alegre (RS/ Brasil).

Para atingir esses objetivos, primeiro desenvolvemos um sistema de avaliação e de intervenção adaptado para a realidade da UTI, consistindo de um estimulador elétrico multifuncional e de um dinamômetro adaptado para utilização em macas hospitalares. Essas duas novas tecnologias nos possibilitaram aplicar a EENM dentro dos parâmetros que consideramos ideais a partir das evidências existentes na literatura para a melhora da estrutura e função musculares. Adicionalmente, nossa tecnologia assistiva também possibilitou definir uma metodologia objetiva de controle da sobrecarga mecânica gerada pela EENM no sistema muscular, e avaliar de forma objetiva os efeitos da EENM sobre o sistema muscular e vascular de pacientes adultos críticos.

As perguntas específicas que queríamos responder são as seguintes: (1) Quais os efeitos de pulsos elétricos com diferentes durações e da EENM com diferentes tempos de repouso-relaxamento sobre o nível ou intensidade máxima de corrente elétrica, sobre o torque evocado, sobre o desconforto e sobre a fadiga muscular em adultos jovens? (2) Qual o efeito do aumento do tempo de contração/relaxamento da EENM sobre a fadiga muscular e respostas cardiovasculares em jovens saudáveis?; e (3) Qual a confiabilidade intra-avaliador, inter-avaliador e inter-analisador das medidas de morfologia (obtida por meio de imagens de ultrassom) e função (torque voluntário e evocado) dos músculos extensores de joelho, em sujeitos jovens saudáveis?

Para responder a essas perguntas, os trêsestudos (capítulos) apresentados a seguir foram redigidos na forma de artigos na língua inglesa. Estes, portanto, tiveram a finalidade específica de avaliar (1) os efeitos da duração do pulso elétrico e do tempo de contração/repouso da EENM, sobre o nível de corrente, torque evocado, desconforto e fadiga muscular; (2) o efeito do tempo de contração/relaxamento da EENM sobre a fadiga muscular e respostas cardiovasculares; e (3) a confiabilidade intra-avaliador, inter-avaliador e inter-analisador das medidas de morfologia e função dos músculos extensores de joelho, em sujeitos jovens saudáveis.

APRESENTAÇÃO: CAPÍTULO 1

Tendo em vista a futura implementação de um programa de EENMpara a reabilitação de adultos críticos internados em unidades de terapia intensiva (UTIs), a determinação de parâmetros adequados da EENM para a melhora da função neuromuscular se faz necessária. O capítulo a seguir procurou avaliar os efeitos de duas diferentes durações de pulso elétrico e de dois diferentes tempos de contração/relachamento da EENM sobre o desconforto, produção de força e fadiga muscular de sujeitos saudáveis, afim de determinar um programa de EENM eficiente para a manutenção ou melhora da função neuromusculardurante a reabilitação destes pacientes.

NEUROMUSCULAR ELECTRICAL STIMULATION: EFFECTS OF TWO DIFFERENT PULSE DURATIONS ON DISCOMFORT AND FATIGUE IN HEALTHY YOUNG SUBJECTS

RESUMO

INTRODUÇÃO: O objetivo do presente estudo foi avaliar os efeitos de diferentes durações de pulso e de diferentes tempos de contração/relaxamento da estimulação elétrica neuromuscular (EENM) sobre a intensidade de corrente, produção de torque, desconforto e fadiga muscular. MÉTODOS: 24 jovens saudáveis (12 ♂ e 12 ♀, 23,8±3,4 anos) realizaram quatro protocolos de EENM (10 min de duração) com parâmetros distintos de corrente elétrica (duração de pulso = 1,2 e 2 ms; tempos de contração-relaxamento = 5/25 s e 10/50 s, respectivamente). Antes do protocolo de EENM, os participantes realizaram três contrações voluntárias máximas isométricas (CVMIs) dos extensores de joelho a 90° de flexão, seguidas de um teste supramáximo de torque evocado (PS_{TE}). A intensidade de corrente (IC) utilizada, torque evocado pela EENM($EENM_{TE}$) eo desconforto produzido (EVA)foram registrados no início e no final dos protocolos de EENM. Imediatamente após o protocolo de EENM, foram realizados um novo PS_{TE} e uma nova CVMI para avaliar o nível de fadiga. RESULTADOS: Menores níveis de IC foram necessários para atingir o nível máximo tolerado de corrente durante os protocolos de EENM com 2 ms de duração de pulso (p=0,001) comparado à duração de 1,2 ms. O desconforto relatado pelos sujeitos diminuiu de forma semelhante (p<0,0001) e não foi diferente entre os quatro protocolos de EENM (efeito observado para: duração do pulso, p=0,203; tempos de contração-relaxamento, p=0,679). Tanto o $EENM_{TE}$ (p<0,0001) quanto a$\Delta EENM_{TE}$ (duração do pulso, p=0,261; tempos de contração-relaxamento, p=0,303) demonstram que a redução da sobrecarga mecânica foi semelhante entre os protocolos de EENM. As CVMIs reduziram significativamente (p<0,0001), e nenhuma diferença foi encontrada na ΔCVMI entre os protocolos de EENM (eduração do pulso, p=0,261; tempos de contração-relaxamento, p=0,303). O PS$_{TE}$ também diminuiu significativamente para todos os protocolos de EENM (p<0,0001), mas o PS$_{TE}$ foi significativamente maior para os protocolos com duração de pulso de 2 ms (efeito observado para duração de pulso: p=0,044). CONCLUSÃO: Embora não tenha havido diferença entre a sobrecarga mecânica produzida durante os protocolos de EENM realizados na máxima IC tolerada, duração maior do pulso permite o uso de menor IC. Além disso, todos os quatro protocolos se mostraram eficientes para gerar sobrecarga mecânica e fadiga nos músculos extensores do joelho, de modo que podem ser utilizados na prática clínica para reabilitação neuromuscular.

Palavras-chaves: Estimulação elétrica neuromuscular, duração de pulso, fadiga muscular.

ABSTRACT

INTRODUCTION: The purpose of the present study was to evaluate the effects of different NMES pulse durations and of different NMES contraction/relaxation (ON/OFF) times on current intensity, torque production, discomfort and muscle fatigue. METHODS: Twenty-four healthy (12 ♂ and 12 ♀, 23.8±3.4 years) performed four 10-min duration NMES protocols with distinct electric current parameters (pulse duration = 1.2 and 2 ms; ON/OFF times = 5s/25s and 10s/50s). Before the NMES protocol, participants performed three maximum voluntary isometric contractions (MVICs) at 90° of knee flexion, followed by a supramaximal evoked torque test (SP$_{ET}$).The current intensity (CI) used,NMES evoked torque(NMES$_{ET}$) and the discomfort produced (VAS)were registered at the beginning and at the end of the NMES protocols. Immediately after each NMES protocol, a new SP$_{ET}$ test and a new MVIC were performed to evaluate the fatigue level. RESULTS: Lower CIs were necessary to reach the maximal tolerated level of current during longer pulse duration (2 ms) NMES protocols (observed effect for pulse-duration: p=0.001). Discomfort reported by the subjects decreased similarly during the four protocols (observed effect for moment: p<0.0001) and was not different between the NMES protocols (observed effect: pulse-duration, p=0.203; ON/OFF times, p=0.679). Both NMES$_{ET}$ (observed effect moment: p<0.0001) as ΔNMES$_{ET}$ (observed effect: pulse-duration, p=0.261; ON/OFF times, p=0.303) demonstrate that mechanical overload reduction was similar between the NMES protocols. MVICs reduced significantly (observed effect for moment: p<0.0001), and no difference was found on ΔMVIC between NMES protocols (observed effect: pulse-duration, p=0.261: ON/OFF times, p=0.303). SP$_{ET}$ also decreased significantly for all NMES protocols (observed effect for moment: p<0.0001), but ΔSP$_{ET}$ was significantly greater for 2 ms pulse duration NMES protocols (observed effect for pulse-duration: p=0.044). CONCLUSION: Although there was no difference in the mechanical overload produced during the four different NMES protocols performed at maximal tolerated CI, a longer pulse duration allows for the use of a lower CI. In addition, all four NMES protocols were eficiente in generating knee extensor mechanical overload and fatigue, and therefore can be used in clinical practice for neuromuscular rehabilitation.

Key-words: Neuromuscular electrical stimulation, pulse duration, muscle fatigue.

1.1. INTRODUCTION

Neuromuscular electrical stimulation (NMES) is used to produce muscle contractions in individuals with motor impairments in order to maintain or restore function and to reduce disuse atrophy (Gerovasili *et al*., 2009; Poulsen *et al*., 2011; Maffiuletti *et al*., 2013). By restoring function and reducing atrophy, NMES can reduce immobility and improve quality of life in individuals unable to perform voluntary exercise (Delitto and Snyder-Mackler, 1990; Maffiuletti, 2010). Unfortunately, the benefits and use of NMES are limited by inconsistency of NMES-based programs, inadequate and expensive equipment, discomfort caused by stimulation, and rapid onset of fatigue (Maffiuletti, 2010). Discomfort generated by NMES limits the increase of electrical current intensity (electrical charge), which has been related to muscular recruitment and force production (Ward *et al*., 2002). Fatigability limits the NMES benefits by reducing the NMES sessions' duration and, consecutively, total mechanical overload (Brass *et al*., 2018). Hence, reducing the discomfort and fatigue generated by NMES evoked contractions can enhance the benefits of NMES and its use in clinical settings.

The greater effectiveness in torque production through NMES depends on the characteristics of the electric current used, and numerous studies were conducted aimed at understanding the effect of modifying NMES parameters in an attempt to make it more effective for muscle contraction (Lieber and Kelly, 1991; Ward and Robertson, 1998; McLoda *et al*., 2000), and to reduce muscle fatigue (Ward *et al*., 2004, Ward and Lucas-Toumbourou, 2007). Conventionally, NMES is delivered to the muscle's motor branches in the form of short electrical pulses (50 to 600 µs of duration) and low-frequency trains (15 to 40 Hz) at relatively higher current amplitudes (Hainaut and Duchateau, 1992). These parameters generate contractions predominantly via peripheral pathways due to both the preferential activation of motor axons and the large antidromic transmission along them (Bergquist *et al*., 2011). For that reason, even though being a key component in rehabilitation, the most commonly accepted disadvantage of conventional NMES is the rapid onset of muscular fatigue due to a random, spatially fixed and temporally synchronous recruitment of motor units produced by electrical stimulus (Vanderthommen *et al*., 2000; Gregory and Bickel, 2005; Maffiuletti, 2010).

An effective way to increase the level of NMES evoked contractions is by increasing the stimulus current intensity or the pulse duration (or both) to depolarize more motor branches and recruit more motor units (Adams *et al*., 1993; Gorgey *et al*., 2006). For a given level of current intensity, pulse duration has been considered the major determinant of evoked force production (Laufer *et al*., 2001; Ward and Lucas-Toumbourou, 2007; Georgey and Dudley, 2008; Scott *et al*., 2009). However, due to the variety of parameters used in these previous studies, it is still not clear which pulse duration will allow for greater force production with less discomfort.

In the last three decades, it has been suggested that contractions evoked by NMES are generated both by peripheral (direct activation of motor axons under the stimulation electrodes) and central (depolarization of sensory nerves producing a reflex response) mechanisms (Collins *et al*., 2002). The central mechanism apparently contributes to motor unit recruitment when using NMES with a longer pulse duration than conventionally used, allowing to deliver the stimulation at lower current intensities (Collins, 2007). The use of relatively long pulse durations (0.5 to 1 ms) increases the recruitment of sensory nerve branches, because of their lower activation thresholds compared to motor axons (Veale *et al.*, 1973). Interestingly, the use of long pulse duration (1 ms), high-frequency (>80 Hz) and low current intensities NMES has been shown to produce much higher isometric forces than conventional NMES (Collins *et al*., 2002; Lagerquist *et al*., 2009), thereby supporting the idea of a relation between large pulse duration and a reflex central mechanism of motor unit recruitment, augmenting the NMES evoked force and muscle mechanical overload.

Considering these findings, it has been suggested that the central contribution to NMES evoked force production might delay the onset of fatigue due to a more physiological recruitment pattern of fatigue-resistant motor units, providing a prospective advantage of long-pulse NMES over the conventional pattern for clinical rehabilitation (Binder-Macleod and Scott, 2001; Gregory *et al*., 2007). However, no informationwas found about the effect of using pulse durations above the recently proposed level (>1 ms) during NMES-based programs. It is not known if such parameter cause a positive or negative effect over NMES evoked contractions on skeletal muscle force production and fatigue. This information is important for physiotherapists or health professionals, providing a clearer criterion for adequate current parameters for NMES prescription and application during rehabilitation programs.

Last but not least, another commonly used way to avoid muscle fatigue during NMES-based programs is to separate subsequent contractions by time intervals in order to recover the muscle fiber sarcolemma homeostasis, and, consequently, the torque produced during successive contractions (Jubeau *et al*., 2008). Cycling pulses ON and OFF(intermittent stimulation) is a common practiceto preserve force development and simultaneouslyincrease comfort for thepatient. Duty cycle describes the actual ONand OFF times of a NMES program, and isusually stated in ratio form, such as 1:2 (10seconds on, 20 seconds off) or percentages (such as 30 percent), indicating time on percentagewhen compared to total ON and OFFtimes combined (Baker *et al*., 2000). As an example, the "Russian current", which was developed to strengthen athletes who often tolerated 100% of MVC through NMES, was traditionally used with a 50% duty cycle (total stimulation duration relative to total session time). However, a 10% duty cycle in the "Russian current" proved to be more efficient for the maintenance of torque production comfortably in contractions with an intensity of approximately 60% of MVC (McLoda and Camarck, 2000). Some studies attribute the level of NMES fatigue to the adopted duty-cycle, and demonstrated that duty-cycles between 10 and 20% would be more efficient in maintaining torque production and delaying the onset of fatigue generated through NMES (McLoda and Camarck, 2000; Ward *et al*., 2004). Also, it has been demonstrated that changing the contraction/relaxation time during conventional NMES with no alteration on duty-cycle has no effect over fatigue (Ward *et al*., 2004). However, no information was found in current literature about the effect of contraction/relaxation time on discomfort or fatigue when a longer pulse (> 1ms) NMES protocol is performed.

1.2. PURPOSE

The purpose of the present study was to evaluate the effects of different NMES pulse durations and of different NMES contraction/relaxation times (ON/OFF times) on current intensity, discomfort and muscle fatigue in healthy young subjects. Therefore, we evaluated the effects of two different large electrical pulse durations (1.2 ms and 2 ms; phase duration of 600 µs and 1 ms, respectively), and two different contraction/relaxation times (5/25 s and of 10/50 s) combined in four 10-min duration protocols over (1) the current intensity and respective neuromuscular efficiency, (2) the level of discomfort generated by the NMES (through the visual analogue scale), (3) the fatigue level produced by NMES (through the evaluation of maximum voluntary torque before and after each NMES protocol), and (4) the level of peripheral fatigue produced by NMES (through the evaluation of the evoked torque by means of supramaximal electric stimuli; Poulsen *et al.*, 2015), before and after each NMES protocol. Our hypothesis was that longer pulse duration NMES current will be more efficient in torque production, while a longer contraction/relaxation time will produce higher levels of fatigue and discomfort during NMES evoked contractions, at the maximal tolerated intensity.

1.3. METHODS

The evaluations took place at the Neuromuscular Plasticity Department of the Exercise Research Laboratory (LAPEX, Porto Alegre, Brazil) of the School of Physical Education, Physiotherapy and Dance (ESEFID - UFRGS). This study used a methodology approved by the University's Research Ethics Committee (CAEE nº 36588914.4.1001.5347). The subjects read the consent form and signed it after clarifying possible doubts with the responsible researcher.

1.3.1. Sample

Twenty-four healthy and physically active subjects (university undergraduate and graduate students) participated in the study (12 men and 12 women), with a mean age of 23.8 ± 3.4 years. Subjects were recruited through announcements in social networks and through an invitation done by the researcher in classrooms. The study did not include subjects who presented (1) lower limb traumatic injury, (2) any cardiovascular disease, (3) any central or peripheral neurological disease, or (4) any clinical contraindications to the tests' performance.The number of subjects was determined through the following equation that indicates the sample size according to the tolerated error

$$n=Z^2*sd^2/e^2$$

of measurement for each evaluated variable, where n is the sample size, Z is the tabled value related to the significance level of this study (1.96 for $\alpha = 0.05$), sd is the standard deviation of the variable in question, obtained through the specific literature, and *e*, the tolerated measurement error (estimated in 10%) and applied over the mean value of the variable in question obtained from the literature. The sample number was obtained through the mean value and standard deviation of maximal knee extension NMES evoked torque (166 ± 41 Nm) from a study that evaluated a similar population (Gorgey *et al.*, 2009).

1.3.2. Procedures

The evaluations were realizedalong four consecutive days for each subject, with an average time of 45 minutes per visit. In each day, subjects were submitted to a 10-min duration NMES protocol, and each protocol, with distinct electric current parameters, wasselected in a randomized fashion. On the first day, the quadriceps motor point was located for the NMES electrodes positioning. Next, the necessary current for the production of a supramaximal stimulus was determined, using single pulses applied with a progressive current intensity increase. Subjects then performed three maximum voluntary isometric contractions (MVICs) at 90° of knee flexion (0° = full extension) followed by the supramaximal evoked torque test (ET).After the determination of the maximal tolerated current by the subjects and its respective discomfort generated, they were submitted to the 10-min NMES protocol, which was performed in the maximal tolerated current, with the pulse duration and the ON/OFF times being drawn at random. The current intensity levels (CI) for each subject during the NMES protocols were registered at the beginning and at the end of the NMES protocol together with the discomfort produced by the electrical stimulus, which was evaluated through the visual analogue scale (VAS). Immediately after the end of the NMES protocol, a new knee extensor supramaximal evoked torque test and a new MVIC were obtained to evaluate the fatigue level produced by the NMES (Figure 1.1).

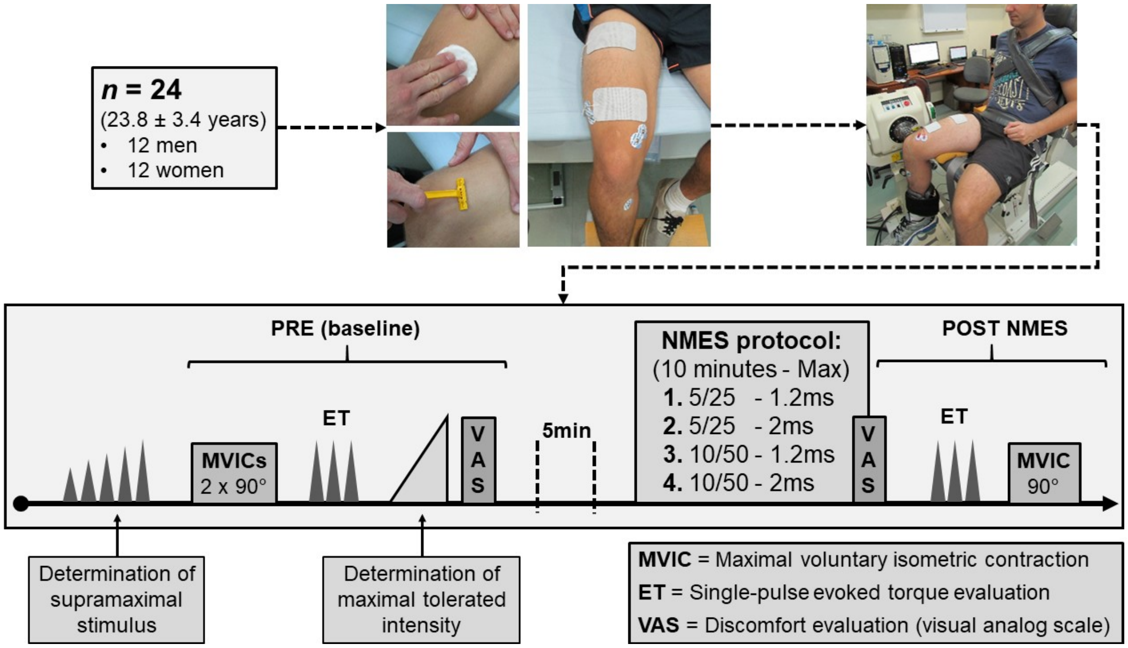

Figure 1.1. Procedures. Sample motor-point localization, skin cleaning, electrode placement and dynamometer positioning (top). Evaluation of supramaximal stimulus, MVIC, ET and discomfort for the four days of NMES protocols (bottom).

