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ISABEL DE ALMEIDA PAZ

IS ALTERNATED CURRENT REALLY MORE EFFICIENT THAN PULSED CURRENT IN TERMS OF NEUROMUSCULAR PARAMETERS, CURRENT INTENSITY AND DISCOMFORT LEVEL?

PORTO ALEGRE - RS
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Master's Dissertation presented to the Graduate Program in Human Movement Sciences of the School of Physical Education, Physiotherapy and Dance of the Federal University of Rio Grande do Sul, as a final requisite for obtaining the title of Master's in Human Movement Sciences.

Supervisor: Prof. Dr. Marco Aurélio Vaz

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Dedico esse trabalho aos meu pais

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RESUMO

A Estimulação Elétrica Neuromuscular (EENM) é amplamente utilizada na reabilitação clínica, especialmente para melhorar a força muscular e prevenir quadros de atrofia. Dois tipos de correntes elétricas são bastante utilizados na prática clínica, a corrente pulsada (CP) e a corrente alternada (CA). A CA foi inicialmente introduzida sob a ideia de que seria capaz de aumentar a capacidade de produção de força em 40%, e, por esse motivo, utilizada na prática clínica sob a ideia de ser mais efetiva do que a CP para o fortalecimento muscular. Contudo, a literatura sobre essa temática é inconclusiva ou contraditória, e os estudos apresentam baixa qualidade metodológica. Em função disso, não é possível determinar qual das duas correntes é mais eficiente. Com a finalidade de responder essa pergunta e de aprofundar o conhecimento sobre esse tema, o objetivo da presente dissertação foi o de avaliar os efeitos agudos da CA comparados aos da CP em parâmetros clínicos (intensidade de corrente e nível de desconforto) e neuromusculares (torque evocado, eficiência neuromuscular, fatigabilidade e trabalho total) em jovens saudáveis. Para atingir esse objetivo realizamos 3 estudos: o primeiro, uma revisão sistemática com metanálise (Capítulo 1), seguido de dois ensaios clínicos randomizados, (Capítulos 2 e 3) que compõem essa dissertação. No Capítulo 1, revisamos sistematicamente a literatura por meio de uma metanálise de ensaios clínicos randomizados com o objetivo de avaliar os efeitos da CA e da CP (a) no torque extensor de joelho evocado pela EENM; (b) no nível de desconforto e (c) no nível de fatigabilidade em jovens saudáveis. CP demonstrou maior torque evocado comparado a CA-2.5 KHz e CA-4.0/4.05 KHz, e similar torque evocado comparado a CA-1.0 KHz. CP demonstrou similar desconforto à CA-1.0 KHz e CA-2.5 KHz. CP apresentou maior redução da contração voluntária máxima isométrica (CVMI) após protocolo com EENM (maior fadiga) comparada CA-2.5 KHz. Contudo, os estudos incluídos demonstraram baixa qualidade metodológica, e a análise de evidências para cada desfecho demonstrou evidências muito baixas a moderadas. Portanto, nos Capítulos 2 e 3 procuramos realizar dois estudos de alta qualidade metodológica com objetivo de preencher as lacunas da literatura. Para ambos os estudos, recrutamos 30 indivíduos saudáveis e fisicamente ativos, os quais foram submetidos a 6 dias de testes: 4 dias de teste para verificar os efeitos da CA e CP em parâmetros neuromusculares, intensidade de corrente e desconforto (Capítulo 2), e 2 dias de teste para avaliar os efeitos da CA e CP na fatigabilidade dos extensores do joelho (Capítulo 3). Portanto, no Capítulo 2 investigamos os efeitos da

CP e CA (a) na intensidade de corrente (mA) necessária para evocar torque submáximo e máximo; (b) no torque extensor de joelho evocado pela EENM; (c) na eficiência neuromuscular; e (d) no nível de desconforto. Para esse estudo, primeiramente utilizamos parâmetros semelhantes de duração de pulso (CP = 2 ms) e de *burst* (CA = 2 ms), bem como uma configuração de pulso comumente estudada e utilizada na prática clínica com as duas correntes (duração de pulso de 0.4 ms para CP e CA, com duração de *burst* de 5 ms para CA). Observamos que a CP gerou maior torque que CA. Adicionalmente, a CP com maior duração de pulso (2 ms) necessitou de menor intensidade de corrente e foi mais eficiente em dois níveis de força submáxima evocada quando comparada às demais configurações, sem diferenças no nível de desconforto entre CA e CP. Além disso, a duração de *burst* de 2.0 ms ou 5.0 ms da CA-2.5 KHz não teve qualquer influência nos parâmetros avaliados. Em relação à fatigabilidade, no Capítulo 1 verificamos que a CP foi mais fatigável que CA-2.5 KHz. Entretanto, somente o índice de queda da força em relação a CVMI pré e pós protocolo de fadiga foi avaliado, e a evidência para esse desfecho foi muito baixa. Além disso, os parâmetros de declínio da CVMI pós protocolo de fadiga, índice de fatigabilidade do torque evocado durante protocolo de fadiga, e análise da integral da curva torque-tempo (i.e., trabalho), não foram avaliados em um único estudo, o que poderia fornecer uma avaliação mais ampla e completa sobre o nível de fatigabilidade entre as correntes. Portanto, o Capítulo 3 teve como propósito comparar os efeitos da CP e da CA-2.5KHz sobre a fatigabilidade e desconforto, através da aplicação de um protocolo submáximo de fadiga isométrica. Os indivíduos foram avaliados em dois momentos: antes e após uma sessão de 20 minutos com CP ou com CA. Os resultados demonstraram que a CA é menos resistente à fatigabilidade que a CP, uma vez que demonstrou rápida queda no torque evocado, menor trabalho total e maior índice de queda da CVMI, mas similar nível de desconforto. Em conclusão, CP é mais eficiente do que a CA devido à menor intensidade de corrente, torque evocado mais elevado, maior eficiência neuromuscular, e menor fatigabilidade com maior trabalho total.

Palavras-chaves: Estimulação elétrica neuromuscular. Eletroterapia. Correntes excitomotoras. Eficiência neuromuscular.

ABSTRACT

Neuromuscular Electrical Stimulation (NMES) is widely used in clinical rehabilitation, especially to improve muscle strength and prevent atrophy. Two NMES types are widely used in clinical practice, pulsed current (PC) and alternating current (AC). AC was initially introduced under the idea that it would have the ability to increase force production capacity by 40%, and was therefore introduced into clinical practice under the idea of being more effective than PC for muscle strengthening. However, the literature involving this theme is inconclusive or contradictory, added to the low methodological quality of previous studies. Thus, it is not possible to determine which of the two currents is more efficient. In order to answer this question and to deepen the knowledge on this theme, the purpose of the present dissertation was to evaluate the acute effects of AC compared to PC on clinical (current intensity and discomfort) and neuromuscular (evoked force, neuromuscular efficiency, fatigability, and total work) parameters in healthy individuals. To achieve these purposes, we conducted 3 studies: the first, a systematic review with meta-analysis (Chapter 1), followed by two randomized clinical trials (Chapters 2 and 3) that encompass this dissertation. In Chapter 1, we systematically reviewed the literature and performed a meta-analysis of randomized controlled trials to assess the effects of AC and PC (a) on the knee extensor evoked torque; (b) on the discomfort level, and (c) on the fatigue level in healthy individuals. PC evoked higher torque compared to AC-2.5 KHz and AC-4.0/4.05 KHz, but similar evoked torque compared to AC-1.0 KHz. PC showed similar discomfort to AC-1.0 KHz and AC-2.5 KHz. PC produced greater maximal voluntary isometric contraction (MVIC) decrease after the NMES protocol (i.e., greater fatigue) compared AC-2.5 KHz. However, the included studies showed low methodological quality, and evidence analysis for each outcome showed very low to moderate evidence. Therefore, in Chapters 2 and 3 we conducted two high quality methodological studies aiming to fill the literature gaps. For both studies, we recruited 30 healthy and physically active individuals who underwent 6 testing days: 4 testing days to verify the AC and PC effects on neuromuscular parameters, current intensity and discomfort (Chapter 2), and 2 testing days to assess the effects of AC and PC on fatigability (Chapter 3). Therefore, on Chapter 2 we investigated the PC and AC effects (a) on the current intensity (mA) required to evoke submaximal and maximum torque; (b) on the knee extensor evoked torque; (c) on the neuromuscular efficiency, and (d) on the discomfort level. For this study, first we used similar pulse duration (PC = 2 ms) and burst (AC = 2 ms) parameters, followed by a pulse configuration commonly studied and used in clinical practice with both currents (PC and AC with 0.4 ms of pulse duration, with AC's burst duration of 5 ms). PC generated higher torque than AC. Additionally, the PC with longer pulse duration (2 ms) required less current intensity and was more efficient at two levels of submaximal evoked force when compared to the other configurations. We also found no differences between PC and AC for the discomfort level. In addition, the AC-2.5 KHz burst durations of 2.0 ms or 5.0 ms had no influence on the evaluated parameters. Regarding fatigability, in Chapter 1 we found that PC was more fatigable than AC-2.5 KHz. However, we evaluated only the force drop index in relation to the MVIC before and after the fatigue protocol, and the evidence for this outcome was very low. In addition, the MVIC decline after the fatigue protocol, the evoked torque fatigue index during the fatigue protocol, and the torque-time curve integral (i.e. work) analysis were not assessed in a single study, which could provide a wider and more detailed evaluation about the between-currents fatigability level. Therefore, Chapter 3 aimed to compare the effects of PC and AC-2.5 KHz on fatigability and discomfort by applying a submaximal isometric fatigue protocol. Participants were evaluated at two times: before and after a 20-minute session with PC or AC. The results showed that the AC was less fatigue resistant than the PC, since it demonstrated a rapid drop in the evoked torque, smaller total work and a higher MVIC drop rate, with similar discomfort level. We conclude that PC is more efficient than AC due to smaller current intensity, better evoked torque, better neuromuscular efficiency and smaller fatigability with higher total work.

Keywords: Neuromuscular electrical stimulation. Electrotherapy. Excitomotor currents. Neuromuscular efficiency.

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LISTA DE ABREVIAÇÕES

AC	Alternating current
SA	Subgroup analysis
BD	Burst duration
BMI	Body mass index
CH1	Channel 1
CH2	Channel 2
COMP	Comparison
DE	Distal electrode
F	Female
GRADE	Grading of Recommendations Assessment
M	Male
MP	Motor point
MTI	Maximally tolerated intensity
MVIC	Maximal voluntary isometric contraction
NA	Not applicable
NEUROEF	Neuromuscular efficiency
NMES	Neuromuscular electrical stimulation
PC	Pulsed current
PD	Pulse duration
PE	Proximal electrode
QUA	Quadriceps
RC	Russian current
RCT	Randomized controlled trials
RF	Rectus femoris
SB	Symmetrical biphasic
TTI	Torque-time integral
MU	Motor unit
VAS	Visual analog scale
VL	Vastus lateralis
VM	Vastus medialis
Y	Years

SUMMARY

1 PREFACE

Neuromuscular Electrical Stimulation (NMES) is a resource capable of generating visible muscle contractions by electrical impulses delivered through surface electrodes positioned on the skin with the purpose of providing a therapeutic effect (MAFFIULETTI *et al.*, 2018). Therefore, NMES has been successfully used in rehabilitation or training of the neuromuscular system in different populations (MAFFIULETTI *et al.*, 2013; DIRKS *et al.*, 2015; HERZIG; MAFFIULETTI; ESER, 2015).

Several approaches have been designed to optimize the benefits of NMES-based programs, among them the current type. Two electric current types are widely used in clinical practice, pulsed current (PC) and alternating current (AC). PC consists in the delivery of singular electric pulses interspersed with intervals between the pulses. PC stimulation frequency determines the number of total pulses per unit of time. AC is characterized by presenting a fixed carrier frequency (e.g., 1.0 KHz, 2.5 KHz) and for delivering successive electrical pulses within a stimulus package/train (burst), which usually is modulated at low-frequency bursts (1 to 120Hz) (WARD, 2009; VAZ; FRASSON, 2018).

Clinical stimulators usually use AC with medium carrier frequencies (between 1000 and 10000 Hz) (ADAMS *et al.*, 2018). Different carrier frequencies led to different names for the ACs. Therefore, the following names have been found in the literature: Aussie current (1.0 KHz), Russian current (RC, 2.5 KHz) and interferential current (1.0 KHz, 2.0 KHz, 4.0 KHz, 8.0 KHz and 10 KHz) (WARD; OLIVER; BUCCELLA, 2006; WARD; LUCAS-TOUMBOUROU, 2007; WARD; CHUEN, 2009; VENANCIO *et al.*, 2013). RC is one of the most common AC types that became popular when Yakov Kots introduced it around 1977, claiming that it produced strength gains of up to 40% in Russian Olympic athletes (WARD, 2009). Therefore, AC became popular and widely used in clinical practice, based on the belief that AC-stimulation was more effective and more comfortable than PC (WARD; LUCAS-TOUMBOUROU, 2007).

However, the literature involving the effects of AC and PC in evoked torque, discomfort level and fatigability demonstrated conflicting results, summed to different evaluation protocols and low methodological quality (DA SILVA *et al.*, 2015; IIJIMA *et al.*, 2018; VAZ; FRASSON, 2018). Therefore, it is unclear which current type causes less discomfort, less fatigue, and is capable of producing greater torque using the lowest current intensity (i.e., is more neuromuscular efficient). The existing evidences do not allow us to establish which current is the most efficient for clinical use.

In order to fill the literature gaps and deepen the knowledge about the effects of AC and PC on neuromuscular parameters, current intensity and discomfort level, we performed a systematic review with meta-analysis and two randomized clinical trials that will be presented in three chapters.

In Chapter 1, entitled ***“Effects of pulsed and alternating currents on quadriceps evoked torque, discomfort and fatigability in healthy individuals: a systematic review and meta-analysis”*** we reviewed randomized controlled trials involving PC and AC. Unlike existing systematic reviews, in this study we stratified AC by its carrier frequency (1.0 KHz, 2.5 KHz and 4.0/4.05 KHz) and compared it with PC in the outcomes of evoked torque, discomfort level and fatigue.

Considering the conflicting evidence involving RC (AC-2.5 KHz) and PC, which are often used by clinicians, in Chapter 2, entitled “***Alternating current is less efficient than pulsed current in healthy individuals: a blinded, randomized crossover trial***”, we performed a randomized controlled trial of high methodological quality to evaluate the effects of these currents on clinical and neuromuscular parameters. In this study we used four current settings, two with similar total charge capacity, that is, similar pulse and burst duration (PC-2 ms pulse duration and AC-2 ms burst duration), and two currents with parameters often used by clinicians and in studies involving AC and PC, with similar charge capacity per pulse (PC and AC with pulse duration of 0.4 ms). Our purpose was to evaluate the effects PC and AC-2.5 KHz in the evoked torque, current intensity, neuromuscular efficiency and discomfort at submaximal and maximal intensity levels.

Additionally, as few studies have evaluated the effects of AC-2.5 KHz and PC on fatigability, and as these studies have used different methodological designs, establishing which current is more fatigable has been a difficult task. Therefore, in Chapter 3, entitled *“**Alternating current is more fatigable than pulsed current in healthy individuals: a blinded, randomized crossover trial**”*, we performed a high quality methodological study using two current settings, both with greater energy capacity than that used in clinical practice (PC-2 ms pulse duration and AC-5 ms burst duration), with the purpose of evaluating the PC and AC effects at the fatigability and discomfort level during a fatigue protocol.

2 DISSERTATION OBJECTIVES

The main purpose of our research was to evaluate the acute effects between PC and AC on clinical (current intensity and discomfort level) and neuromuscular (evoked force, neuromuscular efficiency, evoked fatigue, and total work) parameters in healthy individuals.

To achieve the main purpose, the following specific goals were defined:

1. To systematically review studies that evaluated the PC and AC effects on: (1) knee extensor evoked torque, (2) discomfort level, and (3) fatigue level.
2. To compare the PC and AC-2.5 KHz effects on: (1) current intensity, (2) knee extensor evoked torque, (3) neuromuscular efficiency, and (4) discomfort level at submaximal and maximal intensity levels, through a randomized clinical trial.
3. To evaluate the PC and AC-2.5 KHz effects on fatigability and discomfort level during a submaximal isometric fatigue protocol, through a randomized clinical trial.

3 GENERAL HYPOTHESIS

Based on the literature results, we hypothesized that AC will evoke less torque, will need more current intensity and will be more uncomfortable, at both submaximal and maximal strength levels, compared to PC. We did not find studies evaluating neuromuscular efficiency between PC and AC-2.5 KHz. However, considering that previous studies reported that AC needed higher current intensity and was able to generate a torque similar to PC, we hypothesized that AC-2.5 KHz will be less efficient than PC. Additionally, considering the AC-2.5 KHz’s large number of electrical pulses delivered within each burst, which can lead to action potentials generation failure in motoneurons due to the stimuli high frequency, we hypothesized that AC-2.5 KHz will evoke more (smaller total work) and faster (higher rate of evoked torque loss) fatigue compared to PC.

CHAPTER 1

4 EFFECTS OF PULSED AND ALTERNATING CURRENTS ON QUADRICEPS EVOKED TORQUE, DISCOMFORT AND FATIGABILITY IN HEALTHY INDIVIDUALS: A SYSTEMATIC REVIEW AND META-ANALYSIS.

4.1 ABSTRACT

Introduction: Neuromuscular electrical stimulation (NMES) has been used to recover muscle strength in different populations. However, different electrical current types may influence the NMES protocol efficiency. The most currently used NMES types are the pulsed current (PC) and the alternating current (AC). However, the evidences for their effects on evoked torque, fatigability, and discomfort level are still controversial. **Objective:** To compare the effects of PC and AC on evoked torque, fatigue, and discomfort levels in healthy individuals through a systematic review and meta-analysis of randomized controlled trials (RCTs) (CRD42018110362). **Methods:** RCTs applying NMES to the quadriceps muscle and comparing the PC and AC effects on evoked torque, fatigue and discomfort in healthy individuals were included. Searches were carried out in the following databases: PubMed, Physiotherapy Evidence Database (PEDro), Embase, Cochrane Central, PROQUEST, Web of Science, as well as in thesis and dissertation banks, ClinicalTrials, Brazilian Registry of Clinical Trials (ReBEC) and Google Scholar. As a search strategy, controlled and uncontrolled descriptors for population, intervention and study type were used. Data extraction was performed by two reviewers independently and the main characteristics of included studies, main results and NMES parameters were extracted. For each outcome, the mean and standard deviation values, and the number of both groups' participants were extracted. Qualitative and quantitative analyses of the included studies were carried out. PC was compared to AC with carrier frequencies of 1 KHz, 2.5 KHz and 4.0-4.05 KHz whenever possible. The risk of bias was assessed using the Cochrane tool, and the level of evidence strength was evaluated by the GRADE approach. **Results:** Thirteen studies were included. PC evoked higher torque compared to AC-2.5 KHz and AC-4.0/4.05 KHz, and similar evoked torque compared to AC-1.0 KHz. PC showed similar discomfort to AC-1.0 KHz and AC-2.5 KHz. PC produced greater maximal voluntary isometric contraction (MVIC) decrease after the NMES protocol (i.e., greater fatigue) compared AC-2.5 KHz. Evidence analysis for each outcome demonstrated only very low to moderate evidence. **Conclusion:** There is no significant difference in evoked torque and discomfort between AC-1.0 KHz and PC, suggesting that both currents can be used interchangeably in rehabilitation protocols. PC is more efficient to evoke torque than AC-2.5 KHz and AC-4.0/4.05 KHz. PC and AC-2.5 KHz presented a similar discomfort level. The larger PC-induced mechanical overload leads to larger functional responses, since it showed a greater MVIC decrease after the fatigue protocol. However, these results should be observed with caution, due to the analyzed studies low to moderate evidence quality.

4.2 INTRODUCTION

Neuromuscular electrical stimulation (NMES) is frequently used in clinical practice for atrophy prevention (DIRKS *et al.*, 2015; SAITOH *et al.*, 2016; ESTEVE *et al.*, 2017), functional capacity recovery (HAUGER *et al.*, 2018), and to improve muscle strength in different populations (MAFFIULETTI *et al.*, 2013; HERZIG; MAFFIULETTI; ESER, 2015; ESTEVE *et al.*, 2017; HONG *et al.*, 2018). In order to achieve NMES training goals, electrically evoked force should be considered, since NMES effectiveness is determined by the muscle tension generated by the NMES current (MAFFIULETTI *et al.*, 2018). However, some factors can limit the NMES effectiveness, such as discomfort and early fatigue onset (VAZ; FRASSON, 2018). Patient's tolerance level to NMES may limit the maximum tolerated current intensity (DELITTO *et al.*, 1992), which can affect the evoked torque (ADAMS *et al.*, 1993). In turn, fatigue developed at a NMES-protocol early stage influences the amount of time an evoked force can be sustained and, consequently, the mechanical load produced at the muscle-tendon unit, which determines the rehabilitation programs' neuromuscular adaptation (VAZ; FRASSON, 2018).

In this sense, studies try to establish the NMES current type able to evoke the strongest muscular contraction and to generate the smallest discomfort and fatigability (VAZ; FRASSON, 2018). Two current types have been widely studied in strength training and in clinical practice: the alternating current (AC) and the pulsed current (PC). ACs are characterized by having a biphasic waveform, with no between-pulses gap, and can be delivered continuously or in burst form. On the other hand, PC is characterized by biphasic or monophasic currents, whose pulses are separated by a gap (i.e., an inter-pulse interval) (WARD; LUCAS-TOUMBOUROU, 2007; WARD, 2009).

AC became popular around 1977, from reports by Kots (KOTS; XVILON, 1971) of strength gains of up to 40% in elite athletes using what became to be known as the "Russian current" (RC, AC-2.5 KHz). Apparently, although no scientific evidence was provided through scientific methods, RC became accepted based on the idea that it was an efficient current for muscular strengthening (WARD, 2009). However, other AC carrier frequencies (AC-1.0 KHz and AC-4.0 KHz) are also used clinically, and the effects vary depending on the carrier frequency and burst duration (WARD; ROBERTSON; IOANNOU, 2004; WARD, 2009). The common use of AC in rehabilitation seems to be due to the clinical belief that AC stimulation is more comfortable and more effective in force production compared to PC (WARD; LUCAS-TOUMBOUROU, 2007). However, this higher efficiency of AC compared to PC is still not clear, because several studies have compared the effects of AC and PC on muscular strength production, discomfort and fatigue level, but demonstrated controversial results (LAUFER *et al.*, 2001; LYONS *et al.*, 2005; ALDAYEL *et al.*, 2010; ALDAYEL *et al.*, 2011; VAZ *et al.*, 2012; FUKUDA *et al.*, 2013; DANTAS *et al.*, 2015; LEIN JR; MYERS; BICKEL, 2015; SCOTT *et al.*, 2015; MEDEIROS *et al.*, 2017; DE OLIVEIRA *et al.*, 2018).

In view of the presented divergences, previous systematic reviews have collected and summarized all the available evidences, aiming to present a consistent result on this topic. Reviews with meta-analysis (DA SILVA *et al.*, 2015; IIJIMA *et al.*, 2018) demonstrated similar results for discomfort and evoked torque between the currents. Additionally, Vaz and Frasson (2018) concluded that AC is not superior to PC due to the studies' results, the limited evidence and low-to-moderate studies' quality (VAZ; FRASSON, 2018).

However, there are some methodological issues regarding the existing reviews that should be considered. Reviews with meta-analysis (DA SILVA *et al.*, 2015; IIJIMA *et al.*, 2018) addressed only the strength and discomfort level outcomes. Although these are important variables, the fatigue level is an essential outcome, which must be considered when analyzing a NMES current's efficiency. Although this outcome has been addressed by another review (VAZ; FRASSON, 2018), the authors did not conduct a meta-analysis and, consequently, it is not possible to report quantitatively whether there are between-currents differences. Moreover, Da Silva et al. (2015) demonstrated a large heterogeneity in their meta-analysis for the evoked torque outcome, and the authors did not demonstrate the sensitivity or subgroup analysis to explore the heterogeneity level. In addition, although they included those studies published until 2014, some studies of this period were not included in these reviews (VAZ *et al.*, 2012; FUKUDA *et al.*, 2013).

Although the review of Iijima *et al*. (2018) has included more recent studies, this meta-analysis has several methodological issues that can influence their results. For example, a large heterogeneity was observed. This can be related to the fact that this meta-analysis included (1) studies assessing evoked torque from distinct muscle groups (i.e. wrist extensors and knee extensors), (2) AC studies using different carrier frequencies (AC-1.0 KHz, AC-2.5 KHz, and AC-4.0 KHz), (3) studies with different populations (young healthy and spinal cord injury patients), and (4) different study designs (RCTs and quasi-experimental). Additionally, the process of study selection, data extraction, and risk of bias assessment was performed by a single evaluator. It is also possible to verify that the articles' inclusion was limited to English language, and that the literature search for studies that are not formally published in scientific journals (i.e., gray literature) was not performed. All of these issues may have influenced the presented results (HIGGINS; GREEN, 2011).

In view of the presented problems, it is necessary to systematically review the two NMES currents' (AC and PC) effects on the evoked torque, fatigue and discomfort level, adopting greater methodological rigor, since these outcomes are clinically important and interfere in the NMES efficiency. Therefore, the purpose of this review is to compare the effects of PC and AC on evoked torque, discomfort and fatigability levels in healthy young individuals through a systematic review and meta-analysis of randomized controlled trials (RCTs).

4.3 METHODS

This systematic review and meta-analysis followed the recommendations proposed by the Cochrane Collaboration (HIGGINS; GREEN, 2011) and the PRISMA Statement (LIBERATI *et al.*, 2009), and was registered at PROSPERO (CRD42018110362).

4.3.1 Eligibility Criteria

This review included RCTs that compared PC to AC in the following outcomes: knee extensors evoked torque, discomfort level and evoked fatigue of healthy young subjects. In addition, we only included studies that used biphasic PC. Exclusion criteria were as follows: inclusion of subjects with associated diseases; studies that used drugs or protocol-associated supplementation; studies that associated NMES to exercise; and studies that evaluated AC and PC chronic effects.

4.3.2 Search Strategy

Searches were carried out in the following electronic databases (from inception to November 2018): PUBMED, Cochrane Central Register of Controlled Trials (Cochrane CENTRAL), EMBASE, Physiotherapy Evidence Database (PEDro), PROQUEST, Web of Science, and Brazilian Clinical Trials Registry (ReBEC). In addition, a search in digital thesis and dissertation libraries (LUME, USP), Google Scholar, and the references of the published studies was performed. Controlled and uncontrolled terms for population, intervention and study type were used. In order to establish the study type, previously proposed words for identification of RCTs (ROBINSON; DICKERSIN, 2002) were used. There were no language or publication status restrictions. The complete search strategy used in PubMed is shown in Table 1, and the searches' strategies utilized in the remaining databases are available upon request.

