Study Protocol

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Skeletal muscle atrophy and dysfunction following total knee arthroplasty	3-23-22

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PROTOCOL SUMMARY

Knee osteoarthritis (OA) is the leading cause of disability in older adults. Because non-surgical therapies are inadequate in managing symptoms and do not alter the disease course, total knee arthroplasty (TKA) is an important final treatment option. While TKA alleviates joint pain, objectively-measured physical function does not improve much beyond pre-surgical levels. Moreover, it often takes 1-2 years to recover to function to pre-surgical levels. TKA fails to correct disability, in part, because of the precipitous decline in lower-extremity neuromuscular function in the early, post-surgical period, forcing patients to first overcome the disabling effects of the procedure before redressing pre-surgery functional deficits. Surprisingly, very little is known about the nature of muscle structural and functional adaptations during the early, post-surgical period, aside from a small number of studies at the whole muscle level suggesting decreased neural activation as the major cause of dysfunction. To address these gaps in scientific knowledge and clinical treatment options early following TKA, our goals in this project are to: 1) to comprehensively examine, from the molecular to the whole body level, skeletal muscle structural and functional adaptations following TKA and 2) to assess the utility of neuromuscular electrical stimulation (NMES) to counter early, post-surgical muscle adaptations Based on our preliminary data, we hypothesize that TKA causes profound reductions in skeletal muscle size and intrinsic function during the early, post-surgical period that contribute to long-term functional disability and that these adaptations can largely be prevented by NMES. To accomplish our goals, we will study patients with advanced-stage knee OA prior to and 5 following surgery who are randomized to receive NMES or no intervention during this period. All volunteers will receive standard of care physical rehabilitation during this period. Tissue acquired from skeletal muscle biopsies will be used to characterize skeletal muscle myofilament and mitochondrial structure and function, in combination with assessments of whole muscle and body functional capacity. If successful, our results could shift conventional thinking in this field away from the role of neural adaptations as a cause of post-surgical disability and emphasize the importance of muscle atrophy and intrinsic myofilament function. Additionally, our data on the utility of NMES to prevent such adaptations may provide important mechanistic data to support this modality as a new transitional rehabilitative care intervention to improve long-term functional prognosis in TKA recipients.

PURPOSE AND OBJECTIVES

Knee OA is the leading cause of disability in older adults (1). Because non-surgical therapies are inadequate in managing symptoms (2) and do not alter the disease course, TKA is an important treatment option for knee OA. It is currently the most common elective surgery in the US (3) and its use will rise dramatically (~5-fold) in the future, to ~3.5 million surgeries/yr by 2030 (4) with the expansion of the elderly population. While TKA improves patient-reported function by alleviating joint pain (5), objectively-measured physical function does not improve much beyond pre-surgical levels, which is already severely impaired (6-8), and does not reach healthy control levels even years after surgery (8-11). The inability of TKA to correct functional deficits consigns a large number of older adults to continued disability and greater risk for progression to severe disability (12-14).

TKA fails to correct disability, in part, because of the precipitous decline in lower-extremity neuromuscular function in the early, post-surgical period (8, 15, 16), forcing patients to first overcome the disabling effects of the procedure before redressing pre-surgery functional deficits (6-8). Surprisingly, very little is known about the nature of muscle structural and functional adaptations during the early, post-surgical period, aside from a small number of studies at the whole muscle level (15, 16). Although informative, these studies are unable to define the mechanisms underlying reduced function because of the imprecision of measurements at this anatomic level and the multitude of physiological systems and subjective factors that impact whole muscle function. While the need for early intervention post-TKA to forestall its disabling effects has been consistently recognized (17, 18), there are no widely-accepted, evidence-based interventions that target this early functional decline. We believe that lack of knowledge of the determinants of TKA-induced functional decline and therapeutic interventions to prevent or correct these maladaptations represent critical barriers to more effectively recover physical function in TKA recipients.