1.3.3. Motor point location and electrodes placement

To determine the exact location of the quadriceps muscle motor point, an electrical stimulator with a pen shaped electrode was used (EGF Carci, São Paulo, Brazil). With the subjects seated on the dynamometer chair (knee flexed at 90°), conductive gel was applied on the skin covering the middle portion of the thigh muscles. Next, the pen-shaped electrode was moved through the skin in the middle-proximal region of the muscle belly. Rectangular monophasic current singular pulses, with sufficient current intensity to produce a visible contraction of the knee extensor muscles, were then applied. The location where the pulses produced the most vigorous visual contraction was defined as the quadriceps motor point. After the determination of the motor point, trichotomy and cleaning of the skin with alcohol were performed in the sites where the NMES electrodes were placed. Two 7.5 cm wide and 13 cm long rectangular self-adhesive gelled electrodes (Arktus, Canada) were used. The pair of electrodes was positioned over the motor point and over the distal portion of the quadriceps muscle (5 cm from the patella'supper edge – Figure 1.1). In attempt to allow for identical electrode positioning in subsequent evaluations, a transparent sheet was used to register anatomical structures and skin marks, as well as the electrodes' position(Vaz *et al*., 2013).

1.3.4. Evaluation of the knee extensors mechanical properties

The isometric knee extension torque of the subjects' right lower limb was assessed, through the Biodex System 3 Pro isokinetic dynamometry system (Biodex Medical System, Shirley - NY, USA). Subjects were positioned seated, with the hip joint flexed at an angle of 85° (0° = full hip extension). The dynamometer arm was attached to the evaluated leg with fixing bands 3 cm above the medial malleolus. The lateral femur condyle represented the knee joint center of rotation, and was visually aligned with the dynamometer's axis of rotation. Cushioned straps secured the subjects to the chair at the hips and shoulders regions in order to avoid any undesired movements during the tests. The adjustments made to the chair were recorded for identical positioning in subsequent evaluations (Figure 1.1).

1.3.5. Fatigue evaluation through the maximum isometric voluntary torque

After properly positioned in the dynamometer, subjects performed a warm-up through 10 concentric submaximal contractions of the knee extensors and flexors muscles at an angular velocity of $90°.s^{-1}$. After a 2-min recovery period, they performed two knee extensor muscles MVICs at 90° of knee flexion (full knee extension = 0°), maintaining the maximal efforts for a period of five seconds. Subjects were instructed to perform the contractions without any visual feedback, rapidly increasing the effort until they reached maximal torque production, which should be maintained until a verbal command to cease contraction. A 2-min interval between contractions was provided, and a third contraction was performed to ensure maximum torque production when the difference between the first and second contractions was greater than 10% (Baroni *et al*., 2013). The highest peak torque value recorded during the three contractions (PRE) was determined as $MVIC_{PRE}$. Immediately after the end of the 10-min NMES protocol (POST), the subjects performed a new knee extensor MVIC at 90° of knee flexion in order to verify the presence of fatigue ($MVIC_{POST}$). Fatigue produced during the four NMES protocols performed was determined by the relative reduction of MVIC torque from $MVIC_{PRE}$ to $MVIC_{POST}$ (ΔMVIC), presented in percent values (%). Mean and standard deviation values for $MVIC_{PRE}$, $MVIC_{POST}$ and $\Delta MVIC$, were calculated for the whole group in each NMES protocol.

1.3.6. Evaluation of peripheral fatigue by single-pulse evoked torque

For the assessment of peripheral fatigue by single-pulse evoked torque, a Grass electrical stimulator (S88, Quincy, MA, USA), with a patient unit (SIU8T), was used for the application of supramaximal stimuli, and the Biodex System 3 Pro (Biodex Medical System, Shirley - NY, USA) dynamometer was used for torque evaluation. The stimulator patient unit was connected to the same electrodes used for the NMES protocol. Fatigue was assessed by means of percutaneous electrical stimulation of the motor point, which was applied in the form of monophasic and rectangular single-pulses with a constant voltage of 240 V (maximum) and pulse duration of 0.8 ms. Prior to the tests, electrical pulses of increasing voltage up to a maximum of 240 V were applied over the quadriceps motor point in order to determine the voltage required to generate a supramaximal stimulus and also to familiarize subjects with the procedure. Single-pulse evoked torque (Nm) was determined by the torque produced by three supramaximal stimulus applied at rest, before (SP_{PRE}), and immediately after the NMES protocol (SP_{POST}), with approximately one second interval between contractions. Peripheral fatigue produced by NMES during the four protocols was determined by the relative reduction of single-pulse evoked torque from SP_{PRE} to SP_{POST} (ΔSP_{ET}), presented in percent values (%). Mean and standard deviation values for SP_{PRE}, SP_{POST} and ΔSP_{ET}, were calculated for the entire group in each NMES protocol.

1.3.7. Fatigue protocols

To perform the fatigue protocols, a clinical multifunctional electrical stimulator, developed by the Biomedical Engineering Laboratory of the Porto Alegre Clinics Hospital, was used (Schildt *et al.*, 2016). The NMES was applied using a symmetrical rectangular biphasic pulsed current, with a stimulation frequency of 80 Hz. For each NMES protocol, a combination of both pulse durations of 1.2 ms ($PD_{1.2}$) and 2.0 ms ($PD_{2.0}$), and contraction/relaxation times of 5/25 s ($ON/OFF_{5/25}$) and 10/50 s ($ON/OFF_{10/50}$) were used in a randomized fashion: (1) $PD_{1.2}$ and $ON/OFF_{5/25}$; (2) $PD_{2.0}$ and $ON/OFF_{5/25}$; (3) $PD_{1.2}$ and $ON/OFF_{10/50}$; and (4) $PD_{2.0}$ and $ON/OFF_{10/50}$.

The protocols were performed at the maximum tolerated CI by the subjects, and CI was increased whenever needed during the 10-min protocols in order to maintain the intensity level at the maximum tolerated CI. CI values were recorded before the beginning (CI_{PRE}) of each protocol, and during the last NMES contraction (CI_{END}). The relative change on maximal tolerated current intensities (ΔCI) from CI_{PRE} to CI_{POST} were presented in percent values (%). In order to evaluate the efficiency of current intensity levels used during NMES, neuromuscular efficiency was calculated by means of relative values of both maximal current intensities (NME_{CI}) and estimated current charge (current intensity*pulse duration – NME_{CHARGE}) to maximal NMES evoked torque values obtained from the FIRST and LAST contraction of each protocol. Relative change of NME obtained from the FIRST and LAST contractions (ΔNME), was presented in percent values (%). Mean and respective standard deviations values for CI_{PRE}, CI_{END}, ΔCI, NME_{CI}, NME_{CHARGE} and ΔNME were calculated for the entire group in each NMES protocol.

1.3.8. Assessment of the NMES evoked torque produced by the protocols

During the NMES protocol, subjects were instructed to remain relaxed while positioned on the dynamometer, and the knee extensor NMES evoked torque ($NMES_{ET}$) was recorded. The maximum torques produced by the FIRST and LAST curve of each of the four protocols were used to quantify the level of mechanical overload produced. The relative reduction of the $NMES_{ET}$ from the FIRST to LAST contraction ($\Delta NMES_{ET}$) was calculated and presented in percent values (%). The mean and standard deviation values of $NMES_{ET}$ (FIRST and LAST) and $\Delta NMES_{ET}$ were calculated for the entire group, for each of the four protocols.

1.3.9. Evaluation of NMES-generated discomfort

Subjects were evaluated for the NMES-generated discomfort through a visual analogue scale (VAS). This scale consists of a 10-cm straight line, with the left end referring to the absence of discomfort (0 cm) and the right end referring to the maximum imaginable pain (10 cm). Subjects marked with a pen on the line, indicating the level of discomfort experienced. The level of discomfort was defined by the measurement in centimeters between the zero value and the pen mark made by the subject. Discomfort (VAS) was evaluated before the NMES protocols, after the determination of the maximum tolerated current intensity (PRE) and during the last contraction (END) of each NMES protocol. Relative change of VAS from PRE to END (ΔVAS) was calculated and presented in percent values (%). Mean and standard deviation values for VAS (PRE and END) and ΔVAS were calculated for entire group, for the four protocols.

1.3.10. Statistical analysis

To verify data normality, a Shapiro-Wilk test was used. To verify data sphericity, a Mauchly test was used. For the evaluation of the effects of the different parameters used in the four NMES protocols over CI, NME_{CI}, $\text{NME}_{\text{CHARGE}}$, VAS, NMES_{ET}, MVIC and ET, a three-way repeated measures ANOVA was used, with moments, the ON/OFF times (5/25 s and 10/50 s), and the pulse duration (1.2 ms and 2.0 ms) as factors. For the evaluation of the effects of NMES parameters on ΔCI, ΔNME, ΔVAS, $\Delta\text{NMES}_{\text{ET}}$, ΔMVIC and ΔET, a two-way repeated measures ANOVA was used, using as factors the ON/OFF times and the pulse duration. To verify the clinical relevance of eventual differences found between moments, the variables effect size was calculated by means of Cohen’s *d*, adopting the following criteria: < 0.2: trivial, > 0.2: small; > 0.50: moderate; > 0.80: large (Cohen, 1988 – Table 2.2). A significance level of 5% was used (Software SPSS version 20.0).

1.4. RESULTS

1.4.1. Current intensity relative change and neuromuscular efficiency

The observed effect of moment on maximal CI tolerated by the subjects (p=0.008) showed that the CI was similarly increased during the four protocols (Table 1.1). However, the observed effect for pulse duration on maximal CI (p=0.001) shows that lower CI was necessary to reach the maximal tolerated level of current by the subjects when a longer pulse duration (2.0 ms) was used during the NMES protocols. There was no observed effect for ON/OFF timesfor the maximal tolerated CI (p=0.714). In addition, there was a similar ΔCI (%) tolerated by the subjects among the four NMES protocols, as no effect was observed for pulse duration (p=0.925) and ON/OFF times (p=0.101).

NME_{CI} was also similar among NMES protocols, as no effect was observed for pulse duration (p=0.308) and ON/OFF times (p=0.452 – Table 1.1). Although the NME_{CI}decrease similarly on the four NMES protocols (observed effect for moment, p<0.0001), NME_{CHARGE} was lower for longer pulse NMES protocols (observed effect for pulse duration, p<0.0001). Besides that, NME_{CHARGE}decrease was greater for longer pulse protocols, as an effect was observed for moment (p<0.0001) and an interaction was observed between moment and pulse duration (p=0.019). No effect was observed for ON/OFF times (p=0.538). ΔNME was not different between NMES protocols, as no effect was observed for pulse duration (p=0.510) and ON/OFF times (p=0.594).

1.4.2. NMES Discomfort

The discomfort reported by the subjects decreased during the four protocols, as an effect of moment was observed (p<0.0001). However, no ON/OFFtimes (p=0.79) or pulse duration (p=0.691) effect was observed, suggesting that there was no difference on discomfort between the NMES protocols (Table 1.1). In addition, ΔVAS was not different between NMES protocols, as no effect was observed for pulse duration (p=0.203) and ON/OFF times (p=0.679).

Table 1.1. Evaluation protocols' variables. Maximal tolerated current intensity (**CI**); Relative change in maximal tolerated current intensity (**ΔCI**); Neuromuscular efficiency by means of current intensity (**NME$_{CI}$**); Relative change of **NME$_{CI}$** (**ΔNME$_{CI}$**); Neuromuscular efficiency by current charge (**NME$_{CHARGE}$**); Relative change in **NME$_{CHARGE}$** (**ΔNME$_{CHARGE}$**); Discomfort to NMES (**VAS**); Relative change in **VAS** (**ΔVAS**); NMES evoked torque (**NMES$_{ET}$**); Relative change in **NMES$_{ET}$** (**ΔNMES$_{ET}$**); Maximal voluntary isometric contraction (**MVIC**); Fatigue by means of relative change in **MVIC** (**ΔMVIC**); Single-pulse evoked torque (**SP$_{ET}$**); Fatigue by means of relative change in **SP$_{ET}$** (**ΔSP$_{ET}$**).

		NMES protocols (Days)			
Variables (mean ± sd)	ON/OFF times	**5s / 25 s**		**10 s / 50 s**	
	Pulse duration	**1.2 ms**	**2 ms**	**1.2 ms**	**2 ms**
CI (mA)	FIRST	53.58 ± 18.84	45.33 ± 16.31	55.13 ± 17.20	47.83 ± 16.40
	LAST	59.38 ± 18.93[ab]	50.92 ± 17.68[a]	58.29 ± 18.11[ab]	50.46 ± 16.98[a]
ΔCI(%)		14.32 ± 22.76	15.30 ± 25.06	7.82 ± 23.57	7.72 ± 21.67
NME$_{CI}$(mA/Nm)	FIRST	1.85 ± 1.15	1.73 ± 1.60	2.04 ± 1.37	1.77 ± 0.99
	LAST	2.48 ± 1.26[a]	2.36 ± 1.47[a]	2.63 ± 1.57[a]	2.47 ± 1.29[a]
NME$_{CHARGE}$(C/Nm)	FIRST	2.23 ± 1.38	3.47 ± 3.20	2.45 ± 1.65	3.54 ± 1.98
	LAST	2.99 ± 1.51[a]	4.72 ± 2.95[abc]	3.15 ± 1.89[a]	4.89 ± 2.64[abc]
ΔNME(%)		45.07 ± 47.51	60.41 ± 69.49	45.03 ± 83.88	43.48 ± 45.31
VAS (cm)	PRE	6.30 ± 2.07	6.57 ± 1.60	6.32 ± 1.56	6.49 ± 2.00
	END	5.19 ± 2.13[a]	5.34 ± 2.43[a]	5.35 ± 1.71[a]	5.04 ± 2.17[a]
ΔVAS (%)		-11.05 ± 45.31	-18.00 ± 34.39	-14.19 ± 22.46	-18.52 ± 48.96
NMES$_{ET}$(Nm)	FIRST	39.7 ± 26.77	41.96 ± 30.82	38.06 ± 25.67	39.64 ± 30.37
	LAST	33.43 ± 25.52[a]	32.75 ± 26.62[a]	29.91 ± 21.02[a]	30.20 ± 23.94[a]
ΔNMES$_{ET}$(%)		-17.45 ± 16.48	-22.31 ± 18.67	-21.13 ± 15.84	-24.07 ± 14.63
MVIC (Nm)	PRE	177.5 ± 77.5	184.0 ± 79.1	172.0 ± 72.7	182.0 ± 82.2
	POST	154.9 ± 67.1[a]	164.9 ± 71.9[ab]	150.4 ± 56.4[a]	160.4 ± 71.4[ab]
ΔMVIC(%)		-12.80 ±6.39	-10.78 ± 5.58	-11.29 ± 7.46	-11.84 ± 6.19
SP$_{ET}$(Nm)	PRE	26.43 ± 8.54	29.71 ± 10.18	27.45 ± 9.16	30.50 ± 10.03
	POST	22.69 ± 7.70[a]	24.67 ± 8.94[abc]	23.05 ± 6.74[a]	24.54 ± 7.55[abc]
ΔSP$_{ET}$(%)		-14.31 ± 8.11	-17.09 ± 10.23[b]	-14.67 ± 12.39	-18.27 ± 10.14[b]

[a] Effect observed for moment; [b] Effect observed for pulse duration; [c] Interaction between moment and pulse duration ($p<0.05$). Moments: FIRST to LAST, PRE to END and PRE to POST.

1.4.3. Maximal NMES evoked torque

$NMES_{ET}$ was similar between the four NMES protocols (Table 1.1), and a similar reduction on $NMES_{ET}$ was observed from the FIRST to LAST contraction ($p<0.0001$). There was no effect for ON/OFF times ($p=0.456$) or pulse duration ($p=0.733$). Consecutively, there was no difference in $\Delta NMES_{ET}$ between NMES protocols, as no effect was observed for pulse duration ($p=0.261$) or ON/OFF times ($p=0.303$).

1.4.4. Evaluation of MVIC and ΔMVIC

The absolute values of MVICs reduced significantly for all NMES protocols (Table 1.1), as an effect was observed for moment ($p<0.0001$). Although there was an effect for pulse duration ($p=0.03$), no interaction was observed between moment and pulse duration ($p=0.469$), suggesting that the level of fatigue evaluated by means of MVIC was similar between protocols. No effect was observed for ON/OFF times($p=0.223$). In addition, there was a similar fatigue level, evaluated by means of the ΔMVIC for all NMES protocols (Table 1.1), as no effect was observed for pulse duration ($p=0.261$) or ON/OFF times ($p=0.303$).

Table 1.2. Cohen's *d* values between moments. Maximal tolerated current intensity (**CI**); Neuromuscular efficiency by means of current intensity (**NME$_{CI}$**); Neuromuscular efficiency by estimated current charge (**NME$_{CHARGE}$**); Discomfort to NMES (**VAS**); NMES evoked torque (**NMES$_{ET}$**); Maximal voluntary isometric contraction (**MVIC**); Single-pulse evoked torque (**SP$_{ET}$**).

		NMES protocols (Days)			
	ON/OFF times	**5s / 25 s**		**10 s / 50 s**	
Variables (effect size)	Pulse duration	**1.2 ms**	**2 ms**	**1.2 ms**	**2 ms**
CI		0.30[b]	0.32[b]	0.17[a]	0.15[a]
NME$_{CI}$		0.52[c]	0.41[b]	0.40[b]	0.60[c]
NME$_{CHARGE}$		0.52[c]	0.40[b]	0.39[b]	0.57[c]
VAS		0.52[c]	0.59[c]	0.59[c]	0.69[c]
NMES$_{ET}$		0.23[b]	0.31[b]	0.34[b]	0.34[b]
MVIC		0.31[b]	0.26[b]	0.33[b]	0.30[b]
SP$_{ET}$		0.45[b]	0.52[c]	0.54[c]	0.67[c]

Cohen's *d* (effect size) classification: [a]trivial (<0.2), [b]small (>0.2); [c]moderate (>0.50).

1.4.5. Peripheral fatigue by means of single-pulse evoked torque

The SP$_{ET}$absolute values also decreased significantly for all NMES protocols, but a greater decrease was observed for protocols with longer pulse duration (Table 1.1), with both effects for moment ($p<0.0001$) and pulse duration ($p=0.004$), as well as an interaction between moment and pulse duration ($p=0.012$). No effect was observed for ON/OFF times($p=0.414$).

The level of peripheral fatigue evaluated by means of ΔSP_{ET} (Table 1.1 and Figure 1.2) was significantly greater for longer pulse duration NMES protocols (2 ms), as an effect was observed for pulse duration ($p=0.044$),while no effect was observed for ON/OFF times ($p=0.579$).

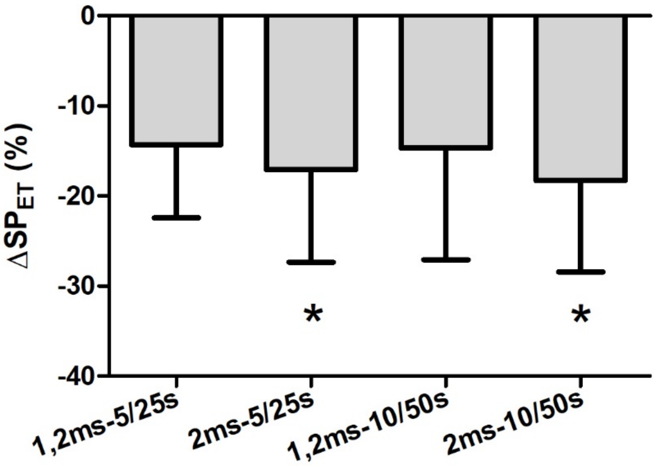

Figure 1.2. Relative change on single-pulse evoked torque (%); *Effect observed for pulse duration (p=0.044).