4.3.3 Study Selection

Two reviewers independently evaluated titles and abstracts of all studies identified by the search strategy. Studies not meeting eligibility criteria according to titles or abstracts were excluded, and those that did not provide sufficient information were selected for full-text evaluation. After this, full-text articles were evaluated by two investigators working independently. In this phase, the reviewers performed their selection according to the eligibility criteria. Disagreements between the reviewers were solved by consensus or by a third reviewer.

4.3.4 Data Extraction

Two reviewers independently conducted data extraction to obtain the studies methodological characteristics, interventions and outcomes. When necessary, authors were contacted for clarification. Differences between reviewers were solved by consensus. The primary analyzed outcome was evoked torque, and the secondary outcomes were discomfort and fatigue level.

4.3.5 Risk of Bias Assessment

Study quality assessment was performed by two reviewers independently using the Cochrane Risk of Bias Tool (HIGGINS; GREEN, 2011). This tool evaluates the following items: adequate sequence generation, allocation concealment, blinding of patients, blinding of outcome assessors, use of intention-to-treat analysis, and description of losses and exclusions. The intention-to-treat analysis was considered if the study reported in the text or tables that the number of randomized participants was the same as the analyzed number. Studies without a clear description of these items were considered unclear.

Table 1- Literature search strategy used for the PUBMED database

#1	“Adult”[Mesh] OR “Adult” OR “Adults” OR “Young Adult”[Mesh] OR “Young Adult” OR “Adult, Young” OR “Adults, Young” OR “Young Adults” OR "Healthy Volunteers"[Mesh] OR "Healthy Volunteers" OR “Healthy Volunteer” OR “Volunteer, Healthy” OR “Healthy Participants” OR “Healthy Participant” OR “Participant, Healthy” OR “Participants, Healthy” OR “Healthy Subjects” OR “Healthy Subject” OR “Subject, Healthy” OR “Subjects, Healthy” OR “Healthy Young” OR "healthy women" OR "healthy man" OR "healthy subjects" OR "healthy individuals" OR “healthy adults”
#2	“Electric Stimulation”[Mesh] OR “Electric Stimulation” OR “Electrical Stimulation” OR “Electrical Stimulations” OR “Stimulation, Electrical” OR “Stimulations, Electrical” OR “Stimulation, Electric” OR “Electric Stimulations” OR “Stimulations, Electric” OR "Electric Stimulation Therapy"[Mesh] OR "Electric Stimulation Therapy" OR “Therapeutic Electrical Stimulation" OR “Electrical Stimulation, Therapeutic" OR “Stimulation, Therapeutic Electrical" OR “Therapeutic Electric Stimulation" OR “Electric Stimulation, Therapeutic" OR “Stimulation, Therapeutic Electric" OR “Electrical Stimulation Therapy" OR “Stimulation Therapy, Electrical" OR “Therapy, Electrical Stimulation" OR “Therapy, Electric Stimulation" OR “Stimulation Therapy, Electric” OR Electrotherapy OR “Interferential Current Electrotherapy" OR “Electrotherapy, Interferential Current” OR "Russian current" OR “Aussie current” OR "Kilohertz current" OR “medium frequency electrical stimulation” OR "Alternating current" OR “Low-frequency pulsed current” OR "Pulsed current" OR “Functional Electrostimulation” OR “neuromuscular electrical stimulation”
#3	"Fatigue"[Mesh] OR “Fatigue” OR "Muscle Fatigue"[Mesh] OR “Muscular Fatigue” OR “Fatigue, Muscular” OR “Fatigue, Muscle” OR “peripheral fatigue” OR “central fatigue” OR "Muscle Strength"[Mesh] OR "Muscle Strength" OR “Strength, Muscle” OR "Torque"[Mesh] OR "Torque" OR “Torques” OR “evoked torque” OR “discomfort”
#4	randomized controlled trial[pt] OR controlled clinical trial[pt] OR randomized controlled trials[mh] OR random allocation[mh] OR double-blind method[mh] OR single-blind method[mh] OR clinical trial[pt] OR clinical trials[mh] OR ("clinical trial"[tw]) OR ((singl*[tw] OR doubl*[tw] OR trebl*[tw] OR tripl*[tw]) AND (mask*[tw] OR blind*[tw])) OR ("latin square"[tw]) OR placebos[mh] OR placebo*[tw] OR random*[tw] OR research design[mh:noexp] OR follow-up studies[mh] OR prospective studies[mh] OR cross-over studies[mh] OR control*[tw] OR prospectiv*[tw] OR volunteer*[tw]) NOT (animal[mh] NOT human[mh]
#5	Search #1 AND #2 AND #3 AND #4

4.3.6 Data Analysis

Qualitative and quantitative data analyses were performed. For the qualitative analysis, the included studies main characteristics and results were presented and discussed. For the quantitative analysis, meta-analysis for each outcome was performed. Mean and standard deviation values for the parameters of interest were used. Studies in which it was not possible to export the mean and standard deviation values, the data was imputed from the presented figures. Calculations in the meta-analysis were performed using a random effects method. A p-value <0.05 was considered statistically significant. Statistical heterogeneity of the intervention effects among studies was assessed using the Inconsistency test (I^2), in which values lower than 25% were considered indicative of low heterogeneity, between 25% and 50% were considered indicative of moderate heterogeneity, and above 50% were considered with high heterogeneity (HIGGINS *et al.*, 2003). All analyses were conducted using the Review Manager software, version 5.3 (HIGGINS; GREEN, 2011). To explore heterogeneity between studies, we performed subgroup analyses, considering PC' and AC's pulse duration, current intensity and NMES application site.

The choice of these criteria for subgroup analysis is justified by the fact that, generally, longer pulse durations generate greater muscle torque at least when PC is used (SCOTT; CAUSEY; MARSHALL, 2009). In this sense, we balanced the PC's pulse duration with the AC's same pulse duration in the analysis. In addition, the current intensity utilized, and the electrodes position for NMES application interfere with both discomfort and force generation (MAFFIULETTI, 2010), and evidence demonstrates the importance of applying NMES at the motor point to minimize the discomfort, and to maximize the NMES evoked force (GOBBO *et al.*, 2011; GOBBO *et al.*, 2014).

4.3.7 *Summary of Evidence*

The Grading of Recommendations Assessment, Development and Evaluation (GRADE) approach was used to assess the evidence quality (SCHÜNEMANN *et al.*, 2008; HIGGINS; GREEN, 2011). For each specific outcome, the evidence quality was based on five factors: [1] risk of bias; [2] inconsistency; [3] indirectness; [4] imprecision; and [5] other considerations (publication bias). The GRADE approach resulted in four levels of quality of evidence: high, moderate, low, and very low, which are defined according to the factors mentioned above and are applied to a body of evidence (BALSHEM *et al.*, 2011).

4.4 RESULTS

4.4.1 Study Selection

Using the search protocol, 3521 (database and additional records identified) articles were identified. Eighty-three of them were identified as potentially eligible (reading in full). After inclusion criteria application, 13 studies were selected for this review and included in the qualitative analysis, and 11 of them were included in the meta-analysis (Figure 1, Tables 2 and 3).

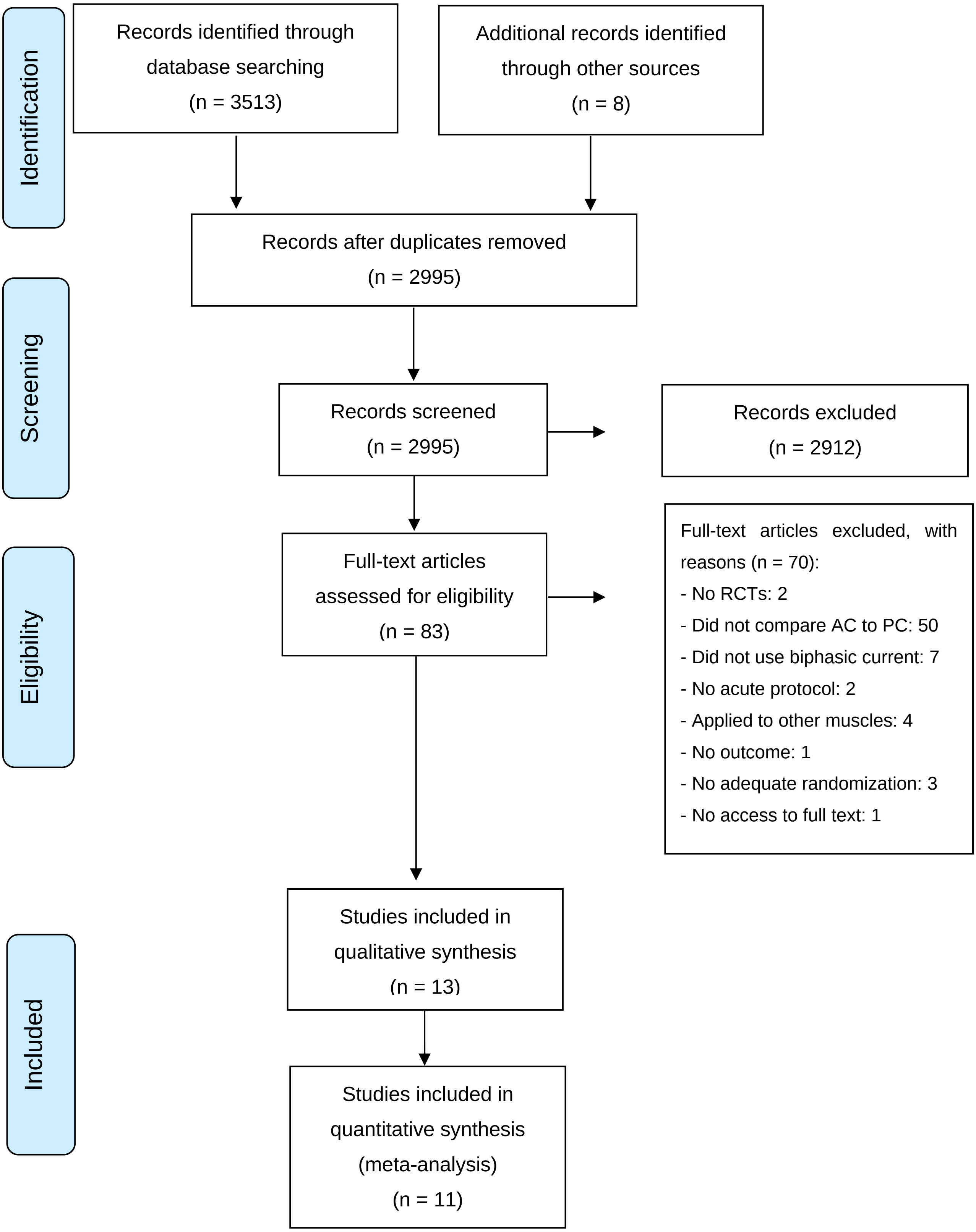


Figure 1. Flowchart for identification and selection of articles for final inclusion (based on the Prisma flowchart template).

Table 2 - Characteristics of the included studies.

Authors	Sample/ gender	Sample Characteristics	Number and Electrodes size	Electrodes position	Position (Knee angle)	Outcomes
Aldayel *et al*. 2010	12/M	Healthy; age: 31.2±5.5 y; weight: 81.4±15.2 kg; height: 174.3±4.8 cm; BMI: 26.9 kg/m^2	2 (5x5 cm) 2 (5x10 cm)	MP: VL and VM Proximal portion QUA	100°	Evoked torque Fatigue
Aldayel *et al*. 2011	9/M	Healthy; age: 34.0±7.0 y; weight: 85.4±14.1 kg; height: 174.0±5.1 cm; BMI: 28.2 kg/m^2	2 (5x5 cm) 2 (5x10 cm)	MP: VL and VM Proximal portion QUA	100°	Evoked torque Fatigue
Dantas *et al*. 2015	21/F	Physically active; age: 21.6±2.5 y; weight: 58.8; 68.5 kg; height: 166.3±7.3 cm; QUA skinfold thickness: 25.2± 4.1 mm; body fat: 24.1±3.9%; BMI: 21.25±15 kg/m^2	4 (5x5 cm)	Ch 1: DE: MP of the VM; PE: 15 cm proximal to the DE on RF Ch 2: DE: VL MP; PE: 15 cm proximal to the DE on RF	60°	Evoked torque Discomfort
Fukuda *et al*. 2013	30/M	Healthy adults; age: 25±3 y; height: 175±6 cm; BMI: 24.2±1.7 kg/m^2	4 (8.5x5 cm)	PE: RF; DE: VM PE: proximal VL; DE: VL	60°	Evoked torque Discomfort
Holcomb *et al*. 2000	5M/5F	Healthy adults; age: 24 y; weight: 64.4 kg; height: 168.4 cm; BMI: 22.8 kg/m^2	4 (5x5 cm)	Ch 1: PE: femoral triangle; DE: VM MP Ch 2: MP: RF and VL.	105º	Evoked torque
Lein *et al*. 2015	12/F	Healthy adults; age: 25.5±9.0 y; weight: 74.4±13.1 kg; height: 175±10.4 cm; BMI: 24.3 kg/m^2	2 (7.5x10 cm)	DE: distal medial thigh PE: proximal lateral thigh	90°	Fatigue
Liebano *et al*. 2013	45/F	Healthy adults; age: 21.8 y	2 (5.08x10.16 cm)	PE: femoral nerve DE: VM MP	90°	Discomfort

Medeiros *et al*. 2017	25/F	Physically active; age: 21±3 y; weight: 59.0±9.0 kg; height: 162±5.0 cm; BMI: 22.2±0.1kg/m^2	2 pairs (5x5 cm)	Ch1: DE: 80% on the line between the anterior superior iliac spine and the anterior border of the medial ligament. PE: 10 to 15 cm proximal to the DE on VM. Ch 2, DE: at 2/3 on the line from the anterior superior iliac spine to the lateral border of the patella on the VL, and the PE:10 to 15 cm proximal to the DE on VL	60°	Evoked torque Discomfort
Oliveira *et al*. 2018	11/M	Healthy adults; age: 24.5±5.4 y; weight: 77.0±8.4 kg; height: 176±4.0 cm; BMI: 24.8±2.6 kg/m^2	2 positive (5x5 cm) negative (10x5 cm)	positive over the VM and VL MP, with negative 3-5cm below the inguinal ligament	60°	Evoked torque Discomfort Fatigue
Petrofsky *et al*. 2009	6M/4F	Age: 25.5±1.3 y; weight: 74.8±18.2 kg; height: 175.7±10.1 cm; BMI: 23.9±3.7 kg/m^2; Skin thickness: 0.08+0.01 cm; Fat thickness: 0.78+0.2 cm; % body fat: 18.7+3.8	2 (2x2 cm) 2 (2x4 cm) For interferential 4 electrodes were used	Over the QUA	90°	Evoked torque Discomfort
Snyder-Mackle *et al*. 1989	11M/9F	Healthy adults; age: 28.7 y	2 (7.6x12.7 cm)	PE: proximal anterior thigh. DE: anteromedial thigh	60°	Evoked torque
Vaz *et al*. 2012	9M/13F	Healthy adults; age: 25±4 y	2 (6x8 cm) 1 (18x3 cm)	PE: MP; PE: distal portion of QUA	90°	Discomfort
Walmsley *et al*. 1984	15/M	Intercollegiate athletes	3 (7.3x3 cm)	over QUA	60°	Evoked torque

M: male; F: female; y: years; BMI: Body Mass Index; QUA: quadriceps; MP: motor point; VL: vastus lateralis; DE: distal electrode; VM: vastus medialis: RF: rectus femoris; PE: proximal electrode; Ch1: channel 1; Ch2: channel 2; NMES: neuromuscular electrical stimulation.

Table 3 - NMES Parameters used in the included studies.

Author	Current Type	Waveform	Carrier Frequency (KHz)	Modulated Frequency (Hz)	Pulse Frequency (Hz)	Burst/ interburst duration (ms)	Burst Duty cycle	Pulse Duration (μs)	ON/OFF (s)	Intensity
Aldayel *et al*. 2010	AC	sinusoidal	2.5	75	-	6.5	50%	400	5/15	Maximal tolerance
	PC	rectangular	-	-	75	-	-	400		
Aldayel *et al*. 2011	AC	sinusoidal	2.5	75	-	6.5	25%	400	5/15	Maximal tolerance
	PC	rectangular	-	-	75	-	-	400		
Dantas *et al*. 2015	AC	sinusoidal	1.0	50	-	4/16	-	500*	10/180	Maximal tolerance
	AC	sinusoidal	2.5	50	-	10/10	_	200*		
	PC	rectangular	-	-	50	-	_	200*		
	PC	rectangular	-	-	50	-	-	500*		
Fukuda *et al*. 2013	AC	sinusoidal	2.5	50	-	10	50%	400	0.2/300	Maximal tolerance
	PC	rectangular	-	-	50	-	-	400		
Holcomb *et al*. 2000	AC	NI	2.5	90	-	5.6	-	-	10/120	Maximal tolerance
	PC	NI	-	-	90	-	-	200		
Lein *et al*. 2015	AC	sinusoidal	2.5	20	-	10	50%	200	1/1	30% of the MVIC
	AC	sinusoidal	2.5	50	-	25	50%	200		
	PC	NI	-	-	20	-	-	500		
	PC	NI	-	-	50	-	-	500		
Liebano *et al*. 2013	AC	rectangular	2.5	50	-	-	-	400	10/180	Full knee extension
	PC	rectangular	-	-	50	-	-	350		
Medeiros *et al*. 2017	AC	sinusoidal	1.0	50	-	2/18	10	500	10/180	Maximal tolerance
	AC	sinusoidal	4.0	50	-	4/16	20	250		
	PC	rectangular	-	-	50	-	-	500		

	PC	rectangular	-	-	50	-	-	250		
Oliveira *et al*. 2018	AC	NI	1.0	100	-	2/18	-	500	6/18	Maximal tolerance
	PC	NI	-	-	100	-	-	500		
Petrofsky *et al*. 2009	AC	Russian	2.5	50	-	-	-	-	2/180	20, 40 and 60mA
	AC	interferential	4.0/4.05	50	-	-	-	-		
	PC	square	-	-	20, 30, 50	-	-	300		
	PC	sine	-	-	20, 30, 50	-	-	300		
Snyder-Mackle *et al*. 1989	AC	NI	2.5	50	-	10/10	-	200*	10/120	Maximal tolerance
	AC	NI	4.0/4.05	-	-	-	-	125*		
	PC	square	-	-	50	-	-	200*		
Vaz *et al*. 2012	AC	sinusoidal	2.5	-	50	10 /10	50%	400	10/600	10% of MIVC
	PC	rectangular	-	50	-	-	-	400		
Walmsley *et al*. 1984	AC	sinusoidal	2.2	50	-	10/10	-	450	6/120	Maximal tolerance
	PC	-	-	-	50	-	-	200		

AC: alternated current; PC: pulsed current; NI: not informed; *phase duration

4.4.2 Studies Methodological Quality

In relation to the studies' methodological quality, 31% described a random generation sequence and presented blinded assessment of outcomes. Only 8% reported allocation concealment, and 23% described losses to follow-up and exclusions, characterizing high bias risk for these items. In addition, only 46% of the studies blinded patients and used the intention-to-treat principle for statistical analyses, characterizing high bias risk for these items (Table 4).

Table 4 - Risk of bias of the included studies.

	Adequate sequence generation	**Allocation concealment**	**Blinding of patients**	**Blinding of outcome assessors**	**Description of losses and exclusions**	**Intention-to-treat analysis**
Aldayel *et al.* 2010	UNCLEAR	NI	YES	NI	NO	NI
Aldayel *et al.* 2011	UNCLEAR	NI	YES	NI	NO	YES
Dantas *et al.* 2015	UNCLEAR	NI	YES	YES	YES	YES
Fukuda *et al.* 2013	YES	YES	NI	YES	NO	NI
Holcomb *et al.* 2000	UNCLEAR	NI	NI	NI	NO	YES
Lein *et al.* 2015	UNCLEAR	NI	NI	NI	NO	YES
Liebano, Alves. 2013	YES	UNCLEAR	YES	NI	NO	NO
Medeiros *et al.* 2017	UNCLEAR	NI	YES	YES	YES	YES
Oliveira *et al.* 2018	UNCLEAR	NI	YES	YES	NO	NI
Petrofsky *et al.* 2009	UNCLEAR	NI	NI	NI	NO	UNCLEAR
Snyder-Mackler *et al.* 1989	YES	UNCLEAR	NI	NI	NO	UNCLEAR
Vaz *et al.* 2012	UNCLEAR	NI	NI	NI	NO	YES
Walmsley *et al.* 1984	YES	UNCLEAR	NI	NI	YES	NO

NI = not informed.

4.4.3 Intervention Effects

4.4.3.1 *Evoked Torque*

Evoked torque was compared in several of the included studies between PC and three AC configurations: AC-2.5 KHz, AC-1.0 KHz and AC-4.0/4.05 KHz. These comparisons were analyzed quantitatively through meta-analysis and are described below in three blocks. Additionally, a comparison between PC and AC-2.2 KHz was done in only one study (WALMSLEY; LETTS; VOOYS, 1984), which did not allow us to perform a meta-analysis. According to this study results, PC evoked a similar torque than AC-2.2 KHz.

4.4.3.1.1 *Evoked Torque between PC vs. AC-2.5 KHz*

Seven studies (SNYDER-MACKLER; GARRETT; ROBERTS, 1989; HOLCOMB; GOLESTANI; HILL, 2000; PETROFSKY *et al.*, 2008; ALDAYEL *et al.*, 2010; ALDAYEL *et al.*, 2011; FUKUDA *et al.*, 2013; DANTAS *et al.*, 2015) evaluated the differences between PC and AC-2.5 KHz for quadriceps evoked torque (Figure 2). There was a slightly higher torque (9.03; 95% CI: 1.36 to 16.69; I^2 92%) for the PC compared to AC (p=0.02) (Figure 2.1.1). When performing subgroup analysis, maintaining the studies that used NMES at the motor point (Figure 2.1.4) and maximum tolerable intensity (Figure 2.1.2), it was possible to decrease the results heterogeneity, and the results were favorable to PC compared to AC-2.5 KHz. However, in the subgroup analysis with studies that used PC's pulse width of 400 μs (Figure 2.1.3), there was no difference between PC and AC. Based on the GRADE approach, the quality of the evidence for this outcome was considered very low (Table 5).

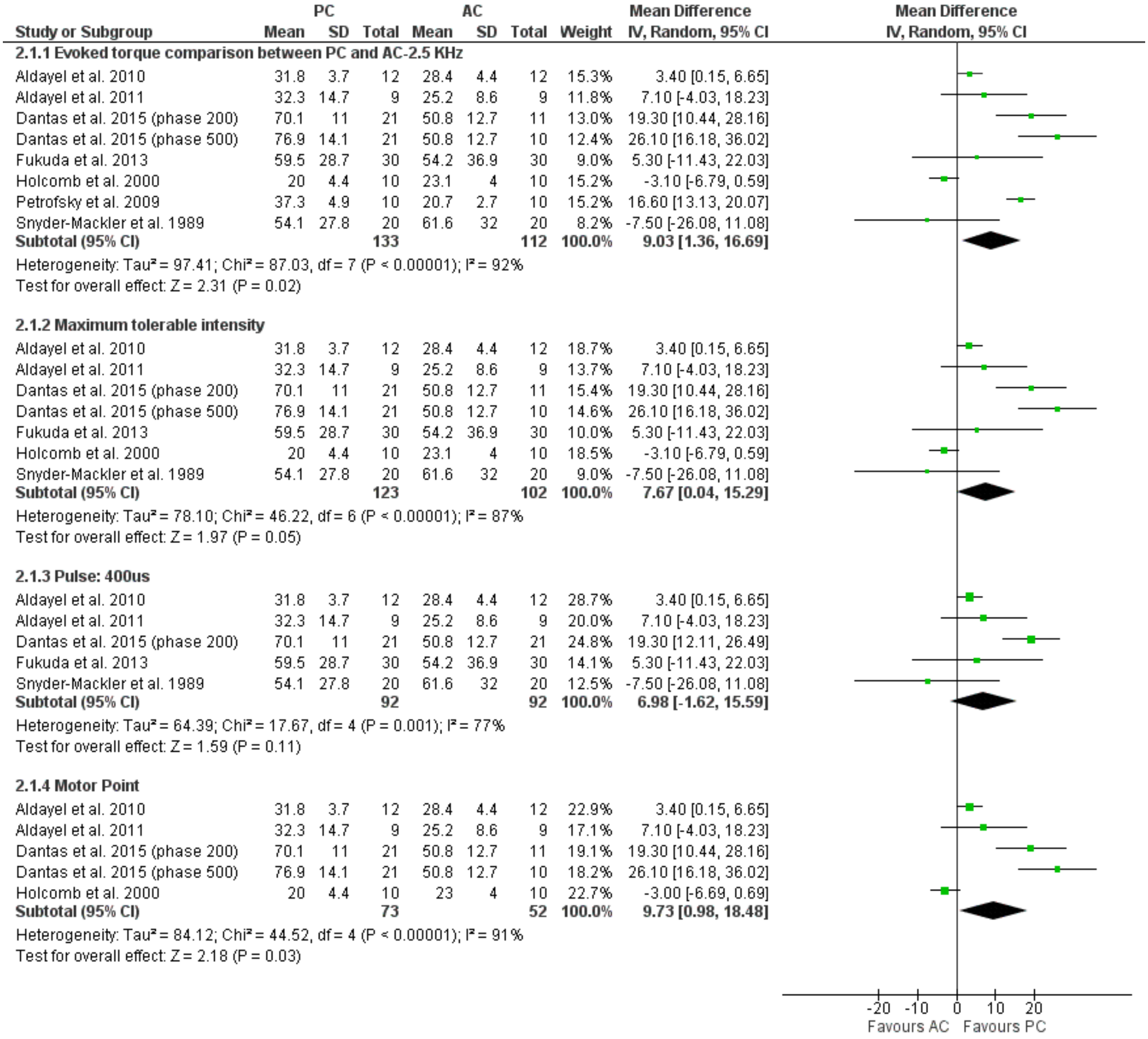
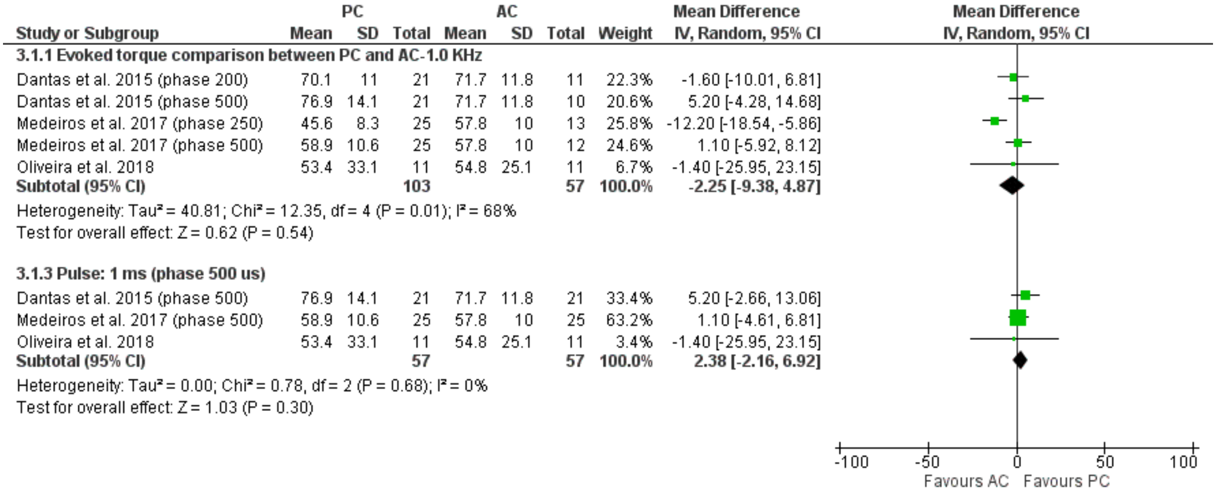


Figure 2 - Evoked torque comparison between PC and AC-2.5 KHz.