The proposed studies address these barriers and their successful completion would advance knowledge of early, post-TKA skeletal muscle adaptations at the cellular, organellar and molecular levels, providing a mechanistic evidence base to inform the development of preventive and rehabilitative interventions. As these muscle deficits contribute to long-term disability in TKA recipients (15, 19, 20), beneficial effects of NMES would highlight its potential as a transitional intervention to bridge a gap in rehabilitative care during the early, post-surgical period. Moreover, as NMES is inexpensive, FDA approved, portable to the clinic and home settings and there are clinicians trained in its use (PTs/OTs), financial, logistical and regulatory barriers for its broader application would be minimal. Finally, beneficial effects of NMES may argue for its broader application to other orthopedic surgical populations that experience post-surgical functional morbidities (21, 22), as well as other older adult populations that suffer muscle atrophy and dysfunction during acute disabling events (14, 23).

Background and Hypothesis for Aim 1

Effects of TKA on skeletal muscle structure and function. Physical function declines rapidly and profoundly in the early, post-surgical period following TKA (8, 24). One determinant of the TKA-related reduction in physical function is lower extremity muscle weakness (15, 16, 25). Data from our labs and others show that reductions are most prominent in the quadriceps, where strength declines >50% early, post-surgery (15, 25-27). Conventional wisdom holds that deficient neural activation is the primary factor driving muscle weakness, with a much smaller contribution from intrinsic muscle adaptations, such as atrophy (15). However, studies at the whole muscle level are insufficient to define muscle-specific adaptations because of methodological limitations (eg, whole muscle imaging techniques underestimate muscle atrophy compared to single fiber assessments) and confounding factors (eg, impaired neural activation and antagonist muscle co-contraction impair whole muscle strength measures). Regarding the latter, using single muscle fibers, we have shown, for the first time, profound (~30-50%) deficits in contractility early following TKA at the cellular level, including reductions in tension (ie, force/CSA) and velocity in MHC IIA and IIAX fibers. Such contractile deficits likely contribute to whole muscle/body functional deficits, as MHC II fibers are important determinants of dynamic muscle function (28).

Muscle size and function are intimately related to cellular energy metabolism (29, 30). Alterations in mitochondrial content, structure and function that alter protein anabolism/catabolism (31, 32) and/or production of metabolites, such as reactive oxygen species (ROS), have been shown to promote atrophy and contractile dysfunction (33-36). Stimuli present in the perioperative and early, post-surgical period, such as inflammation, hypoxia and muscle disuse, can provoke mitochondrial loss and dysfunction and, in turn, muscle atrophy and weakness (35, 37, 38). Thus, mitochondria may serve to both sense these stimuli and transduce their signal throughout the cell. In this context, the early, post-TKA period is characterized by detrimental mitochondrial adaptations that may not only impair energy provision to support physical function (39, 40), but may contribute to muscle atrophy and dysfunction (33, 38, 41). Collectively, available literature and our preliminary data in TKA recipients suggest that it is reasonable to hypothesize that: *TKA will reduce single skeletal muscle fiber size and contractility and mitochondrial content and function, while increasing mitochondrial ROS generation*.

Background and Hypothesis for Aim 2

Effects of NMES on skeletal muscle structure and function following TKA. During the early, post-surgical period, rehabilitation of TKA recipients is impeded by pain, healing of the surgical site and limited range of motion. Because of these impediments, more anabolic/strengthening exercises are not started until several weeks post-surgery. NMES has been forwarded as an adjunct to classical resistive-type exercises begun at this later date to overcome neural activation deficits to allow activation of a greater quantity of muscle mass (42). A recent trial, which has been the largest to date using NMES in TKA recipients, showed that NMES as an adjunct to resistance training, with both *starting at 4 weeks post-surgery*, had no additional benefit over resistance training alone (43), a result confirmed in other orthopedic surgical populations (44). In stark contrast to these results, *use of NMES alone early, post-TKA* improves physical function (45). In fact, work from our labs showed that NMES begun 48 hrs post-surgery and maintained for 6 wks reduced quadriceps strength loss and lessened reductions in physical function (46). The timing of the start of the NMES intervention is critical because, although we acknowledge that use of NMES in orthopedic populations is not new, its use early post-surgery is. More specifically, NMES use early post-surgery to counter skeletal muscle atrophy and intrinsic dysfunction

may offer a promising transitional intervention to bridge the gap in rehabilitation care and improve long-term muscle functionality.