1.5. DISCUSSION

The purpose of the present study was to evaluate the NMES effects of pulse duration ($PD_{1.2}$ and $PD_{2.0}$) and of contraction/relaxation times ($ON/OFF_{5/25}$ and $ON/OFF_{10/50}$) over the CI level, mechanical overload, discomfort and muscle fatigue in healthy young subjects. Our hypothesis was in part confirmed, as $PD_{2.0}$ was effective in producing similar levels of evoked torque with lower maximal tolerated CI. Despite similar levels of mechanical overload were produced during the four NMES protocols, and no difference on fatigue was identified by means of MVICs, $PD_{2.0}$ resulted in a higher level of fatigue evaluated by means of ET. Furthermore, contraction/relaxation times during NMES had no effect over CI, discomfort, torque production or fatigue.

As previously stated, muscle response to conventional NMES application depends fundamentally on the anatomical properties of motor neuron distribution and branching (Lieber and Kelly, 1991). As axons have membranes with lower impedance than muscle fibers, and once they reach their threshold of excitability, an action potential will be triggered (Cairns *et al*., 2007). However, the nerves are constituted byneurons with axons of different diameters, and, consequently, different excitability thresholds. If stimulation parameters are kept constant, NMES imposes spatially fixed stimulation on the same population of axonal branches and muscle fibers, more superficial and close to the electrodes (Vanderthommen *et al.*, 2000).Therefore, in order to increase the number of fibers to be recruited, and, consecutively, the NMES mechanical overload, there must be an increase in the electrical charge delivered to the muscle (Liebano and Alves, 2009).However, increasing pulse amplitude will increase the current intensity in a more localized area, close to the stimulating electrodes, and recruit more nerve fibers with smaller diameters than a longer pulse low-intensity current (Grill and Mortimer, 1996).

When comparing the effects of pulsed currents, with pulse durations of 50 and 200 µs, on maximum CI tolerated NMES evoked torque, Scott et al. (2009) observed that a longer pulse duration was able to generate greater muscle torque with a lower CI. Similar results were presented by Gorgeyand Dudley (2008) when comparing two NMES protocols(100 Hz and 450 µs versus 60 Hz and 250 µs). In the same direction, the present study also demonstrates that NMES with longer pulse duration needs a much lower CI for torque production than NMES with shorter pulse duration. However, despite similar ΔCI and ΔNME were observed among the NMES protocols, NME_{CHARGE} was higher during $PD_{2.0}$ protocols. Hence, as no difference was observed between protocols with respect to $NMES_{ET}$(mechanical overload), these resultsshow that the use of $PD_{2.0}$was less efficient than $PD_{1.2}$ for torque production, as we had more electrical charge with the 2.0 ms pulse duration for a similar ET than that of the 1.2 ms pulse duration.Therefore, our results seems to agree with previous studies (Liebano and Alves, 2009; Scott *et al.*, 2009) that demonstrated that the torque production and level of discomfort during NMES were related to electrical charge, and they also show that there seems to exist a limit to the increase in pulse duration, after which further increases do not produce any additional benefit.

NMES application over the skin also excites(depending on current amplitude) pain receptors, such asfree nerve endings (A-delta and C fibres) located in dermal andsubdermal connective and adipose tissues, causing discomfort that limits mechanical overload increase (Alon and Smith, 2005). Liebano *et al*. (2013), evaluating discomfort produced by different pulse durations (800 µs, 1.4 ms and 2 ms, at 50 Hz) during NMES evoked contractions that produced similar mechanical overload (relative to 40% of

MVIC), found that discomfort slightly increased when a longer pulse duration was used. In disagreement to these findings, there was no difference in discomfort (VAS) reported by our subjects during the different NMES protocols in the present study, which produced similar $NMES_{ET}$. Furthermore, in the present studyVAS decreased (ΔVAS) in a similar degreefrom the beginning to the end of NMES protocols, despite CI was increased (ΔCI).This reduction in the subjects' reported discomfort is explained by a previous study that clearly demonstrated that subjectscan accommodate for the discomfort after repeated exposureto NMES by increasing pain receptors activation thresholds, and,consequently, increase their tolerance to CI levels(Alon and Smith, 2005).

In the present study, although there was no difference in the mechanical overload ($NMES_{ET}$) produced between the four NMES protocols, our results demonstrate that $PD_{2.0}$ allowed to achieve maximal evoked torques with lower CI levels. Also, as previously mentioned, despite the lower CI levels used with $PD_{2.0}$, similar levels of discomfort were observed during the four NMES protocols. Possibly, if the CI was kept constant, the increase in pulse duration from 1.2 to 2.0 ms would result in a proportional increase in the chargeper pulse, and hence, greater recruitment of motornerve fibers and higher $NMES_{ET}$ would occur (Gregory *et al*., 2007).However, CI increase should also determinea greater recruitment ofpain receptors and increased input through afferent fibers.Increase in pulse duration might cause a similar effect as that of increasing CI, as there is an increase in the total electrical charge or electrical energy when pulse duration is augmented. In practice, because $NMES_{ET}$ is dictatedby discomfort associated with the stimulation, CImight have decreased significantly with $PD_{2.0}$ possibly due to the increased electrical energy.However, as no difference in discomfort level was observed between the different pulse duration protocols, muscle inhibition through sensory mechanisms apparently did not play a role in our NMES protocols. In agreement to our findings, Liebano *et al*. (2013) found no difference in ET between NMES with different large pulse durations (800 µs, 1.4 ms and 2 ms), when NMES was performed at the maximal tolerated CI. Hence, it appears that, over the range of pulse durationsexamined in the present study, the similarty indiscomfort between the differentpulse durationssuggests that central inhibition did not play a role in $NMES_{ET}$.

Although a short pulse duration along with a long contraction time (5 to 10 s) has been previously proposed as being efficient in producing muscle strength (Lauffer *et al*., 2001), Georgey and Dudley (2008), when evaluating both the effects of pulse duration (250 and 450 μs) and different stimulation contraction/relaxation times (ON/OFFtimesof 3/3s and 10/20 s) on torque production, observed that the pulse duration, not the contraction/relaxationtimes, would be responsible for maximizing the torque during NMES. In the present study, during NMES protocols performed at the maximal tolerated CI, neither long pulse duration(> 1 ms) nor contraction/relaxation times had effect over $NMES_{ET}$.

On the other hand, our results are in agreement with several previous studies (McLoda and Camarck, 2000; Ward *et al*., 2004; Jubeau *et al*., 2008), as the different contraction/relaxation times (5/25 s and 10/50 s) used during NMES protocols with the samefixed duty-cycles (16%), had no observed effect over the level of fatigue. Despite the similar overload observed along NMES protocols, $PD_{2.0}$ produced higher levels of fatigue when evaluated by means of ET. One possible explanation would be that$PD_{2.0}$ electrical charge delivered to motor-neuron branches wasinsufficient to maximize the recruitment generated by NMES, but sufficientlylargeto causehigher peripheral neurotransmitter depletion (Ward *et al*., 2004). This finding is also supported by the NME_{CHARGE} values observated in the present study,which showless efficiency of $PD_{2.0}$, aseven a higher level of electrical energy delivered to the muscle was ineffective in increasing $NMES_{ET}$.

Currently, the independent effect of pulse duration on muscle fatigue is not clear, yet it has been shown that pulse duration modulation has less effect on muscle fatigue than frequency modulation (Kesar *et al*., 2008). Previously, the effect of the product of the frequency and pulse duration was tested on muscle fatigue and, after matching the initial peak torques, a combination of 20 Hz and 500 μs produced less fatigue compared to 50 Hz and 200 μs (Gregory *et al*., 2007). On the other hand, Gorgey *et al*. (2009) compared pulse durations of 450 μs and 150 μs and concluded that the longerpulse duration modestly increased torque per muscle active area but not muscle fatigue. However, that was not the case in the present study, as $PD_{2.0}$ delivered at a fixed frequency (80 Hz), produced the same level of $NMES_{ET}$ at a maximum tolerated (but lower) CI compared to $PD_{1.2}$, while a slightly higher level of fatigue was observed on PD_2 protocols by means of ET evaluation.

These findings could be explained by understanding the effect of pulse duration increase on the recruitment of motor units. It has been suggested that larger motor units could be incrementally recruited between 100 and 400 μs, resulting in a steep torque rise, followed by a gradual torque rise at longer pulse durations (Chou and Binder-Macleod, 2007). Jeon and Griffin (2018) found that during intermittent contractions of moderate forcelevels (25% MVC) at moderate stimulation frequencies(30 HZ), the use of long pulse durations(1 ms) with low current amplitude reduces fatigueand pain, and improves the recovery rate to a greaterextent than shorter pulse durations (200 μs) withhigher current amplitudes.However, it is still not clear whether even longer pulse durations(>1 ms) would be effective in torque production during NMES programs. So in the present study, despite a longer pulse duration of 2.0 ms (phase duration of 1.0 ms) produced higher levels of fatigue compared to a pulse duration of 1.2 ms (phase duration of 600 μs), 10-minute NMES protocols produced similar levels of mechanical overload possibly because both $PD_{1.2}$ and $PD_{2.0}$ were at the “plateau region”(>400 μs) of the pulse duration x evoked force relationship (Ward *et al.*, 2004).

As suggested by Collins et al. (2007), longer pulse durations combined with high frequency stimulation (>80 Hz) would increase NMES evoked force by contribution of central mechanisms to motor unit recruitment, allowing to deliver the stimulation at lower current intensities. Unfortunately,as no central mechanism was directly measured during the present study (M-wave and H-reflex response), is not possible to know if central mechanisms in fact participated during NMES on increasing muscle recruitment, and consecutively, torque production.

The present study was conducted in healthy subjects without motor impairments, which limits its generalization to clinical rehabilitation. Further NMES studies should aim to include impaired populations who could actually benefit from a NMES-based program. Furthermore, NMES investigations should evaluate the effect of submaximal NMES protocols with longer pulse durations over evoked torque and fatigue.

1.6. CONCLUSION

Although there was no difference between mechanical overload produced during NMES protocols performed at maximal tolerated CI, $PD_{2.0}$ allowed for the use of a lower CI. That would be a useful information regarding the limitations of maximal CI delivered by some equipment designed for clinical purposes. Lastly, $PD_{2.0}$ produced higher levels of fatigue evaluated by means of ET, which is not desired if the goal of a NMES program is to produce continuous mechanical overload with lower metabolic cost. Therefore, we suggest that 1.2 ms pulse duration should be used instead of 2.0 ms pulse duration NMES protocols due to its lower neuromuscular efficiency e higher muscle fatigue.

FUNDING

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APRESENTAÇÃO: CAPÍTULO 2

No capítulo anterior foram testados os efeitos da duração de pulso e do tempo de contração-relaxamento da EENM sobre a produção de torque, desconforto e fadiga muscular de indivíduos saudáveis, com o objetivo de melhorar a eficiência de programas de reabilitação a serem realizados com pacientes adultos críticos. Contudo, informações a respeito dos efeitos cardiovasculares da EENM ainda são escassos. Muitos dos pacientes internados em unidades de terapia intensiva sofrem de doenças cardiovasculares que podem ser beneficiadas pelo efeito sistêmico provocado pela EENM. Aumento da função cardiovascular decorrente de protocolos de reabilitação de NMES pode facilitar a condução e administração de determinado medicamento nos diferentes tecidos do organismo, assim como facilitar a eliminação de produtos do metabolismo indesejáveis e que não deveriam permanecer por muito tempo em nosso organismo. Além disto, sabe-se que o adulto crítico sofre perda gradativa da função cardiovascular e do controle autonômico durante o período em que permanece acamado na unidade de terapia intensiva.Portanto, avaliar os efeitos da EENM sobre o sistema cardiovascular em sujeitos saudáveis é fundamental (e talvez o primeiro passo) a fim de que possamos entender todos os possíveis benefícios dessa modalidade terapêutica na reabilitação de pacientes que se encontram em repouso no leito. O capítulo apresentado a seguir tem como objetivo avaliar os efeitos de parâmetros temporais (contração/relaxamento) da EENM sobre o controle autonômico de indivíduos saudáveis.

EFFECT OF NEUROMUSCULAR ELECTRICAL STIMULATION CONTRACTION/RELAXATION TIMES ON HEART RATE VARIABILITY IN HEALTHY SUBJECTS

RESUMO

INTRODUÇÃO: O presente estudo teve como objetivo avaliar o efeito de dois protocolos de estimulação elétrica neuromuscular (EENM) sub-máximos com tempos de contração/relaxamento (tempos ON/OFF) distintos,sobre a variabilidade da frequência cardíaca (VFC) em indivíduos sadios. MÉTODOS: 24 participantessaudáveis (12 ♂ e 12 ♀, 26,6±4,8 anos) foram submetidos a dois protocolos de EENM (20 min de duração) com tempos ON/OFF de 5/25 s (EENM$_{5/25}$) e 1/5 s (EENM$_{1/5}$), realizados bilateralmenteem uma UTI simulada. Um monitor cardíaco digital foi colocado na região do tórax e cada sujeito descansou por 10 minutos, a fim de estabelecer os valores basais da VFC. Antes dos protocolos de EENM, cada participante realizou a avaliação da força máxima dos músculos extensores do joelho, tanto pela contração isométrica voluntária máxima (CVMI), quanto pelo torque evocado supramáximo pelo duplo-pulso (TE). O nível de corrente da EENM foi ajustado para produção e manutenção do torque igual a 50% do TE previamente avaliado. A VFC foi avaliada e registrada ao longo dos protocolos de EENM, por meio da análise de períodos de 5 min obtidos da segunda metade do período de repouso (10 min) e da segunda metade dos protocolos de EENM (20 min). Para a análise na VFC no domínio do tempo, calculou-se a média da frequência cardíaca (MFC) e a média dos intervalos RR (MRR). Para a análise no domínio de frequência, os componentes de potência de baixa frequência (BF) e alta frequência (AF), seguidos de sua razão (BF/AF), foram calculados. Imediatamente após os protocolos de EENM, o TE e a CVMI de cada membro inferior foram novamente avaliados para determinar o nível de fadiga produzido. A intensidade de corrente (IC) e o respectivo desconforto produzido (EVA) foram registrados ao início e ao final dos protocolos de EENM. RESULTADOS: A MFC aumentou, enquanto a MRR reduziu (efeito observado para momento: p=0,049 e p<0,0001, respectivamente) nos protocolos de EENM. Houve aumento daBF durante ambos os protocolos (efeito observado para momento: p=0,001), mas o aumento de BF foi maior durante o EENM$_{5/25}$ (tempos ON/OFF: p<0,0001). O componente de AF apresentou diminuição tanto para a EENM$_{5/25}$ quanto para a EENM$_{1/5}$, mas observou-se maior decréscimo para a EENM$_{5/25}$ (efeito observado: momento, p=0,001; tempos ON/OFF, p<0,0001). Consequentemente, BF/AF aumentou tanto em NMES$_{5/25}$ quanto em EENM$_{1/5}$, mas o aumento maior foi encontrado para EENM$_{5/25}$ (efeito observado: momento, p=0,008; tempos ON/OFF, p=0,001). Para ambos os protocolos de EENM, a IC foi consideravelmente aumentada durante ambos protocolos de EENM para ambos os membros inferiores (efeito observado para momento: p<0,0001). EVA relatada pelos sujeitos aumentou ligeiramente do início para o final dos protocolos (efeito observado para momento: p<0,0001). O torque absoluto produzido durante os dois protocolos de EENM foi maior para o membro direito dos indivíduos (efeito observado para membro inferior:

p=0,004). A CVMI diminuiu significativamente após ambos os protocolos (efeito observado para momento: p<0,0001), mas a redução foi maior para o membro direito (efeito observado para membro inferior: p<0,0001). ΔCVMI foi maior para $EENM_{5/25}$ em comparação com $EENM_{1/5}$ (efeito observado para tempos ON/OFF: p=0,022). Os valores absolutos de TE diminuíram significativamente para ambos os protocolos de EENM, com maior redução para o membro inferior esquerdo (efeito observado: momento, p<0,0001; membro inferior, p=0,003). ΔTE não foi diferente entre os protocolos $EENM_{5/25}$ e $EENM_{1/5}$ (efeito observado para tempos ON/OFF: p=0,109), mas foi maior para o membro direito em ambos os protocolos de EENM (efeito observado para membro inferior: p<0,0001). CONCLUSÃO: O aumento do tempo de contração/relaxamento da EENM de 1/5 s para 5/25 s aumentou a razão BF/AF, sugerindo um aumento na atividade simpática em indivíduos saudáveis.
Palavras-chaves: Estimulação elétrica neuromuscular, variabilidade da frequência cardíaca.

ABSTRACT

INTRODUCTION: The present study aimed to evaluate the effect of two submaximal NMES protocols with contraction/relaxation times (ON/OFF times) of 5/25 s and 1/5 s, respectively, over the heart rate variability (HRV) of healthy subjects. METHODS: Twenty-four healthy participants (12 ♂ and 12 ♀, 26.6±4.8 years) were submitted to two 20-min NMES protocols with ON/OFF times of 5/25 s ($NMES_{5/25}$) and 1/5 s ($NMES_{1/5}$), performed bilaterally in a simulated ICU setup. A digital heart rate monitor was placed in the chest region and each subject rested for 10 minutes in order to establish baseline HRV values. Before NMES protocols, each subject performed the evaluation of the maximum knee extensor muscles strength, both through the maximal voluntary isometric contraction (MVIC) as through the supramaximal doublet-pulse evoked torque (ET). NMES current level was adjusted for a production and maintenance of torque equal to 50% of the ET previously evaluated. HRV was assessed and recorded throughout the NMES protocols analyzed though 5-min periods obtained at the second half of the 10-min rest period and at the second half of 20-min NMES protocols. For HRV time domain analysis, mean heart rate (MHR) and mean RR intervals (MRR) were calculated. For HRV frequency domain analysis, low frequency (LF) and high frequency (HF) power components, followed by their ratio (LF/HF), were calculated. Immediately after the NMES protocols, the ET and MVIC of each lower limb were again evaluated in order to evaluate the fatigue level produced. The discomfort produced at the beginning and at the end of NMES protocols was evaluated using the visual analogue scale (VAS). RESULTS: MHR was increased (moment: p=0.049), while MRR was reduced (moment: p<0.0001) during the NMES protocols. There was an increase in LF power component during both $NMES_{5/25}$ and $NMES_{1/5}$ (observed effect for moment: p=0.001) with a greater increase on LF for $NMES_{5/25}$(ON/OFF times: p<0.0001). The HF component showed a decrease for both $NMES_{5/25}$ and $NMES_{1/5}$, but a higher decrease for $NMES_{5/25}$ was observed (observed effect: moment, p=0.001; ON/OFF times, p<0.0001). LF/HF increased for both $NMES_{5/25}$ and $NMES_{1/5}$, but a much larger increase was found for $NMES_{5/25}$ (observed effect: moment, p=0.008; ON/OFF times, p=0.001). For both NMES protocols, the CI was considerably increased from

PRE to END for both lower limbs (observed effect for moment: $p<0.0001$). The VAS reported by the subjects during NMES slightly increase from PRE to END in a similar extent for both lower limbs (observed effect for moment: $p<0.0001$). The absolute torque produced during both NMES protocols was slightly higher for the subjects right limb (observed effect for lower limb: $p=0.004$). MVIC decreased significantly in both protocols (observed effect for moment: $p<0.0001$), but in a greater extent for the right limb (lower limb: $p<0.0001$). ΔMVIC was greater for NMES$_{5/25}$ compared to NMES$_{1/5}$ protocol (observed effect for ON/OFF times: $p=0.022$). Absolute ET values decrease significantly for both NMES protocols, but in a greater extent for the left lower limb (observed effect: moment, $p<0.0001$; lower limb, $p=0.003$). ΔET was not different between NMES$_{5/25}$ and NMES$_{1/5}$ protocols (observed effect for ON/OFF times: $p=0.109$), but was higher for the right limb in both NMES protocols (observed effect for lower limb: $p<0.0001$). CONCLUSION: The increase in NMES contraction/relaxation times from 1s/5s to 5s/25s largely increased LF/HF ratio, suggesting an increase on sympathetic activity in healthy subjects.

Key-words: Neuromuscular electrical stimulation, heart rate variability.