4.4.3.1.2 *Evoked Torque between PC vs. AC-1.0 KHz*

Three studies (DANTAS *et al.*, 2015; MEDEIROS *et al.*, 2017; DE OLIVEIRA *et al.*, 2018) evaluated the differences between PC and AC-1.0 KHz for the quadriceps evoked torque, and found no differences between them (-2.25; 95% CI: -9.38 to 4.87; I^2 68%; p=0.54; Figure 3.1.1). Subgroup analyses maintaining only studies with PC pulse width of 1 ms (2.38; 95% CI: -2.16 to 6.92, I^2 0%; p=0.30; Figure 3.1.3) demonstrated no between-currents difference for evoked torque. Based on the GRADE approach, the evidence quality for this outcome was considered very low for the analysis involving all studies. When we analyze this outcome based on the subgroup analysis maintaining studies with the PC pulse width balanced with the AC pulse width, the evidence quality became moderate (Table 5).

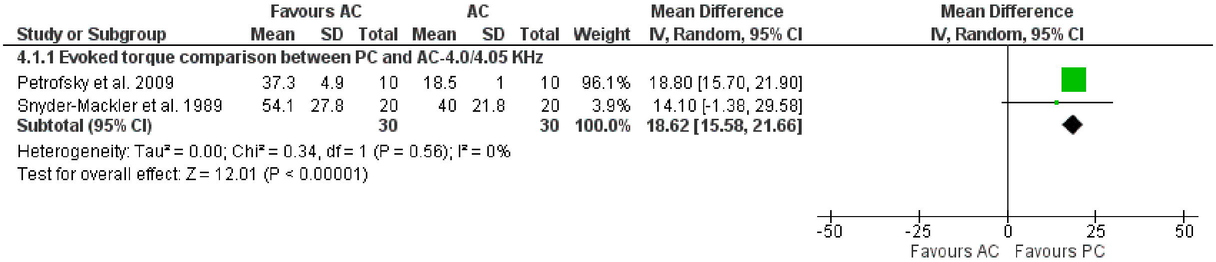


Study or Subgroup	PC Mean	PC SD	PC Total	AC Mean	AC SD	AC Total	Weight	Mean Difference IV, Random, 95% CI
3.1.1 Evoked torque comparison between PC and AC-1.0 KHz								
Dantas et al. 2015 (phase 200)	70.1	11	21	71.7	11.8	11	22.3%	-1.60 [-10.01, 6.81]
Dantas et al. 2015 (phase 500)	76.9	14.1	21	71.7	11.8	10	20.6%	5.20 [-4.28, 14.68]
Medeiros et al. 2017 (phase 250)	45.6	8.3	25	57.8	10	13	25.8%	-12.20 [-18.54, -5.86]
Medeiros et al. 2017 (phase 500)	58.9	10.6	25	57.8	10	12	24.6%	1.10 [-5.92, 8.12]
Oliveira et al. 2018	53.4	33.1	11	54.8	25.1	11	6.7%	-1.40 [-25.95, 23.15]
Subtotal (95% CI)			**103**			**57**	**100.0%**	**-2.25 [-9.38, 4.87]**
Heterogeneity: Tau² = 40.81; Chi² = 12.35, df = 4 (P = 0.01); I² = 68%								
Test for overall effect: Z = 0.62 (P = 0.54)								
3.1.3 Pulse: 1 ms (phase 500 us)								
Dantas et al. 2015 (phase 500)	76.9	14.1	21	71.7	11.8	21	33.4%	5.20 [-2.66, 13.06]
Medeiros et al. 2017 (phase 500)	58.9	10.6	25	57.8	10	25	63.2%	1.10 [-4.61, 6.81]
Oliveira et al. 2018	53.4	33.1	11	54.8	25.1	11	3.4%	-1.40 [-25.95, 23.15]
Subtotal (95% CI)			**57**			**57**	**100.0%**	**2.38 [-2.16, 6.92]**
Heterogeneity: Tau² = 0.00; Chi² = 0.78, df = 2 (P = 0.68); I² = 0%								
Test for overall effect: Z = 1.03 (P = 0.30)								

Figure 3 - Evoked torque comparison between PC and AC-1.0 KHz.

4.4.3.1.3 *Evoked Torque between PC vs. AC-4.0/4.05 KHz*

Two studies (SNYDER-MACKLER; GARRETT; ROBERTS, 1989; PETROFSKY *et al.*, 2008) evaluated the differences between PC and interferential AC on the quadriceps evoked torque. There was a slightly higher torque (18.62; 95% CI: 15.58 to 21.66; I^2 0%; p<0.00001) with PC compared to AC-4.0/4.05 Hz, and a low heterogeneity (Figure 4). Based on the GRADE approach, the evidence quality for this outcome was considered low (Table 5).



Study or Subgroup	Favours AC Mean	Favours AC SD	Favours AC Total	AC Mean	AC SD	AC Total	Weight	Mean Difference IV, Random, 95% CI
4.1.1 Evoked torque comparison between PC and AC-4.0/4.05 KHz								
Petrofsky et al. 2009	37.3	4.9	10	18.5	1	10	96.1%	18.80 [15.70, 21.90]
Snyder-Mackler et al. 1989	54.1	27.8	20	40	21.8	20	3.9%	14.10 [-1.38, 29.58]
Subtotal (95% CI)			**30**			**30**	**100.0%**	**18.62 [15.58, 21.66]**
Heterogeneity: Tau² = 0.00; Chi² = 0.34, df = 1 (P = 0.56); I² = 0%								
Test for overall effect: Z = 12.01 (P < 0.00001)								

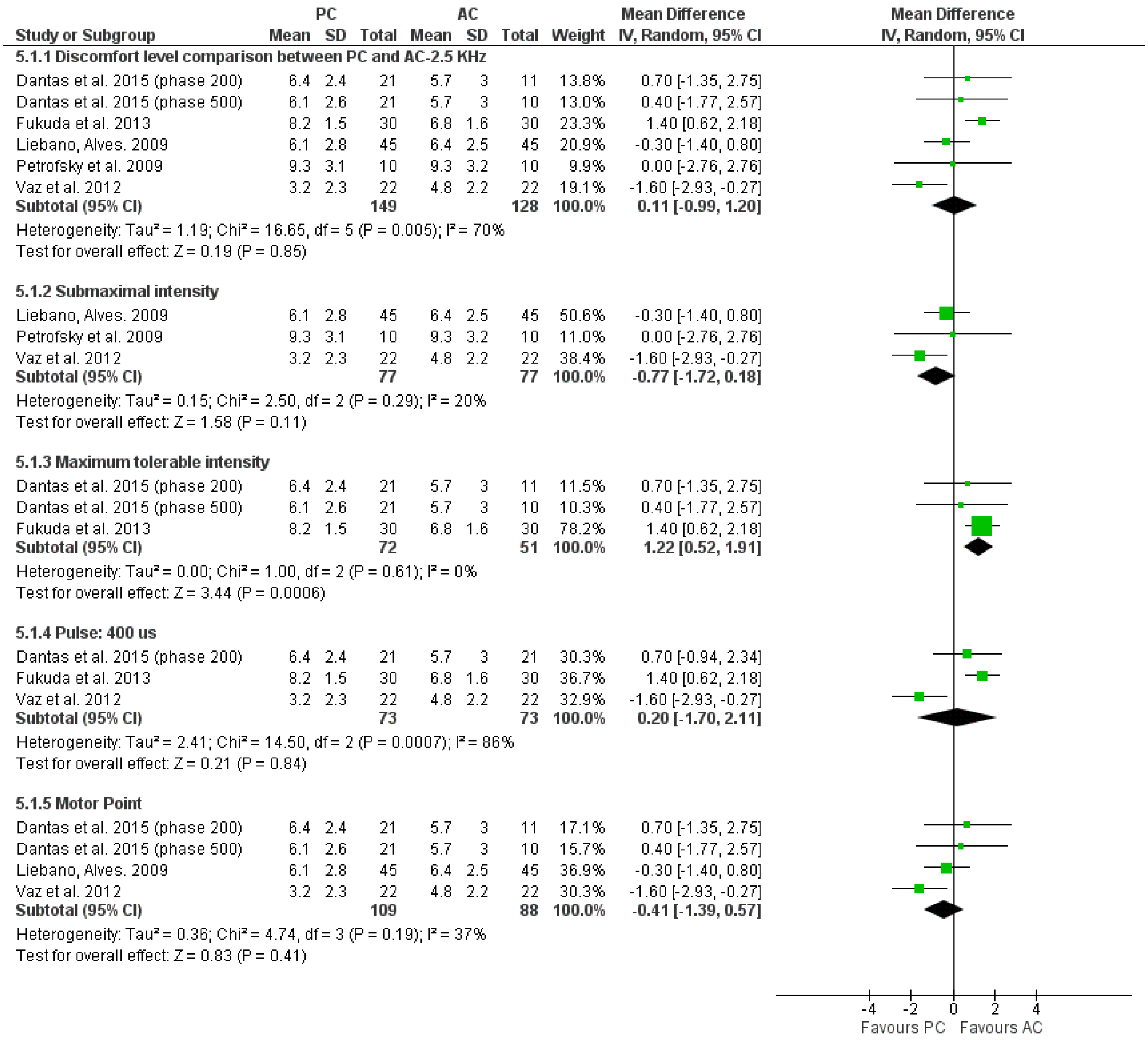
Figure 4- Evoked torque comparison between PC and AC-4.0/4.05 KHz.

4.4.3.2 *Discomfort*

4.4.3.2.1 *Discomfort between PC vs. AC-2.5 KHz*

Five studies (PETROFSKY *et al.*, 2008; LIEBANO; ALVES, 2009; VAZ *et al.*, 2012; FUKUDA *et al.*, 2013; DANTAS *et al.*, 2015) evaluated the differences between PC and AC-2.5 KHz for self-reported discomfort level. There was no significant difference between PC and AC for discomfort level (0.11; 95% CI: -0.99 to 1.20, I^2

70%; p=0.85; Figure 5.1.1). Subgroup analysis was performed, maintaining only the studies that used submaximal intensity, and again there was no significant difference (-0.77; 95% CI: -1.72 to 0.18, I^2 20%; p=0.11; Figure 5.1.2). Similar results were observed for the subgroup analysis of the studies that applied NMES at the motor point (-0.41; 95% CI: -1.39 to 0.57, I^2 37%; p=0.41; Figure 5.1.5) or which used the pulse duration of 400 µs (0.20; 95% CI: -1.70 to 2.11, I^2 86%; p=0.84; Figure 5.1.4). However, in the subgroup analysis maintaining only the studies that evaluated the discomfort at maximal intensities (Figure 5.1.3) it is possible to see that AC caused less discomfort (1.22; 95% CI: 0.52 to 1.91, I^2 0%; p=0.0006) than PC. All analyzes for this outcome showed very low quality of the evidence, based in the GRADE approach (Table 5).

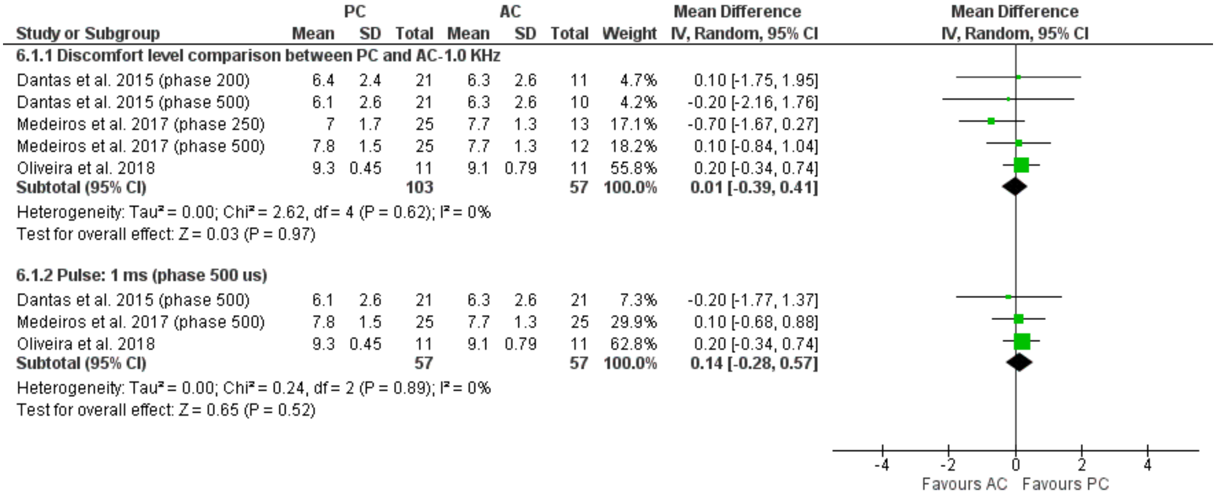


	PC			AC				Mean Difference
Study or Subgroup	**Mean**	**SD**	**Total**	**Mean**	**SD**	**Total**	**Weight**	**IV, Random, 95% CI**
5.1.1 Discomfort level comparison between PC and AC-2.5 KHz								
Dantas et al. 2015 (phase 200)	6.4	2.4	21	5.7	3	11	13.8%	0.70 [-1.35, 2.75]
Dantas et al. 2015 (phase 500)	6.1	2.6	21	5.7	3	10	13.0%	0.40 [-1.77, 2.57]
Fukuda et al. 2013	8.2	1.5	30	6.8	1.6	30	23.3%	1.40 [0.62, 2.18]
Liebano, Alves. 2009	6.1	2.8	45	6.4	2.5	45	20.9%	-0.30 [-1.40, 0.80]
Petrofsky et al. 2009	9.3	3.1	10	9.3	3.2	10	9.9%	0.00 [-2.76, 2.76]
Vaz et al. 2012	3.2	2.3	22	4.8	2.2	22	19.1%	-1.60 [-2.93, -0.27]
Subtotal (95% CI)			**149**			**128**	**100.0%**	**0.11 [-0.99, 1.20]**
Heterogeneity: $Tau^2 = 1.19$; $Chi^2 = 16.65$, df = 5 (P = 0.005); $I^2 = 70\%$								
Test for overall effect: Z = 0.19 (P = 0.85)								
5.1.2 Submaximal intensity								
Liebano, Alves. 2009	6.1	2.8	45	6.4	2.5	45	50.6%	-0.30 [-1.40, 0.80]
Petrofsky et al. 2009	9.3	3.1	10	9.3	3.2	10	11.0%	0.00 [-2.76, 2.76]
Vaz et al. 2012	3.2	2.3	22	4.8	2.2	22	38.4%	-1.60 [-2.93, -0.27]
Subtotal (95% CI)			**77**			**77**	**100.0%**	**-0.77 [-1.72, 0.18]**
Heterogeneity: $Tau^2 = 0.15$; $Chi^2 = 2.50$, df = 2 (P = 0.29); $I^2 = 20\%$								
Test for overall effect: Z = 1.58 (P = 0.11)								
5.1.3 Maximum tolerable intensity								
Dantas et al. 2015 (phase 200)	6.4	2.4	21	5.7	3	11	11.5%	0.70 [-1.35, 2.75]
Dantas et al. 2015 (phase 500)	6.1	2.6	21	5.7	3	10	10.3%	0.40 [-1.77, 2.57]
Fukuda et al. 2013	8.2	1.5	30	6.8	1.6	30	78.2%	1.40 [0.62, 2.18]
Subtotal (95% CI)			**72**			**51**	**100.0%**	**1.22 [0.52, 1.91]**
Heterogeneity: $Tau^2 = 0.00$; $Chi^2 = 1.00$, df = 2 (P = 0.61); $I^2 = 0\%$								
Test for overall effect: Z = 3.44 (P = 0.0006)								
5.1.4 Pulse: 400 us								
Dantas et al. 2015 (phase 200)	6.4	2.4	21	5.7	3	21	30.3%	0.70 [-0.94, 2.34]
Fukuda et al. 2013	8.2	1.5	30	6.8	1.6	30	36.7%	1.40 [0.62, 2.18]
Vaz et al. 2012	3.2	2.3	22	4.8	2.2	22	32.9%	-1.60 [-2.93, -0.27]
Subtotal (95% CI)			**73**			**73**	**100.0%**	**0.20 [-1.70, 2.11]**
Heterogeneity: $Tau^2 = 2.41$; $Chi^2 = 14.50$, df = 2 (P = 0.0007); $I^2 = 86\%$								
Test for overall effect: Z = 0.21 (P = 0.84)								
5.1.5 Motor Point								
Dantas et al. 2015 (phase 200)	6.4	2.4	21	5.7	3	11	17.1%	0.70 [-1.35, 2.75]
Dantas et al. 2015 (phase 500)	6.1	2.6	21	5.7	3	10	15.7%	0.40 [-1.77, 2.57]
Liebano, Alves. 2009	6.1	2.8	45	6.4	2.5	45	36.9%	-0.30 [-1.40, 0.80]
Vaz et al. 2012	3.2	2.3	22	4.8	2.2	22	30.3%	-1.60 [-2.93, -0.27]
Subtotal (95% CI)			**109**			**88**	**100.0%**	**-0.41 [-1.39, 0.57]**
Heterogeneity: $Tau^2 = 0.36$; $Chi^2 = 4.74$, df = 3 (P = 0.19); $I^2 = 37\%$								
Test for overall effect: Z = 0.83 (P = 0.41)								

Figure 5 - Discomfort level comparison between PC and AC-2.5 KHz.

4.4.3.2.2 *Discomfort between PC vs. AC-1.0 KHz*

Three studies (DANTAS *et al.*, 2015; MEDEIROS *et al.*, 2017; DE OLIVEIRA *et al.*, 2018) evaluated the differences between PC and AC-1.0 KHz for self-reported discomfort level. There was no significant difference between PC and AC-1.0 KHz for discomfort level (0.01; 95% CI: -0.39 to 0.41; I^2 0%; p=0.97; Figure 6.1.1). Subgroup analyzes (Figures 6.1.2) demonstrated no differences in discomfort level between the currents. Based on the GRADE approach, this outcome's quality of evidence was considered moderate (Table 5).



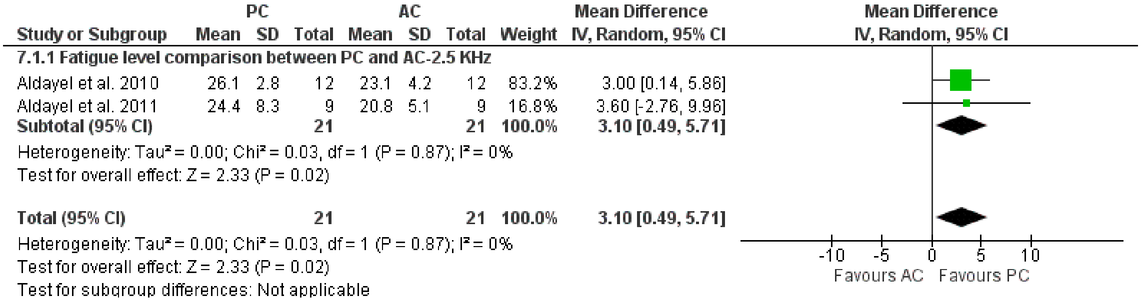
	PC			AC				Mean Difference
Study or Subgroup	**Mean**	**SD**	**Total**	**Mean**	**SD**	**Total**	**Weight**	**IV, Random, 95% CI**
6.1.1 Discomfort level comparison between PC and AC-1.0 KHz								
Dantas et al. 2015 (phase 200)	6.4	2.4	21	6.3	2.6	11	4.7%	0.10 [-1.75, 1.95]
Dantas et al. 2015 (phase 500)	6.1	2.6	21	6.3	2.6	10	4.2%	-0.20 [-2.16, 1.76]
Medeiros et al. 2017 (phase 250)	7	1.7	25	7.7	1.3	13	17.1%	-0.70 [-1.67, 0.27]
Medeiros et al. 2017 (phase 500)	7.8	1.5	25	7.7	1.3	12	18.2%	0.10 [-0.84, 1.04]
Oliveira et al. 2018	9.3	0.45	11	9.1	0.79	11	55.8%	0.20 [-0.34, 0.74]
Subtotal (95% CI)			**103**			**57**	**100.0%**	**0.01 [-0.39, 0.41]**
Heterogeneity: Tau² = 0.00; Chi² = 2.62, df = 4 (P = 0.62); I² = 0%								
Test for overall effect: Z = 0.03 (P = 0.97)								
6.1.2 Pulse: 1 ms (phase 500 us)								
Dantas et al. 2015 (phase 500)	6.1	2.6	21	6.3	2.6	21	7.3%	-0.20 [-1.77, 1.37]
Medeiros et al. 2017 (phase 500)	7.8	1.5	25	7.7	1.3	25	29.9%	0.10 [-0.68, 0.88]
Oliveira et al. 2018	9.3	0.45	11	9.1	0.79	11	62.8%	0.20 [-0.34, 0.74]
Subtotal (95% CI)			**57**			**57**	**100.0%**	**0.14 [-0.28, 0.57]**
Heterogeneity: Tau² = 0.00; Chi² = 0.24, df = 2 (P = 0.89); I² = 0%								
Test for overall effect: Z = 0.65 (P = 0.52)								

Figure 6 - Discomfort level comparison between PC and AC-1.0 KHz.

4.4.3.3 *Fatigue*

4.4.3.3.1 *Fatigue between PC and AC-2.5 KHz*

Regarding fatigability, only two studies (ALDAYEL *et al.*, 2010; ALDAYEL *et al.*, 2011) evaluated the maximum voluntary isometric contraction (MVIC) before and after a fatigue protocol and were included in the meta-analysis (Figure 7). The results demonstrate a greater reduction in the MVIC after the fatigue protocol with PC (3.10; 95% CI: 0.49 to 5.71, I^2 0%; p=0.02). Based on the GRADE approach, the quality of the evidence for this outcome was considered low (Table 5).



Study or Subgroup	PC Mean	PC SD	PC Total	AC Mean	AC SD	AC Total	Weight	Mean Difference IV, Random, 95% CI
7.1.1 Fatigue level comparison between PC and AC-2.5 KHz								
Aldayel et al. 2010	26.1	2.8	12	23.1	4.2	12	83.2%	3.00 [0.14, 5.86]
Aldayel et al. 2011	24.4	8.3	9	20.8	5.1	9	16.8%	3.60 [-2.76, 9.96]
Subtotal (95% CI)			**21**			**21**	**100.0%**	**3.10 [0.49, 5.71]**

Heterogeneity: Tau² = 0.00; Chi² = 0.03, df = 1 (P = 0.87); I² = 0%

Test for overall effect: Z = 2.33 (P = 0.02)

Total (95% CI)			**21**			**21**	**100.0%**	**3.10 [0.49, 5.71]**

Heterogeneity: Tau² = 0.00; Chi² = 0.03, df = 1 (P = 0.87); I² = 0%

Test for overall effect: Z = 2.33 (P = 0.02)

Test for subgroup differences: Not applicable

Figure 7- Fatigue level comparison between PC and AC-2.5 KHz.

Table 5 - Quality of evidence using the GRADE approach.

	Certainty assessment					N		Absolute	Certainty
OUTCOME	**N (RCTs)**	**Risk of Bias**	**Inconsistency**	**Indirectness**	**Imprecision**	**Interv**	**Comp**	**(95% CI)**	
EVOKED TORQUE									
PC vs. AC-2.5 KHz	8	very serious [a]	very serious [c]	not serious	very serious [e]	133	112	9.03 [95% CI 1.36, 16.69]	Very low
SA: Maximum intensity	7	very serious [a]	very serious [c]	not serious	very serious [e]	123	102	7.67 [95% CI 0.04, 15.29]	Very low
SA: Pulse 400 us	5	very serious [a]	very serious [c]	not serious	very serious [e]	92	92	6.98 [95% CI -1.62, 15.59]	Very low
SA: Motor Point	5	very serious [a]	very serious [c]	not serious	very serious [e]	73	52	9.73 [95% CI 0.98, 18.48]	Very low
PC vs. AC-1.0 KHz	5	serious [b]	very serious [c]	not serious	very serious [e]	103	57	-2.25 [95% CI -9.38, 4.87]	Very low
SA: Pulse 1 ms	3	serious [b]	not serious	not serious	not serious	57	57	2.38 [95% CI -2.16, 6.92]	Moderate
PC vs. AC-4.0/4.05 KHz	2	very serious [a]	not serious	not serious	not serious	30	30	18.62 [95% CI 15.58, 21.66]	Low
DISCOMFORT									
PC vs. AC-2.5KHz	6	very serious [a]	very serious [c]	not serious	not serious	149	128	0.11 [95% CI -0.99, 1.20]	Very low
SA: Submaximal intensity	3	very serious [a]	not serious	not serious	very serious [e]	77	77	-0.77 [95% CI -1.72, 0.18]	Very low
SA: Maximum intensity	3	serious [b]	not serious	not serious	very serious [e]	72	51	1.22 [95% CI 0.52, 1.91]	Very low
SA: Pulse 400 us	3	very serious [a]	very serious [c]	not serious	very serious[e]	73	73	0.20 [95% CI: -1.70, 2.11]	Very low
SA: Motor Point	4	serious [b]	serious [d]	not serious	very serious [f]	109	88	-0.41 [95% CI -1.39, 0.57]	Very low
PC vs. AC-1.0 KHz	5	serious [b]	not serious	not serious	not serious	103	57	0.01 [95% CI -0.39, 0.41]	Moderate
SA: Pulse 1 ms	3	serious [b]	not serious	not serious	not serious	57	57	0.14 [95% CI -0.28, 0.57]	Moderate
FATIGUE:									
PC vs. AC-2.5 KHz	2	very serious [a]	not serious	not serious	not serious	21	21	3.10 [95% CI 0.49, 5.71]	low

SA: Subgroup analysis; a:≥4 items classified as high risk of bias in the methodological quality analysis; b: ≤3 items classified as high risk of bias in the methodological quality analysis; c: High heterogeneity (over 50%); Comp: Comparison; d: Moderate heterogeneity (30 e 50%); e: Large confidence interval (CI); f: Moderate confidence interval (CI); Interv: Intervention.