For NMES to improve disability in TKA recipients, it should address the mechanisms underlying persistent functional deficits. While impaired neural activation is widely believed to drive early, post-surgical reductions in muscle strength (15), these deficits regress rapidly, with near complete correction within 3-6 months (46). In contrast, intrinsic muscle adaptations are thought to remediate more slowly, contributing disproportionately to long-term functional deficits (15, 19, 20). Thus, while NMES may improve early functional deficits due to impaired neural activation (47, 48), as our data show (46), to impact long-term disability it needs to prevent atrophy and intrinsic contractile dysfunction during the early, post-surgical period. To this end, our preliminary data provide the first direct evidence of effects of NMES on muscle fiber size and function in TKA recipients. We found that early use of NMES diminishes fiber atrophy in MHC I and II fibers and improves myofilament protein function in MHC II fibers, with a surprising improvement in contractile velocity (+23%) after 5 wks. The combined effect of improved tension and velocity may mitigate reductions in whole muscle power output (power= force X velocity) that predispose to disability (49). These effects of NMES on single muscle fiber size and MHC II fiber function, particularly improvements in contractile velocity, which are increasingly thought to be at the root of deficits in muscle power that promote disability (50), may ameliorate the precipitous decline in lower extremity function in the early post-surgical period following TKA.

How does NMES preserve muscle size and function? As NMES maintains neuromuscular activation in the face of post-operative muscle disuse, it likely functions similar to standard exercise, which maintains myocellular size and function during catabolic conditions (51, 52). Several pathways stimulated by exercise influence muscle size/function, in part, by preserving mitochondrial content and function (53-58). In support of this notion, we found that NMES not only preserved skeletal muscle IMF mitochondria, but dramatically increased their content. In addition to these structural effects, NMES nearly prevented TKA-induced reductions in ADP-stimulated, oxygen consumption rate in isolated mitochondra. These effects of NMES on mitochondrial biology may partially explain its ability to mitigate muscle atrophy, in accord with genetic and pharmacological interventions in pre-clincial models that seek to preserve mitochondrial content and funciton and have been shown to prevent atrophy (33, 35, 59, 60). To our knowledge, however, this hypothesis has never been evaluated in humans, much less over time in response to a clinical events that predipose to disability progression in older adults (14, 23). Collectively, our preliminary data describing the effects of early NMES use following TKA on myofilament and mitochondrial content and function suggest that it is reasonable to hypothesize that: *NMES will diminish TKA-induced single muscle fiber atrophy, declines in contractile and mitochondrial function and increases in ROS production, while increasing mitochondrial content.*

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Objectives:

Our *goals in this application* are two-fold: 1) to comprehensively examine, from the molecular to the whole body level, skeletal muscle structural and functional adaptations following TKA to identify factors contributing to functional disability and 2) to assess the utility of neuromuscular electrical stimulation (NMES) to counter early, post-surgical muscle adaptations and improve muscle size and functionality at these same anatomic levels. Our rationale is that the early post-surgical period is critical for development of skeletal muscle structural and functional adaptations that contribute to disability in TKA recipients and that preventive/rehabilitative interventions that specifically target these muscle adaptations are needed to improve long-term functional recovery. Based on our preliminary data, we *hypothesize* that TKA causes profound reductions in skeletal muscle size and intrinsic function during the early, post-surgical period that contribute to functional disability and that these adaptations can largely be prevented by NMES. We propose two aims to test our hypothetical model:

Aim 1: Define the effects of TKA on skeletal muscle myofilament and mitochondrial structure and function during the early, post-surgical period from molecular to the whole body level.

Hypothesis: TKA will impair whole body and muscle function, in part, through reductions in skeletal muscle fiber size, with preferential atrophy in MHC II sub-types, reduced single fiber tension (force/CSA) and velocity and decreased mitochondrial content and function.

Aim 2: Define the utility of NMES to prevent early, postsurgical skeletal muscle structural and functional adaptations to TKA.

Hypothesis: NMES will lessen TKA-induced muscle fiber atrophy and contractile dysfunction and preserve or increase mitochondrial content and respiration while lessening mitochondrial ROS production.