2.1. INTRODUCTION

Neuromuscular electrical stimulation (NMES) is applied to muscles motor-points to prevent intensive care unit (ICU) acquired weakness in hospitalized critically ill patients (Gruther *et al*., 2010; Karatzanos *et al*., 2012; Hough *et al*., 2013; Maffiuletti *et al*., 2013). In order to improve the effectiveness of NMES-based rehabilitation programs, most studies have been carried out aiming to understand the effects of the NMES current parameters over muscle evoked force (Gregory *et al*., 2007; Gorgey & Dudley, 2008), discomfort (Ward & Lucas-Toumbourou, 2007), muscle fatigue (Chou *et al*., 2007; Gorgey *et al*., 2009) and metabolic demand (Muthalib *et al*., 2016). However, although several studies have observed an acute cardiovascular systemic response to NMES in healthy subjects (Lyons *et al*., 2002; Moloney*et al*., 2006; Theurel *et al*., 2006; Aldayel *et al*., 2010; Hsu*et al*., 2011) and ICU patients (Gerovasili *et al*., 2009), the NMES parameters' effects are still not clear due to the variety of parameters used in these studies. Understanding which parameters would be more adequate and safe is fundamental for the development of more effective NMES-based rehabilitation programs, especially for patients suffering from cardiovascular disease.

As the heart receives double innervation corresponding to the sympathetic and parasympathetic nervous systems, heart rate variability (HRV) is modulated by information from baroreceptors, chemoreceptors, respiratory system, vasomotor system, thermoregulatory system and renin-angiotensin-aldosterone system (Marâes, 2009). This complex interaction of the cardiac autonomic nervous system regulates the cardiovascular system to respond to various physiological and pathological stimuli, increasing the HRV in order to maintain the body's homeostasis (Ernst, 2017). It has been recently reviewed that conventional exercise can benefit heart failure affected individuals (Pearson & Smart, 2018), improving autonomic activity evaluated through HRV. Previously, methods of HRV analysis have also been used to evaluate the acute effect of NMES on HRV of both healthy subjects (Kang & Hyong, 2014) as well as heart disease patients (Nicolodi *et al*., 2016; Tanaka *et al*., 2016).

HRV analysis measures the variability of the intervals between each heart beat obtained by analyzing successive series of RR intervals from periods of at least 5-min of the electrocardiographic signal. As a periodic biological signal, its analysis can be performed in time and frequency domains. In the time domain, it is possible to calculate the mean heart rate values (HRM - bpm / min) and mean RR interval (MRR - ms). The frequency domain analysis allows obtaining the power spectral density, which consists of the decomposition of the RR signal into a sum of sinusoidal waves of different amplitudes and frequencies (Ernst, 2017). Some studies divide the power spectrum into two regions: the low frequency component (LF) ranges from 0.04 to 0.15 Hz and expresses the intensity of the sympathetic modulation on the heart, while the high frequency component (HF) have limits ranging from 0.15 to 0.40 Hz and represents the vagal modulation acting on the sinoatrial nodule (Marães, 2009). Taking these two components together, the LF/HF ratio is commonly used in order to present autonomic control balance in a single value (Pearson & Smart, 2018). According to the Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology standard, LF/HF ratio values acquired from 5-min measurements during supine resting ranges from 1.5 to 2.0 in healthy subjects (Heart Rate Variability, 1996).

To date, only three studies that evaluated the acute effect of NMES over HRV were found (Kang & Hyong, 2014; Nicolodi *et al*., 2015; Tanaka *et al*., 2016). Evaluating heart failure patients, the study conducted by Nicolodi *et al*. (2016) showed that NMES applied to the quadriceps muscle led to a significant increase in the MRR and a significant reduction MHR followed by a decrease in the autonomic balance (LF/HF), due to a decrease in sympathetic nervous system activity. In disagreement, Tanaka *et al*. (2016) evaluated patients with acute myocardial infarction and showed that the LF component of HRV increased significantly during a NMES session performed at maximal tolerable current intensity. However, no significant changes were observed in the HRV-HF component. These findings suggest that sympathetic activity was enhanced during NMES. In the only study found evaluating the NMES effects over healthy subject's HRV, Kang and Hyong (2014) stated that low intensity NMES would slightly increase the LF/HF ratio, but they failed to find significant changes. According to their results, the NMES-evoked muscle strengthening exercise is safe as apparently it does not affect the sympathetic or parasympathetic nervous systems in healthy subjects. Overall, besides the lack of agreement, the aforementioned studies not only used different NMES parameters (electrode placement, contraction/relaxation time, current wave form, frequency, intensity and pulse duration), as had no concern to the level of force or fatigue produced, which is fundamental for proper dosage of NMES interventions (Maffiuletti *et al.*, 2010). This inconsistency found in literature makes impossible to conclude which are the best parameters for safe muscle strengthening NMES-based programs, either with the goal of promoting a cardiovascular response or avoiding cardiovascular overload in unstable critically ill patients.

2.2. PURPOSE

The present study aimed to evaluate the effect of two submaximal NMES protocols with contraction/relaxation times (ON/OFF times) of 5/25 s and 1/5 s, respectively, over the HRV of healthy subjects in a simulated ICU setup. In order to control the influence of possible intervenient variables, this study also evaluated: (1) muscle fatigue assessed by maximal voluntary isometric contractions (MVIC); (2) muscle fatigue assessed by the torque evoked by supramaximal electrical stimuli (ET); (3) the current intensity used during NMES (CI); and (4) the respective discomfort perceived during NMES (Visual Analogue Scale - VAS). The hypothesis of the present study is that the reduction of both contraction/relaxation times (ON/OFF times), and the consequent increase in the number of contractions produced during the 1/5s NMES protocol, should result in a greater change in HRV parameters, even with no differences observed in the level of current intensity, discomfort, mechanical overload and muscle fatigue produced.

2.3. METHODS

The evaluations took place in the Neuromuscular Plasticity Department of the Exercise Research Laboratory (LAPEX, Porto Alegre, Brazil) of the School of Physical Education, Physiotherapy and Dance (ESEFID - UFRGS). This study used a methodology approved by the University’s Research Ethics Committee (CAEE nº 36588914.4.1001.5347). The subjects read the consent form and signed it after clarifying possible doubts with the responsible researcher.

2.3.1. Sample

Twenty-four healthy physically active young subjects participated in the study (12 men and 12 women, mean age 26.6 ± 4.8 years). Participants were enrolled in the university's undergraduate and graduate courses where the study was conducted. Recruitment occurred by means of dissemination in social networks and by invitation made by the researchers in classrooms. The study did not include subjects who presented (1) lower limb traumatic injury, (2) any cardiovascular disease, (3) any central or peripheral neurological disease, or (4) any clinical contraindications to the tests' performance. The number of subjects was chosen through the following equation that indicates the sample size according to the tolerated measurement error for variables evaluated, where *n* is

$$n=Z^2*dp^2/e^2$$

the sample size, *Z* is the tabled value related to the significance level of this study (1.96 for $\alpha = 0.05$), *dp* is the standard deviation of the variable in question, obtained through the specific literature, and *e*, the tolerated measurement error (estimated in 5%) and applied over the mean value of the variable in question obtained from the literature. The obtained sample number, through the HRV index mean and standard deviation values (14.3 ± 1.1 Nm) from a study that evaluated a similar population (healthy men, 22 ± 2 years old) (Kang & Hyong, 2014), was doubled as the present study aimed to evaluate both men and woman. The calculated sample size was increased in 20% for eventual sample loss.

2.3.2. Procedures

The subjects attended the laboratory on three separate days, a week apart (Figure 2.1). On the first day, participants performed a familiarization protocol with the tests. After being placed on the stretcher and fixed in the dynamometry system, subjects performed three maximal voluntary isometric contractions (MVICs) of the knee extensor muscles of both lower limbs. The quadriceps muscle motor point of both lower limbs was then located by NMES, and assessed for the NMES current level required to generate a supramaximal electrical stimulus delivered in the form of doublet-pulses (interpulse interval = 0.8 ms). After determining the current level, supramaximal stimuli were applied to evaluate the evoked torque (ET) basal level by three doublet-pulses electrical stimulation. Immediately after the test, subjects were submitted to a bilateral NMES protocol in the form of symmetrical biphasic rectangular pulsed current (80 Hz frequency, 1 ms pulse duration, 5s/25 s ON/OFF time) applied on the knee extensor muscles, with a 10 min duration and with sufficient current intensity to produce contractions that represented 50% of the knee extensors ET. At the end of the NMES protocol, fatigue was assessed by means of a new evaluation of MVIC and doublet-pulse ET.

On the second and third days, the subjects were submitted to two NMES protocols with different stimulus and rest times (time ON/OFF = 5/25 s and 1/5 s, respectively) performed in a randomized fashion with a 20-minutes duration. Once positioned on the stretcher, a digital heart rate monitor was placed in the chest region and each subject rested for 10 minutes in order to establish HRV baseline values. Each subject performed again the evaluation of the knee extensor muscles maximum strength both through the MVICs and through the ET. The subjects were then submitted to one of the two NMES protocols, in which the current level was adjusted to produce and maintain a torque level equal to 50% of the ET previously evaluated during the supramaximal doublet-pulse ET. HRV was assessed and recorded throughout the NMES protocol on the two evaluation days. Immediately after the NMES protocols, the ET and MVIC of each lower limb were again evaluated in order to evaluate the level of fatigue produced. The discomfort produced at the beginning and at the end of NMES protocols was evaluated using the Visual Analogue Scale (VAS).

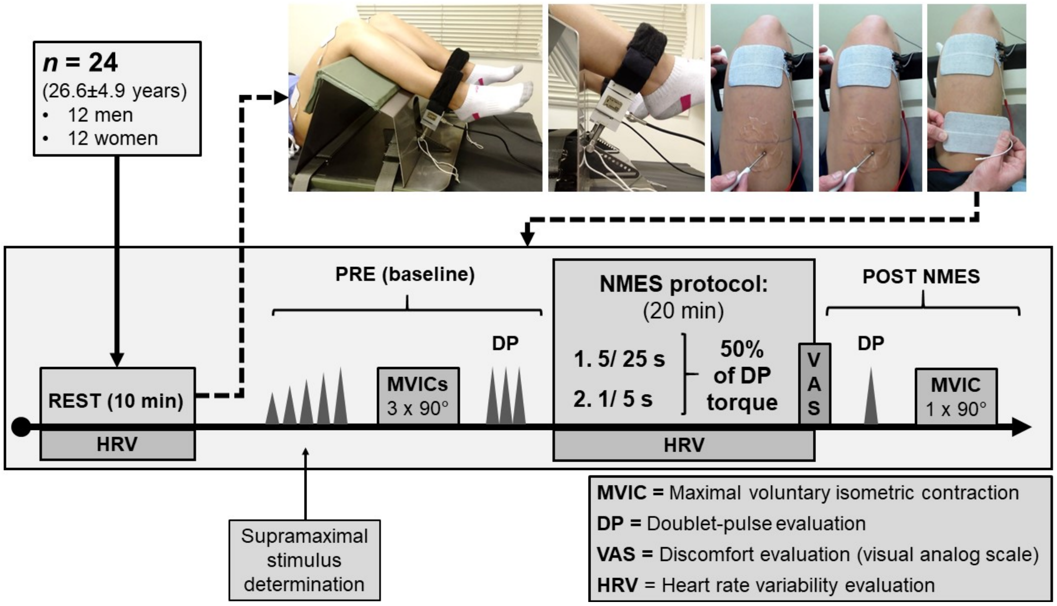

Figure 2.1. Procedures. Sample size, motor-point localization, electrode positioning, dynamometer fixation (top) and evaluation protocol (bottom).

2.3.3. Evaluation of heart rate variability

Heart rate variability (HRV) evaluation was performed by recording the heart rate at the beginning of the tests, where the participant was rested for 10 min for HRV levels to return to baseline values, and during the 20 mins NMES protocols. To obtain the records, a Polar heart rate monitor (POLAR RS800CX, USA) was placed on the subjects' precordium region, and fastened by a belt, with an elastic strap to its back, for proper fixation. The data was recorded and later emitted by infrared light to its interface, which allowed the connection between the monitor and the computer. Through dedicated software (Polar Precision Performance, POLAR, USA) it was possible to extract the HRV data. Data analysis was done by studying the normal RR intervals extracted from the records of the heartbeats imported into *Kubios* software (version 3.0.2, Finland). From these records, analyzes were performed in the domains of (1) time, by means of both the mean heart rate (MHR – beats/min) and the mean values of the intervals between the R-waves (MRR - ms);and (2) frequency, by calculating both the spectral power values of the low frequency (LF) and high frequency (HF) components (normalized unit; n.u.), as the LF/HF ratio obtained from the RR intervals. Windows with a duration of 5 min were analyzed, drawn from both the second half of the REST period, at the beginning of the tests (MRR_{REST}, MHR_{REST}, LF_{REST}, HF_{REST} and LF/HF_{REST}) and 5 min within the second half of the NMES protocols (MRR_{NMES}, MHR_{NMES}, LF_{NMES}, HF_{NMES}, and LF/HF_{NMES}).

2.3.4. Evaluation of the knee extensors mechanical properties

The force production capacity of the femoral quadriceps muscle was evaluated in the present study through isometric tests, using a stainless steel structure instrumented with a pair of load cells from the *Miotool* data acquisition system (Miotec, Brazil -Figure 2.1) which possesses a dedicated software. The steel structure was fixed to the stretcher with special bands. The subjects were placed in dorsal decubitus with knees at 90° and hips at 45° flexion (0 ° = full extension). A pair of special clips were attached to the load cells and attached to the distal ends of both legs, perpendicular to the tibia's surface, 5 cm above the medial malleolus (Figure 2.1).

Subjects were attached to the stretcher by a special band in the waist region (below the iliac crest) so that no movement occurred during contractions. The lateral femur condyle represented the knee joint center of rotation, and its distance from the dynamometer support point was recorded for the calculation of the produced extensor torque (Nm). The angles in which the hip and knee joints were positioned were checked by means of manual goniometry to ensure identical positioning on the two testing days, and all other adjustments made to the patient's positioning on the stretcher were recorded and reused in subsequent evaluations to avoid measurement errors.

2.3.5. Evaluation of maximum voluntary isometric contraction

The assessment of maximal voluntary isometric contraction (MVIC) was performed before and immediately after the NMES protocols, on the three evaluation days. At the beginning of the evaluations, the subjects performed three knee extensor MVICs, with each lower limb, maintaining the contractions for a period of 5 s. The subjects were instructed to perform the contractions without any visual feedback, rapidly increasing the effort until they reached maximal torque production, which should be maintained until a verbal command to cease the contraction was given. The contractions were performed alternately between the limbs, with a 2 min interval between contractions. The highest torque value recorded during the three MVICs was adopted as the basal maximum torque ($MVIC_{PRE}$) for each evaluated limb. Immediately after the end of the NMES protocol, the subjects performed a new knee extensor MVIC ($MVIC_{POST}$), in order to verify the presence of fatigue. Mean and standard deviation absolute values of $MVIC_{PRE}$ and $MVIC_{POST}$ values were calculated for the whole group. Fatigue was also determined by the relative reduction of MVIC torque (ΔMVIC) presented in percent values (%).

2.3.6. Motor point location and NMES electrodes positioning

The motor point location, ET evaluation and NMES protocols were performed with a clinical electrical stimulator developed by the Laboratory of Biomedical Engineering of the Porto Alegre Clinical Hospital (Schildt *et al*., 2016). Rectangular self-adhesive electrodes with saline gel (Arktus, Canada), measuring 7.5 cm in width and 13 cm in length, were positioned on the motor point of the quadriceps muscle and on the distal portion of the muscle (five centimeters from the upper edge of the patella).To determine the exact quadriceps muscle motor point location, a pen-shaped electrode was first connected to the proximal pole of the stimulator. After applying saline gel to the skin covering the mid-proximal portion of the thigh muscles, the pen-shaped electrode was placed in contact with the skin, applying a current of 1 Hz, pulse duration of 100 µs and sufficient intensity to produce a measurable contraction of the knee extensor muscles (Figure 2.1).

The subjects performed the procedure lying on the stretcher, with the lower limbs on the dynamometry system, with knee positioned at 90º of flexion and ankles attached to the load cells. The location where the electric pulse generated the greatest torque production seen in the acquisition system was defined as the quadriceps motor point.The electrodes positioning (Figure 2.1) was recorded on an acetate sheet for identical positioning on the two NMES evaluation days.

2.3.7. Fatigue evaluation by evoked torque

For the assessment of fatigue through evoked torque (ET), knee extensor NMES-evoked contractions were produced by double-pulses, with a pulse duration of 2 ms (duration of 1 ms phase) and 8 ms between-pulses interval. Firstly, doublet-pulses were applied gradually increasing the current intensity (maximum 180 mA), providing intervals of 3 to 5 s between the contractions, until an increase of evoked torque was no longer observed. The NMES current level needed to reach the peak ET was increased by 10% in order to guarantee the application of a supramaximal stimulus during the ET evaluation. Three doublet-pulses were applied before the start and one immediately after the NMES protocol. The subjects were instructed not to perform voluntary contractions during the tests. The mean peak value of the three doublet-pulses obtained before fatigue (ET_{PRE}) was calculated, and the peak value of the doublet-pulse applied immediately after the NMES protocols (ET_{POST}) was used. The absolute mean and standard deviation values of ET_{PRE} and ET_{POST} were then calculated for the entire group, for each of the two tested protocols. For determination of fatigue, the relative reduction of ET from ET_{PRE} to ET_{POST} (ΔET) was also calculated and presented in percent values (%).

2.3.8. NMES protocols

In order to perform the NMES protocols, the electrical stimulator was adjusted to emit alternating symmetrical biphasic current with 80 Hz stimulation frequency and 1 ms pulse duration (phase duration of 500µs). NMES was applied bilaterally to the quadriceps muscle motor point, in an alternately way for 20 min. The current intensity (mA) was increased until 50% of the supramaximal doublet-pulse ET was reached. The difference between the NMES protocols parameters consisted of changing the stimulus contraction and relaxation times (ON/OFF times). In protocol 1, contractions lasting 5 s (accompanied by 2 s ramp up intensity increase and 1 s relaxation ramp down) were performed, followed by 25 s rest intervals ($NMES_{5/25}$). In protocol 2, contractions of 1 s duration were produced (accompanied by 1 s ramp up intensity increase and 1 s relaxation ramp down) followed by a 5 s rest interval ($NMES_{1/5}$). The $NMES_{5/25}$ and $NMES_{1/5}$ protocols were performed in randomized order, and both protocols generated a total stimulus time of 10 s/min for each lower limb. The mean absolute torque ($NMES_{ABS}$) and relative to MVIC torque ($NMES_{REL}$) values produced during $NMES_{5/25}$ and $NMES_{1/5}$, followed by their respective standard deviation values, were calculated for the whole group. In addition, the current intensity (mA) values determined before (CI_{PRE}) and recorded at the end (CI_{END}) of $NMES_{5/25}$ and $NMES_{1/5}$ fatigue protocols were recorded. The mean and standard deviation CI_{PRE} and CI_{END} values were calculated for the entire group for both NMES protocols.

2.3.9. Evaluation of the discomfort generated by the NMES

Subjects were evaluated for the NMES discomfort through a visual analogue scale (VAS), after the determination of current intensity before NMES protocols (VAS_{PRE}) and at the end of both $NMES_{5/25}$ and $NMES_{1/5}$ (VAS_{END}). VAS consists of a straight line of 10 centimeters, the left end being no discomfort (0 cm) and the right end being the maximum pain imaginable (10 cm). The subjects marked on the line with a pen, indicating the level of discomfort experienced for each of the lower limbs. The level of discomfort was defined by the measure in centimeters between the zero value and the pen mark made by the subject. The mean and standard deviation values of VAS_{PRE} and VAS_{END} of all participants were calculated for $NMES_{5/25}$ and $NMES_{1/5}$.