4.5 DISCUSSION

This meta-analysis is the first to compare PC to AC with different carrier frequencies on evoked torque, discomfort and fatigue level in healthy individuals. Considering that previous studies demonstrated that the AC carrier frequencies interfere with torque production and discomfort level (WARD; ROBERTSON, 1998; PARKER; KELLER; EVENSON, 2005), it is important to analyze the effects of PC and AC categorizing them by their carrier frequencies. PC provided larger quadriceps evoked torque compared to AC-2.5 KHz and AC-4.0/4.05 KHz, and similar evoked torque compared to AC-1.0 KHz. Additionally, PC produced a similar discomfort to AC-1.0 KHz and AC-2.5 KHz. Regarding fatigability, PC produced a greater decrease in MVIC after the NMES protocol, which may be explained by the higher evoked torque.

The NMES evoked torque is one of the most important variables in strength training and rehabilitation, since it determines the produced mechanical load, which is related to the neuromuscular adaptation in strength and rehabilitation programs (VAZ; FRASSON, 2018). In this sense, studies have utilized different NMES parameters in an attempt to optimize the force production for both AC and PC. However, the ability to evoke maximum torque depends on the neurophysiological responses of different nerve fiber types to NMES. This response can be different with AC stimulation, and depends on the chosen configurations (WARD, 2009).

Ward and Robertson (1998) demonstrated that the evoked torque increases with decreasing AC carrier frequency, and the greatest evoked torque was produced with AC-1.0 KHz. In addition, in another study (WARD; OLIVER; BUCCELLA, 2006) they showed that AC-1.0 KHz produces similar torque to that of PC. Medeiros *et al.* (2017) showed similar results, as AC-1.0 KHz and PC with the same pulse duration induced similar evoked torque, regardless of the current type. Our meta-analysis results agree with these previous results, as AC-1.0 KHz was similar to PC for both the evoked torque and the discomfort level.

However, regarding AC-2.5 KHz, previous results (WARD; OLIVER; BUCCELLA, 2006) demonstrated that this AC current is less effective than AC-1.0 KHz and PC in terms of wrist extensor torque production. Similarly, a recent study (DANTAS *et al.*, 2015) demonstrated that AC-2.5 KHz evoked significantly lower knee extensor torque compared with PC and AC-1.0 KHz. Therefore, the statements that AC-2.5 KHz is optimal for muscle strengthening is uncertain (DANTAS *et al.*, 2015), because the evidence shows that either AC-1.0 KHz or PC are more effective than AC-2.5 KHz (WARD; OLIVER; BUCCELLA, 2006).

These findings can be explained by the burst size influence on the summation process (WARD; ROBERTSON; IOANNOU, 2004), since it has been proposed that successive pulses within a burst can summate, leading the nerve fiber membrane closer to its excitation threshold until an action potential is produced (WARD; ROBERTSON, 2000; WARD; ROBERTSON; IOANNOU, 2004). However, the burst size of AC-2.5 KHz, as well as the one from AC-4.0 KHz, is long, and although it has been proposed that fibers could fire at some multiple of the burst frequency (WARD; ROBERTSON, 2001; WARD; ROBERTSON; IOANNOU, 2004), apparently there is no sufficient time for the motoneuron’s membrane recovery. In this case, the evoked torque is reduced either by neurotransmitter depletion or by the action potential propagation failure through the muscle fiber (WARD; ROBERTSON, 2001; WARD; OLIVER; BUCCELLA, 2006), which might lead to neuromuscular fatigue.

Thus, a possible explanation for the similar torque generation between AC-1.0 KHz and PC is the fact that both currents do not allow time for multiple firing to occur, because there are lower number of pulses (PC) and cycles per burst (AC-1.0 KHz) in these two currents compared to AC-2.5 KHz and AC-4.0 KHz. This also supports the evoked torque results presented in our meta-analysis where the PC is more efficient than both the 2.5 and 4.0 KHz ACs.

Similarly, the stimulus burst duration seems to influence the reported discomfort during AC stimulation. Ward et al. (2004) demonstrated that a burst duty cycle of about 20% is less uncomfortable compared to a larger burst duty cycle (50%) (WARD; ROBERTSON; IOANNOU, 2004). In addition, AC was shown to be more comfortable compared to PC (WARD; OLIVER; BUCCELLA, 2006). However, this study evaluated the number of uncomfortable events during NMES. In addition, these authors used monophasic PC, which might be more uncomfortable than biphasic PC, since in the biphasic PC the first phase or stimulating phase is used to elicit the desired physiological effect such as initiation of an action potential, and the second phase, or reversal phase, is used to reverse electrochemical processes occurring during the stimulating pulse (MERRILL; BIKSON; JEFFERYS, 2005).

In our meta-analysis we used only biphasic PCs, and all comparisons showed similar between-currents discomfort level results, except for the subgroup analyses with studies that evaluated the discomfort at maximum tolerable intensity, in which AC was less uncomfortable than PC. However, in this analysis, one of the included studies (FUKUDA *et al.*, 2013) had a large weight on the results. In addition, the data from this study (FUKUDA *et al.*, 2013) influenced the meta-analysis' heterogeneity (70%). When we removed it from the analysis, the heterogeneity decreased to 17%, thereby showing that PC displayed a similar discomfort level to that of AC. The large weight of this study (FUKUDA *et al.*, 2013), in both meta-analysis and subgroup analysis, can be explained by the included population, or by electrode size and positioning, or by both. The authors used only men, unlike the other studies that used both sexes. It has already been established that the sensory threshold is lower in women than in men, and VAS pain scores can be higher in women than in men at the motor threshold (MAFFIULETTI *et al.*, 2008). Additionally, they used larger electrodes that were positioned longitudinally over the muscular belly, unlike most studies included in the meta-analysis that used the electrodes positioned transversely over the motor point. Therefore, it is possible that these methodological differences may have influenced the discomfort level between AC and PC. However, the evidence level for this outcome with AC-2.5 KHz was very low, which demonstrates that these results should be interpreted with caution.

Few studies have evaluated the fatigue level between PC and AC, and they used different ways to assess this outcome. Therefore, the meta-analysis was possible considering only the MVIC reduction after the fatigue protocol with PC and AC-2.5 KHz as the outcome, since we found only one study (DE OLIVEIRA *et al.*, 2018) that compared the fatigue level between PC and AC-1.0 KHz.

Oliveira *et al*. (2018) developed a clinical-like NMES session, and demonstrated that both NMES currents (PC and AC-1.0 KHz) induced similar fatigue levels with both peripheral and central alterations. Therefore, no difference in NMES-evoked torque during the fatigue protocol was observed between the two conditions. As previously explained, the similar fatigue level results between PC and AC-1.0 KHz can be explained by the smaller number of cycles within the burst delivered by AC-1.0 KHz, which does not allow time for multiple firing to occur, allowing the membrane to recover in a similar way as that of PC (WARD; ROBERTSON; IOANNOU, 2004; WARD; OLIVER; BUCCELLA, 2006). Since the rate of fatigue induced by the AC is related to the total number of pulses delivered (LAUFER; ELBOIM, 2008), longer burst duration (i.e., AC-2.5 KHz) apparently allows time for multiple motoneurons' firing, and consequently high-frequency fatigue (WARD; OLIVER; BUCCELLA, 2006).

Regarding the comparison between PC and AC-2.5 KHz, our findings demonstrated that PC caused greater fatigue, since PC showed a greater MVIC decrease after the fatigue protocol compared to AC-2.5 KHz. However, contrary results were presented in other studies (LAUFER *et al.*, 2001; LAUFER; ELBOIM, 2008; LEIN JR; MYERS; BICKEL, 2015), suggesting that PC is less fatiguing than AC-2.5 KHz. However, these studies used other ways to analyze the fatigue protocol, using the area under the curves analysis (i.e., total work; LAUFER *et al.*, 2001; LAUFER; ELBOIM, 2008), the electrically induced strength analysis during the fatigue protocol (LAUFER *et al.*, 2001; LAUFER; ELBOIM, 2008), the number of contractions before the torque fell below 50% of the initial force (LAUFER *et al.*, 2001; LAUFER; ELBOIM, 2008; LEIN JR; MYERS; BICKEL, 2015), and the percent difference in the torque from the first to the last contraction (LEIN JR; MYERS; BICKEL, 2015).

Lein, Myers and Bickel (2015), using the current amplitude necessary to produce an evoked torque that achieved approximately 30% of the participant's peak MVIC, demonstrated that PC with low-frequency (20 Hz) stimulation generated less fatigue than AC stimulation through the analysis of the evoked torque reduction during the NMES session. In addition, a significant between-currents difference was observed for the number of contractions required to reach a 50% reduction of the torque generated by the first contraction. For AC at 20 and 50 Hz, this reduction in evoked torque was observed at contractions 16 (±2.5) and 13 (±1.7), respectively, while for PC at 20 and 50 Hz this torque reduction was observed at contractions 63 (±6.4) and 29 (±3.6), respectively. Thus, the two

ACs showed a higher fatigability level, since the 50% force drop occurred faster with the ACs compared to the two PCs.

Therefore, the differences between our meta-analysis results and the existing literature can be explained by the different methodologies used to investigate fatigue. Additionally, we found only 2 studies (ALDAYEL *et al.*, 2010; ALDAYEL *et al.*, 2011) that evaluated the MVIC before and after the fatigue protocol with PC and AC-2.5 KHz. These studies evaluated fatigue at the maximum tolerated intensity, which was adjusted along the protocol (ALDAYEL *et al.*, 2010; ALDAYEL *et al.*, 2011), unlike the methodology from the pre-existing literature.

Considering that the MVIC changes after NMES reflect the functional impact of the stimulation protocol on the force-generating capacity of an individual (MATKOWSKI; LEPERS; MARTIN, 2015), it is plausible to suppose that the greatest MVIC reduction with PC in our meta-analysis is due to its higher mechanical stress generated compared to that of AC. As demonstrated by one of the included studies (ALDAYEL *et al.*, 2011), the decrease in minimum tissue oxygenation index amplitude was significantly greater for PC than for AC, at the last (13th) contraction. This could be associated to a smaller muscle volume being recruited and a smaller evoked torque during AC-stimulation, compared to PC, as suggest by the authors.

These results are important for clinical practice, since the mechanical load produced by NMES will determine possible adaptations in both muscle structure and muscle function (VAZ; FRASSON, 2018). Additionally, during the NMES protocol, the mechanical stress that is imposed can be the main cause of muscle micro-damage in NMES-evoked contractions (NOSAKA *et al.*, 2011). Thus, micro-damage at the myofiber and sarcomere levels (i.e., macrophages infiltration and z-line disruption) can be NMES-induced (MACKEY *et al.*, 2008), and may be necessary to maximize muscle hypertrophy and strength gain (NOSAKA *et al.*, 2011).

Finally, the quality analysis revealed the need of studies with better methodological quality and with well-described parameters. In addition, fatigue studies should evaluate fatigue not only by the post-fatigue MVIC decrease, but should also look at the work generated during a NMES fatigue protocol, as well as the evoked torque decrease during the plateau of the force generation curve. These outcomes might give further insight into the NMES-induced fatigue mechanisms. Additionally, it would be interesting to control the stimulus intensity level, since the use of the maximal tolerated intensity may have influenced the observed results, as the participants might have experienced different stimulation levels due to the fact that the maximum tolerated intensity is subjective and may be influenced by personal factors (i.e., induced discomfort). Therefore, studies with better methodological design are needed, in order that we can answer the question whether PC causes more fatigue than the AC-2.5 KHz and, consequently, improve the existent evidence for this outcome.

4.6 STUDY LIMITATIONS

This review evaluated only studies that applied both types of current in healthy individuals and using an acute protocol. Therefore, the findings may not be generalizable, because different responses may be possible in special populations or in the presence of different pathologies, as well as during the application of chronic protocols.

4.7 CONCLUSIONS

We conclude that PC showed greater evoked torque than the AC-2.5 KHz (Russian current) and AC-4.0/4.05 KHz. However, there is no significant difference in torque production between PC and AC-1.0 KHz, suggesting that both currents can be used interchangeably in rehabilitation protocols. Regarding discomfort, PC showed similar results compared to AC-1.0 KHz and AC-2.5 KHz. Regarding fatigue, PC showed a greater decrease in MVIC after the fatigue protocol compared to AC-2.5 KHz. However, these results should be observed with caution, due to the low methodological quality of the analyzed studies, and the fact that evidence analysis for each outcome demonstrated only very low to moderate evidence. We believe that well-designed RTCs, comparing the NMES effects between AC-2.5 KHz and PC on neuromuscular parameters and fatigability, with greater methodological rigor, are necessary, in order to allow a conclusion about the efficacy of these two therapeutic modalities.

OTHER INFORMATION

PROSPERO: CRD42018110362

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CHAPTER 2

In chapter 1, we observed that most studies involving PC and AC-2.5 KHz demonstrate poor methodological quality, use different NMES parameters (i.e. frequency, burst and pulse duration), and few studies used parameters that maximized the evoked torque. The force-frequency relation for the quadriceps muscle of healthy young individuals demonstrates that frequencies between 80 Hz and 100 Hz produce a higher peak force than lower stimulation frequencies (LEE; RUSS; BINDER-MACLEOD, 2009). Thus, for muscle strengthening, the use of frequencies higher than 80 Hz would be more indicated. However, only one study included in the meta-analysis used frequencies in this range. In addition, pulse and burst duration can influence force generation and discomfort level, since they determine the electrical charge delivered to the muscle. However, the optimization of these parameters was only defined in one study that compared PC and AC-2.5 KHz. Therefore, resolving methodological issues and optimizing NMES parameters is of utterly importance, as they may influence the responses between AC and PC, which may have an important impact on NMES use in clinical practice. Therefore, we conducted a randomized, blinded, crossover trial to fill the literature gaps, aimed at comparing the PC and AC-2.5 KHz effects on current intensity, evoked torque, discomfort level, and neuromuscular efficiency.

5. ALTERNATING CURRENT IS LESS EFFICIENT THAN PULSED CURRENT IN HEALTHY INDIVIDUALS: A BLINDED, RANDOMIZED CROSSOVER TRIAL

5.1 ABSTRACT:

Background: Pulsed current (PC) and alternating current (AC) are two types of neuromuscular electrical stimulation (NMES) often used by clinicians that show contradictory results in their effects on evoked torque and discomfort level. The low methodological quality and different NMES parameters and protocols might explain their inconclusive results. Additionally, these currents neuromuscular efficiency (i.e., the NMES current type that evoke the highest torque with the lowest current intensity) has not been established yet. **Purpose:** To compare current intensity, evoked torque, neuromuscular efficiency, and discomfort at submaximal and maximal levels between PC and AC-2.5 KHz (Russian Current) in healthy individuals. **Design:** blinded, randomized crossover trial. **Methods:** Thirty healthy men (age 23.23±4.59 years) participated in the study. Each participant was randomized to 4 current settings: 2-ACs with carrier frequency of 2.5 KHz, similar pulse duration (0.4 ms) and burst frequencies (100 Hz), but with different burst duty cycle/burst duration (AC20 = 20% and 2 ms; AC50 = 50% and 5 ms); and 2-PCs with similar pulse frequency (100 Hz) and different pulse duration (PC1 = 2 ms; PC2 = 0.4 ms). All NMES currents were applied in separate sessions (with a 7-day interval) to the quadriceps femoris motor-point of the dominant limb of each subject. The current intensity needed to reach submaximal force levels and maximal tolerable intensity, evoked torque, neuromuscular efficiency and discomfort level were evaluated. Participants and evaluators were blinded to the waveform type being used. **Results:** Current intensity at both submaximal and maximal force levels was lower for PC1 compared to AC20, AC50 and PC2. Both PC's generated higher evoked torque than the ACs. PC1 showed better neuromuscular efficiency than AC20, AC50 and PC2 at submaximal and maximal intensity levels. PC2 demonstrated better neuromuscular efficiency than AC50 and AC20 at the maximal intensity level. AC and PC generated similar discomfort levels both at the submaximal and maximally tolerated current intensity levels. Altering the AC-2.5 KHz burst duty cycle did not influence the evaluated neuromuscular parameters. **Conclusions:** PC is able to evoke higher torque, has a higher neuromuscular efficiency and produces similar discomfort compared to AC-2.5KHz. PC is the optimal NMES current when the goal is to produce a high level of evoked torque with the smallest current intensity. PC1 seems to be the best choice for clinicians when elaborating clinically relevant NMES protocols aimed at producing a significant mechanical load to the quadriceps muscle-tendon unit.

5.2 INTRODUCTION

Neuromuscular electrical stimulation (NMES) is an important tool that has been used in rehabilitation (MAFFIULETTI *et al.*, 2013; HERZIG; MAFFIULETTI; ESER, 2015; HONG *et al.*, 2018) as a resource to improve muscle function (BAX; STAES; VERHAGEN, 2005). NMES therapy's clinical success is related to the evoked force (MAFFIULETTI *et al.*, 2018), which can be maximized by increasing the current intensity (GORGEY *et al.*, 2006). However, current intensity is limited by the participants' tolerance to NMES (DELITTO *et al.*, 1992; LAUFER *et al.*, 2011), and the discomfort can affect the NMES evoked force and NMES-effectiveness (SCOTT; CAUSEY; MARSHALL, 2009).

NMES efficiency is defined as the inverse relation between current intensity and the corresponding amount of generated force (VAZ; FRASSON, 2018). Two different NMES currents, the low frequency pulsed current (PC) and the medium frequency alternating current (AC), are commonly used clinically (WARD, 2009; DA SILVA *et al.*, 2015; IIJIMA *et al.*, 2018). However, it is not yet clear which NMES-type is the most efficient for clinical use. The rationale for using AC in rehabilitation seems to be based on the idea that AC delivers several pulses in the same burst, providing a greater stimuli summation. In addition, AC would recruit more motor units due to its greater penetration in the soft tissues (i.e. in the nerve), evoking greater force and, therefore, being more effective than PC (WARD; LUCAS-TOUMBOUROU, 2007; WARD, 2009).

Nonetheless, studies comparing the AC and PC effects on evoked torque and discomfort do not support the previous claims. Various stimulation parameter combinations have been tested using maximally tolerated NMES intensity (WALMSLEY; LETTS; VOOYS, 1984; LAUFER; ELBOIM, 2008; ALDAYEL *et al.*, 2010; FUKUDA *et al.*, 2013; MEDEIROS *et al.*, 2017) to evaluate the effects on evoked torque and discomfort. However, these studies demonstrated divergent results for these outcomes. Few studies have used submaximal stimulation (LIEBANO; ALVES, 2009; VAZ *et al.*, 2012), which is clinically relevant since many daily-living activities are predominantly performed at submaximal force intensities (BAPTISTA *et al.*, 2009; MAU-MOELLER *et al.*, 2017), and similar discrepancies between PC and AC effects were found during submaximal stimulation protocols. The divergences among studies can be attributed to low methodological quality, variability in their methodologies and differences in their NMES parameters (VAZ; FRASSON, 2018). Therefore, there is no consensus on which is the best current type, and it is still unknown which is the most efficient NMES current at both submaximal and maximal stimulation levels.

Additionally, few studies comparing PC and AC determined which the optimal NMES parameters are. Pulse duration and burst duty cycle play an important role regarding clinical efficacy (WARD; ROBERTSON; IOANNOU, 2004; GORGEY; DUDLEY, 2008). For instance, large pulse duration can generate stronger contractions, requires lower current intensity, and is associated with lower electrical charge to stimulate the nociceptive fibers, which may lead to a more comfortable NMES intervention (GORGEY; DUDLEY, 2008). Similarly, in AC stimulation, lower burst duration and smaller burst duty cycle may prevent the excessive depolarization of nerve fibers and evoke higher torque (WARD; ROBERTSON; IOANNOU, 2004; LIEBANO; WASZCZUK JR; CORRÊA, 2013) with minimum discomfort (WARD; ROBERTSON; IOANNOU, 2004). However, currents with different phase durations (LIEBANO *et al.*, 2013) or burst durations (LAUFER; ELBOIM, 2008) produced similar evoked torque, and only a single study (LEIN JR; MYERS; BICKEL, 2015) reported higher evoked torque with longer burst duration. Therefore, it is still unclear if higher pulse or lower burst duration are clinically more effective.

Furthermore, it is unclear how AC pulse charge and burst duty cycle affect the evoked torque and effectiveness compared to PC. Additionally, we were unable to find any study reporting whether PC and AC with similar total energy charge capacity (similar pulse and burst duration) differ on the force generation and discomfort. Similarly, we did not find studies evaluating neuromuscular efficiency between PC and AC-2.5 KHz (Russian Current). In addition, the neuromuscular responses between PC and AC with the same pulse duration at maximal and submaximal force levels are unclear. These gaps may compromise clinical decision-making in protocols involving NMES. Since evidence-based practice has been widely used by therapists (GRECO *et al.*, 2018), providing evidence with good quality is of uterly importance for the best decision-making regarding the NMES use in clinical practice.

Therefore, the purpose of this study was to fill the literature's gaps and conduct a high methodological quality study, where allocation, assessors, therapists and participants blinding, baseline similarity control, adequate sequence generation and intention-to-treat analysis were carefully controlled. In addition, parameters with similar total electrical charge capacity (PC-2 ms pulse duration and AC-2 ms burst duration), as well as the AC and PC parameters commonly used in clinical practice (WARD; LUCAS-TOUMBOUROU, 2007), involving similar charge-per-pulse capacity (i.e., PC and AC with 0.4 ms of pulse duration) were compared to determine the NMES parameters that generated the highest evoked torque with the lowest current intensity, thereby displaying the highest neuromuscular efficiency and the lowest possible discomfort at submaximal and maximal intensity levels in healthy individuals.

We hypothesize that PC with large pulse duration will need lower current intensity to generate higher evoked torque, will produce greater neuromuscular efficiency and smaller discomfort, at both submaximal and maximal levels, compared to AC.

5.3 MATERIALS AND METHODS

This study is a blinded, randomized crossover trial, that was approved by the University's Research Ethics Committee (3.064.351), followed the CONSORT recommendations (DWAN *et al.*, 2019), and was registered at the Clinical trials (NCT03796117). Participants read and signed an informed consent form after they had all questions about the tests to be performed answered by the responsible researcher. 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). The study was conducted from January to June 2019.

5.3.1 Participants

Healthy, physically active men from the University and local community were recruited. Inclusion criteria were determined as young (age between 18 and 35 years), healthy and physically active men, with normal knee function and range of motion, and without pain complaints or presence of pathology in the dominant lower limb. Exclusion criteria included: presence of injury, cardiovascular and/or neurologic disease, acute musculoskeletal impairment (e.g. ligament or meniscal injuries) or presence of knee pain at the time of testing, and being treated with NMES in the lower limb in the last 3 months. Participants were asked to avoid stimulants (e.g. alcohol, caffeine, chocolate) and exercise two days before the tests.

Subjects self-reported activity levels using the International Physical Activity Questionnaire - IPAQ (CRAIG *et al.*, 2003).

5.3.2 Sample size

Sample size was determined a priori using G*Power (version 3.1.9.4, Universitat Kiel, Germany), with the significance level set at $p<0.05$ and power (1-β) 0.95, and to detect an effect size ($f>0.42$). We used data from a previous study (ALDAYEL *et al.*, 2010) to calculate the effect size to be used for the dependent variable evoked torque. Based on these a priori calculations, a sample of 22 individuals was defined as the minimum number of subjects. However, as a previous study reported the exclusion of approximately 33% of the selected sample for eligibility assessment (MEDEIROS *et al.*, 2017), additional eight subjects were recruited to consider those respondents who would fail to attend the follow-up session. Therefore, we recruited 30 participants.

5.3.3 Procedures and measurements

Participants were tested on 4 separate occasions, at the same time of the day, in the same room with room temperature kept about constant at 22±2°C. In the first visit, subjects were familiarized with the NMES and the testing equipment, and the anthropometric measurements (height and body mass) and physical activity level were assessed. After a 10-minute interval, warm-up trials and maximal voluntary isometric contraction (MVIC) tests were performed. Next, the participants were tested with the first randomized current, which was applied in the dominant limb knee extensor muscles. Current intensity and discomfort level were evaluated at two different submaximal strength levels (20% of the MVIC and 40 Nm), and at maximal level (maximal tolerated intensity at the 90° knee joint angle - 0° = full knee extension) for each current and set configuration (Figure 8).

A five-minute interval was given between the tests to avoid fatigue. In the second, third and fourth days, the tests were performed with the other NMES configurations (Table 6). To avoid the carry-over effect, a 7-day interval between evaluations was used. In addition, to assess the similarity at baseline, the MVIC was tested during all testing sessions.

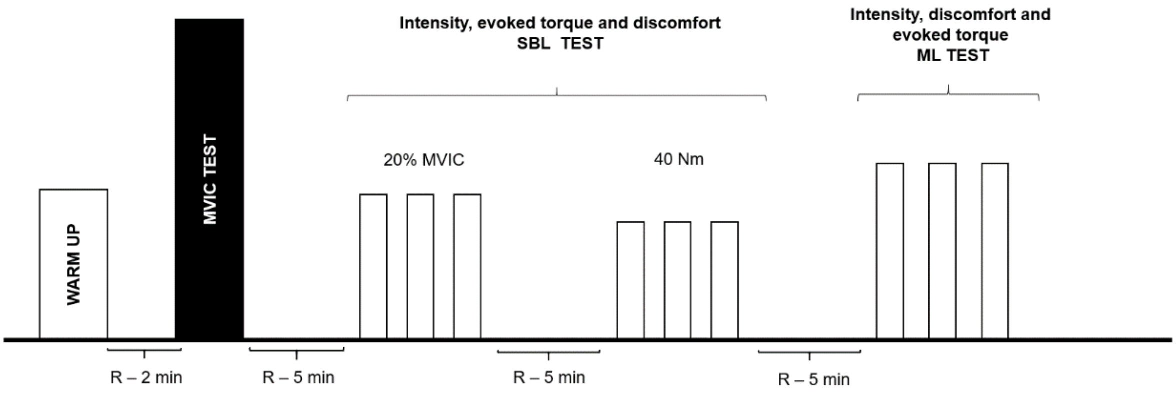

Figure 8 - Representation of the experimental protocol. MVIC: Maximum Voluntary Isometric Contraction; R: rest; SBL: submaximal level; ML: maximal level.