METHODS AND PROCEDURES

We will use a randomized, controlled design, in which older adult patients with knee OA being evaluated for TKA will be recruited and undergo baseline evaluations (Pre-surgery Testing) prior to surgery. Patients will then be randomized (1:1) to a 5 week intervention of NMES or control (n=32/group, expecting 25% attrition rate to arrive at n=24/group) using a covariate-adaptive approach, which will incorporate age, BMI, sex and surgeon into the randomization scheme. After completing the intervention, they will be re-tested at 5 weeks (5 wk Post-surgery Testing) following surgery. Volunteers can opt into an optional 2 week follow-up testing period. Primary outcomes will be tested at the 5 week evaluation, with 2 wk testing including assessment of ancillary mechanistic hypotheses. Assessment of physical activity by accelerometry (5 d each throughout the 5 week post-surgical period) will monitor variation in physical activity between groups. Each phase of the protocol is described below.

Pre-surgery testing will include assessments of whole muscle size and function, accelerometry to assess habitual weight-bearing activity level, single muscle fiber size and mechanical function and mitochondrial content and function. Detailed descriptions of these assessments are provided below.

Treatment intervention Volunteers will be randomized to NMES or control groups for the 5 weeks following TKA surgery (described below in detail).

5 wk Post-surgery testing will include measurements identical to Baseline Testing, with post-training biopsies performed ≥3 d following strength testing/NMES to eliminate any residual effects, per our usual protocol in our past IRB-approved training studies in other clinical populations (heart failure, end-stage knee osteoarthritis).

2 wk Post-surgery testing (optional) will consist of a skeletal muscle biopsy to assess single muscle fiber size, mitochondrial content and function and anabolic catabolic signaling pathways via standard biochemical/immunolabeling techniques.

6 month Post-surgery testing will include assessments of whole muscle size and function and whole body functional measures to provide data on whether hypothesized preservation in single muscle fiber size/function with NMES will relate to longer-term improvements in whole muscle size/function and whole body functionality.

Procedures:

All of the procedures and interventions on volunteers recruited for these studies are carried out solely for research purposes, as none are part of standard therapy in TKA patients. We will describe procedures that will be performed for each of the Aims.

Aim 1

Primary outcomes, which will be assessed at baseline and 5 wk post-intervention, include: skeletal muscle fiber cross-sectional area (CSA) and function, mitochondrial content, morphology and function and weight-bearing activity. *Secondary outcomes* include: whole muscle size/function and accelerometry. Procedures that will be performed on volunteers to obtain these primary and secondary outcome measures are described below.

Pre-surgery testing

Percutaneous skeletal muscle biopsy of the vastus lateralis (VL) will be performed on the leg scheduled to undergo TKA, per procedures used by our research group in a range of patient populations, including knee OA patients (1, 2). Tissue will be proportioned for mechanical (single fiber function), immunohistochemical (IHC), mitochondrial functional (oxygen consumption rate and hydrogen peroxide production) and electron microscopy (EM) measurements, with remaining tissue frozen in liquid N_2. The VL was chosen because of its importance in determining functional disability (3) and its structural and functional adaptations to TKA (4). The following measures will be assessed from muscle tissue.

Single fiber function will be assessed on segments of chemically-skinned muscle fibers, as used routinely by our lab. *Cellular function* will be measured on chemically-skinned, fiber segments, including force, velocity and power, as described by us (1) and *molecular function* (myosin-actin cross-bridge kinetics/mechanics) by sinusoidal analysis, as pioneered in human skeletal muscle by our lab (5). These measures will be available on the most abundant fiber types: MHC I, IIA and IIAX (>90% of fibers in cancer patients (6)).

Single fiber cross-sectional area (CSA) will be measured by IHC, as described (1), with the addition of MHC IIA and IIX antibodies to delineate MHC II sub-types, as described (7), as these comprise the majority of fibers (MHC I, IIA, IIAX; >90%) in cancer patients (2).

Mitochondrial content/morphology (sub-sarcolemmal and intermyofibrillar: % content, size, # per unit area/sarcolemmal length) and myofibrillar ultrastructure (myofibrillar % area, A-band length) will also be assessed by EM, as described by us (2, 6).