2.3.10. Statistical Analysis

To verify the data normality, a Shapiro-Wilk test was used. To verify data homogeneity and sphericity, Levene and Maucly’s test were used, respectively. To verify if there were differences in the mechanical overload ($NMES_{ABS}$ and $NMES_{REL}$) produced during $NMES_{5/25}$ and $NMES_{1/5}$, a two-way repeated measures ANOVA was used, using the NMES protocols ON/OFF times and the evaluated lower limb (right and left) as factors. In order to verify the effects of $NMES_{5/25}$ and $NMES_{1/5}$ mechanical overload on MRR, MHR, LF, HF and LF/HF, a two-way repeated measures ANOVA was performed, using ON/OFF times and moments (REST and NMES) as factors. A three-way ANOVA was used to evaluate the effect of $NMES_{5/25}$ and $NMES_{1/5}$ mechanical overload over CI, VAS, MVIC and ET using as factors ON/OFF times, moments (PRE and END or

PRE and POST) and evaluated lower limb (right and left). To evaluate the effect of $\text{NMES}_{5/25}$ and $\text{NMES}_{1/5}$ mechanical overload over ΔMVIC and ΔET, a repeated measures two-way ANOVA, using as factors mechanical overload and lower limb evaluated, was performed. To verify the clinical relevance of eventual differences found between moments, the effect size by meand of Cohen's *d*was calculated adopting the following criteria: < 0.2: trivial; > 0.2: small; > 0.50: moderate; and > 0.80: large (Cohen, 1988). A significance level of 5% was used (Software SPSS version 20.0).

2.4. RESULTS

2.4.1. Heart rate variability to NMES

On the time domain analysis, MHR was increased (Figure 2.2), as an effect was observed for moment (p=0.049), while concomitantly, MRR (Figure 2.3) was reduced (effect observed for moment, p<0.0001). No effect was observed for ON/OFF times for MHR (p=0.353) or MRR (p=0.244) comparisons. Effect sizes between moments were large for both MHR and MRR (Table 2.2).

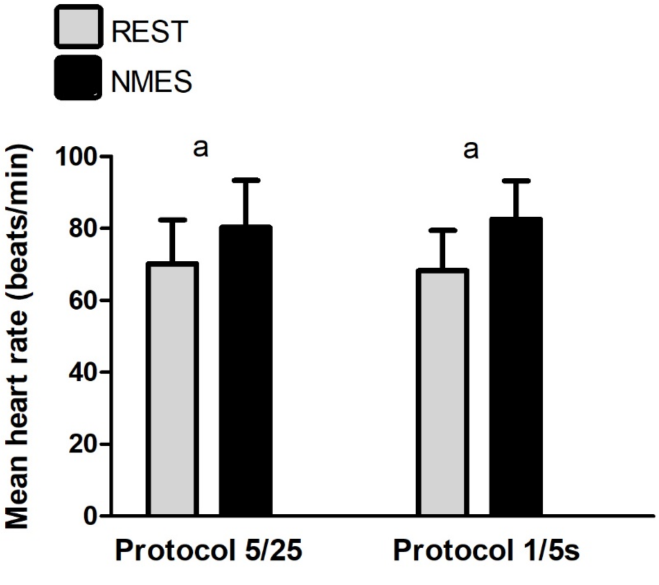

Figure 2.2. Mean heart rate (beats/min). [a]Effect observed for moment (p=0.049).

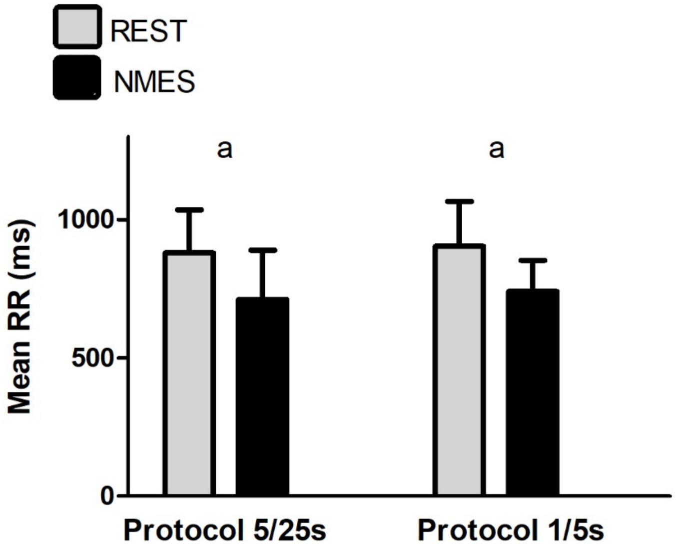

Figure 2.3. Mean RR (ms). [a]Observed effect for moment ($p<0.0001$).

On the frequency domain analysis, there was an increase in LF power component during both $NMES_{5/25}$ and $NMES_{1/5}$ (Figure 2.4), as an effect was observed for moment ($p=0.001$). However, $NMES_{5/25}$ showed a greater increase on LF power (Table 2.2), as both an effect for ON/OFF times ($p<0.0001$) and an interaction between moment and ON/OFF times ($p=0.027$) were observed.

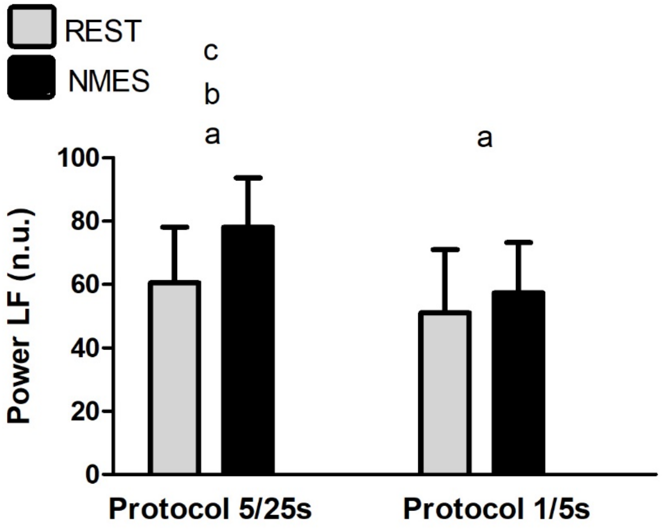

Figure 2.4. Power LF (n.u.). [a]Effect observed for moment ($p=0.001$); [b]Effect observed for ON/OFF times ($p<0.0001$); [c]Interaction between moment and ON/OFF times ($p=0.027$).

Concomitantly, the HF component showed a decrease for both $NMES_{5/25}$ and $NMES_{1/5}$, but a higher decrease for $NMES_{5/25}$ was observed (Figure 2.5), as an effect both for moment ($p=0.001$) and ON/OFF times ($p<0.0001$), followed by an interaction between moment and ON/OFF times ($p=0.028$) were observed. Those results were confirmed by the large and small effect sizes found between moments for $NMES_{5/25}$ and $NMES_{1/5}$, respectively, for both LF and HF power components (Table 2.2).

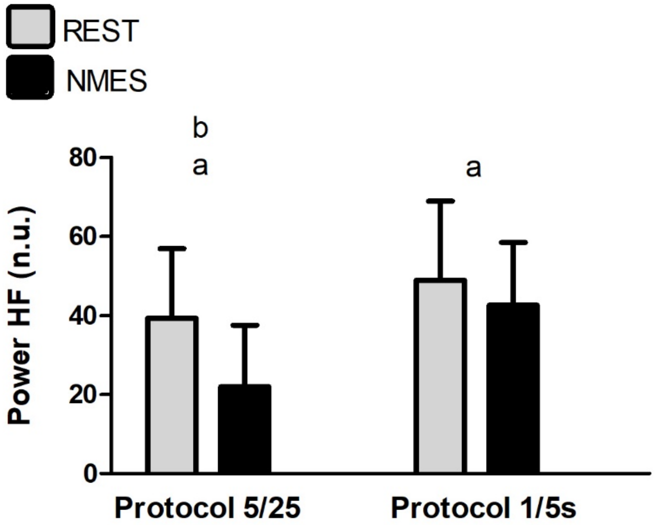

Figure 2.5. Power HF (n.u.). [a]Effect observed for moment ($p=0.001$); [b]Effect observed for ON/OFF time ($p<0.0001$). [c]Interaction between moment and ON/OFF times ($p=0.028$).

Taking LF and HF power components together, LF/HF increased for both $NMES_{5/25}$ and $NMES_{1/5}$, but a much larger increase was found for $NMES_{5/25}$ (Figure 2.6), as an effect for moment ($p=0.008$) and ON/OFF times ($p=0.001$), followed by an interaction between moment and ON/OFF times ($p=0.017$), were observed. In addition, a large effect size was observed for $NMES_{5/25}$, while a trivial effect size was observed for $NMES_{1/5}$ between moments (Table 2.2).

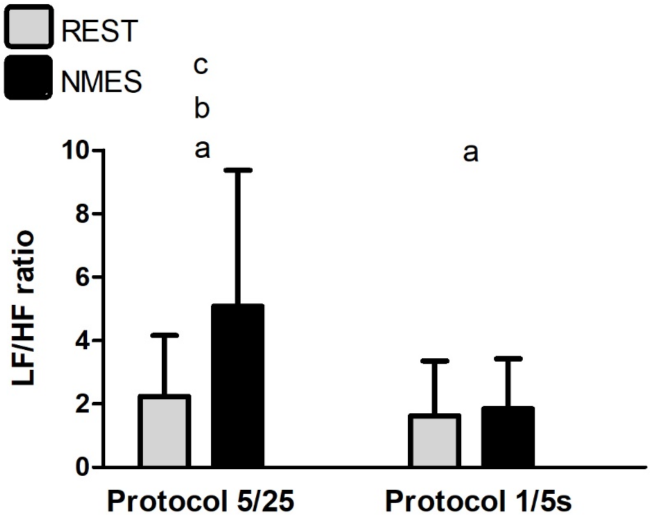

Figure 2.6. LF/HF ratio. [a]Effect observed for moment (p=0.008); [b]Effect observed for ON/OFF time (p=0.001); [c]Interaction between moment and ON/OFF times (p=0.017).

2.4.2. NMES current intensity

For both NMES protocols, the CI was considerably increased from PRE to END and for both lower limbs (effect for moment, p<0.0001 - Table 2.1) as showed by the large effect sizes observed for $NMES_{5/25}$ and $NMES_{1/5}$ (Table 2.2). No effect for both ON/OFF times (p=0.69) and lower limb (p=0.136) were observed.

2.4.3. Discomfort to NMES

The VAS reported by the subjects during NMES slightly increase from PRE to END in a similar extent for both lower limbs (Table 2.1), as an affect was observed for moment (p<0.0001), but no effect was observed for ON/OFF times (p=0.233) or lower limb (p=0.498). That was confirmed by the effect sizes observed between moments, which were small for both NMES protocols and lower limbs (Table 2.2).

Table 2.1. Current intensity (**CI**), discomfort generated by NMES (**VAS**), absolute NMES evoked torque (**NMES$_{ABS}$**), relative NMES evoked torque (**NMES$_{REL}$**), maximal voluntary isometric contraction (**MVIC**), relative decrease in MVIC (**ΔMVI**C), absolute doublet-pulse evoked torque (**ET**), and relative doublet-pulse evoked torque (**ΔET**).

		NMES protocols (Days)			
	ON/OFF times	**5 s / 25 s**		**1 s / 5 s**	
Variables (mean ± sd)	Lower Limb	**Right**	**Left**	**Right**	**Left**
CI(mA)	PRE	47.0 ± 9.5	47.9 ± 10.1	46.7 ± 8,6	48.8 ± 8.6
	END	57.0 ± 11.7[a]	59.2 ± 12.1[a]	55.5 ± 11.2[a]	58.4 ± 12.0[a]
VAS (cm)	PRE	3.50 ± 2.65	3.67 ± 2.60	3.36 ± 2.49	3.52 ± 2.67
	END	4.55 ± 3.35[a]	4.85 ± 3.12[a]	4.23 ± 3.18[a]	4.55 ± 3.05[a]
NMES$_{ABS}$(Nm)		50.96 ± 16.52[c]	47.58 ± 14.41	48.85 ± 12.61[c]	45.07 ± 12.64
NMES$_{REL}$ (%)		20.36 ± 3.76	21.27 ± 4.68[f]	20.14 ± 3.97	20.38 ± 3.88
MVIC (Nm)	PRE	252.0 ± 72.7	229.1 ± 71.3	250.2 ± 79.5	224.9 ± 66.0
	POST	210.2 ± 69.3[ace]	194.6 ± 64.2[a]	210.9 ± 68.5[ace]	198.0 ± 61.7[a]
ΔMVIC(%)		-17.27 ± 6.74[bce]	-15.18 ± 6.99	-15.51 ± 6.35[bce]	-12.19 ± 7.37
ET (Nm)	PRE	119.2 ± 34.2	114.5 ± 30.3	118.7 ± 33.3	115.0 ± 32.19
	POST	101.9 ± 30.7[a]	94.3 ± 26.7[ace]	102.8 ± 27.2[a]	96.4 ± 25.7[ace]
ΔET(%)		-14.69 ± 5.62	-17.95 ± 4.46[c]	-12.97 ± 4.12	-15.72 ± 4.32[c]

[a] Effect observed for moment; [b] Effect observed for ON/OFF times; [c] Effect observed for lower limb; [d] Interaction between moment and ON/OFF times; [e] Interaction between moment and lower limb; [f] Interaction between ON/OFF times and lower limb ($p<0.05$).

Table 2.2. Cohen's *d* values (effect size) between moments. Current intensity (**CI**), discomfort generated by NMES (**VAS**), maximal voluntary isometric contraction (**MVIC**), absolute doublet-pulse evoked torque (**ET**), mean heart rate (**MHR**), mean RR interval (**MRR**), low frequency power component (**LF**), high frequency power component (**HF**) and power components ratio (**LF/HF**).

	NMES protocols (Days)			
	5 s / 25 s		**1 s / 5 s**	
Variables (effect size)	**Right**	**Left**	**Right**	**Left**
CI	0.93^{d}	1.01^{d}	0.88^{d}	0.91^{d}
VAS	0.34^{b}	0.41^{b}	0.30^{b}	0.35^{b}
MVIC	0.58^{c}	0.50^{c}	0.52^{c}	0.42^{b}
ET	0.53^{c}	0.70^{c}	0.52^{c}	0.63^{c}

Variables (effect size)	**5 s / 25 s**	**1 s / 5 s**
MRR	1.01^{d}	1.14^{d}
MHR	0.80^{d}	1.28^{d}
LF	1.05^{d}	0.34^{b}
HF	1.04^{d}	0.34^{b}
LF/HF	0.85^{d}	0.13^{a}

Cohen's *d* (effect size) classification: [a]trivial (<0.2); [b]small (>0.2); [c]moderate (>0.50); [d]large (>0.80).

2.4.4. NMES mean evoked torque

The absolute torque produced during both NMES protocols ($NMES_{ABS}$) was slightly higher for the subjects right limb (effect observed for lower limb, p=0.004). However, the relative NMES evoked torque ($NMES_{REL}$) showed a slightly higher mechanical overload produced by the left limb during $NMES_{5/25}$ protocol (Table 2.1). Despite no effect was observed for ON/OFF times (p=0.654) or lower limb (p=0.158), an interaction between ON/OFF times and lower limb (p=0.005) was observed.

2.4.5. Fatigue by means of MVIC

MVIC absolute torque values decreased significantly in both protocols (effect observed for moment, p<0.0001), but in a greater extent for the right limb, as an effect was observed for lower limb (p<0.0001) followed by an interaction between moment and lower limb (p<0.0001 – Table 2.1). In addition, effect sizes between moments ranged from small to moderate for both NMES protocols and lower limbs (Table 2.2).

The level of fatigue evaluated by means of ΔMVIC was greater for $NMES_{5/25}$ compared to $NMES_{1/5}$ protocol (Table 2.1), as an effect for ON/OFF times was observed (p=0.022). In addition, an effect observed for lower limb (p=0.001) suggests that torque decrease was grater for the right lower limb on both conditions. However, no interaction was observed between moment and lower limb (p=0.451).

2.4.6. Fatigue by means of doublet evoked torque

Absolute ET values decrease significantly for both NMES protocols, but in a greater extent for the left lower limb (Table 2.1), as effects both for moment (p<0.0001) and lower limb (p=0.003) were observed, followed by an interaction between moment and lower limb (p=0.006). Even so, effect sizes between moments were moderate for both NMES protocols and lower limbs (Table 2.2).

The level of fatigue evaluated by means of ΔET shows that there was no difference in reduction between $NMES_{5/25}$ and $NMES_{1/5}$ protocols, as no effect was observed for ON/OFF times (p=0.109). However, the effect observed for lower limb (p<0.0001) showed significant higher decrease in ET production for the right limb in both NMES protocols (Table 2.1).

2.5. DISCUSSION

The main purpose of the present study was to evaluate the effect of NMES contraction/relaxation times over HRV in healthy subjects. The present study results shows a similar increase of HRM and concomitant similar reduction of MRR, followed by an increase of the LF component with concomitant decrease of HF component in both NMES protocols. Thus, our results suggests that there was an increase in the sympathetic activity during NMES applied bilaterally over the quadriceps muscles. However, our hypothesis was not confirmed as a larger increase on LF/HF ratio during $\text{NMES}_{5/25}$, while a trivial increase during $\text{NMES}_{1/5}$ protocol were observed, the opposite of what we expected.

In disagreement to our findings, Nicolodi et al. (2016) found that the NMES protocol led to a significant increase in the MRR intervals and a significant reduction of MHR in heart failure patients. In addition, a decrease in the autonomic balance (LF/HF), caused by a reduction of the sympathetic component (LF) was also observed (Nicolodi *et al*., 2016). On the other hand, our findings partially agree with Tanaka *et al*. (2016), which found that LF component increased significantly during the NMES with no significant change in the HF component, although no MHR change was observed. Together with these findings, our results suggest that sympathetic activity is enhanced during NMES. However, these studies evaluated heart disease affected subjects, so any comparison to our results should be made with caution. The only previous study that evaluated the effect of NMES over HRV of healthy subjects found conflicting results (Kang and Hyong, 2014), as no change was observed in HRV during NMES. Despite the different populations tested possibly explain the literature inconsistency, the variety of methods and NMES parameters used on these studies should also be taken in consideration.

In the present study, a single pair of electrodes was placed bilaterally over the mid-belly quadriceps motor-point and 5 cm above the patella, respectively. Both Kang and Hyong (2014) and Nicolodi et al. (2016) placed two pairs over the vastus lateralis and vastus medialis muscles, while Tanaka et al. (2016) simultaneously stimulated the quadriceps and gastrocnemius muscles. As previously demonstrated (Dobsák *et al*., 2006), lower leg muscles stimulation is efficient increasing blood flow in heart failure patients, which could explain the augmented response of sympathetic activity in the latter study. Also, Kang and Hyong (2014) and Nicolodi et al. (2016)

used low frequency (20 – 30 Hz) medium pulse duration (300 – 500 µs) NMES, while Tanaka et al. (2016) used a 50 Hz burst (10-milliseconds on and 10-milliseconds off) frequency that was modulated from an alternating sinusoidal current at 2.5-kHz carrier frequency. A previous study already demonstrated that during transcutaneous electrical nerve stimulation (TENS), low frequency (10 Hz) increases and high frequency (100 Hz) reduces the sensitivity of venoconstriction and α-adrenergic receptors, modifying venous responsiveness (Franco *et al.*, 2014). Therefore, the high frequency used in the present study (80 Hz) could in part explain why the autonomic system response to NMES was similar to the previously observed in heart failure patients (Tanaka *et al.*, 2016).

Another possible explanation is with regard to NMES current intensity used in previous HRV studies. Nicolodi et al. (2016) adjusted NMES current intensity individually, considering the patient's ability to perform knee extension with comfort during contractions. On the study performed by Tanaka et al. (2016), patients received NMES at the highest tolerable intensity without feeling discomfort or pain. On the other hand, Kang and Hyong (2014) adjusted the NMES intensity in the range of 20 to 30 mA, until the subject's knee joint was fully extended. Despite none of these studies measured the NMES evoked force, it has been demonstrated before that NMES current intensity has a positive relationship with energy expenditure, which consecutively increase cardiovascular response (Caulfield *et al.*, 2011). That could explain why the low NMES intensity (20 to 30 mA) used by Kang and Hyong (2014) was unable to cause any change in LF/HF in healthy subjects. However, it does not explain differences found between conditions tested in the present study, as the level of current intensity was similar at the baseline and equally increased during NMES protocols in order to maintain constant mechanical overload (~20% of MVIC). Furthermore, in the present study VAS was also similar between protocols, so it is unlikely that different levels of pain would explain the higher LF/HF ratio values observed during $\text{NMES}_{5/25}$ compared to $\text{NMES}_{1/5}$ protocol (Kim *et al.*, 2017).