5.3.4 Randomization and Allocation Concealment

The NMES current types order was randomized by a researcher blinded to the study, using the http://randomization.com/ website. Subsequently, the same researcher organized the randomization data in opaque and sealed envelopes, to ensure the allocation blinding. Thus, the therapist had access to the applied current types only on the evaluation day.

5.3.5 Blinding

Data collection was performed by one therapist and one evaluator. The therapist was responsible for the NMES parameters definition and system operation. The evaluator operated the data acquisition equipment, instructed the patient on the use of the visual analog scale (VAS), and collected the self-perceived discomfort. The stimulator was positioned behind the dynamometer chair so that the stimulator parameters were not visible to the subject and to the evaluator. In addition, an opaque cover was placed on the NMES equipment during the application process to guarantee the blinding procedure. The therapist was blinded to the VAS and evoked torque data acquisition. Participants were blinded to the current type being used and to the torque output. The collected data were blinded by codes, so the data analyzers were blinded to the current type and participants. In addition, the waveforms testing order was randomly determined by a researcher blinded to the study, as previously described.

5.3.6 Outcomes

The evaluated outcomes were current intensity level, evoked torque, neuromuscular efficiency and discomfort. The assessment and analysis of each outcome are described below.

5.3.7 Maximum Voluntary Isometric Contraction (MVIC)

The MVIC tests were carried out on an isokinetic dynamometer (Biodex 3; Biodex, Shirley, New York). Participants were seated in the chair with their hips flexed at 85° and knees flexed at 90° (full knee extension = 0°), and velcro straps were applied tightly across the thorax and pelvis with the distal right leg fixed to the dynamometer lever arm. The lateral femoral condyle, which was used to estimate the knee joint anatomical axis, was aligned to the dynamometer's rotation axis. Initially, warm-up trials were performed through 10 submaximal concentric contractions of the knee extensor and flexor muscles, at an angular velocity of $90°.s^{-1}$. After a 2-min recovery period, participants performed three MVICs with the knee flexed at 90°, holding each contraction for 5 seconds, and with a 120-seconds resting period between consecutive contractions. Torque was then measured and recorded instantaneously, and MVIC was defined as the highest peak torque produced from the three maximum-effort trials. The MVIC testing was always performed on the participant's dominant limb. For the subsequent NMES tests, the submaximal intensity relative to each participant's MVIC was determined.

5.3.8 Interventions - NMES Procedure

The NMES tests were carried out on an isokinetic dynamometer (Biodex 3; Biodex, Shirley, New York). Participants were positioned as previously described. The NMES tests were performed with a Myomed 932® (Enraf-Nonius, The Netherlands) stimulator, which was used to generate the 4 NMES waveforms (Table 6): 2 ACs, defined commercially as Russian current, modulated in the duty cycles of 20% (AC20) and of 50% (AC50), and 2 low-frequency PCs, named as PC1 (PD = 2ms) and PC2 (PD = 0.4 ms). All NMES parameters were checked using a digital oscilloscope (model DSOX2014A, Keysight Technologies, Malaysia) prior to intervention. Self-adhesive NMES electrodes measuring 8×13 cm (ValuTrode®, Axelgaard Mfg. Co., Ltd., Fallbrook, CA, USA) were used. Trichotomy was performed in the electrode placement region, and the skin was cleaned with alcohol (MEDEIROS *et al.*, 2017). Electrodes were positioned as follows: one proximally, over the quadriceps muscle motor point, and one distally, 5 cm above the patella upper edge (MELO *et al.*, 2015). During the tests, participants were instructed to remain as relaxed as possible and to not perform any voluntary effort during NMES application.

PC2 (0.4 ms) was chosen based on its great use in several studies (ALDAYEL *et al*., 2010; ALDAYEL *et al*., 2011; VAZ *et al*., 2012*;* FUKUDA *et al*., 2013*;* DANTAS *et al.*, 2015) and for having the same PD (0.4 ms) of the 2 ACs. However, the literature suggests that long pulses, associated with high frequencies, increase the central contribution to evoked contractions (LAGERQUIST; COLLINS, 2010). Therefore, we also chose PC1 with greater PD (2 ms). For comparison purposes, we chose AC with 50% of duty cycle because it is the most used in clinical practice (WARD; ROBERTSON; IOANNOU, 2004), and because it was used in previous studies that compared PC to AC. However, evidence considers that a lower duty cycle (10-20%) appears to be optimal when evoked torque, comfort and stimulation efficiency are considered (WARD; ROBERTSON; IOANNOU, 2004), the reason why we also chose the 20% duty cycle for the comparison. In addition, when using a 2.5 KHz carrier frequency, the burst duration of 2 ms can be used for maximum muscle torque production (WARD, 2009). Therefore, burst-duration of the AC20, when using 100 Hz of burst frequency and a burst duty cycle of 20%, is similar to the pulse duration of PC1 (2 ms), with similar total charge capacity (PC- 2 ms pulse duration and AC- 2 ms burst duration). Furthermore, parameters commonly used in clinical practice were balanced to similar pulse charge capacity (PC and AC with 0.4 ms of pulse duration).

Table 6 - Neuromuscular electrical stimulation parameters for PC and AC.

	PC		**AC**	
	PC1	**PC2**	**AC20**	**AC50**
Pulse frequency (Hz)	100	100	NA	NA
Pulse duration (ms)	2	0.4	0.4	0.4
Carrier frequency (KHz)	NA	NA	2.5	2.5
Burst frequency (Hz)	NA	NA	100	100
Burst duty cycle (%)	NA	NA	20%	50%
Burst duration (ms)	NA	NA	2	5
Cycle time (ms)	10	10	10	10
Stimulus on time (rise time/fall time)	5 s (1/1)	5 s (1/1)	5 s (1/1)	5 s (1/1)
Waveform	SB - REC	SB - REC	SB - REC	SB - REC

NA: Not applicable. SB: symmetrical biphasic; REC: rectangular

5.3.9 NMES Current Intensity Assessment

NMES current intensity was assessed at submaximal and maximal levels. Tests were carried out on an isokinetic dynamometer (Biodex 3; Biodex, Shirley, New York). Participants were positioned seated as previously described, at 90° of knee flexion angle. For the two submaximal levels, a target was placed on the dynamometer's screen in the position corresponding to 20% of MVIC and at 40 Nm, respectively. The current intensity was increased until reaching the target submaximal evoked torque, after which it was recorded. At maximal levels, NMES intensity was gradually increased until participants indicated that their tolerance limit had been reached.

The 20% of MVIC submaximal level was chosen based on the fact that most daily life activities generally require similar submaximal levels of force production (BAPTISTA *et al.*, 2009). In addition, submaximal intensities (10-20% of MVIC) are well tolerated by frail populations and healthy subjects (VAZ *et al.*, 2012), and by hospitalized patients (KITAMURA *et al.*, 2018), who usually have more difficulty tolerating high stimulation intensities. Therefore, this assessment was chosen based on its functional and clinical relevance. Previous studies have also suggested that NMES current amplitudes should be assessed at particular torque outputs (SELKOWITZ; ROSSMAN; FITZPATRICK, 2009). Therefore, we opted to assess the current intensity required to achieve a fixed torque at 40 Nm, because it is an evoked torque level that could be reached by the different NMES current types we chose, and, furthermore, because it is within the range of 10% to 20% MVIC, as reported above.

5.3.10 Evoked Torque Assessment

The evoked torque tests were carried out on an isokinetic dynamometer (Biodex 3; Biodex, Shirley, New York). Participants were positioned seated as previously described at a 90° knee flexion angle. Evoked torque was assessed at submaximal levels and at the maximally tolerated current intensity level. After reaching the submaximal intensity level, three contractions were recorded, in order to ensure that the subjects were being evaluated at a similar level throughout the protocol. After assessing the participant's maximal tolerated NMES intensity, three evoked torques were recorded. The highest evoked torque (peak torque) of the 3 evoked contractions was recorded for analysis and normalized with respect to the MVIC obtained at 90° of knee flexion.

5.3.11 Discomfort Assessment

NMES-induced discomfort level was measured using a visual analog scale (VAS). The scale corresponds to a line, ranging from 0 to 10 cm, at which 0 cm corresponds to a complete absence of discomfort and 10 cm to the maximal discomfort level tolerated by the participant (DANTAS *et al.*, 2015). Participants were asked to show their discomfort level by making a vertical tick mark on the scale line. Self-reported discomfort levels were obtained by measuring the distance (in cm) to the mark made by the participant.

5.3.12 Neuromuscular Efficiency

Stimulation efficiency is determined by the stimulus intensity needed to generate a given amount of torque (WARD; ROBERTSON; IOANNOU, 2004). This relationship is also reported as neuromuscular efficiency (VAZ; FRASSON, 2018). In order to investigate neuromuscular efficiency, the evoked torque values (Nm) were divided by their corresponding NMES current intensity values (mA) required to generate the evoked torque, resulting in a torque (Nm)/NMES (mA) ratio (LIEBER; KELLY, 1991; WIEST *et al.*, 2019). The higher the ratio values, the higher the neuromuscular efficiency.

5.3.13 Data Analysis

The Shapiro-Wilk test was used to assess the data normality. Evoked torque values were normalized to the peak MVIC obtained at the 90° of knee flexion (%MVIC). To compare similarity at baseline, a one-way repeated-measures analysis of variance (ANOVA) was used. To compare the possible differences across NMES types, a one-way repeated-measures ANOVA was conducted for evoked torque, current intensity, discomfort levels and neuromuscular efficiency. When appropriate, Bonferroni post-hoc test for multiple comparisons was used to determine significant differences. All analyses were carried out using SPSS software (Version 21, IBM Corp., Armonk, NY, USA, 2012). Significance level was set at $p<0.05$ for all procedures. Additionally, effect size was calculated using Cohen's Equation (COHEN, 1988). Effect sizes (d) were categorized as trivial (<0.20), small (0.20-0.49), moderate (0.50-0.79), large (0.80-1.29), and very large (>1.30) effect (ROSENTHAL, 1996).

5.4 RESULTS

Thirty healthy, physically active men, age (mean±SD) 23.2±4.6 years; weight 74.9±9.2 kg; height 177.5±5.7 cm; BMI 23.8±2.6 participated in the study. According to IPAQ metabolic equivalents (MEITs), 16 participants were classified in the high physical activity level and 14 participants at the moderate level. All 30 individuals were assessed for eligibility and received the interventions. There were no losses or exclusions, and all data were analyzed for the 30 participants (Figure 9).

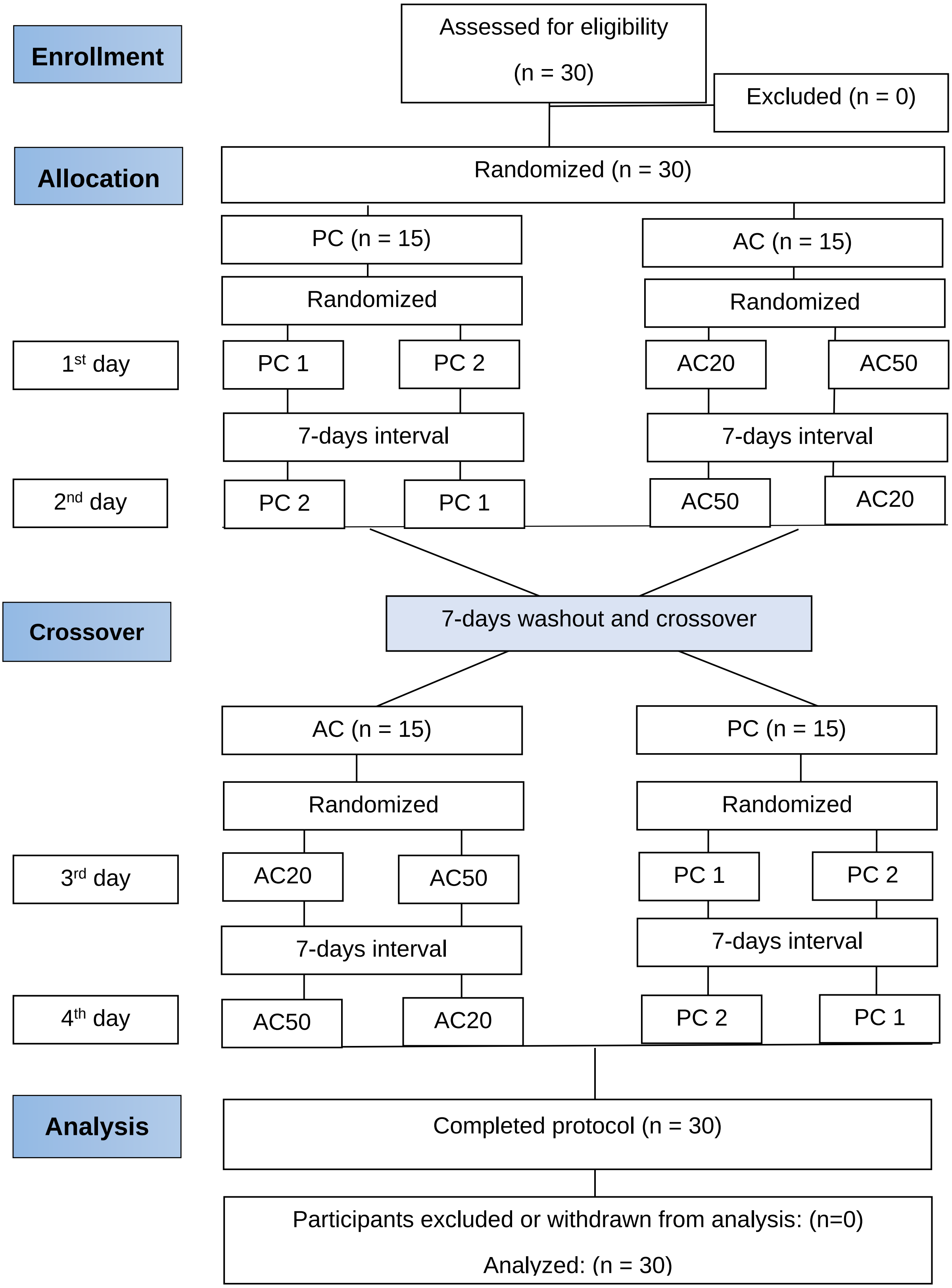

Figure 9 - Flowchart of the blinded randomized crossover trial.

5.4.1 Similarity at Baseline

To ensure the participants similarity throughout the protocol, MVIC was evaluated in all sessions. Additionally, the submaximal evoked torque levels were controlled throughout the protocol. Both analyzes showed that participants were in similar conditions during the evaluations (Table 7).

Table 7 - Similarity at baseline.

Variables (mean±SD)	**PC1**	**PC2**	**AC20**	**AC50**	**p-value (ANOVA)**
MVIC (Nm)	271.4±70.4	274.3±67.5	268.4±68.4	267.5± 67.8	p=0.243
Evoked torque at 20% MVIC (Nm)	54.2±13.3	54.8±13.7	53.0±13.0	53.6± 13.8	p=0.125
Evoked torque at 40 Nm (Nm)	40.6±2.3	40.3±2.3	40.6±1.9	40.9±1.8	p=0.426

MVIC: Maximum voluntary isometric contraction; PC: pulsed current; AC: alternated current.

5.4.2 Intensity Level

5.4.2.1 Submaximal Intensity Level

NMES currents were different for the submaximal intensity level (Figure 10) to reach the 20% of MVIC [F(3,87)=350.717; $p<0.0001$)] and the 40 Nm [F(3,87)=508.279; $p<0.0001$)] levels. The Bonferroni post hoc test demonstrated that lower current intensity was needed to reach the submaximal force level with PC1 compared to PC2, to AC20 and to AC50 (Figure 10), with a large effect size (Table 9).

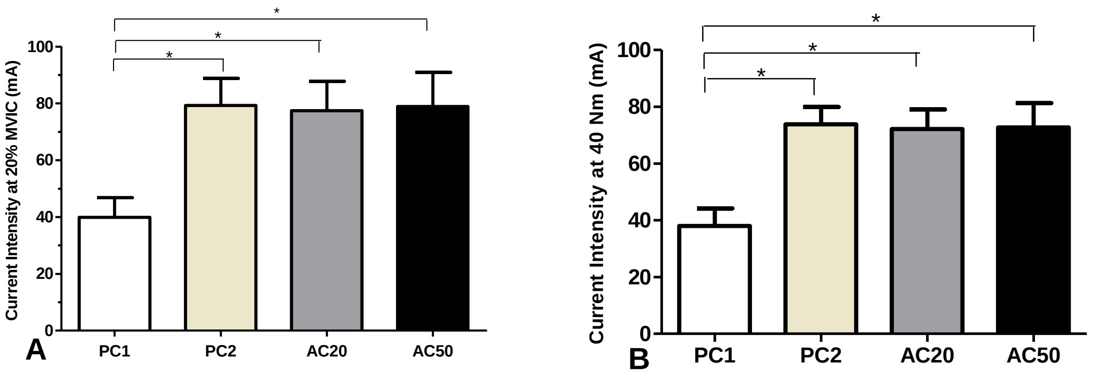

Figure 10 - Current Intensity level. **A:** Intensity level at 20% MVIC. **B:** Intensity level at 40 Nm. * $p<0.0001$ (Bonferroni Post hoc).

5.4.2.2 Maximal Intensity Level

NMES currents were also different for the maximally tolerated NMES intensity [F(1.957, 56.762)=96.969; p<0.0001)]. The Bonferroni post hoc test demonstrated that the maximally tolerated intensity level was reached with a lower NMES current intensity for PC1 compared to PC2, AC20 and AC50 (Figure 11), with a large and a very large effect size (Table 9). Additionally, a higher current intensity was necessary with PC2 compared to AC20, AC50 and PC1 to reach the maximally tolerated current intensity (Figure 11), with a very large effect size in both comparisons (Table 9).

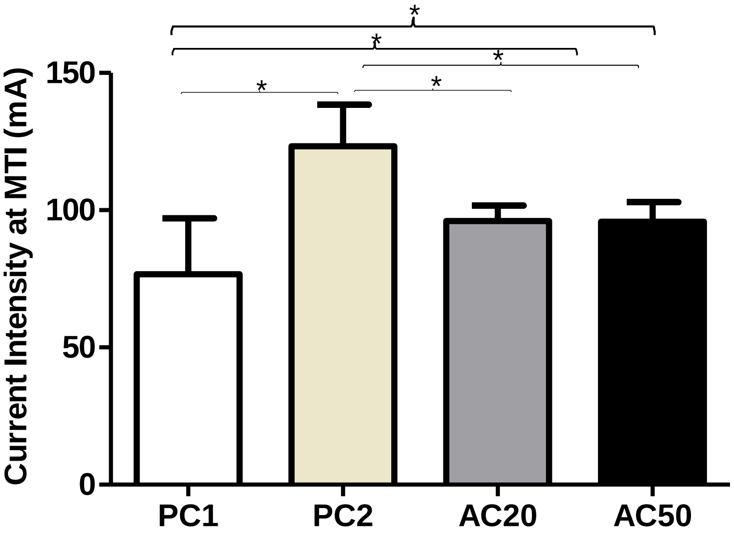

Figure 11- Maximal tolerated intensity level. * p<0.0001 (Bonferroni Post hoc).

5.4.3 Evoked Torque

As expected, evoked torque at both submaximal levels (20% MVIC and 40 Nm) was similar between NMES currents (Table 7), but different at maximal levels [F(1.91,55.64)=45.304; p<0.0001)]. The Bonferroni post hoc test demonstrated that both PC's were able to evoke greater torque than both AC's (Figure 12), demonstrating large and very large effect sizes (Table 9). Additionally, we observed that PC1 was able to evoke greater torque than PC2 (small effect size – Table 9).

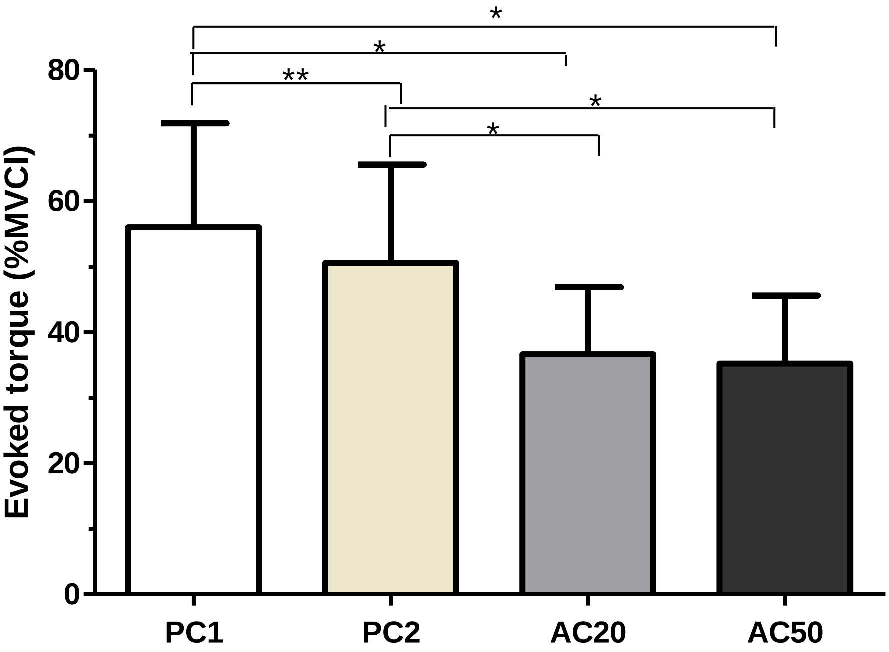

Figure 12 - Evoked torque at maximal intensity level. * p<0.0001 and ** p=0.031 (Bonferroni Post hoc).

5.4.4 Neuromuscular Efficiency

5.4.4.1 Neuromuscular efficiency at submaximal levels

Neuromuscular efficiency was different for the 20% of MVIC [F(1.230,35.68)=180.586; $p<0.0001$)] and at the 40 Nm [F(1.318,38.22)=242.163; $p<0.0001$)]. At the 20% of MVIC and at the 40 Nm levels, the Bonferroni post hoc test demonstrated that PC1 was more efficient compared to AC20, AC50 and PC2 (Figure 13), with very large effect size in both comparisons (Table 9).

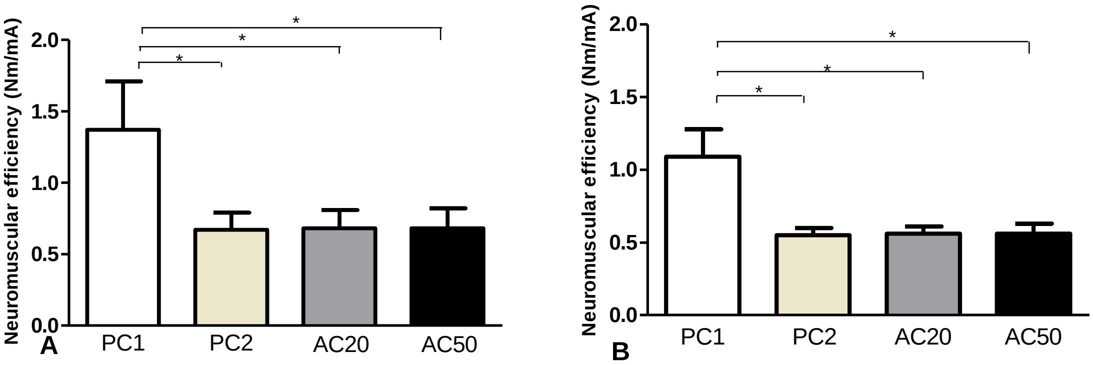

Figure 13 – Neuromuscular efficiency at submaximal levels. **A:** Neuromuscular efficiency at 20% MVIC. **B:** Neuromuscular efficiency at 40 Nm. * p<0.0001 (Bonferroni Post hoc).

5.4.4.2 Neuromuscular efficiency at maximally tolerated current level

Neuromuscular efficiency was different for the maximally tolerated intensity analysis [F(1.446,41.94)=104.61; p<0.0001)]. The Bonferroni post hoc test showed that PC1 was able to generate higher neuromuscular efficiency compared to AC20, AC50 and PC2 (Figure 14), with very large effect size in both comparisons (Table 9). In addition, PC2 showed higher neuromuscular efficiency compared to AC20 and to AC50 (Figure 14) with a small and moderate effect sizes, respectively (Table 9).

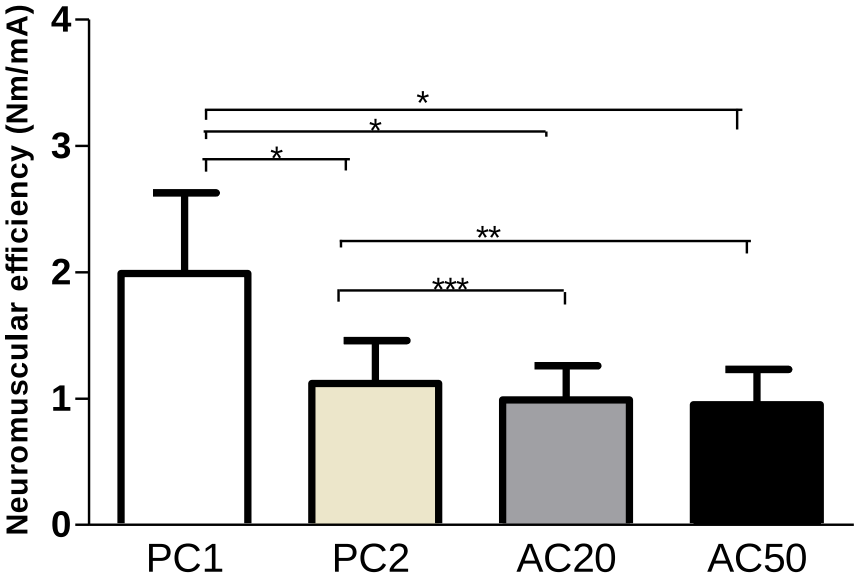

Figure 14 - Neuromuscular efficiency at maximal level. * p<0.0001, ** p=0.009, ***p=0.021(Bonferroni Post hoc).

5.4.5 NMES Discomfort level

5.4.5.1 NMES Discomfort at submaximal level

Both PCs and ACs showed similar discomfort when NMES reached the intensity to evoke 20% MVIC and 40 Nm (Table 8).