The presented studies also used different contraction/relaxation times: Nicolodi et al. (2016) used 5s/10s for 30 min, Tanaka et al. (2016) used 5s/5s during 30 min and Kang and Hyong (2014) used 10s/10s for 15 min - which consecutively could result in different total stimulus time and total mechanical overload. Although none of these studies evaluated NMES muscle evoked force or fatigue, it is well documented that NMES contractions produce more metabolic demand than voluntary contractions (Theurel *et al*., 2006; Muthalib *et al*., 2016), so the total time of NMES performed also could play a role in the inconsistency observed between studies results. In the present study, NMES protocols had the same total time of stimulus (16% of duty-cycle for 20 min) and the lack of observed effect for ON/OFF times over $NMES_{ABS}$, $NMES_{REL}$ suggests that the large difference in LF/HF ratio between $NMES_{5/25}$ and $NMES_{1/5}$ was not influenced by total mechanical overload. Furthermore, although it was previously suggested that short and frequent contractions produced by NMES were more efficient in promoting venous return (Cauldfield *et al*., 2011), curiously, less frequent longer contractions (5s/25s) were more efficient than more frequent short contractions (1s/5s) on increasing sympathetic activity. A possible explanation for our results would be that longer contractions would produce higher metabolic demand and fatigue, which in turn would stimulate chemo-receptors and greatly increase sympathetic activity (Muthalib *et al*., 2016). However, despite there was a slight difference in ΔMVIC between $NMES_{5/25}$ and $NMES_{1/5}$, ΔET was not different between protocols. Hence, the much larger increase in LF/HF during $NMES_{5/25}$ do not seems to be caused by the slightly higher muscle fatigue observed through ΔMVIC evaluation.Another possible explanation is that longerisometric contractionsmight elicit a stronger chemoreflex response, asblood flow within and the release of metabolites from themuscle is limited. The chemoreflex not only elevates blood pressure bya sympathetic vasoconstriction (Rowell and O'Leary, 1990), but also seems to affectsympathetic heart rate modulation (Iellamo*et al*., 1999). Similarly to our findings, Taylor et al. (2017) also showeda reciprocal increase in LF anddecrease and HF, which resulted in anincrease in theLF/HF ratio during voluntary submaximal isomectric contractions.

Unfortunately, the present study was not designed to access directly the physiological mechanisms that could be responsible for the observed increase in the autonomic system sympathetic activity. Another limitation is that the present study evaluated healthy individuals, so the present findings extrapolation to unhealthy subjects needs further investigation. Previous studies have reported that systolic blood pressure elevation (within 10 mmHg) during NMES in critically ill ICU patients caused no significant adverse events (Gerovasili *et al*., 2009). Therefore, we believe that NMES can be safely performed in ICU patients without inducing unstable hemodynamicconditions such as excessive elevations in blood pressure and heart rate, although it did enhance sympathetic activity. Overall, as NMES contraction/relaxation times (ON/OFF times) had a large effect over the level of autonomic control activity (LF/HF), it has to be taken in consideration when prescribing NMES-based rehabilitation programs for critically ill patients affected by cardiovascular conditions.Although we have not yet a clear clinical application for this finding, we believe that the modification of these parameters should be performed when necessary, so that it is possible both to avoid excessive cardiovascular overload in unstable patientsand to progressively increase cardiovascular overload as a form of training.

2.6. CONCLUSION

Our results showed that the increase in NMES contraction/relaxation times (ON/OFF times) from 1s/5s to 5s/25s largely increased LF/HF ratio, suggesting an increase on sympathetic activity in healthy subjects.

FUNDING

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APRESENTAÇÃO: CAPÍTULO 3

Nos dois capítulos anteriores foram avaliados os efeitos de diferentes parâmetros da EENM sobre a função muscular e cardiovascular de indivíduos saudáveis, afim de determinar parâmetros adequados para um programa de reabilitação de adultos críticos internados em unidades de terapia intensiva. Para avaliar os possíveis efeitos deste programa de reabilitação sobre a morfologia e função muscular destes pacientes, medidas que sejam sensíveis, confiáveis e reprodutíveis se fazem necessárias. O capítulo a seguir teve como objetivo avaliar a confiabilidade intra-avaliador, inter-avaliador e inter-analizador das medidas de morfologia e função dos músculos extensores de joelho de sujeitos saudáveis.

RELIABILITY OF ULTRASSOUND ACQUIRED MUSCLE MORPHOLOGY AND MUSCLE FUNCTION IN A SIMULATED ICU SETTING

RESUMO

CAPÍTULO 3: CONFIABILIDADE DE MEDIDAS DE ULTRASSONOGRAFIA DA MORFOLOGIA MUSCULAR E DA FUNÇÃO MUSCULAR EM UM AMBIENTE DE UTI SIMULADA. INTRODUÇÃO: O objetivo do presente estudo é de avaliar a confiabilidade intra-avaliador, inter-avaliador e inter-analisador de medidas obtidas por meio de ultrassonografia da morfologia do músculo quadríceps e das medidas de força voluntária e evocada dos extensores de joelho em uma UTI simulada. MÉTODOS: 32 indivíduos saudáveis (16 ♂ e 16 ♀; idade: 26,6 ± 4,9 anos) realizaram a duas baterias de avaliações, realizadas em dois dias separados por uma semana. Em um dos dias de teste, os participantes foram avaliados por um único avaliador experiente, que repetiu a avaliação no dia de testes seguinte, juntamente com outros dois avaliadores. As avaliações foram realizadas sem a colaboração dos participantes (exceto durante as contrações voluntárias). Os indivíduos foram colocados deitados na maca em decúbito dorsal, apoiando o membro inferior direito em um sistema de dinamometria, com o tornozelo fixado por meio de uma presilha a uma célula de carga acoplada à estrutura do sistema. A morfologia muscular foi avaliada por meio de ultrassonografia para obtenção da área de secção transversa do músculo reto femoral (RF_{AST}), espessura muscular do quadríceps femoral (Q_{EM}), reto femoral (RF_{EM}), vasto intermédio (VI_{EM}), vasto medial (VM_{EM}) e vasto lateral (VL_{EM}), o comprimento dos fascículos das fibras musculares do vasto lateral (VL_{CF}) e seu respectivo ângulo de penação (VL_{AP}). Em seguida, foi avaliado o torque máximo produzido por meio das contrações voluntárias máximas (CVMIs) e pela força evocada por meio de estímulo supramáximo na forma de duplo-pulso (FE). Para a determinação da confiabilidade, foram calculados o coeficiente de correlação intra-classe (CCI), erro padrão de medida (EPM) e mínima mudança detectável (MMD). RESULTADOS: As comparações intra-avaliador mostraram um alto valor de CCI (>0,90). Os valores de EPM e MMD para esta comparação foram baixos, com exceção da RF_{AST} (0,25cm^2 e 0,49cm^2, respectivamente; média=3,83±1,39cm^2), que foi um pouco maior em relação aos valores médios obtidos no estudo. Nas comparações inter-avaliadores, os parâmetros Q_{MT}, VL_{MT}, VM_{MT}, RF_{MT} e VI_{MT} apresentaram CCIs elevados (0,87 à 0,96), enquanto os parâmetros VL_{AP} e VL_{CF} apresentaram um CCI moderadamente elevados (0,66 e 0,69, respectivamente). Os valores de EPM e MMD foram baixos para todas as medidas de espessura muscular (0,06cmà 0,15cm e 0,13cm à 0,29cm, respectivamente; média=1,52±0,39cm à 4,09±0,57cm), enquanto para as medidas de RF_{AST}, VL_{AP} e VL_{CF} foram ligeiramente altas em relação às suas respectivas médias (0,49cm^2, 0,83cm e 0,89°, respectivamente; média=4,13±1,39cm^2, 11,42±1,54° e 12,91±1,93cm, respectivamente). Para as comparações entre os analisadores das medidas de RF_{AST}, o CCI foi alto (0,98). Consecutivamente, os valores de EPM e MMD foram baixos (0,18cm^2 e 0,35cm^2, respectivamente; média=3,83±1,39cm^2) em relação aos respectivos valores médios. Para as comparações intra-avaliadores das avaliações de força, os CCIs foram altos tanto para CVMI quanto para FE (0,91 e 0,94, respectivamente), seguidos por valores mais altos de EPMs e MMDs para as medidas de CVMI em relação aos seus valores médios (5,78 and 11,33kgf, respectivamente; média=71,82±21,85kgf). Nas comparações inter-avaliadores das medidas de força, os CCIs foram menores do que aqueles para as comparações intra-avaliadores, mas ainda considerados altos tanto para a CVMI quanto para a FE (0,89 e 0,86, respectivamente). Os valores de EPM e MMD foram semelhantes aos encontrados para comparações intra-avaliadores (6,18 e 1,65 kgf, 12,12 e 3,24 kgf; média=70,62±20,08 e 30,03±7,64kgf, respectivamente). CONCLUSÃO: As medidas ultrassonográficas são precisas na avaliação da morfologia muscular, quando realizadas pelo mesmo avaliador em diferentes momentos. A alta confiabilidade inter-avaliadores e inter-analistas encontrada para a RF_{AST} e medidas de espessura muscular mostra que essas medidas também são confiáveis quando realizadas por diferentes avaliadores e analistas. A confiabilidade moderadamente alta encontrada para os parâmetros VL_{AP} e VL_{CF} nas comparações inter-avaliadores sugere que esses parâmetros são avaliador-dependentes. A confiabilidade das medidas de força foi alta tanto para a CVMI quanto para a FE, mas maior para a FE, o que reforça seu uso para avaliação de pacientes na UTI.

Palavras-chaves: Confiabilidade, ultrassonografia, morfologia muscular, função muscular.

ABSTRACT

INTRODUCTION: The goal of the present study is to evaluate the intra-rater, inter-rater and inter-analyst reliability of ultrasonography muscle morphology measurements, and of voluntary and evoked knee extensor torque measurements in a simulated ICU. METHODS: Thirty-two healthy (16 ♂ and 16 ♀; 26.6±4.9 years)were submitted to two batteries of evaluations, performed on two separate days, separated by a week. In one of the testing days, subjects were evaluated by a single experienced rater who repeated the evaluation on the next testing day along with two other raters. The evaluations were performed without the cooperation of the subjects (except for the voluntary contractions). The individuals were placed lying on the stretcher in a dorsal decubitus position, resting the right lower limb on a dynamometry system and the ankle was fixed with a clip to a load cell coupled to the system structure. Muscle morphology was evaluated by means of ultrasonography in order to obtain the rectus femoris muscle cross-sectional area (RF_{CSA}), the muscle thickness of the quadriceps femoris (Q_{MT}), rectus femoris (RF_{MT}), vastus intermedius (VI_{MT}), vastus medialis (VM_{MT}), and vastus lateralis (VL_{MT}), the fascicle length of vastus lateralis muscle fibers (VL_{FL}) and its respective pennation angle (VL_{PA}). Then, the maximum torque produced by means of both maximal voluntary contractions (MVICs) and by supramaximal doublet-pulse evoked force (EF) were evaluated. RESULTS: Intra-rater comparisons showed a high intra-class correlation (ICC) value (>0.90). The SEM and MDC values for this comparison were low, except for the RF_{CSA} (0.25cm^2 and 0.49cm^2; mean=3.83±1.39cm^2) that was slightly higher in relation to the mean values obtained in the study. In the inter-rater comparisons, the parameters Q_{MT}, VL_{MT}, VM_{MT}, RF_{MT} and VI_{MT} showed high ICCs (0.87 to 0.96), while the VL_{PA} and VL_{FL} parameters presented moderately high ICCs (0.66 and 0.69, respectively). The SEM and MDC values were low for all muscle thickness measurements (0.06cm to 0.15cm and 0.13cm to 0.29cm; mean=1.52±0.39cm to 4.09±0.57cm), while the RF_{CSA}, VL_{PA} and VL_{FL} measurements were slightly higher in relation to their mean values (0.49cm^2, 0.83cm and 0.89°; mean=4.13±1.39cm^2, 11.42±1.54° and 12.91±1.93cm, respectively). For inter-analyst comparisons of RF_{CSA} measurements, ICC was high (0.98). Consecutively, SEM and MDC values were low (0.18cm^2 and 0.35cm^2, respectively; mean= 3.83±1.39cm^2) in relation to the mean values. For force evaluations intra-rater comparisons ICCs were high for both MVIC and EF (0.91 and 0.94, respectively), followed by higher SEMs and MDCs values for MVIC measurements in relation with its mean values (5.78 and 11.33kgf, respectively; mean=71.82±21.85 kgf). In the inter-rater comparisons of force measures, ICCs were lower than those for the intra-rater comparisons, but still considered high for both MVIC and EF (0.89 and 0.86, respectively). The SEM and MDC values were similar to those found for intra-rater comparisons (6.18 and 1.65 kgf, 12.12 and 3.24 kgf; mean=70.62±20.08 and 30.03±7.64 kgf). CONCLUSION: Ultrassound measurements are accurate in the muscle morphology evaluation, when performed by the same rater at different moments. Inter-rater and inter-analyst high reliability found for RF_{CSA} and muscle thickness measures shows that these measures are also accurate when performed both by different evaluators and analysts. The moderately high reliability found for VL_{AP} and VL_{FL} parameters in the inter-rater comparisons suggests that these parameters are evaluator-dependent. The reliability of force measures was high for both MVIC and EF, but higher for EF, which reinforce its use for evaluation of ICU patients.

Key-words: Reliability, ultrassound, muscle morphology, muscle function.

3.1. INTRODUCTION

In the evaluation of physiological responses, different techniques have been used to explain the mechanisms responsible for changes in functional capacity. To verify if these measures are reproducible, it is essential to identify the changes that can occur due to a rehabilitation programwith accuracy. Common metrics to quantify reliability are intra-class correlation coefficients (ICC), standard error of measurements (SEM) and minimal detectable change (MDC) (Weir, 2005). Although the terminology is not constant between studies, and there are different kinds of reliability, three are commonly found in the literature. The intra-rater reliability compares the data collected by the same rater in different moments (Vieira *et al*., 2016). The inter-rater reliability compares the data collected by different raters in the same day (Zaidman *et al*., 2014). The inter-analyst reliability uses images regardless of who collected them, comparing the analysis done by different analyzers (Sarwal *et al*., 2015).

Prolonged reduction of muscle mass and strength are important shortcomings acquired by the critical patient (Hough, 2013), and interventions aimed at preventing or minimizing these deficiencies have been increasingly the object of study (Denehy *et al*., 2013). Muscle loss occurs early and rapidly during hospitalization in intensive care units (ICUs) (Puthucheary *et al*., 2013), and muscle imaging evaluation allows the quantification of the muscular morphology related to both muscular strength and to the development of ICUs acquired muscular weakness (Hough, 2013). By means of ultrasonography, it is possible to evaluate the muscular architecture (muscle volume, muscle thickness, fascicle length and angle of pennation of its fibers), which is determinant of muscle function and force production (Henriksson-Larsen *et al*., 1992; Lieber and Fridén, 2000). Comparison of muscle architecture changes among synergistic muscles in response to disuse or to a training program may provide a greater understanding of their contribution during functional recovery and for the capacity of force production.

In 2011, Baldwin et al. published an observational methodological study evaluating the reliability of muscle thickness of mid-upper arm, mid-forearm, and mid-thigh, in 13 healthy subjects, supine positioned as would be expectedin the ICU. They found intra-rater ICCs ranging from 0.99 to 1.0, but the study was limited by lacking the inter-rater reliability evaluation and measures performed in different days.

In a prospective study of muscle wasting in the critically ill patients, using ultrasonography to measure rectus femoris cross-section area (Puthucheary *et al*., 2013), reliability was evaluated using two blinded, independent raters, and the resulting ICC was 0.97. This study was limited by the fact that only one measure at one muscle site was performed. Despite these previous results, and although ultrasonography is the most commonly used technique for the visualization of morphological parameters, the methodological quality of the majority of the studies that evaluated the muscle morphology measures reliability in ICU settings is still low (Bunnell *et al*., 2015).

Neuromuscular incapacity precedes muscle mass loss due to ICU acquired polyneuropathy (Apostolakis *et al*., 2015), which impairs muscle force production. Muscle strength reliability has also been evaluated in clinical trials. Adsuar et al. (2011) and Ferri-Morales et al. (2014) found similar reliability results for evaluations of knee extensor maximal voluntary contractions (ICC = 0.91), observed both in fibromyalgia patients and healthy young women. However, despite the high reliability observed by the authors, this type of evaluation is inadequate for ICU patients who are unable to perform voluntary strength tests during the first days of hospitalization due to sedation. Volitional strength production is complex, containing information on the ability to perform the task (cognition), coordination, visual information processing and central motor control, as well as the activation of signaling pathways from the motor cortex to the muscle (Sherwood, 2004). As the assessment of voluntary strength requires that the patient is alert and able to cooperate with the tests, this assessment is performed at a later stage (before discharge) in ICU patients. Therefore, methods that use supramaximal electrical stimulus have been used to evoke muscle contractions in non-cooperating patients (Poulsen *et al*., 2015).

Nathaniel et al. (2014) found a high intra-rater ICC (0.92) for supramaximal evoked force evaluation in healthy subjects. However, muscle evoked strength measurements require clinician specialization and special equipment to be performed with ICU patients (Ginz *et al*., 2008). In addition, when designing this equipment type for critically ill patients muscle strength evaluation during bed rest, it is important to control the patient positioning and fixation by the evaluator. Probably, due to the abovementioned difficulties in evaluating the evoked capacity of muscle force production, no study regarding the reliability of ICU patients evoked force measures was found. However, before conducting a reliability study with critically ill patients, evoked force reliability should be previously tested in healthy subjects in order to determine the new ICU-friendly equipment accuracy and for determining normal healthy values to be used as a goal to be achieved in ICU rehabilitation programs.

3.2. PURPOSE

The main goal of the present study is to evaluate the intra-rater (measurements performed on different days by the same rater), inter-rater (measurements performed on the same day by three independent raters) and inter-analyst (images analyzed digitally by two different analysts) reliability of ultrasonography muscle morphology measurements, and of voluntary and evokedknee extensor torque measurements in a simulated ICU. To accomplish this, we evaluated: (1) the rectus femoris muscle cross-sectional area (RF_{CSA}); the muscle thickness of the (2) quadriceps femoris (Q_{MT}), (3) rectus femoris (RF_{MT}), (4) vastus intermedius (VI_{MT}), (5) vastus medialis (VM_{MT}), and (6) vastus lateralis (VL_{MT}); (7) the fascicle length of vastus lateralis muscle fibers (VL_{FL}) and (8) its respective pennation angle (VL_{PA}); (9) maximum voluntary isometric contraction (MVIC); and (10) the evoked force (EF) by means of supramaximal electrical stimulus in healthy young adult subjects. The hypothesis raised in the present study is that all measures will have good reliability as an adequate training of the raters team was carried out before the tests.

3.3. METHODS

The evaluations took place at the Neuromuscular Plasticity Department of the Exercise Research Laboratory (LAPEX, Porto Alegre, Brazil) of the Physical Education, Physiotherapy and Dance Faculty (ESEFID - UFRGS). This study used a methodology approved by the University’s Research Ethics Committee (CAEE nº 36588914.4.1001.5347). Participants read and signed the informed consent form after they had all questions about the tests to be performed answered by the responsible researcher.

3.3.1. Sample

The sample consisted of 32 healthy and physically active university students (16 men and 16 women; age: 26.6 ± 4.9 years). Subjects were invited to participate in the study through social networks and visits to the classrooms of the institution where the study was conducted. Subjects (1) who presented injury to the evaluated lower limb, (2) who had any cardiovascular disease, (3) who had central or peripheral neurological disease, or (4) who had any clinical contraindications to the execution of the tests were not included in the study. The number of subjects was chosen through the following equation that indicates the sample size according to the tolerated measurement error for each evaluated variable ($n=Z^2*dp^2/e^2$). In the equation, n is the sample size, Z is the study tabled value related to the significance level (1.96 for $\alpha = 0.05$), dp is the standard deviation of the variable in question, obtained through the specific literature, and e, the tolerated measurement error (estimated in 10%) and applied over the variable mean obtained from the literature. The sample size was obtained through the mean and standard deviation values of the VL_{MT} (2.61 ± 0.24 cm) from a previous study by our research group, which evaluated a similar population (healthy adults with age between 20 and 35 years) and that used similar methodology (Franke *et al.*, 2014). The calculated number of 16 individuals was defined as the minimum number of subjects for each group. Because in this study we wanted to evaluate the measurements reliability in both sexes, 16 subjects from each sex were recruited totalizing 32 individuals.

3.3.2. Procedures

After reading the consent form, the subjects were submitted to two batteries of evaluations, performed on two separate days, separated by a week (Figure 3.1). In one of the testing days, subjects were evaluated by a single experienced rater (6 years of experience with ultrasound and force measurements) who repeated the evaluation on the next testing day along with two other raters, who were duly trained by the experienced rater to perform the procedures (during one year and three months, respectively). Both the evaluations' order between the raters and the order of the day in which the subject was evaluated by a single rater or by the three raters were intentionally randomized, in order to discard the influence of gender, moment in which the subjects were evaluated and the possible establishment of the fatigue process throughout the tests.

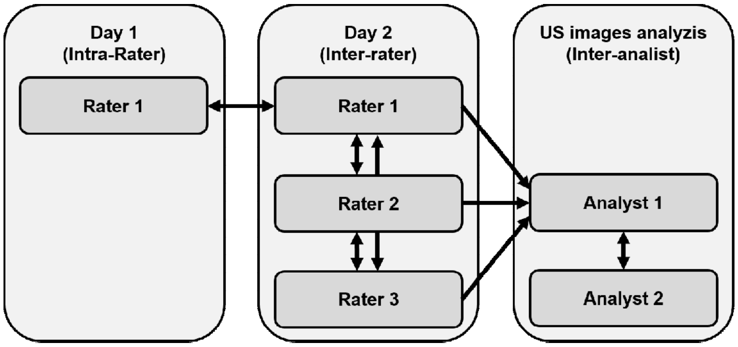

Figure 3.1. Experimental procedures for the intra-rater, inter-rater and inter-analyst comparisons.

The equipment was installed in a small room equipped with a stretcher. The evaluations were performed without the cooperation of the subjects (except for the voluntary contractions), in order to simulate a hospital ICU environment. Firstly, the individuals were placed lying on the stretcher in a dorsal decubitus position, resting the right lower limb on a dynamometry system specifically developed for the assessment of the knee extensor torque of bedridden patients (Figure 3.2). After resting for 20 minutes for the redistribution of body fluids, the measurements of muscle morphology were performed by means of ultrasonography. The subjects' right ankle was fixed with a clip to a load cell coupled to the structure of the dynamometry system. Then, the maximum torque produced with MVICs and with EF were evaluated.

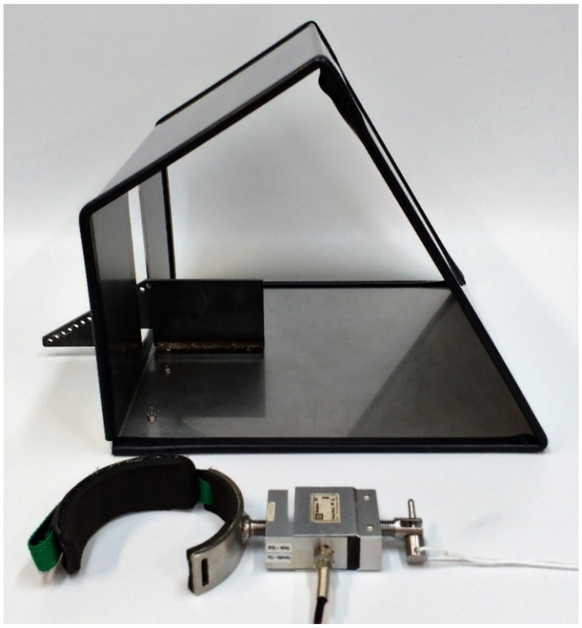

Figure 3.2. Dynamometry system instrumented with a load cell for the evaluation of muscle strength at the intensive care units' beds.

3.3.3. Evaluation of muscular morphological properties

Two-dimensional real-time ultrasonography (Vivid-I, General Electric) was used for the evaluation of muscle morphology using a linear array probe with a 9MHz sampling frequency and a 4 cm depth. The probe was soaked in a water-soluble transmission gel promoting acoustic contact without depressing the skin surface. Three images were obtained from each muscle. The ultrasound robe positioning on the evaluated muscles was marked on the skin by the experienced rater who later transferred the positioning to an acetate sheet, also identifying signs, scars and bone protuberances to enable the same positioning of the probe in the subsequent evaluation.

Images were analyzed through the *Image-J* software (National Institute of Health, USA), which allows for the manual demarcation of the muscle structures (aponeuroses and fibers) for muscle architecture parameters calculations. For each morphological variable evaluated, the mean values and their respective standard deviations were calculated for the whole group for each rater and evaluation moment.

3.3.4. Evaluation of the rectus femoris muscle cross-sectional area

For the RF_{CSA} evaluation, images of the rectus femoris muscle were obtained at rest *in vivo*. The ultrasound procedure was done by capturing images in the transverse plane, at the 50% level of the muscle belly (Figure 3.2A). The probe was positioned transversely to the orientation of the muscle belly and perpendicular to the orientation of its fibers, with the image depth adjusted so that both the RF muscle aponeuroses and the femur were visualized. The RF_{CSA} was obtained from the mean values from the analysis of three transverse images. Also, analysis of RF_{CSA} images was performed by two analyzers in order to calculate inter-analyst reliability. RF_{CSA} values were chosen to calculate inter-analyst reliability, since its collection requires a manual analysis of greater complexity.

3.3.5. Evaluation of the quadriceps muscles thickness

Q_{MT}, FR_{MT} and VI_{MT} were obtained by analyzing the same images obtained for the RF_{CSA} evaluation. For each image, a single thickness measurement of each muscle was performed in the central portion of their respective belly (Figure 3.2B). For the VM_{MT} and VL_{MT} evaluation, the ultrasound procedure was done by capturing images in the sagittal plane. The probe was positioned perpendicular to the muscles surface, at 70% of the length of the vastus medialis muscle (Figure 3.2C) and 50% of the length of the vastus lateralis muscle (Figure 3.2D). Muscle thickness was obtained through image analysis, where five equidistant measurements of the vastus medialis muscle and three equidistant measurements of the vastus lateralis muscle were performed, measuring the distance between the superficial and deep aponeuroses.

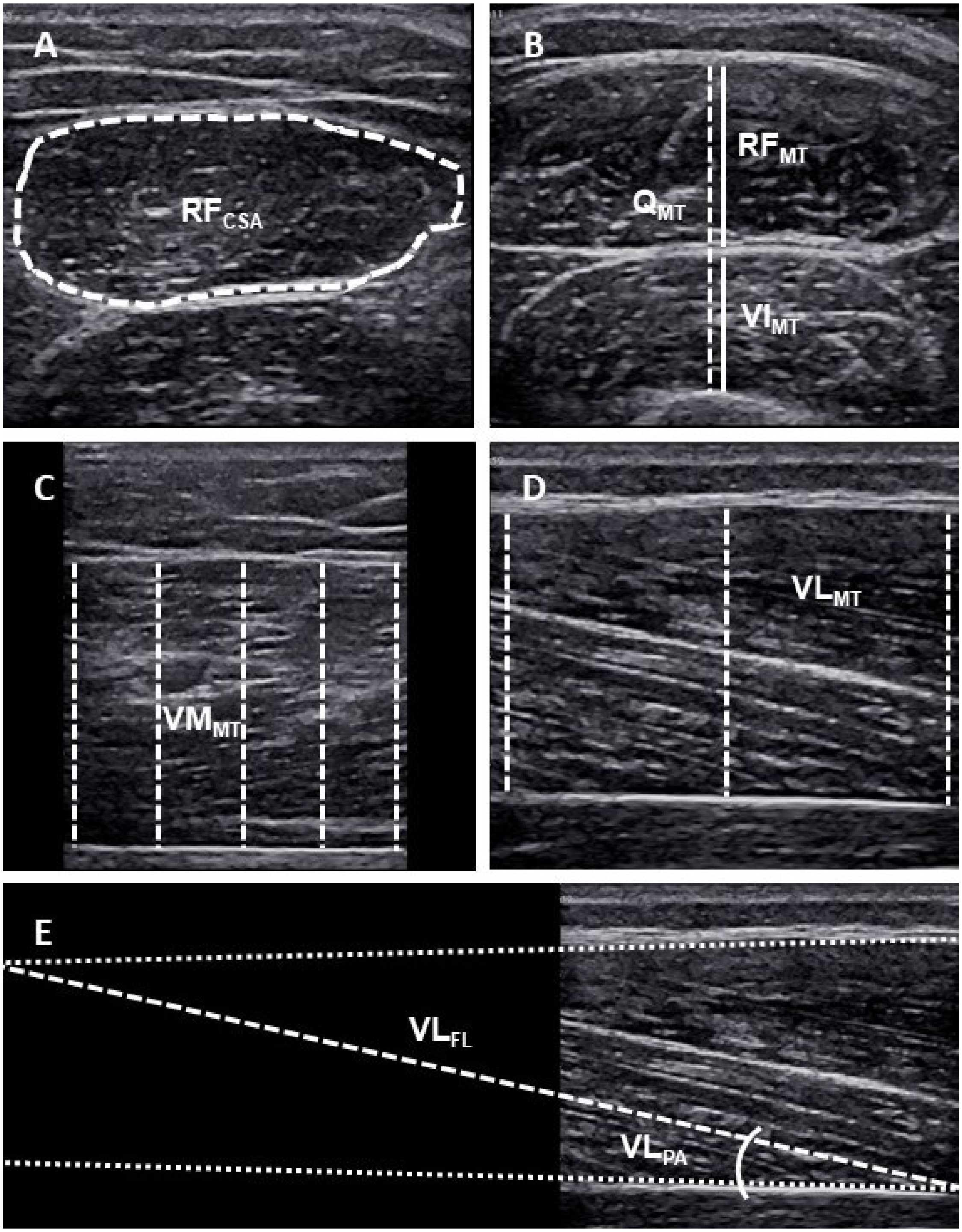

Figure 3.3. A. Rectus femoris cross-section area (RF_{CSA}); **B**. Quadriceps muscle tickness (Q_{MT}), rectus femoris muscle thickness (RF_{MT}) and vastus intermedius muscle thickness (VI_{MT}); **C**. vastus medialis muscle thickness (VM_{MT}); **D**. vastus lateralis muscle thickness (VL_{MT}); **E**. vastus lateralis fiber length (VL_{FL}) and pennation angle (VL_{PA}) analisys. Images were obtained from a single representative subject.

3.3.6. Evaluation of the vastus lateralis fascicle length and pennation angle

For the evaluation of the VL_{FL} and the VL_{PA}, the same images obtained for the evaluation of the VL_{MT} were analyzed, with one muscle fiber being selected for each of the three images. Since the used probe had a small scanning area (4 cm), trigonometry was used to calculate the VL_{FL} using the VL_{PA}, and mathematically extrapolating the trajectories of the structures out of the image (Figure 3.2E). The mean and standard deviation values of the VL_{FL} and VL_{PA} were calculated through the analysis of the three images.

3.3.7. Evaluation of the knee extensors mechanical properties

The quadriceps femoris muscle force production capacity was assessed in this study by isometric tests using a dynamometer system especially developed for the evaluation of the knee extensor torque in bedridden patients, built in stainless steel (Figure3.2) and instrumented with a load cell (Miotec, Brazil). The dynamometry system was fixed to a stretcher with special bands and the subjects were placed lying down with the knees bent at 90° and hips flexed at 60° (0° = full extension). A special clip fixed to the load cell was attached to the distal end of the subjects' legs, perpendicular to the tibia surface, 3 cm above the medial malleolus (Figure 3.4).

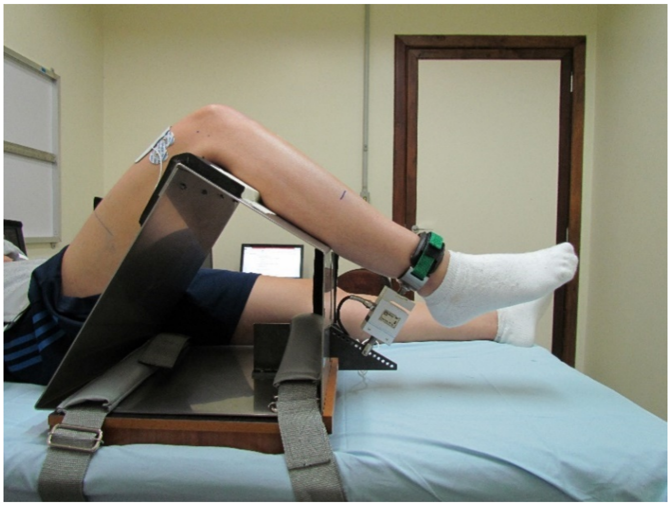

Figure 3.4. Attachment of the stainless steel structure to the stretcher and of the load cell to the distal end of the subject's lower limb.

The subjects were also fixed by a special band to the stretcher in the hip region in order to avoid compensatory movements during the tests. Both the distance between the clip fixed at the ankle and the knee rotation axis, as the knee and hip angles in which the subjects were positioned on the stretcher were recorded in order to identify the cause of possible measurement errors.

3.3.8. Evaluation of the maximum voluntary isometric contraction

On the first evaluation day, once positioned, the subjects performed a brief familiarization consisting of two to three knee extensors MVICs at a 90° angle. After properly recovered, subjects performed two to three knee extensor MVICs, maintaining the contraction during a period of approximately 5 sec. A third contraction was performed whenever the difference between the first and second contractions exceeded 10%, in order to guarantee the maximum torque. Two- minute intervals were given between contractions. Subjects were instructed to perform the contractions without any visual feedback, increasing the effort until the maximal torque production was achieved within 2 or 3 s, which should then be maintained until a verbal command to cease contraction was given. The peak force value (kgf) obtained from the contractions was used as the MVIC. The mean and the standard deviation values for the whole experimental group were calculated for each rater and moment of evaluation.

3.3.9. Location of the motor point and positioning of the electrodes

The motor point location and EF evaluation were performed with a clinical electrical stimulator developed by the Biomedical Engineering Laboratory of the Hospital de Clínicas of Porto Alegre (Schildt *et al*., 2016). EF was obtained using rectangular self-adhesive electrodes with saline gel (Arktus, Canada) measuring 7.5 cm in width and 13 cm in length, which were positioned on the motor point of the quadriceps muscle and on the distal portion of the muscle (five centimeters from the upper edge of the patella). To determine the exact location of the quadriceps' motor point, a pen-shaped electrode was first connected to the stimulator proximal pole. After applying saline gel to the skin covering the mid-proximal portion of the thigh muscles, the pen-shaped electrode was placed in contact with the skin, applying a current of 1 Hz, pulse duration of 100 μs and sufficient intensity to produce a measurable contraction. During the procedure, the subjects were lying on the stretcher with the lower limbs on the dynamometry system with the knee positioned at 90º of flexion and ankles attached to the load cells (Figure 3.5).

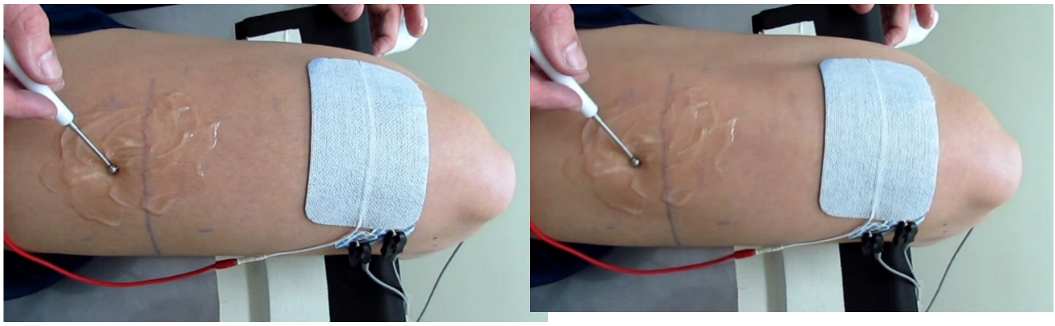

Figure 3.5. Location of the motor point (upper view). Note that the muscle is relaxed on the left image, while on the right one the muscle contraction can be observed.

The place where the electric pulse generated the greatest torque production, visually assessed in the data acquisition system, was defined as the quadriceps motor point. The electrodes positioning (Figure 3.6) was recorded on an acetate sheet for identical positioning on the two evaluation days.

Figure 3.6. Electrode positioning: proximally, over the quadriceps motor point, and distally, 5 cm above the patella.

3.3.10. Evoked force evaluation

The evoked force (EF) evaluation was performed by means of neuromuscular electrical stimulation (NMES). EF was obtained by means of supramaximal stimuli delivered as double pulses with 2 ms duration (1 ms phase duration) and 8 ms intervals between the pulses. In order to determine the current intensity required for the supramaximal stimulus, a test with increasing current intensity (maximum of 180 mA) was first performed until an increase in the knee extensors torque was no longer observed. The current intensity value observed at the EF plateau was increased by 10% in order to guarantee a supramaximal stimulus during the test. The test consisted in the application of three double pulses with a 3 s interval between the contractions, by means of a trigger system manually activated by the raters. The mean peak force (kgf) produced in each of the three contractions was adopted as the EF value.

3.3.11. Statistical analysis

For the evaluation of intra-rater, inter-rater and inter-analyst reliability (1) the intra-class correlation coefficients (ICCs) and their respective confidence intervals (CIs), (2) the standard error of measurement (SEM),and (3) the minimum detectable change (MDC) were calculated for each of the evaluated variables. A significance level of 5% was adopted for all analyzes (SPSS Software version 20.0).

3.4. RESULTS

All parameters' intra-rater comparisons, performed by the same evaluator on different days, showed a high ICC value (> 0.90 – Table 3.1). The SEM and MDC values for this comparison were low, except for the RF_{CSA} that was slightly higher in relation to the mean values obtained in the study.

In the inter-rater comparisons (Table 3.2), the parameters Q_{MT}, VL_{MT}, VM_{MT}, RF_{MT} and VI_{MT} showed high ICCs (0.87 to 0.96), while the VL_{PA} and VL_{FL} parameters presented moderately high ICCs (0.66 and 0.69, respectively). The SEM and MDC values were low for all muscle thickness measurements (0.13 cm to 0.25 cm), while the RF_{CSA}, VL_{PA} and VL_{FL} measurements were slightly higher in relation to their mean values (0.49 cm2, 0.83 cm and 0.89°, respectively). For inter-analyst comparisons of RF_{CSA} measurements, ICC was high 0.98. Consecutively, SEM and MDC values were low (0.18cm^2 and 0.35cm^2, respectively) in relation to the mean values.

For force evaluations intra-rater comparisons, ICCs were high for both MVIC and EF (0.91 and 0.94, respectively), followed by higher SEMs and MDCs values for MVIC measurements (Table 3.3) in relation with its mean values. In the inter-rater comparisons of force measures (Table 3.4), ICCs were lower than those for the intra-rater comparisons, but still considered high for both MVIC and EF (0.89 and 0.86, respectively). The SEM and MDC values were similar to those found for intra-rater comparisons.

Table 3.1. Mean and standard deviation values of ultrasound measurements acquired in two different days by the same evaluator. Standard error of measure (SEM). Minimal detectable change (MDC). Intra-class correlation coefficient (ICC) and 95% Confidence intervals (CI).

INTRA-RATER						
Ultrasound measurements	**1st Day** (Mean ± sd)	**2nd Day** (Mean ± sd)	**SEM**	**MDC**	**ICC**	**95% CI**
RF_{CSA}	3.72 ± 1.35 cm^2	3.83 ± 1.39 cm^2	0.25 cm^2	0.49 cm^2	0.97	0.93 - 0.98
Q_{MT}	3.70 ± 0.67 cm	3.84 ± 0.70 cm	0.13 cm	0.25 cm	0.97	0.93 - 0.98
RF_{MT}	2.10 ± 0.35 cm	2.15 ± 0.38 cm	0.07 cm	0.13 cm	0.95	0.91 - 0.97
VI_{MT}	1.56 ± 0.40 cm	1.53 ± 0.38 cm	0.7 cm	0.14 cm	0.96	0.92 - 0.98
VM_{MT}	4.09 ± 0.57 cm	4.06 ± 0.56 cm	0.13 cm	0.25 cm	0.95	0.90 - 0.98
VL_{MT}	2.47 ± 0.32 cm	2.50 ± 0.33 cm	0.08 cm	0.16 cm	0.94	0.87 - 0.97
VL_{FL}	12.59 ± 1.8 cm	12.44 ± 1.72 cm	0.42 cm	0.83 cm	0.94	0.89 - 0.97
VL_{PA}	11.07 ± 1.36°	11.43 ± 1.53°	0.45°	0.89°	0.90	0.81 - 0.95

RF_{CSA} = rectus femoris muscle thickness; Q_{MT} = quadriceps muscle thickness; VI_{MT} = vastus intermedius muscle thickness; VM_{MT} = vastus medialis muscle thickness; VL_{MT} = vastus lateralis muscle thickness; VL_{FL} = vastus lateralis fiber length; and VL_{PA} = vastus medialis pennation angle.