5.4.5.2 Discomfort at the maximally tolerated NMES current intensity level

Discomfort measured at the maximally tolerated NMES intensity level was similar between the currents at maximal current intensity levels (Table 8).

Table 8 - Discomfort level

	PC1 (mean±SD)	**PC2** (mean±SD)	**AC20** (mean±SD)	**AC50** (mean±SD)	**p (ANOVA)**
Discomfort at 20% (cm)	4.00±2.55	3.22±2.40	3.49±2.53	3.32± 2.15	p=0.405
Discomfort at 40 Nm (cm)	3.73±2.35	2.92±1.85	3.01±2.05	2.93±1.81	p=0.130
Discomfort at MTI (cm)	6.73±2.46	6.56±2.40	6.14±2.49	5.98±2.51	p=0.079

MTI: maximal tolerable intensity

Table 9 - Effect size analysis

Variables	**PC1 vs. PC2**	**PC1 vs. AC20**	**PC1 vs. AC50**	**PC2 vs. AC20**	**PC2 vs. AC50**	**AC20 vs. AC50**
Current Intensity at 20% MVIC	**4.74**[e]	**4.25**[e]	**4.00**[e]	0.18[a]	0.03[a]	0.14[a]
Current Intensity at 40 Nm	**5.82**[e]	**5.18**[e]	**4.59**[e]	0.26[b]	0.16[a]	0.06[a]
MTI (mA)	**2.60**[e]	**1.29**[d]	**1.25**[d]	**2.39**[e]	**2.32**[e]	0.02[a]
Evoked torque at 20% MVIC	0.04[a]	0.08[a]	0.04[a]	0.13[a]	0.08[a]	0.04[a]
Evoked torque at 40 Nm	0.16[a]	0.00[a]	0.15[a]	0.16[a]	0.34[b]	0.18[a]
Evoked torque at MTI	0.35[b]	**1.45**[e]	**1.55**[e]	**1.09**[d]	**1.19**[d]	0.13[a]
NEUROEF at 20% MVIC	**2.75**[e]	**2.68**[e]	**2.65**[e]	0.07[a]	0.07[a]	0.00[a]
NEUROEF at 40 Nm	**3.89**[e]	**3.82**[e]	**3.70**[e]	0.20[b]	0.16[a]	0.00[a]
NEUROEF at MTI	**1.70**[e]	**2.03**[e]	**2.10**[e]	0.42[b]	0.55[c]	0.14[a]
Discomfort at 20% MVIC	0.32[b]	0.20[b]	0.28[b]	0.10[a]	0.04[a]	0.07[a]
Discomfort at 40 Nm	0.38[b]	0.33[b]	0.38[b]	0.04[a]	0.00[a]	0.04[a]
Discomfort at MTI	0.07[a]	0.24[b]	0.30[b]	0.17[a]	0.24[b]	0.06[a]

a: Trivial effect size; b: small effect size; c: moderate effect size; d: large effect size; e: very large effect size; MTI: maximal tolerable intensity; MVIC: Maximum voluntary isometric contraction NEUROEF: Neuromuscular efficiency.

5.5 DISCUSSION

The purpose of this study was to compare the effects of PC and AC with different pulse/burst durations at submaximal and maximal NMES current intensity levels on the evoked torque, current intensity, neuromuscular efficiency and discomfort. The results of our study partially confirmed our hypotheses. PC1 needed less current intensity to reach both the submaximal and maximally evoked torque levels compared to PC2, AC20 and AC50. However, at the maximally tolerated intensity level, PC2 required more current intensity to reach this level compared to the other NMES currents. Regarding the evoked torque, both PC's were able to produce greater torque than both AC's. Similarly, with regard to the neuromuscular efficiency, PC1 was more efficient than AC20 and AC50 at both submaximal and maximally tolerated levels. PC2 was also more efficient than AC20 and AC50 at the maximally tolerated intensity level. Finally, with respect to the discomfort level, there was no difference between AC and PC in both submaximal and maximally tolerated current intensity levels.

NMES Current Intensity Level

Current intensity is a very important parameter during a NMES session, since it is commonly adjusted to maximize the evoked torque (MAFFIULETTI, 2010). However, several factors may affect the maximally tolerated current intensity or the current intensity required to evoke a submaximal force. Among these factors are the skin and the subcutaneous fat tissue (PETROFSKY *et al.*, 2008), which act as capacitive barriers to the current flow. However, it has been reported that, by using AC, less electrical energy would dissipate peripherally, due to the lower impedance observed for the passage of a large number of electrical pulses with this NMES current type, and a higher electrical energy would be available to stimulate nerves in the underlying tissue with AC compared to PC, thereby resulting in less current intensity being needed by AC compared to PC (WARD, 2009; VAZ; FRASSON, 2018).

However, our findings do not support this theory, since PC1 needed a lower current intensity compared to AC20 and AC50 to reach the same force level at the two submaximal evoked force levels. In addition, PC2 (with PD=0.4 ms) needed a similar current intensity to evoke the same force level compared to AC20 and AC50, showing evidence that the previously assumed advantage of AC compared to PC does not hold true.

Vaz *et al.* (2012) evaluated the current intensity needed to produce 10% MVIC, using AC (2.5 KHz, 50 Hz, PD=0.4 ms) and PC (50Hz, PD=0.4 ms). Similar to our findings, their results showed lower current intensity for PC (64.32±10.49 mA) compared to AC (74.59±15.78 mA). However, when we compare only the NMES currents with similar PD (i.e., 0.4 ms), there was no difference in current intensity between PC and AC. The difference between our results and those of Vaz *et al.* (2012) might be explained to the different pulse shapes used in their study (rectangular for PC and sinusoidal for AC), which might have led to different activation effects.

We also found that altering the AC-2.5 KHz's burst duration generated no impact at the current intensity to reach the submaximal or the maximal force levels. These results agree with previous evidences that demonstrated no differences in the maximally tolerated intensity using 20% or 50% of burst duty cycle with the AC-2.5 KHz (LIEBANO; WASZCZUK JR; CORRÊA, 2013). On the other hand, our results support previous findings (JEON; GRIFFIN, 2018) that increasing the pulse duration in PC had an impact on current intensity. More specifically, as the pulse duration became longer, lower current amplitude was required to cause motor stimulation (ALON; ALLIN; INBAR, 1983). This is supported by our results, since PC1 required less intensity to generate the two submaximal force levels compared to PC2, AC20 and AC50. Similarly, at maximum intensity levels, PC1 required lower intensity, and was able to evoke a higher motor response compared to AC20 and AC50, with a very large effect size (Table 9).

Similar results were shown by Medeiros *et al*. (2017), who evaluated the maximally tolerated intensity by two PCs (50 Hz, PDs = 500 μs and 250 μs) and two ACs with different carrier frequencies [AC-1.0 KHz (50 Hz, PD = 500 μs), and AC-4.0 KHz (50 Hz, PD = 250 μs)]. Although the carrier frequency used in this study (MEDEIROS *et al.*, 2017) is different from our study, the results are partly similar. These authors demonstrated that larger pulse duration (500 μs) needed less current amplitude and was capable of generating more force compared to shorter pulse duration (250 μs). No differences in current intensity were observed between currents with similar pulse duration. Our results for the two submaximal evoked force levels also agree with their results, as all NMES currents with the same pulse duration (i.e., 400 μs - PC2, AC20 and AC50) showed similar current intensities.

But perhaps the main result observed in our study is that longer pulse duration requires lower stimulation intensities to activate peripheral motor axons and achieve a desired force output. On the other hand, when using shorter pulse durations, greater current intensity will be required to reach similar excitation levels as those with longer pulse durations (BICKEL; GREGORY; DEAN, 2011). Therefore, our results suggest that, at both submaximal and maximally tolerated intensity levels, PC with larger pulse duration is the best current, since it requires less energy compared to the other current configurations.

Evoked torque

Evoked torque during NMES treatment/intervention determines the mechanical output, or the mechanical load to which the muscle-tendon unit is subjected to, and therefore, the desired adaptations to the NMES treatment (VAZ; FRASSON, 2018). Thus, NMES effectiveness is proportional to the evoked force (MAFFIULETTI *et al.*, 2018).

Our results showed that PC was more effective for producing knee extensor muscle torque, with large and very large effect sizes for the differences between PCs and ACs for this outcome (Table 9). These results are clinically relevant, since evoked force is related to the NMES therapy clinical success (MAFFIULETTI *et al.*, 2018).

In addition, our results support previous evidences that pulse duration influences the NMES-generated torque (GORGEY; DUDLEY, 2008; SCOTT; CAUSEY; MARSHALL, 2009; SCOTT *et al.*, 2014; MEDEIROS *et al.*, 2017). Recent research has shown lower knee extensor muscle torque with AC-2.5 KHz, with short phase duration (200 μs) (29.8±12.4%) compared to a PC with large phase duration (500 μs) (49.5±19.6%) (SCOTT *et al.*, 2015). Scott *et al.* (2015) demonstrated that PC is more effective than AC, and our findings are similar, and the between-currents difference demonstrated a large effect size (1.20).

Indeed, the PC1 results might have been favored by the longer pulse duration compared to that of AC-2.5 KHz. However, the other studies that used a similar pulse duration for both currents found that AC-2.5 KHz produced similar torque to PC (ALDAYEL *et al.*, 2010), or found that this current was less effective for eliciting maximally evoked muscle torque compared to PC (LAUFER *et al.*, 2001; WARD; OLIVER; BUCCELLA, 2006; DANTAS *et al.*, 2015).

Aldayel *et al.* (2010) assessed the evoked torque by PC and AC (2.5 KHz) with similar pulse/burst frequency (75Hz) and pulse duration (400 μs). They found similar normalized evoked torque for AC (28.4±4.4%, relative to MVIC) and for PC (31.8±3.7, relative to MVIC). However, these results can be explained by the fact that this study evaluated the evoked torque during a NMES protocol that consisted of 45 evoked isometric contractions. The torque output increased in the first 15 contractions and reached a maximal torque plateau, despite the continuous increases in stimulation intensity throughout the protocol. The authors suggested that no further increases in motor unit recruitment were observed, indicating that muscle fatigue occurred. This could explain the similarity in evoked torque by both currents, since the protocol induced fatigue.

In contrast, different results were obtained by Laufer *et al.* (2001), who compared the knee extensor evoked torque between 2 PCs (MP -monophasic and BP-biphasic) and one AC-2.5 KHz. Both current types were similarly configured (50 Hz, and 200 μs of pulse phase duration, at the maximally tolerated NMES intensity). The authors demonstrated that both PCs were able to evoke higher torque (BP: 38.0±16.6%, MP: 36.6±17.1%, normalized to the MVIC) compared to AC (30.9±12.6%). Therefore, this study shows evidence that, at the maximally tolerated intensity, AC elicited weaker contractions than PC, similar to our results.

Similar results were obtained by Dantas *et al. (2015)*, who demonstrated that AC-2.5 KHz (50% - burst duty cycle) is less effective than PC stimulation in terms of torque production, and supports the results from Ward *et al.* (2006). In both studies, the authors suggest that the lower torque evoked by AC may be related to pulses number and burst duration. Long burst duration allows time for multiple motor unit firing, resulting in high frequency fatigue (WARD; OLIVER; BUCCELLA, 2006; DANTAS *et al.*, 2015) due to neurotransmitter depletion and propagation failure, and consequently reducing the evoked torque (JONES, 1996; WARD; ROBERTSON, 2001; WARD; OLIVER; BUCCELLA, 2006).

However, we used both a short (2 ms) and long (5 ms) burst duration, but we found that altering the AC-2.5 KHz burst duration had no clinically relevant impact on the evoked torque at MTI. Indeed, our results at MTI should be interpreted with caution because some participants reached the stimulator’s maximum NMES current intensity with AC (see the limitations section below). Nevertheless, despite this limitation, our results agree with the previously reported results, and demonstrate that changing the burst duration did not influence the AC evoked torque, which was lower compared to PC. Furthermore, the behavior of the two ACs appears to be similar, with a trivial effect size.

On the other hand, when analyzing the differences between PC1 and both AC's, the effect size was very large (Table 9), further emphasizing the importance of using a PC with a long pulse duration to obtain the best evoked torque. Considering that there is a positive relationship between the amount of the elicited torque and the muscle strength gains after a NMES protocol (SELKOWITZ, 1985; LAI; DE DOMENICO; STRAUSS, 1988), our study suggest that PC is more indicated than AC-2.5 KHz when the NMES intervention goal is strength gain.

Neuromuscular efficiency

Neuromuscular efficiency is defined as the inverse relation between current intensity and evoked force. Therefore, the most efficient current is the one that produces the highest amount of evoked force with the smallest current intensity or that needs less current intensity to generate a predetermined amount of evoked force (VAZ; FRASSON, 2018).

Our results demonstrated that PC with a long pulse duration is more efficient than AC-2.5 KHz. At the two different submaximal levels, PC1 needed lower current intensity to reach a specific strength level and, consequently, it proved to have a better neuromuscular efficiency than AC20 and AC50, with a very large effect size at both submaximal levels (20% MVIC and 40 Nm, Table 9). These findings are clinically relevant, as NMES should be used in clinical practice with the NMES current and specific settings with the highest efficiency, which is expected to produce the best clinical results.

Although we have not found studies that have evaluated neuromuscular efficiency with AC-2.5 KHz to compare with our results, analyzing the data presented in previous studies (LIEBANO; ALVES, 2009; VAZ *et al.*, 2012), we can indirectly assume that AC was less efficient than PC. Studies that compared AC and PC at submaximal force levels (LIEBANO; ALVES, 2009; VAZ *et al.*, 2012) demonstrated that the NMES current intensity needed to produce a pre-established force was higher for AC than for PC, which further supports the idea that AC is less efficient in submaximal force levels than PC, and which we demonstrated with our results.

Similarly, at force levels evoked with the maximally tolerated NMES current intensity, previous results (FUKUDA *et al.*, 2013) provided evidence in support of our assumptions. Fukuda and colleagues (2013) compared the knee-extensor evoked torque between AC-2.5 KHz (50 Hz, PD = 400μs) and PC (50 Hz, PD = 400μs). A higher current intensity (74.7±14.5mA) was observed for AC compared with the PC (59.7±10.9mA). However, the evoked torque results were similar between PC (59.5±28.7%) and AC (54.2±36.9%). Considering that for similar strength level AC required higher current intensity, we can assume that it was not as efficient as PC.

Our results at maximally tolerated NMES intensity levels demonstrate that AC20 and AC50 required more current intensity compared to PC1, with a large effect size for the MTI level (Table 9). However, even using higher current intensity, AC20 and AC50 evoked a lower force compared to PC1. As a result, PC1 demonstrated greater neuromuscular efficiency than AC20 and AC50 at maximally tolerated intensity levels.

Therefore, the main result observed in our study is that longer pulse duration is more efficient than short pulse duration in both maximal and submaximal intensity levels. There was a very large effect size when PC1 was compared to PC2, AC20 and AC50, demonstrating that this difference was clinically significant. The higher efficiency of PC1 in relation to the other currents may be related to the fact that increasing pulse duration increases the activated area of the stimulated muscle, thereby maximizing torque output (GORGEY *et al.*, 2006). Another possible explanation is that PC1 was able to generate greater total charge, which is defined as the product of the frequency and pulse duration, which has been strongly suggested to play a role in maximizing evoked torque (GREGORY; DIXON; BICKEL, 2007).

Our results suggest that AC-2.5 KHz is less efficient than PC. However, few studies comparing AC-2.5 KHz and PC reported the intensity level that was used, and we did not find studies that evaluated neuromuscular efficiency between PC and AC-2.5 KHz, which makes direct comparisons between our results and the literature results impossible. Nevertheless, as previously reported, when selecting the NMES type one should always analyze the amount of evoked torque for a given current intensity, since this will determine the NMES efficiency. Therefore, PC with longer pulse duration is the most efficient NMES current, and our results suggest that it is the best tool to be used for strength training and/or rehabilitation in clinical practice.

Discomfort Level

Discomfort caused by NMES has been reported as one of the main limiting factors for muscle strengthening by electrical stimulation (LIEBANO *et al.*, 2013). A large discomfort level at the NMES site may inhibit the corresponding muscle activation through a reflex inhibition pathway, therefore compromising the NMES efficacy (SCOTT *et al.*, 2014). Our results demonstrated no discomfort difference between AC-2.5 KHz and PC, both at submaximal and maximally tolerated intensity levels, showing evidence that discomfort did not play a role as an inhibitor of the evoked torque.

It has been reported that the individual's ability to tolerate high intensities during a NMES protocol may be related to unpleasant sensations related to the NMES-evoked contractions than by the unpleasant sensation related to the current amplitude (LAUFER *et al.*, 2011). Therefore, assessing discomfort at submaximal intensity levels may be a good method to assess current-related discomfort. However, few studies have evaluated PC and AC discomfort at submaximal levels (LIEBANO; ALVES, 2009; VAZ *et al.*, 2012), and our results suggest similar discomfort between AC and PC, agreeing with previous findings (LIEBANO; ALVES, 2009).

Liebano and Alves (LIEBANO; ALVES, 2009) assessed the discomfort level during the current intensity needed to produce full knee extension with PC (50Hz, PD = 350 μs) and AC (2.5kHz, 50Hz, PD = 400 μs). Although the authors demonstrated higher current intensity with AC, the discomfort was similar between AC (6.4±0.37 cm) and PC (6.1±0.42 cm).

Contrarily, Vaz *et al.* (2012) evaluated the discomfort sensation between PC (50 Hz, PD = 400 μs) and AC (2.5kHz, 50 Hz, PD = 400 μs) at the moment that the evoked torque reached 10% MVIC. The authors found lower discomfort levels with PC (3.2±2.3 cm) than with AC (4.8±2.2 cm).

Divergent results between the present study and Vaz et al.’s (2012) can be explained by the methodological differences. Vaz et al. (2012) used two different pulse waveforms: rectangular for PC and sinusoidal for AC. Evidence shows that the amount of discomfort caused by rectangular and sinusoidal pulse waveforms are different. Specifically, sinusoidal AC with 50% of burst duty cycle was more uncomfortable than the rectangular PC stimulation (SZECSI; FORNUSEK, 2014). A lower chronaxie time has been reported for the rectangular waveform (SAHIN; TIE, 2007), that is, it can cause greater peripheral nerve excitability compared to the sine waveform (SZECSI; FORNUSEK, 2014). Therefore, this may have led to a greater effectiveness for the rectangular waveform, that is, less phase charge was required to elicit similar torques (ADAMS et al., 2018), which might have led to a higher activation of skin receptors by AC due to the higher current intensity, thereby eliciting a higher discomfort with AC compared to PC. Additionally, previous findings demonstrated that quadriceps stimulation with a symmetrical biphasic pulse waveform was perceived as more comfortable than conventional sinusoidal AC (50% burst duty cycle) at 50 Hz (BAKER; BOWMAN; MCNEAL, 1988).

In Vaz *et al.* (2012) study, the force of the electrically induced contractions was low (10% MVIC). Evidence shows that the discomfort associated with the current amplitude and with evoked muscle contractions seemed to be equivalent at low force levels (DELITTO et al., 1992). However, in our study the submaximal level was twice that of Vaz *et al.* (i.e., 20% MVIC), enough to cause visible contractions, and the discomfort associated with muscle contractions can be higher than that of the skin sensory receptors, which could be a confounding factor for sensation related purely to electrical stimulation. However, these are only assumptions, since we have not found studies that have evaluated these issues between PC and AC.

It has been reported that as the current intensity increases, the magnitude of the evoked contractions increases (SPRINGER; SHAPIRO, 2017), which may limit the NMES tolerance (DELITTO *et al.*, 1992). Although our results showed differences in current intensity level between NMES currents at the maximally tolerated level, this factor apparently did not influence the discomfort level, as we did not find between-currents differences in discomfort. Our results agree with previous studies that found discomfort similarity between PC and AC (LYONS *et al.*, 2005; DANTAS *et al.*, 2015), but disagree with other findings that reported lower discomfort with AC-2.5 KHz compared to PC at MTI (WARD; OLIVER; BUCCELLA, 2006; FUKUDA *et al.*, 2013).

Dantas *et al.* (2015) compared the discomfort levels between four NMES types: two PCs (50 Hz, PDs = 200 and 500 μs), and two ACs with different carrier frequencies [AC-2.5 KHZ (50 Hz, PD = 200 μs), and AC-1.0 KHz (50 Hz, PD = 500 μs)]. Discomfort level was evaluated after reaching the maximally tolerated NMES intensity. Although the authors used different pulse durations and different carrier frequencies, all currents produced similar discomfort levels. Our results were similar to theirs, as different pulse durations and different NMES current types showed no differences in the discomfort level.

In contrast, Ward *et al.* (2006) compared AC-2.5 KHz to monophasic PCs, and demonstrated less discomfort for AC. However, the use of monophasic current could explain the higher discomfort with PC. As the monophasic electrical current has unidirectional current flow in the circuit, it can lead to an alkaline buildup at the positive electrode and an acidic one at the negative electrode, which can also cause skin irritation and more discomfort (FARY; BRIFFA, 2011). Furthermore, this study (WARD; OLIVER; BUCCELLA, 2006) reported discomfort while recording an unpleasant feeling during the build up to the maximally tolerated current intensity, which differs from the evaluation used by most studies, where discomfort is evaluated during the plateau of the evoked force-time curve.

Therefore, it is difficult to compare the results of the NMES-induced discomfort, since the studies used different parameters and evaluation protocols. Additionally, self-reported discomfort during different NMES protocols is subjective (MEDEIROS *et al.*, 2017), which makes evaluation difficult, since, between-individuals variability is relatively high.

Limitations

Among the study’s limitations, is the restriction of the selected sample, being composed only of young, active and healthy men, which limits the inference of the obtained results for other populations. However, this experimental design was chosen because it allows greater control of the outcome variables, and we adopted a higher methodological quality than the majority of the studies at the NMES field.

We need to highlight that the assessments at the maximal current intensity should be interpreted with caution, since 43% (13/30 participants) of the participants reached the maximal current intensity output of the device using AC stimulation, so this may limit our findings. In addition, the stimulator’s maximal output current was different among the currents (PC1: 110 mA; PC2: 140 mA; AC20 and AC50: 100 mA), which possibly explains the fact that PC2 displayed the highest current intensity at the maximally tolerated intensity compared to the other three NMES currents, and the two

ACs were limited by the smallest maximal NMES current intensity values. More specifically, about 43% of the participants reached the maximal stimulator output (i.e., maximal current intensity) during the AC session, and they might have tolerated additional current intensity increases. However, this is a limitation of the Enraf Nonius stimulator, which was not possible to change. Nevertheless, considering that the discomfort level at the maximum tolerated intensity generated by the two PC's was similar to that generated by the two ACs, the subjects supposedly would not tolerate a large increase in current intensity with AC20 and AC50 to the point of reaching the evoked torque levels generated by the PCs. Despite this limitation, we found similar results to those presented in the literature, in which they used the same current intensity level in both currents. This suggests that, despite the possibility of further current intensity increases, similar results as those here presented probably would be observed. However, we recommend that future studies also evaluate the evoked torque at a fixed and predetermined NMES intensity level for all currents, as this would allow to definitely resolve this limitation. Thus, it would be possible to evaluate if, for a fixed current intensity, there is difference in the evoked torque by the two currents types.

Finally, this study was designed to evaluate the acute response by different NMES modalities, and perhaps in chronic NMES protocols the currents' effects might be different from those presented in this study.

Clinical applicability

Our results demonstrate that if the goal is to gain strength, PC is more indicated than AC, because it is capable of generating more force using lower current amplitude (PC1), that is, it is more efficient than AC. Using short pulse durations (i.e., 0.4 ms) determines that a greater NMES current amplitude is required to reach a pre-established evoked force, and it is possible that we reached the NMES amplitude limit of the electrical stimulator, as occurred with 43% of our sample for AC. This can constitute a big disadvantage, because it can limit the training/rehabilitation program progression. Therefore, using a longer pulse duration would be a strategy to ensure that greater force is evoked over the course of the training/rehabilitation sessions, requiring less current intensity, and allowing further current increases throughout the rehabilitation program. Finally, we designed a study with the best possible quality at the PEDRo scale, in order to assure that most confounding factors did not play a role in our results, which is, in our opinion, very important for clinical practice.

5.6 CONCLUSION

Our results demonstrate that PC is efficient in generating greater evoked force compared to AC-2.5 KHz. PC with long pulse duration requires less current intensity to achieve submaximal and maximal force levels, generating higher neuromuscular efficiency. AC-2.5 KHz and PC generated similar discomfort level at submaximal and maximally tolerated current intensity levels. Therefore, our study shows evidence that AC is less effective for eliciting muscle torque at submaximal and maximally tolerated levels, has lower neuromuscular efficiency, and should be replaced by PC in clinical practice.

OTHER INFORMATION

Clinical trials: NCT03796117

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CHAPTER 3

In chapter 1 we observed that very few studies evaluated fatigability between PC and AC-2.5 KHz (Russian Current). Additionally, these studies demonstrated low methodological quality, which influences the evidence quality for this outcome and, consequently, may lead to an inadequate clinical decision making. In addition, they used different NMES protocols, which make between-studies comparisons difficult. Increasing the current intensity to the maximum tolerated throughout the protocol, starting at the maximum tolerated current intensity or using a submaximal current intensity were some of these methodological differences. We found only one study that assessed fatigue between PC and AC at submaximal strength levels, which is clinically relevant, since our daily living activities are performed at submaximal strength levels. In addition, the studies used different methodologies for the outcomes' evaluation. While some studies evaluated the evoked peak force drop from the beginning to the end of the fatigue protocol, others evaluated the area under the force-time curve, number of contractions for the force to drop to 50% of the session initial value, and only two studies evaluated the MVIC before and after the fatigue protocol. Additionally, assessments of the discomfort level during the fatigue protocol are scarce. Considering these differences in protocols and methodologies, it is fair to say that it has not yet been well established which of the two current types is the most fatigable one, and, therefore, the less efficient. Based on these literature gaps, we conducted a blinded, randomized crossover trial in an attempt to fill these gaps by comparing the PC and AC-2.5 KHz NMES effects on fatigability and discomfort in healthy young participants.