Table 3.2. Mean and standard deviation values of ultrasound measurements acquired in the same day by three different evaluators. Standard error of measure (SEM). Minimal detectable change (MDC). Intra-class correlation coefficient (ICC) and 95% Confidence intervals (CI).

INTER-RATER							
Ultrassound measurements	**Rater 1** (Mean ± sd)	**Rater 2** (Mean ± sd)	**Rater 3** (Mean ± sd)	**SEM**	**MDC**	**ICC**	**95% CI**
RF_{CSA}	3.69 ± 1.34 cm^2	3.82 ± 1.26 cm^2	4.13 ± 1.39 cm^2	0.39 cm^2	0.77 cm^2	0.91	0.85 - 0.95
Q_{MT}	3.83 ± 0.67 cm	3.85 ± 0.66 cm	3.81 ± 0.71 cm	0.12 cm	0.23 cm	0.97	0.95 - 0.98
RF_{MT}	2.14 ± 0.35 cm	2.13 ± 0.39 cm	2.15 ± 0.38 cm	0.06 cm	0.13 cm	0.96	0.91 - 0.97
VI_{MT}	1.56 ± 0.39 cm	1.52 ± 0.39 cm	1.56 ± 0.39 cm	0.07 cm	0.13 cm	0.96	0.93 - 0.98
VM_{MT}	4.09 ± 0.57 cm	4.04 ± 0.51 cm	3.98 ± 0.56 cm	0.15 cm	0.29 cm	0.93	0.88 - 0.96
VL_{MT}	2.49 ± 0.34 cm	2.53 ± 0.31 cm	2.48 ± 0.29 cm	0.12 cm	0.24 cm	0.87	0.78 - 0.93
VL_{FL}	12.57 ± 1.61 cm	12.91 ± 1.93 cm	12.51 ± 1.84 cm	1.00 cm	1.95 cm	0.69	0.52 - 0.82
VL_{PA}	11.15 ± 1.39°	11.42 ± 1.54°	11.37 ± 1.41°	0.84°	1.64°	0.66	0.49 - 0.80

RF_{CSA} = rectus femoris muscle thickness; Q_{MT} = quadriceps muscle thickness; VI_{MT} = vastus intermedius muscle thickness; VM_{MT} = vastus medialis muscle thickness; VL_{MT} = vastus lateralis muscle thickness; VL_{FL} = vastus lateralis fiber length; and VL_{PA} = vastus medialis pennation angle.

Table 3.3. Mean and standard deviation values of MVIC and EF evaluation in two different days by the same evaluator. Standard error of measure (SEM). Minimal detectable change (MDC). Intra-class correlation coefficient (ICC) and 95% Confidence intervals (CI).

INTRA-RATER						
Force evaluation	**1st Day** (kgf ± sd)	**2nd Day** (kgf ± sd)	**SEM** (kgf ± sd)	**MDC** (kgf ± sd)	**ICC**	**95% CI**
MVIC	70.79 ± 18.19	71.82 ± 21.85	5.78	11.33	0.91	0.83 - 0.95
EF	29.82 ± 6.75	31.14 ± 8.13	1.76	3.45	0.94	0.88 - 0.97

MVIC = maximal voluntary isometric contraction; EF = evoked force.

Table 3.4. Mean and standard deviation values of MVIC and EF evaluation in the same day by three different evaluators. Standard error of measure (SEM). Minimal detectable change (MDC). Intra-class correlation coefficient (ICC) and 95% Confidence intervals (CI).

INTER-RATER							
Force evaluation	**Rater 1** (kgf ± sd)	**Rater 2** (kgf ± sd)	**Rater 3** (kgf ± sd)	**SEM** (kgf ± sd)	**MDC** (kgf ± sd)	**ICC**	**95% CI**
MVIC	70.62 ± 20.08	68.78 ± 19.07	67.78 ± 19.40	6.18	12.12	0.89	0.82 - 0.94
EF	30.03 ± 7.64	29.39 ± 6.71	29.57 ± 7.54	1.65	3.24	0.86	0.77 - 0.92

MVIC = maximal voluntary isometric contraction; EF = evoked force (kgf)

3.5. DISCUSSION

The main purpose of the present study was to evaluate intra-rater, inter-rater and inter-analyst reliabilities of ultrasound and force measurements. All ICC values obtained in the intra-rater comparisons were considered high (ICC > 0.90), demonstrating that these measures are highly reliable when performed by the same rater at different times. In addition, the ICC values for muscle thickness parameters in the intra-rater comparisons were almost identical to the values of previous studies (Raj *et al*., 2012; Baroni *et al*., 2013; Ema *et al*. 2013). For the inter-rater comparisons, only muscle thickness measures and RF_{CSA} showed high reliability, while VL_{FL} and VL_{PA} showed to be moderately reliable when different raters obtain these variables. Force measurements by means of MVIC and EF were both highly reliable.

While the present study found an ICC of 0.97 for Q_{MT}, previous studies evaluating the same parameter obtained ICCs of 0.96 (Baroni *et al*., 2013) and 0.98 (Ema *et al*. 2013). For the VL_{MT}, the ICC score found was 0.94, a value very similar to those found in the studies of Baroni et al. (2013) and Raj et al. (2012), who found ICCs of 0.91 and 0.96 for the same parameter, respectively. The ICC of the VM_{MT} was 0.95, similar to the other muscle thickness variables. However, as no studies were found that evaluated this specific muscle and presented intra-rater ICC calculations, it is not possible to compare this parameter with the previously reviewed literature. Nevertheless, the high reliability found shows that VM_{MT} can be evaluated in different days by the same rater.

The comparison of RF_{CSA} measures presented an ICC for the intra-rater comparison of 0.97, slightly above, but according to the literature values, which observed ICCs of 0.88 and 0.87 for two different positions of the probe (Lima *et al*., 2012). In the present study, a different probe position was used, maintaining it at 70% of the thigh length, while in the study by Lima et al. (2012), two positioning sites were used, one with the probe at 50% of the thigh length, and the other from a fixed distance from the patella upper edge (15 cm). The positioning used in the present study was chosen because in this position the probe (4 cm) was able to capture the entire RF muscle area in all subjects, and was relative to the subject’s thigh length. Therefore, this relative and more distal position apparently resulted in a higher RF_{CSA} reliability, and encompasses the entire muscle CSA.

The VL_{PA} intra-rater reliability showed an ICC of 0.90 and SEM of 0.45°, being similar to the values of previous studies that found ICC values between 0.90 and 0.99 (Blazevich *et al*., 2006), 0.90 (Baroni *et al*., 2013) and 0.95 (Henriksson-Larsen *et al*., 1992), with SEM values of 0.24° and 1.22° (Blazevich *et al*., 2006).For VL_{FL} measurements, an ICC of 0.94 was obtained, slightly above the values of previous studies for intra-rater reliability. In these studies, the observed ICC values were 0.91 (Baroni *et al*., 2013) and 0.74 (Henriksson-Larsen *et al*., 1992).

In the inter-rater comparisons, the muscle thickness measurements ICC values (0.97) were also similar to the studies previously found in the literature (0.95 - Tillquist *et al*., 2014; 0.99 - Hadda *et al*., 2017). No study evaluated the inter-rater reliability of the VL_{MT} and VM_{MT} muscle thickness measures, making comparisons impossible. However, ICC values of 0.86 and 0.93 for VL_{MT} and VM_{MT}, respectively, are considered high and appear to be reliable when performed by different evaluators.

For RF_{CSA} measurements, an ICC of 0.91 and an SEM of 0.39 cm^2 was obtained in the inter-rater comparisons, a value considered high. The present study reproducibility results were better than in the study by Hammond and colleagues, who compared the reproducibility of the same measure between an experienced and a novice assessor (ICC = 0.79 and SEM = 0.42 cm^2). However, our results were slightly below the results of the same study comparing two experienced evaluators, where ICC values of 0.99 and SEM of 0.08 cm^2 were found (Hammond *et al*., 2014). As our evaluators had different time experience in using the force and ultrasound equipment, this might explain our intermediate results between novice and experienced evaluators.

The ICC for the VL_{PA} measurements was considered moderately high (0.66) with an SEM of 0.84°, with a mean value of 11°. No other study was found evaluating the VL inter-rater reproducibility of this measure. The only study evaluating this variable was the study by Konig et al. (2014), but evaluated it in the medial gastrocnemius muscle, obtaining a high ICC of 0.90, but with an SEM similar to that found in the present study (1.1°).

The VL_{FL} parameter, which is directly influenced by the pennation angle, also obtained a moderately high ICC of 0.69, with SEM of 0.99 cm. The only study found that evaluated the fascicle length measurements inter-rater reliability used the medial gastrocnemius muscle, and the authors observed a similar ICC (0.77) and a lower SEM when compared to our study (0.4 cm) (Konig *et al*., 2014).

Another important factor for measuring muscular architecture parameters is data analysis, here performed in *Image-J* (National Institute of Health, USA) software. Data analysis is challenging, especially when evaluating the RF_{CSA} parameter, where the muscle contour should be determined, excluding the aponeuroses. Our RF_{CSA} inter-analyst comparisons showed a high ICC of 0.98, followed by low SEM ($0.18cm^2$) and MDC ($0.35cm^2$) values, which were considered low in relation to the obtained mean values. These results indicate a great RF_{CSA} images reliability when analyzed by different analysts, suggesting that analysis can be safely done by different analyzers. Sarwal et *al*. (2015) found similar values for quadriceps and abdominal muscles, finding ICC values ranging from 0.84 to 0.99 while evaluating a critically ill population.

When a same rater repeats the evaluations at different times, a map can be used so that the probe is positioned in exactly the same way as in the first evaluation. This map is produced using a transparent leaf where anatomical points such as skin signs, scars and bony protrusions are recorded as a reference. Despite the use of the map, other factors are more difficult to accurately reproducing, such as the angle of the probe relative to the skin surface, which may make it difficult to find exactly the same structures in both evaluations. However, even with these limitations, the present study demonstrated, through ICC, indices above 0.90 for all parameters, showing evidence that these measures are reproducible when performed by the same rater at different moments. In this study, to perform the inter-raters evaluations, raters were not present during the evaluations performed by the other evaluators, and all pen-markings on the skin were erased in order to not influence the next rater assessments. In addition, for the 32 individuals evaluated, the evaluators’ order was intentionally randomized, so the evaluation order did not influence the results. As maps were not used in these comparisons, the identification of the probe position at the subjects’ thigh was made through the distance measures between anatomical points, localized by palpation by each rater. Therefore, the raters’ ability to identify the anatomical points also influenced the measurements reliability.

The raters' experience also plays a very important role in the reliability of the measurements, since experienced raters easily identify these anatomical points and the structures that need to be obtained during the evaluation of the ultrasonography muscle morphology measurements. In the present study, the three raters had different levels of experience. While rater 1 had four years of experience with the assessment technique, rater 2 had one year of experience and rater 3 worked with the technique for only three months. When comparing the reproducibility obtained in comparisons between the most experienced rater and the other two raters individually, we observed that only the VL_{FL} and VL_{PA} were less reliable when we compared the evaluator 1 with the evaluator 2 (0.853 and 0.717) and when comparing evaluator 1 with evaluator 3 (0.646 and 0.599). However, when comparing evaluator 1 with himself, the ICC values were 0.942 and 0.903, respectively, so these differences may have been caused by the rater's different level of experience (6 years, 1 year and 3 months, respectively). Therefore, it is fundamental that the raters have a proper training with the technique before carrying out the evaluations, which should endure, at least, a period greater than 3 months.

The reproducibility of the majority of the muscular morphology parameters, expressed by the ICC, was high, and only the VL_{PA} and VL_{FL} variables presented smaller values considered moderately high. As was expected, both parameters showed similar values, since the VL_{PA} is one of the variables used to calculate the VL_{FL}. The VL_{PA} is a very sensitive parameter, being affected by the rotation and the inclination of the probe during evaluation and by the parallel alignment of the deep aponeurosis with respect to superficial aponeurosis. In addition, especially for untrained raters, it is challenging to clearly visualize the muscle fibers, making their angulation calculation even more difficult. Therefore, the acquisition of the vastus lateralis muscle ultrasound images, from which the VL_{PA} and VL_{FL} parameters are obtained, must be performed with caution.

In the present study, both intra- and inter-rater comparisons of force evaluations were considered to be highly reliable, with higher values observed for intra-rater comparisons. ICC values for MVIC were 0.91 and 0.89, while for EF were 0.94 and 0.86, for intra- and inter-rater comparisons, respectively. Our results corroborate with a study conducted by Ferri-Morales et al. (2014), where a high intra-rater reproducibility (ICC = 0.91) was observed in the evaluation of torque during knee extensor maximal voluntary contractions of healthy young women. Our findings also corroborate with the study by Adsuar et al. (2011), which found identical intra-rater reproducibility (ICC = 0.91) in the evaluation of knee extensor maximal isometric torque in patients with fibromyalgia, followed by a SEM of 7.36 Nm. Our results are also in agreement with Nathaniel et al. (2014), who found a high (0.92) intra-rater ICC for EF evaluation, followed by a SEM of 6.13 Nm and MDC of 16.99 Nm. Therefore, our results are evidence that EF evaluation provides a highly reliable measure of healthy subjects muscle force, and, for this reason, shows to be a promising variable for ICU patient evaluation, as they are not able to perform voluntary muscle contraction.

The measurement of muscle strength through isometric NMES contractions proved to be an objective and useful tool for assessing knee extensor force in a simulated clinical environment. Therefore, evaluating the intra- and inter-rater reliability of dynamometry tests during a standardized performance was fundamental, as there is a need for precision in the measurements performed. Understanding reliability by means of voluntary and evoked muscle contractions may contribute to future studies in the selection of these variables to consistently study central and peripheral factors of neuromuscular function. The identification of variables that show less reliability should lead to new procedures to improve their reliability or to their exclusion as an outcome variable if reliability cannot be improved.

Finally, the data produced in this study defined the MDC required for each of the dependent variables in order to consider whether changes on measurements are real. This should benefit researchers and practitioners who wish to know when a patient's score actually changed as a result from an intervention. Our data also can be used as normative reliable data of a healthy condition for both men and women when determining the goals to be achieved in rehabilitation programs with critically ill patients.

3.6. CONCLUSION

The high reliability observed for all intra-rater parameters demonstrates that these measurements are accurate in the muscle morphology evaluation, when performed by the same rater at different moments. High reliability found for RF_{CSA} and muscle thickness measures in inter-rater and inter-analyst comparisons demonstrates that these measures are also accurate in the evaluation of musculoskeletal morphology, when performed both by different evaluators and analysts. The moderately high reliability found for VL_{AP} and VL_{FL} parameters in the inter-rater comparisons suggests that these parameters are evaluator-dependent, so that a more precise assessment needs to be performed. The bias that can influence these measures must be identified, so its reliability can be increased in order to reduce possible measurement errors. The reliability of force measures was high for both MVIC and EF, but higher for EF, which reinforce its use for evaluation of ICU patients.

FUNDING

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CONSIDERAÇÕES FINAIS

Os três capítulos apresentados na presente tese tinham como objetivos avaliar (1) os efeitos da duração do pulso elétrico e do tempo de contração/repouso da estimulação elétrica neuromuscular (NMEE), sobre o nível de corrente, desconforto e fadiga muscular; (2) o efeito do tempo de contração/relaxamento da EENM sobre a fadiga muscular e respostas cardiovasculares; e (3) a confiabilidade intra-avaliador, inter-avaliador e inter-analizador das medidas de morfologia e função dos músculos extensores de joelho, em sujeitos jovens saudáveis.

No primeiro capítulo, concluímos que uma maior duração de pulso (2 ms) permitiu o uso de um intensidade de corrente menor, o que seria umainformação útil dada as limitações de intensidade máximaalcançada por alguns equipamentos projetados para fins clínicos. Além disto,o pulso mais longo (2 ms) produziu níveis mais elevados de fadiga quando estes foram avaliados por meio do torque evocado, o que não é desejado se o objetivo de um programa de NMES for produzir sobrecarga mecânica contínua com menor custo metabólico. Portanto, visto que o estimulador desenvolvido para o presente projeto atinge níveis suficientes de corrente (180 mA), uma corrente bifásica simétrica com frequência de 80 Hz e duração de pulso de 1 ms parece adequada para a reabilitação de pacientes críticos.

No segundo capítulo, nossos resultados mostraram que o aumento dos tempos de contração/relaxamento da EENM de 1s/5s para 5s/25s (com mesma sobrecarga mecânica) aumentou a atividade simpática em indivíduos saudáveis. Embora ainda não tenhamos uma aplicação clínica clara para este achado, acreditamos que a modificação destes parâmetros deve ser realizada quando necessário, de forma que é possível tanto evitar a sobrecarga cardiovascular excessiva de pacientes instáveis, quanto aumentar progressivamente a sobrecarga cardiovascular como forma de treinamento. Além disto, durante a realização deste experimento foram desenvolvidos e testados dois sistemas de dinamometria para a avaliação e intervenção de indivíduos acamados. O desenvolvimento deste sistema possibilitou também determinar a dosagem adequada da EENM com base na força evocada pelo duplo-pulso, sem a colaboração dos sujeitos. Desta forma é possível realizar um programa de EENM para a reabilitação individualizada, relativizando a sobrecarga mecânica ao nível funcional do paciente.

No terceiro capítulo, observou-se uma alta confiabilidade para todas as medidas de ultrassonografia, quando estas foram realizadas pelo mesmo avaliador em diferentes momentos. A maior parte dessas medidas também foramaltamente reprodutíveis quando realizadas por diferentes avaliadores e analistas, tendo sido observada uma confiabilidade moderadamente alta somente para as medidas de comprimento fascicular e ângulo de penação, sugerindo que essas medidas são dependentes do avaliador que as realiza. Por fim, a confiabilidade das medidas de força foi alta para a CVMIe aindamaior para a força evocada, o que reforça seu uso para avaliação de pacientes na UTI que estão impossibilitados de realizar contrações voluntárias.Além disto, os valores de erro padrão de medida (SEM) e de mínima mudança detectável (MDC) fornecem critérios para a clara identificação das alterações na estrutura e da função dos músculos extensores de joelho dos pacientes, garantindo assim medidas reais das adaptações provocadas pela EENM.

Finalmente, deve-se salientar que os resultados encontrados na presente tese não devem ser diretamente extrapolados para pacientes, visto que todos os estudos apresentados anteriormente foram realizados com sujeitos saudáveis. Ainda assim, estes estudos forneceram informações fundamentais para o desenvolvimento de um programa de reabilitação seguro, com dosagem adequadae que não necessita da colaboração do paciente da UTI.

DIREÇÕES FUTURAS

O presente projeto de tese faz parte de um projeto maior que tem como objetivo o desenvolvimento e aplicação de um estimulador elétrico especial para a reabilitação e avaliação de pacientes adultos críticos internados em UTIs. A próxima etapa deste projeto envolve a realização de um ensaio clínico randomizado que tem como objetivo avaliar os efeitos da EENM sobre a morfologia e função dos músculos extensores de joelho de pacientes críticos internados na unidade de terapia intensiva do Hospital de Clínicas de Porto Alegre. Esta etapa será realizada de forma integrada ao Serviço de Fisioterapia do hospital, e já conta com a participação de diversos professores da instituição, alunos de iniciação científica, de mestrado e doutorado.Durante os últimos meses foi realizado o treinamento intensivo dos alunos que irão compor as equipes de intervenção e avaliação do projeto, que compreendeu desde a operação de todos os equipamentos até os conhecimentos clínicos necessários para a realização dos procedimentos no leito da UTI. Após o treinamento, foram formadas equipes de avaliação e intervenção independentes.

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