6 ALTERNATING CURRENT IS MORE FATIGABLE THAN PULSED CURRENT IN HEALTHY INDIVIDUALS: A BLINDED, RANDOMIZED CROSSOVER TRIAL

6.1 ABSTRACT:

Background: Tolerance level and the rapid fatigue onset are limitations involving the use of Neuromuscular Electrical Stimulation (NMES) as an electrotherapeutic resource in rehabilitation and strength training protocols. Due to different pulse characteristics, pulsed (PC) and the alternating (AC) currents produce different fatigability and discomfort. **Purpose:** To compare fatigability and discomfort between PC and AC during a submaximal fatigue protocol in healthy individuals. **Design:** Blinded, randomized crossover trial. **Methods:** Thirty healthy men (age 23.23±4.59 years) were randomized to 2 different submaximal fatigue protocols: AC50 (2.5 KHz, pulse duration = 0.4 ms, burst frequency = 100 Hz) and PC1 (pulse duration = 2 ms, pulse frequency = 100 Hz). NMES currents were applied in separate sessions (with 7-days interval) to the subjects' dominant limb's quadriceps femoris motor-point. Participants and evaluators were blinded to the NMES type. Maximal voluntary isometric contraction (MVIC), fatigue index (evoked torque decline), number of contractions for a 50% evoked-torque decline from the initial torque, total evoked torque-time integral (TTI), decline in the TTI, and discomfort level were evaluated immediately post-fatigue. **Results**: AC-2.5 KHz demonstrated higher MVIC decline post-fatigue, higher fatigue index, less contractions for the 50% evoked torque decline from the initial torque, smaller total TTI, and higher TTI decline compared to PC ($p<0.05$). No between-currents difference was observed in mean discomfort ($p>0.05$). **Conclusion:** PC is less fatigable than AC-2.5 KHz, being therefore the best NMES choice when the NMES goal is to generate higher muscle work, higher mechanical load and smaller fatigability during training or rehabilitation.

6.2 INTRODUCTION

Neuromuscular electrical stimulation (NMES) consists in a rehabilitation modality aimed at preserving (BENAVENT-CABALLER *et al.*, 2014; HASHIDA *et al.*, 2016), restoring (SPECTOR *et al.*, 2016), and improving neuromuscular function (ESTEVE *et al.*, 2017; MAFFIULETTI *et al.*, 2018; MEKKI *et al.*, 2018). However, its therapeutic effects are limited by the patient’s tolerance level and the rapid fatigue onset (THRASHER; GRAHAM; POPOVIC, 2005; MAFFIULETTI, 2010; DOUCET; LAM; GRIFFIN, 2012; IBITOYE *et al.*, 2016). A high discomfort level reduces the NMES tolerance, thereby reducing its ability to produce the desired training effects (LYONS *et al.*, 2005). Fatigue is related to a reduction in the neuromuscular system’s total force generating capacity (BIGLAND-RITCHIE; WOODS, 1984). Therefore, during a NMES session, the early fatigue onset leads to smaller mechanical load intervention (VAZ; FRASSON, 2018), and therefore a smaller and undesired adaptation result. Thus, reducing the NMES fatigability can maximize the benefits of this therapeutic modality in rehabilitation programs (BARSS *et al.*, 2018; VAZ; FRASSON, 2018).

Studies have examined the effect of different NMES current types in fatigability, by comparing the fatigue effects between low frequency pulsed current (PC) and medium frequency alternating current (AC-2.5 KHz, Russian current). PC is characterized by the delivery of successive pulses separated by an inter-pulse interval (VAZ; FRASSON, 2018), whereas in AC stimulation the pulses are applied within bursts, and there is no time gap between the pulses, but there is a time gap between the bursts (WARD; CHUEN, 2009).

Due to this larger number of electrical pulses, AC-2.5 KHz has been suggested to generate multiple nerve fiber action potentials per burst that would produce smaller discomfort and elicit greater force than PC (VAZ; FRASSON, 2018). However, the longer duration of a burst compared to the shorter PC pulse duration leads to multiple nerve firings with AC-2.5 KHz, which may lead to a rapid fatigue onset (WARD; OLIVER; BUCCELLA, 2006; DANTAS *et al.*, 2015). This higher fatigability may reduce the evoked force-maintenance capacity during AC-2.5 KHz protocols (LAUFER; ELBOIM, 2008). In other words, a smaller mechanical load should be produced during NMES with AC-2.5 KHz compared to PC, thereby limiting its effectiveness.

Nonetheless, few studies evaluated the discomfort level and fatigability between PC and AC-2.5 KHz, and they show inconsistent results. AC-2.5 KHz has been reported to induce a higher (LAUFER *et al.*, 2001; LAUFER; ELBOIM, 2008; LEIN JR; MYERS; BICKEL, 2015) or a similar fatigue response (ALDAYEL *et al.*, 2010; ALDAYEL *et al.*, 2011) compared to PC. Regarding discomfort level during (LYONS *et al.*, 2005) or after a fatigue protocol (LAUFER; LBOIM, 2008), the results showed between-currents similarities.

These divergent fatigability results may be related to the methodological differences used to assess fatigue and the different fatigue protocols (i.e., maximum tolerated intensity or submaximal intensity) used by the studies (VAZ; FRASSON, 2018). Differences in the NMES on-off times have been used in different studies. While Laufer *et al.* (2001) and Laufer and Elboim (2008) used longer on-time durations (7:2s, 7:3s), Aldayel *et al*. (2010; 2011) used longer off-time durations (5:15s), and Lein Jr *et al.* (2015) used similar on:off time durations (1:1s), which makes between-studies fatigability comparison difficult.

In addition, most fatigue studies applied NMES at the maximum tolerated current intensity. However, determining NMES fatigability at submaximal intensity levels seems more clinically relevant, since most functional activities are executed at submaximal contractions (CONWIT *et al.*, 2000). In addition, frail populations cannot tolerate high NMES training intensities (MAFFIULETTI, 2010), and a higher adherence to NMES may be obtained with smaller discomfort and with submaximal NMES.

We found only one study that evaluated fatigue between PC and AC-2.5 KHz at submaximal NMES intensity levels (LEIN JR; MYERS; BICKEL, 2015). However, Lein and colleagues (2015) did not evaluate several outcomes that are clinically relevant, such as the decrease in maximum voluntary isometric contraction (MVIC), and the torque-time integral (TTI), which is used as a measure of isometric work (ROZAND *et al.*, 2015). Work has been linearly related to an isometric contraction’s energetic cost (ORTEGA *et al.*, 2015), which has a direct relation to muscle fatigability, and it has been suggested that an earlier reduction in TTI indicates greater muscle fatigue (NEYROUD *et al.*, 2014b). Therefore, it is unclear whether there is a difference between PC and AC-2.5 KHz regarding fatigability and muscle isometric work performed during a NMES submaximal fatigue session.

According to Vaz and Frasson (2018), the evidences for fatigability between PC and AC are limited, and there is a risk of bias involving eligibility criteria, blinding, intention-to-treat analysis and reporting of key-outcomes among the available studies. These methodological criteria are very important for the evidence quality of the assessed outcomes (BALSHEM *et al.*, 2011), which may influence clinical decision making involving NMES (GRECO *et al.*, 2018).

Therefore, the purpose of this study is to fill the literature's methodological gaps by comparing the effects of AC-2.5 KHz and PC on knee extensor fatigability and discomfort during submaximal evoked contractions in healthy subjects. We hypothesize that fatigability will be higher for the AC-2.5 KHz compared to the PC fatigue protocol. In addition, AC-2.5 KHz will present a greater reduction in the evoked force and work generated between the beginning and the end of the fatigue protocol. AC-2.5 KHz will also show a greater decrease in MVIC after fatigue compared to PC. Additionally, the total work performed during the fatigue protocol with AC-2.5 KHz will be smaller than that performed by PC. Finally, the discomfort level generated during the submaximal fatigue protocol will be higher with AC-2.5 KHz compared to PC.

6.3 MATERIALS AND METHODS

This study was a blinded, randomized, crossover trial. The study was approved by the University's Research Ethics Committee (3.064.351), followed the CONSORT recommendations (DWAN *et al.*, 2019), and was recorded at the Clinical Trials (NCT03796117). Participants read and signed the informed consent form after they had all questions about the tests to be performed answered by the responsible researcher. 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).

6.3.1 Participants

Participants were recruited from the University and local communities. Inclusion criteria were determined as: young men, physically active and healthy, age between 18 and 35 years, normal knee function and range of motion, without complaints of pain or presence of pathology in the dominant lower limb. Participants were excluded in the presence of injury, cardiovascular, neurologic, acute musculoskeletal impairment (e.g., ligament or meniscal injuries) or presence of pain at the knee region at the testing time. Participants were asked to avoid stimulants (e.g., alcohol, caffeine, chocolate) and exercise two days before the testing days. Subjects self-reported their activity levels using the International Physical Activity Questionnaire - IPAQ (CRAIG *et al.*, 2003).

6.3.2 Sample Size

Sample size was determined a priori using G*Power (version 3.1.9.4, Universitat Kiel, Germany), with the significance level set at $p<0.05$ and power at $(1-\beta)$ 0.95 to detect an effect size for $f>0.42$. Data from a previous study (ALDAYEL *et al.*, 2010) was used to calculate the effect size for the dependent variables evoked torque and MVIC after the NMES protocol. Based on these a priori calculations, a sample of 22 individuals was defined as the minimum number of subjects for the trial. However, a previous study reported the exclusion of approximately 33% of the selected sample for eligibility assessment (MEDEIROS *et al.*, 2017). Therefore, additional eight subjects were recruited due to the possible drop-out from respondents who would fail to attend the follow-up session. Therefore, we recruited 30 participants for the study.

6.3.3 Procedures and Measurements

Participants were tested on 2 separate occasions, with a minimum of 7 days between sessions. In the first visit, subjects were familiarized with NMES and the testing equipment. In addition, initial assessment, anthropometric measurements and physical activity level were determined. After a 10-minute interval, MVIC tests were performed, after which the first randomized current was applied at the knee extensor muscles of the participants' dominant limb. Before the fatigue test, the intensity to evoke 20% MVIC was determined, which was followed by the fatigue protocol (see below). To avoid a carry-over effect, a 7-day interval between fatigue evaluations was observed. In addition, to assess the similarity at baseline, the MVIC was measured during the two testing sessions.

6.3.4 Randomization

A researcher, blinded to the study, performed the current types order randomization, using the http://randomization.com/ website. The randomization was organized in opaque and sealed envelopes by the same researcher, and therefore the therapist had access to the NMES current type to be used only on the evaluation day.

6.3.5 Blinding

Therapists, evaluators, analysts and participants were blinded. Data collection was performed by one therapist and one evaluator. The therapist was responsible for the NMES-parameters definition and application, whereas the evaluator was responsible for collecting the visual analog scale (VAS) and the evoked torque data. The therapist was positioned behind the dynamometer chair so that the stimulator parameters were not visible to the participant and evaluator. Additionally, an opaque cover was placed on the equipment during the application process to maintain the blinding procedure. The therapist was blinded to VAS and evoked torque data acquisition. The evaluator operated the data acquisition equipment, instructed the patient on the VAS use and obtained the self-perceived discomfort rating for each trial. Participants were blinded to the current type being used and to the muscle torque output. The data analyzers were blinded to the current type and participants by substitution of the participants' names by codes on the collected data files. In addition, the waveform order to be tested was randomly determined by a researcher blinded to the study.

6.3.6 Outcomes

The evaluated outcomes were fatigability and discomfort. Fatigability was analyzed by the change in MVIC from pre to post-fatigue, fatigue index analysis, contraction number to achieve 50% of force drop with respect to the first contraction, total TTI analysis and decline in TTI. Discomfort was evaluated throughout the fatigue protocol, as explained below.

6.3.7 Maximum Voluntary Isometric Contraction (MVIC)

MVIC tests were carried out on an isokinetic dynamometer (Biodex 3; Biodex, Shirley, New York). Participants were seated on the dynamometer chair with their hips flexed to 85° and knees flexed to 90° (full knee extension = 0°). Velcro straps were applied tightly across the thorax and pelvis, with the distal right leg fixed to the dynamometer lever arm. The dominant limb's lateral femoral condyle was used to determine the knee anatomical joint axis and was aligned to the dynamometer axis of rotation. Initially, warm-up trials were performed through 10 concentric submaximal contractions of the knee extensor and flexor muscles at an angular velocity of $90°.s^{-1}$. After a 2-min recovery period, participants performed three 5-second MVICs with the knee flexed at a 90° joint angle, and a 120-sec resting period was observed between contractions. Torque was then measured and recorded instantaneously, and MVIC was defined as the highest peak torque produced from the 3 maximum-effort trials. The submaximal intensity for the NMES fatigue protocol was determined relative to each participant's MVIC. The MVICs were performed before and after the fatigue protocol.

6.3.8 NMES Protocol

Participants were positioned on the isokinetic dynamometer for the fatigue tests as previously described. The fatigue protocol was performed with a Myomed 932® stimulator (Enraf-Nonius, The Netherlands), which was used to generate the two NMES waveforms: medium-frequency alternating current, defined commercially as Russian current, modulated in a duty cycle of 50% (AC50), and low-frequency pulsed current with 2 ms of pulse duration (PC1). The stimulator's physical parameters used in the two electrical current types (Table 10) were checked using a digital oscilloscope (model DSOX2014A, Keysight Technologies, Malaysia) prior to the experiment. These two NMES configurations were chosen because they were supposed to cause a higher pulse (PC1: 2.0 ms) and burst (AC50: 5ms) load, or a higher current charge. In addition, AC-2.5 KHz, with 50% duty cycle, is often used in clinical practice (WARD; ROBERTSON; IOANNOU, 2004), and has been used in previous studies comparing the two currents (LAUFER *et al.*, 2001; LAUFER; ELBOIM, 2008; VAZ *et al.*, 2012; FUKUDA *et al.*, 2013; LEIN JR; MYERS; BICKEL, 2015).

During the NMES fatigue protocol, a fixed current intensity was used for both tested currents. The current amplitude was set to produce an evoked torque of 20% of the participant's MVIC. The current intensity was maintained constant throughout the fatigue protocol to ensure that the changes in evoked force were exclusively a result from the NMES current type, which was the only changed parameter between testing days. The NMES current intensity to generate this submaximal evoked force level is generally well tolerated, and is similar to that of previous studies (MATKOWSKI;

LEPERS; MARTIN, 2015; GROSPRÊTRE *et al.*, 2017). The NMES fatigue test consisted of 80 evoked contractions, and lasted 20 min. During the tests, participants were instructed to leave their leg as relaxed as possible and to not perform any voluntary contraction during NMES application. Self-adhesive NMES electrodes measuring 8×13 cm (ValuTrode®, Axelgaard Mfg. Co., Ltd., Fallbrook, CA, USA) were used. Trichotomy at the electrode placement region was performed and the skin was cleansed with alcohol (MEDEIROS *et al.*, 2017). Electrodes were positioned as follows: proximally, over the quadriceps motor point (determined with a pen-shaped electrode as the skin point over the quadriceps muscle that evoked the strongest contraction), and distally 5 cm above the patella upper edge (MELO *et al.*, 2015).

Table 10- Neuromuscular electrical stimulation parameters for AC and PC.

	PC1	**AC50**
Pulse frequency (Hz)	100	NA
Pulse duration (ms)	2	0.4
Burst duration (ms)	NA	5
Carrier frequency (KHz)	NA	2.5
Burst frequency (Hz)	NA	100
Burst duty cycle	NA	50%
Stimulus ON time (rise time/fall time)	5 s (1/1)	5 s (1/1)
Stimulus OFF time	10 s	10 s
Waveform	SB - REC	SB – REC

NA: Not applicable; SB: Symmetric Biphasic; REC: Rectangular.

6.3.9 Evoked Torque Data Acquisition and Analysis

Evoked torque signals during the NMES fatigue-protocols were acquired using LabChart 8 software (PowerLab System; ADInstruments, Australia) at a 2-KHz analog-digital sampling frequency and saved to a computer disk. These signals were used to evaluate the peak torque decline and the torque-time integral (TTI).

6.3.10 Decline in MVIC

The decrease in MVIC values was determined using pre- and post-fatigue values. The absolute values before and after the fatigue protocol were analyzed to verify the within and between-currents' difference. The pre-to-post fatigue change in MVIC was considered as an index of global muscle fatigue (NEYROUD *et al.*, 2014a), which corresponds to the percentage reduction of MVIC.

6.3.11 Fatigue Index - Decline in evoked torque

Decline in NMES evoked torque represents failing to maintain a force level, and is a method used to asses fatigue (PAPAIORDANIDOU *et al.*, 2014). Therefore, in our study, NMES-induced fatigue was assessed by measuring the decline in NMES evoked torque throughout the 20-min fatigue protocol. In order that between-subjects comparison could be made, we expressed the peak torque produced during all 80 NMES evoked contractions for each test session as a percent decline relative to the peak torque produced during the initial NMES evoked contraction of each test. The evoked torque percent decline from the onset to the protocol end was evaluated through the fatigue index analysis, which was calculated from the ratio of the average peak torque elicited during the last five contractions to the average peak torque elicited during the first five contractions (ARPIN *et al.*, 2019).

6.3.12 Fatigability - Torque Drop to 50% of the Initial Evoked Torque

The evoked torque values from the fatigue tests were normalized to the first evoked contraction and plotted for each contraction per NMES current type. The number of contractions until the evoked force dropped to 50% of the initial contraction was determined between the different stimulation conditions (LAUFER; ELBOIM, 2008; LEIN JR; MYERS; BICKEL, 2015). When participants did not reach the level of 50% drop at the initial torque, the relative level of the evoked torque drop was determined and the number of 79 contractions after the initial evoked torque was used for analysis.

6.3.13 Torque-Time Integral (TTI)

Torque-Time integral (TTI) is an approach used to analyze the neuromuscular alterations following sustained isometric contractions, and reflects the isometric work (ROZAND *et al.*, 2015). TTI was defined as the integrated area under the torque-time curve (SAYENKO *et al.*, 2014). We evaluated the TTI for all 80 electrically evoked contractions using the torque curves from the recordings in the LabChart software (ADInstruments, Australia). The total TTI was considered the total amount of isometric work performed under each NMES fatigue protocol, and, as such, also represented a fatigue-related outcome. The total TTI for each condition was calculated by summing the individual TTIs from the two NMES fatigue protocols (NEYROUD *et al.*, 2019).

6.3.14 Decline in Torque-Time Integral (TTI)

A decline in the TTI during a NMES protocol has also been used as index of NMES-induced fatigue (NEYROUD *et al.*, 2014a). Therefore, we expressed the TTI of each NMES-induced contraction during the two testing sessions as a work percent decline relative to that induced at the initial contraction of each NMES fatigue test. TTI decline was calculated from the ratio of the average TTI obtained during the last five contractions to the average TTI elicited during the first five contractions.

6.3.15 Self-Reported Discomfort

Visual analog scale (VAS) was used to measure self-reported discomfort levels during each NMES fatigue protocol. EVA corresponds to a 10-cm horizontal line, in which 0 cm corresponds to "no discomfort" and 10 cm to the "maximum tolerated discomfort level” (DANTAS *et al.*, 2015). Participants were asked to draw their discomfort level by making a vertical tick mark on the line. We obtained self-reported discomfort levels by measuring the distance (in cm) from the line start to the mark made by the participant. EVA was evaluated during all fatigue protocol evoked contractions. For statistical analysis, the mean discomfort throughout the fatigue protocol was analyzed. In addition, to assess if there was a between-currents difference during the fatigue protocol, the mean discomfort level for every 5 consecutive evoked contractions was analyzed.

6.3.16 Statistical Analysis

Statistical methods were used to calculate mean and standard deviation (SD) for each parameter, and normality was checked using the Shapiro-Wilk test. Two-factor repeated measures ANOVA was used to compare MVIC force reduction between the different conditions (PC vs. AC-2.5 KHz) and time (pre vs. post), and to evaluate the current type effect on discomfort (current type vs. evoked contractions between the beginning and protocol end). When appropriate, Bonferroni post-hoc tests were used. To evaluate the fatigue index, percent decline in TTI, percent decline in MVIC and the number of evoked contractions to reach a 50% drop in evoked torque from the initial evoked contraction, paired t-tests were used. All analyses were carried out using SPSS software version 21 (IBM Corp., 2012). Significance level was set at $p<0.05$ for all procedures. Additionally, effect size was calculated using Cohen's Equation (COHEN, 1988). Effect sizes (*d*) were categorized as trivial (<0.20), small (0.20-0.49), moderate (0.50-0.79), large (0.80-1.29), and very large (>1.30) effect (ROSENTHAL, 1996).

6.4 RESULTS

Thirty healthy and physically active men [aged (mean±SD) 23.23±4.59 years; weight 74.90±9.16 kg; height 177.53±5.72 cm; BMI±23.75±2.63] participated in the study. According to the IPAQ's (CRAIG *et al.*, 2003) metabolic equivalents (METs), 16 participants were classified at the high and 14 participants at the moderate physical activity levels. All 30 individuals were assessed for eligibility and received the interventions. There were no losses or exclusions and all data were analyzed for the 30 participants (Figure 15).

6.4.1 Similarity at Baseline

To ensure the participants similarity during the protocol, MVIC was evaluated in all sessions. The comparisons between PC1 (276.17±65.15 Nm) and, AC50 (273.00±58.98 Nm) showed no significant between-days difference (p=0.342).

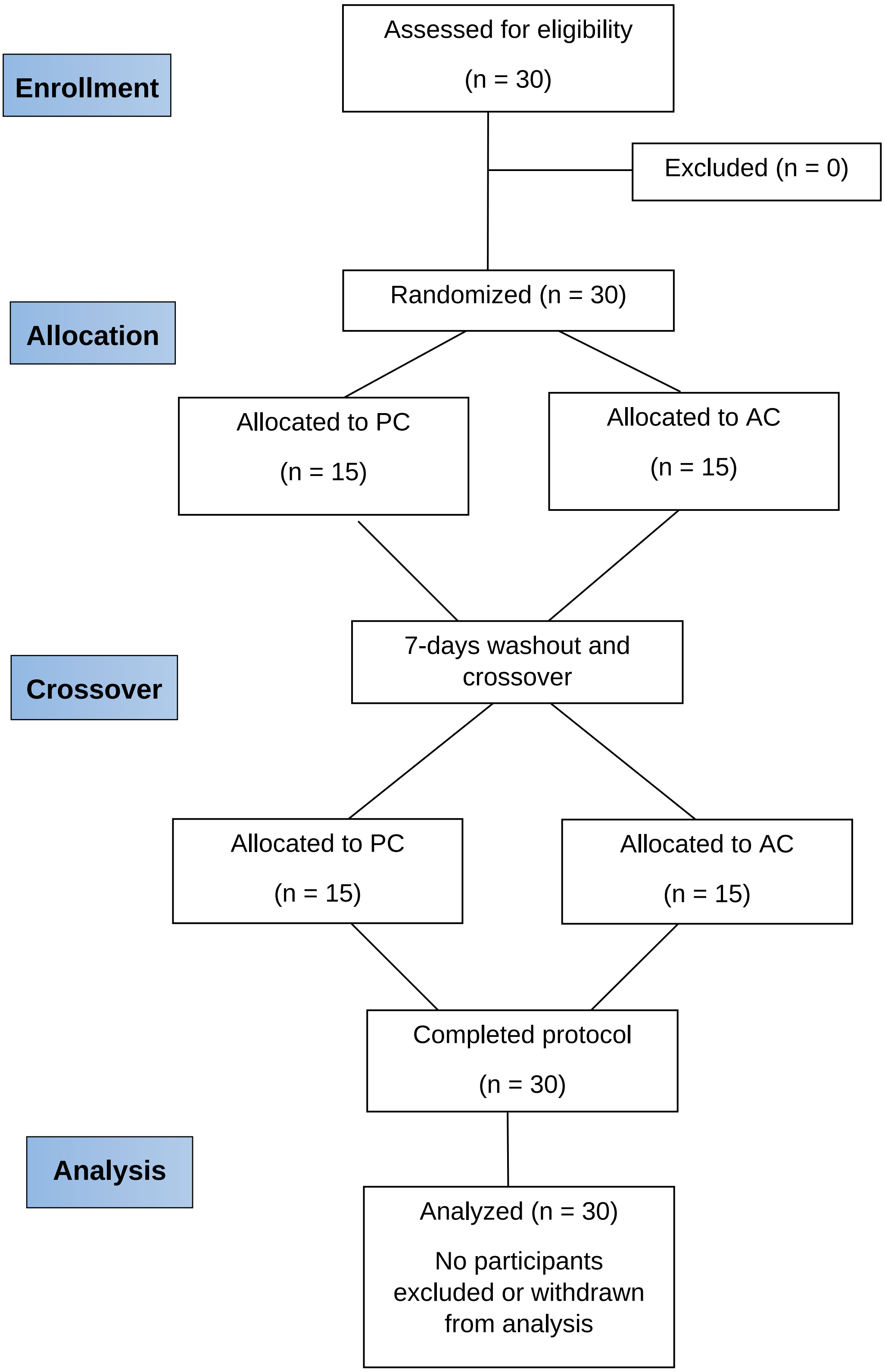

Figure 15. Flowchart of the blinded randomized crossover trial.

6.4.2 MVIC Decline

MVIC significantly decreased in PC1 and AC50 after the NMES sessions (Figure 16). ANOVA showed a significant time effect [(F1,29) = 127.26; p<0.0001] and current effect [(F1,29) = 5.2221; p=0.030], as well as a significant interaction effect between current and time factors [(F1,29) = 10.498; p=0.003]. The Bonferroni post hoc test revealed a statistically significant difference between AC50 and PC1 in the post-fatigue time (p=0.006), but similarity for the MVIC torque in the pre-fatigue time (p=0.342), demonstrating the between testing days similarity at baseline. Percent decline analysis demonstrated a larger MVIC reduction (T29: 3.367; p=0.002) for AC50 (-19.16±8.0%) compared to PC1 (-15.50±6.03%).

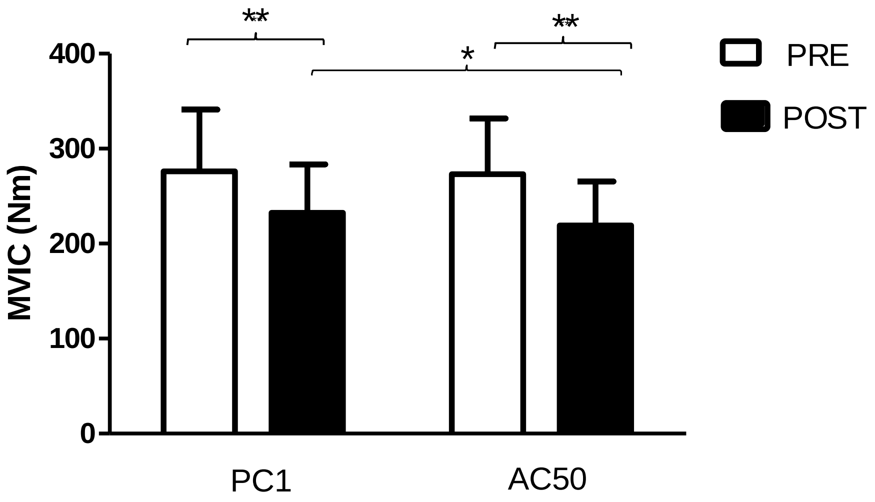

Figure 16- MVIC before (pre) and after (post) the two NMES fatigue sessions. *p=0.006; **p<0.0001 (Bonferroni test).

6.4.3 Fatigue Index - Evoked Torque

The PC1 protocol resulted in a lower fatigue index, as shown by the smaller percent decline in the evoked torque compared to AC50 (figure 17). This between-currents difference was significant (T_{29}: -2.498, p=0.018), with a moderate effect size (Table 11).

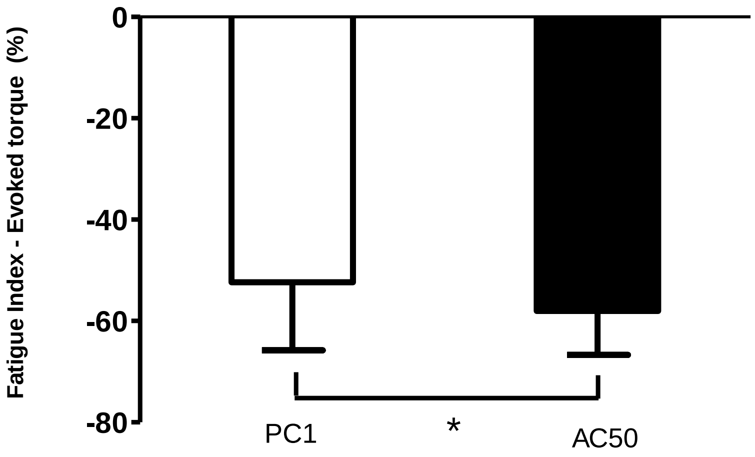

Figure 17 - Fatigue Index related to the decline in evoked torque from the first 5 to the last 5 contractions of the fatigue trial for the two NMES currents. * p=0.018.

6.4.4 Fatigability - Torque Drop to 50% of the Initial Evoked Torque

A significant difference (T_{29}: 3888, p=0.001) between PC1 and AC50, with a moderate effect size (Table 11), was observed for the average number of contractions for the evoked torque to drop to 50% of the torque evoked by the first contraction. AC50 demonstrated a faster evoked torque reduction (22.53±10.71 contraction) compared to PC1 (32.83±17.84 contraction). As shown in Figure 18, one participant did not reach a 50% drop in the evoked torque by PC1 (his maximum decrease in the evoked torque was 39%). However, excluding him from the analysis did not change the results. Considering that we developed a study with intention-to-treat analysis, we considered appropriate to keep him in the statistical analysis. Therefore, for this participant we considered the maximum number of performed contractions (80 contractions) for the analysis of the 50% evoked force drop.

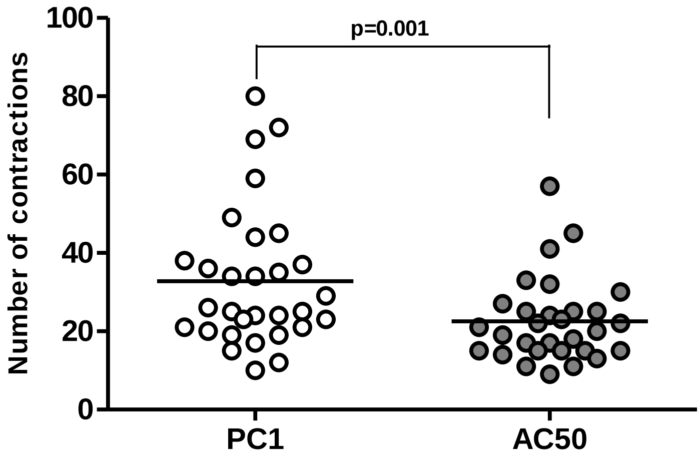

Figure 18 - Number of contractions for the evoked torque to drop to 50% of the initial evoked torque. Circles indicate individual data; black line indicate mean values.

6.4.5 Total Torque-Time Integral (TTI)

The total TTI for each NMES protocol was calculated by summing the individual TTI from each evoked contraction (Figure 19). PC1 produced more isometric work compared to AC50 (T_{29}: 5769, p<0000.1), with a large effect size (Table 11).

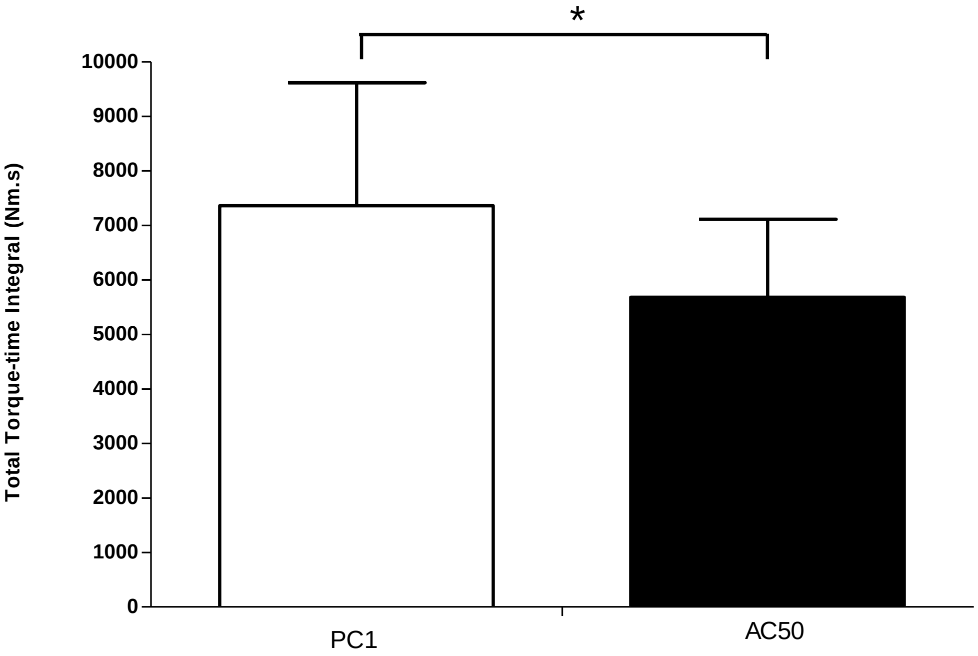

Figure 19 - Total Torque-time Integral (TTI) for PC1 and AC50. * p<0000.1.

6.4.5 Percent Decline in TTI

PC1 led to a lower TTI percent decline (-61.9±10.1%) compared to AC50 (-67±9.5%) (T_{29}: -3.097, p=0.004). This difference between PC1 and AC50 revealed a moderate effect size (Table 11).

6.4.6 Discomfort

Mean discomfort from the VAS scores was similar (T_{29}: p=.805) between PC1 and AC50 throughout the NMES fatigue protocols (PC1-VAS: 2.73±2.3; AC50-VAS: 2.63±2.3). Similar results were observed at specific points from each fatigue protocol (mean value for each group of 5 consecutive evoked contractions). ANOVA indicated no current effect ($F_{1.0,\ 29.00}$: 0.994; p=0.792) and contraction effect ($F_{1.64,\ 47.68}$: 0.071; p=0.792) on the perceived discomfort, and no between-NMES interaction effect was observed ($F_{4.07,118,.02}$:0.288; p=0.888) (Figure 20).

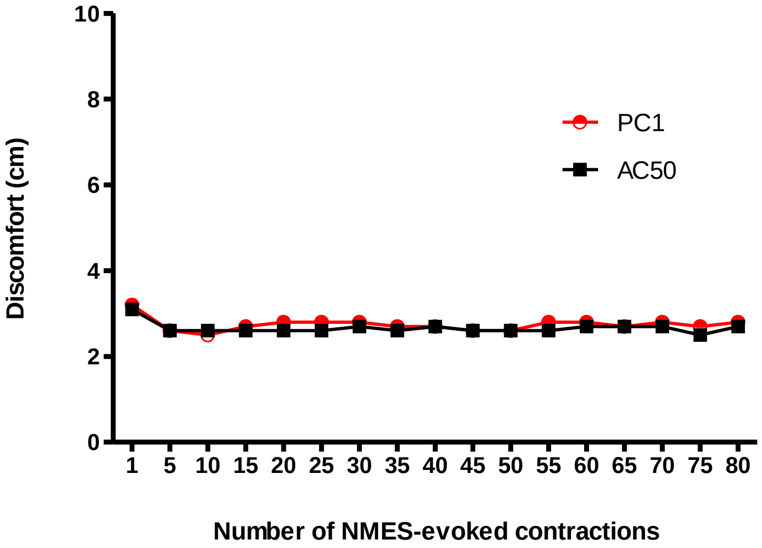

Figure 20 - Discomfort analysis during the NMES fatigue protocols. Standard error bars are not displayed for clarity.

Table 11- Effect size analysis.

	PC1 vs. AC50	**Classification**
Percent decline- MVIC	0.52	Moderate
Fatigue Index – evoked torque	0.50	Moderate
Total-TTI	**0.89**	Large
Percent decline – TTI	0.52	Moderate
Drop of 50% - evoked torque	0.70	Moderate
Discomfort	0.04	Trivial

MVIC: Maximum Voluntary Isometric Contraction; PC1: Pulsed Current 1000; TTI: Total torque-time Integral.

6.5 DISCUSSION

The purpose of this study was to compare the effects of AC50 and PC1 on fatigability and discomfort during a NMES fatigue protocol in healthy subjects. Our results partially confirmed our hypotheses. AC50 was more fatiguing than PC1, since it demonstrated a greater MVIC percent decline, and a greater fatigue index compared to PC1. Additionally, a lower number of contractions for the evoked torque to drop to 50% of the initial evoked torque was observed for AC50 compared to PC1, with a higher TTI relative decline. A smaller total TTI was observed with AC50 compared PC1. Therefore, our study findings indicate that PC1 was significantly less fatigable and more efficient in the knee extensors' mechanical loading when compared to AC50. However, for the discomfort level, both currents presented similar results, therefore not supporting our initial hypothesis that AC50 should lead to a higher discomfort compared to PC1.

Few studies have evaluated the fatigability between PC and AC. Additionally, previous studies used a variety of methodologies to evaluated fatigability, such as decline in evoked torque, number of contractions for the evoked torque to drop to 50% of the torque initial, area under the evoked torque time analysis, and decline in MVIC force. However, none of them evaluated all these outcomes in a single study.

Although MVIC is considered the "gold standard" to detect muscle fatigue (VØLLESTAD, 1997), we found only two studies (ALDAYEL *et al.*, 2010; ALDAYEL *et al.*, 2011) evaluating the declines in MVIC after a fatigue protocol using PC and AC-2.5KHz. Declines in MVIC were reported to be 24±8.3% (ALDAYEL *et al.*, 2011) and 26.1±2.8% (ALDAYEL *et al.*, 2010) for PC, compared to 20.8±5.1% (ALDAYEL *et al.*,

2011) and 23.1±4.2% (ALDAYEL *et al.*, 2010) for AC, with no between-currents differences being observed.

Contrary to the abovementioned studies, our results demonstrated a higher (p=0.002) decrease of MVIC for AC50 (19.16±8.0%) compared to PC1 (15.50±6.03%), with a moderate (0.52) effect size. The differences between our findings and these studies' findings may be attributed to methodological differences, such as the NMES parameters and current intensity used. In both Aldayel *et al's* studies, NMES intensity was adjusted throughout the protocol to the maximum tolerated intensity, as opposed to ours where current intensity was set to a specific submaximal level (20% MVIC). In addition, these studies used 15s off-times and fatigue protocols with 30 (ALDAYEL *et al.*, 2011) and 45 contractions (ALDAYEL *et al.*, 2010), as opposed to our shorter off-time and longer fatigue protocol (10s off and 80 contraction).

Additionally, considering that we used a frequency of 100 Hz, our protocol with AC50 delivered more burst frequency than the 75 Hz burst frequency in these previous studies (ALDAYEL *et al.*, 2010; ALDAYEL *et al.*, 2011). It is possible that the larger number of pulses within the burst, added to the largest number of contractions in our protocol, has led to a greater fatigue by AC50, since a higher number of pulses results in repetitive activation of motoneurons, and leads to a decrease in their excitability or response to excitatory synaptic input, which contributes to the higher force loss (TAYLOR *et al.*, 2016; WAN *et al.*, 2017).

Moreover, the longer pulse duration used with PC1 in our study (2 ms) may have led to a central mechanism of motor unit (MU) recruitment (COLLINS; BURKE; GANDEVIA, 2002). In this central mechanism, sensory axons' depolarization leads to MU recruitment via reflex pathways (COLLINS; BURKE; GANDEVIA, 2001; COLLINS; BURKE; GANDEVIA, 2002; BERGQUIST *et al.*, 2011). This MU recruitment would be done through Henneman's size principle (i.e., from smaller fatigue resistant to larger fast fatigable MUs), which might have reduced fatigability in the PC1 case (BARSS *et al.*, 2018). However, as no central mechanism was directly evaluated during our study, it is not possible to determine if this mechanism in fact occurred during the PC1 fatigue protocol.

The drop in the evoked torque from the start to the end of the fatigue protocol (i.e., the fatigue index) was higher with AC (58±8.7%) than with PC1 (52.4±13.4%). This higher fatigability with AC compared to PC was partly similar to what was observed in previous findings (LEIN JR; MYERS; BICKEL, 2015). Lein *et al.* (2015) also performed a fatigue protocol at a submaximal level (30% MVIC). The authors compared two PCs (stimulation frequencies = 20 Hz and 50 Hz, pulse duration = 500µs) and two AC-2.5KHz (burst frequencies = 20 Hz and 50 Hz, pulse duration = 400µs, 50% duty cycle). The evoked force reduction from the beginning to the end of the protocol was smaller with PC-20 Hz (45±5%) compared to PC-50Hz (65±5%), AC-20 Hz (67±4%) and AC-50Hz (75±3%). However, there were no significant differences between PC-50Hz and the two ACs. The frequencies of 20 Hz and 50 Hz, used in this study, are in distinct portions of the knee extensors force-frequency relationship (LEE; RUSS; BINDER-MACLEOD, 2009), which means that the two frequencies imposed different energy demands per contraction. Stimulation with higher frequencies requires higher energy costs, and may result in greater muscle fatigue compared to lower stimulation frequencies (GREGORY; DIXON; BICKEL, 2007). This possibly explains the lower fatigue induced by PC-20 Hz compared to the other currents. However, this study also used AC-20 Hz, which was more fatiguing than PC-20 Hz. Although the authors did not find a significant difference between PC-50 Hz and the AC currents, the effect size analysis showed a very large difference (2.42) between PC-50Hz and AC-50Hz.These results suggest that the currents' behavior is different and, possibly, is related to the larger number of pulses per burst in AC-2.5 KHz, which could lead to an undesirable increase in the rate of fatigue (VAZ; FRASSON, 2018).

Although NMES sessions using PC and AC induce fatigue by both peripheral and central mechanisms (DE OLIVEIRA *et al.*, 2018), it has been reported that AC protocols demonstrated the presence of "high frequency fatigue" (LAUFER; ELBOIM, 2008). High frequency fatigue has been related to action potential propagation failure along the muscle fibers. Additionally, it is characterized by an extensive force decrease at high stimulation frequencies, and a rapid recovery when stimulation is reduced. Although high frequency fatigue can produce considerable force reduction, it is unclear whether this is a normal mechanism of fatigue (JONES, 1996).

Therefore, one might ask if there is any benefit to train a muscle with a current that leads to this fatigue-type (LAUFER; ELBOIM, 2008), as muscle evoked contractions by AC are not as strong and stable as those produced during PC stimulation (ALDAYEL *et al.*, 2011). In addition, during the NMES protocol, the evoked force decrease (i.e., the fatigability) occurred faster with AC than with PC stimulation (LAUFER *et al.*, 2001).

These statements are supported by our results, since AC-stimulation demonstrated less resistance to fatigue compared to PC1. The 50% drop in the evoked force from the protocol start occurred faster with AC50 (mean contraction number = 22.53±10.71) than with PC1 (32.83±17.84). Similar results were observed by Lein *et al.* (2015), who also observed a smaller number of contractions to reach a 50% reduction in the evoked torque with AC compared to PC. For AC-20 Hz and AC-50 Hz, this reduction was observed at contractions 16 (±2.5) and 13 (±1.7), respectively, while for PC-20 Hz and PC-50 Hz, this torque reduction was observed at contractions 63 (±6.4) and 29 (±3.6), respectively.

Similar results were observed when the protocol was generated at the maximum tolerated intensity. Laufer and Elboim (2008) demonstrated that the number of contractions before the evoked torque decreased 50% from the initial value was smaller for AC-2.5 KHz with a duty cycle of 50% (3.5±1.5) compared to PC (7.2±4.2), both modulate in 50 Hz and with a pulse phase duration of 200 μs.

Consistent with these findings, a faster torque decay was reported to occur following AC stimulation. Lower measurements were obtained for the area under the AC evoked torque curve compared to PC (LAUFER *et al.*, 2001). This evoked torque-time curve area, also defined as the torque-time integral (TTI), reflects the work of an isometric contraction (ROZAND *et al.*, 2015), and has been used as a surrogate for the recruited MUs fatigue (NEYROUD *et al.*, 2014a).

Successive stimulations with PC resulted in a gradual decline in the TTI values, whereas AC stimulation demonstrated a dramatic decrease and greater fluctuations in the TTI compared to PC (LAUFER; ELBOIM, 2008). In agreement with these results, we observed higher TTI reduction for AC compared to PC. Considering that there is a relationship between TTI and the work performed by the contracting MUs (CELICHOWSKI; GROTTEL; BICHLER, 1998), our results suggest a reduced MU mechanical output with AC compared to PC.

The main goal of a rehabilitation program is to generate the highest mechanical load with the smallest fatigability, as this may lead to larger neuromuscular adaptations. This decrease in TTI implied that the total work generated during the AC protocol was smaller compared to that of PC, suggesting a higher fatigue level of the recruited MUs with AC. Considering that during a NMES protocol the optimal stimulation is obtained when the maximal area of the evoked contraction is reached (GOBBO *et al.*, 2011), our results show that PC1 induced a significantly greater workload than AC50, which was further supported by the large effect size (Table 11).

When analyzing the NMES settings for PC1 and AC50, one could argue that PC1 was favored by the large pulse duration, as wide-pulse and high-frequency NMES can induce higher total TTI for the PC stimulation responders (WEGRZYK *et al.*, 2015). However, other studies that balanced pulse duration (400 us) between currents (LAUFER *et al.*, 2001), or that used smaller pulse duration for PC (250 us) compared to AC (400 us) (LYONS *et al.*, 2005), also agreed with our results, demonstrating greater total TTI with PC than with AC.

Lyons *et al.* (2005) evaluated TTI in contractions evoked by PC (75 Hz, PD = 250 μs) and AC-2.5 KHz (75 Hz, PD = 400 μs) at the maximum tolerated intensity. PC produced a higher torque integral value (988.6±330.4 Nm.s) compared with that of AC (822.7±292.6 Nm.s). Similar results are demonstrated by Laufer *et al*. (2001) using PC (MP-monophasic and BP- biphasic) and AC-2.5 KHz, both similarly configured (stimulation frequency of 50 Hz, and phase duration of 200 μs). MP and BP PCs induced greater TTI (5,631.16±2,824.2 and 5,572.66±2,465.0, respectively) as compared with the AC-2.5 KHz (2,362.46±1,227.5).

Therefore, these findings suggest that AC-stimulation inhibits the muscle ability to sustain contractile force. These results are clinically relevant, because the greater the fatigability, the lower the stimulated tissues' overload, and, therefore, the lower are the NMES-induced adaptations (VAZ; FRASSON, 2018).

Despite AC and PC currents have shown differences in their fatigability levels, they appear to induce a similar discomfort level. Assessments of the discomfort level during a submaximal fatigue protocol with PC and AC-2.5 KHz are scarce. However, our results are similar to the little existing literature involving discomfort during long protocols (≥10 contractions). PC and AC demonstrated modest and similar discomfort levels during and/or after electrically elicited contractions (LYONS *et al.*, 2005; LAUFER; ELBOIM, 2008). These findings are important, since the discomfort level influences the ability to tolerate the NMES adequate stimulus to produce the desired training effect (LYONS *et al.*, 2005), and, in our case, discomfort did not affect our evoked force fatigue results.

In summary, our results reinforce the evidence in support of the use of PC when the goal is to produce a high muscle work intensity with high resistance to fatigue during a NMES training and/or a rehabilitation protocol.

Limitations

Among the study's limitations, the selected sample, which was composed only by young, active and healthy men, limits the inference of the obtained results for other populations. However, this experimental design was chosen because it allows for a greater control of the involved variables. In addition, the absence of electromyographic parameters and twitch interpolation technique, which could provide information about central and peripheral fatigue involving PC and AC can also be considered a limitation of our study, and should be evaluated in future studies. Additionally, the between-currents difference in pulse duration, which suggests a different energy charge, can also be considered a limitation of our study. PC1 was set to 2 ms pulse while AC50 had a relatively short pulse duration of 0.4 ms. However, pulse duration of the AC cannot be changed because it is inherent to its 2.5-KHz carrier frequency.

Clinical applicability

Clinically, fatigue studies are important because they assist clinicians in decision making when choosing the best NMES protocols for therapeutic interventions. Our results demonstrate that if the training/rehabilitation goal is to generate a high muscle work intensity with the smallest fatigability, PC with 2.0 ms of pulse duration is more indicated than AC-2.5 KHz. Since smaller fatigability allows for a longer NMES protocol time and for a greater mechanical overload, greater soft tissues (i.e. muscle, tendon) adaptation might be obtained with the ideal current for rehabilitation or training protocols, in our case, PC.

6.6 CONCLUSION

AC-2.5 KHz produced a smaller TTI, higher fatigue index, higher relative decline in TTI, and higher MVIC force loss compared to PC. The discomfort level during the AC and PC fatigue protocols was similar. PC is more efficient than AC-2.5 KHz, as it generates higher mechanical load with less fatigue.

OTHER INFORMATION

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7 GENERAL DISCUSSION

Our research was designed to compare the effects between PC and AC on the evoked torque, current intensity, neuromuscular efficiency, fatigability and discomfort level. Therefore, we hypothesized that AC would evoke lower torque, would need higher intensity, would generate less neuromuscular efficiency, would be less fatigue-resistant and would produce more discomfort compared to PC. These hypotheses were partially proven throughout the three studies developed in this dissertation.

In chapter 1 we observed that the responses between PC and AC depended of the used carrier frequency, since it influences the duration and number of pulses within the burst in AC NMES. Therefore, AC with lower carrier frequency (1.0 KHz) showed similar results to PC, for evoked torque and discomfort level. Additionally, AC with higher carrier frequency (4.0/4.05 KHz) was less efficient than PC for torque production. Regarding AC-2.5 KHz, the meta-analysis results demonstrated that it was able to evoke less torque and generate similar discomfort than PC. However, the subgroups analyses showed less or similar torque production and discomfort with AC-2.5 KHz compared to PC.

These results' differences are possibly influenced by the different protocols, variability and poor methodological quality between studies involving AC-2.5 KHz and PC. Therefore, we developed two high quality methodological randomized trials involving mainly blinding criteria, which can influence the results, and we evaluated the effects of AC-2.5 KHz and PC on neuromuscular and clinical parameters.

Thus, regarding evoked torque by PC and AC-2.5 KHz, Chapter 2 results agree with the results of the meta-analysis and subgroups analysis (maximum tolerable intensity and motor point) of Chapter 1, that demonstrated that AC-2.5 KHz is able to evoke less torque than PC. Additionally, regarding the discomfort level generated by PC and AC-2.5 KHz, Chapter 2 results agree with the meta-analysis and subgroups analysis results (submaximal intensity, motor point and pulse duration) that demonstrated similar discomfort between AC-2.5 KHz and PC in Chapter 1. But it disagrees with the subgroup analysis (maximum tolerated intensity), which shows that AC is less uncomfortable than PC.

Therefore, Chapter 2 results showed that, although the NMES currents produced similar discomfort, PC was capable of evoking greater torque at maximum tolerated intensities. Thus, our experimental study proves our hypothesis that AC is capable of evoking lower torque compared to PC. But it does not confirm the hypothesis that AC would be capable of generating greater discomfort at both submaximal and maximal intensity levels.

Additionally, in Chapter 2, PC with longer pulse duration (PC1 - 2ms) required lower intensity at submaximal and maximum force levels, and produced greater neuromuscular efficiency at both intensity levels compared to AC-2.5 KHz. Therefore, our initial hypothesis that PC needs lower current intensity at submaximal and maximum strength levels and would be more efficient than AC-2.5 KHz was confirmed.

Regarding fatigue, in Chapter 1 results, PC showed a greater decrease in MVIC after the fatigue protocol compared AC-2.5 KHz. On the other hand, in Chapter 3, AC-2.5 KHz demonstrated a greater decrease in MVIC after the fatigue protocol, compared to PC. These results' differences can be explained by the fatigue protocol and NMES parameters differences used in our study and those included in Chapter 1's meta-analysis. We performed an isometric fatigue protocol at a fixed submaximal intensity and used PC with long pulse duration and high frequency, as opposed to Chapter 1's studies, which performed fatigue protocols at the maximum tolerated intensity that was adjusted throughout the protocol, and shorter pulse duration and stimulation frequency compared to our study.

Additionally, in Chapter 3, we evaluated evoked torque reduction analysis (fatigue index), 50% force drop regarding the first contraction, total work produced (total torque-time integral), and work reduction from the beginning to the end of the fatigue protocol between PC and AC. AC-2.5 KHz demonstrated higher fatigue index to evoked torque, faster force drop at the 50% force with respect to the initial contraction, smaller total work and higher percentage work decline compared to PC. Therefore, our initial hypothesis was confirmed, as AC-2.5 KHz demonstrated less fatigue resistance compared to PC.

8 CLINICAL APPLICABILITY

Our results provide information that can assist clinicians in decision making involving NMES protocols. When aiming to perform a protocol with greater fatigue-resistance and to induce greater strength gains, PC is more indicated than AC-2.5 KHz. Additionally, as some people do not tolerate high current intensity during NMES training, PC with larger pulse duration can be a resource used in these situations, as it requires lower current intensity, is more efficient and more fatigue-resistant at submaximal NMES current intensity levels compared to AC-2.5 KHz. Therefore, PC seems to be an excellent tool for strength training or rehabilitation protocols.

CONCLUSION

PC is able of evoking higher torque than AC-2.5 KHz. PC with large pulse duration needs less current intensity and is more efficient in both submaximal and maximal strength levels, and it is more fatigue-resistant compared to AC-2.5 KHz. Regarding the discomfort level, the two NMES currents are similar.

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