Accelerometry will be performed (5 d each): at baseline and ~weekly throughout the 5 week intervention, as described (12). This will be accomplished using an Actigraph waist-worn accelerometer.

Thigh muscle size and body composition will be measured, as described (13), to track changes in body composition and quadriceps muscle CSA by CT to characterize differential changes between legs, as described (2).

Thigh muscle function will be assessed by isokinetic/isometric dynamometry, as described (12), to characterize changes in whole muscle function (isometric: 70°; isokinetic: 60 and 180 °/sec), adjusted for quadriceps CSA, with central neural activation assessed by the interpolation technique, as described by us (14).

Whole body function will be assessed using the 30 second sit-to-stand test, timed up and go (TUG), stair climb (SCT) (16) and the Short Physical Performance Battery (SPPB) (17, 18), as described by us (19, 20).

Blood samples will be taken to examine circulating factors that may contribute to muscle atrophy and/or dysfunction.

Questionnaires will consist of the Western Ontario and McMaster Universities Arthritis Index (WOMAC) and Medical Outcomes Short Form (SF-36; Appendix 1) form to assess the symptom severity of the volunteer's knee OA, as well as their functional status and quality of life to be used as basic data to characterize the cohort so that, upon publication, readers can assess the external validity of our cohort.

NMES

NMES will be conducted on the quadriceps of the operative leg using a portable stimulation device (Empi Continuum; DJO Global), starting within 48-72 hrs of surgery, as described by us (21), and used in our preliminary studies at UVM (CHRMS 12-184). Briefly, the operative leg will be immobilized on a support board at a neutral angle (30º) or using a knee positioning bolster and ankle weight, with electrodes affixed to the anterior surface of the thigh. Symmetrical, biphasic pulses (400 μs duration at 50 Hz) will be used, with a duty cycle of 25% (10 s on, 30 s off), with patient-selected stimulation intensity to cause visible contractions below pain threshold. NMES sessions will occur 5 d/week, twice daily for 45 min (5 min warm-up) for 5 wks. Our prior trial (21) had excellent compliance, with 91 ± 2% of sessions completed at 5 weeks post-TKA, with compliance not related to age (P>0.5) or number of comorbidities (P>0.5). Studies utilizing muscle biopsies have yielded a similarly high compliance with

NMES (93 ±4%). Thus, we have assumed an attrition rate of 10% due to non-compliance. To track the NMES dose, volunteers will self-report device intensity (mA), as well as knee pain (see Appendix 2), with reports reviewed weekly by the study coordinator. Compliance will be confirmed using the compliance monitoring feature built-in to the device software and we will measure NMES dose (% pre-surgery MVIC) directly at 5 weeks post-TKA, as described (22).

Control

Volunteers randomized to the control group will not receive an NMES device and will be instructed to not undertake any structured exercise regimen outside of standard post-TKA rehabilitation. Controls will receive weekly phone calls to check on their rehabilitation and to standardize interaction with study personnel to the NMES group and will record knee pain similar to volunteers in the NMES group. We chose not to utilize a sham control NMES intervention (eg, electrical stimulation below the threshold to elicit muscle contraction) to mitigate the possibility that patients will increase stimulation intensities sufficient to produce muscle contractions if there is perceived benefit. Rigorous control over who receives NMES was considered preferable to controlling for any effects of sub-threshold, cutaneous electrical stimulation.

5 week Post-surgery testing will be identical to pre-surgery testing, as described above. The only addition to the pre-surgery regimen will be the use of NMES during strength testing in order to assess the intensity of muscle contraction elicited by the NMES stimulus, as described by us (22). Post-training muscle biopsies will be performed ≥3 following strength testing or the last bout of training to eliminate any residual effects, as described by us (23).

6 month Post-surgery testing will include whole muscle strength testing, body composition analysis and whole body functional assessments. The purpose of these assessments is not to assess whether NMES is effective at improving longer-term functional recovery, as our co-I's research has demonstrated (21), but instead to determine whether the effects of NMES to mitigate muscle atrophy and dysfunction during the early, post-surgical period predicts longer-term improvements in function.

2 week Post-surgery testing will be optional. Volunteers can opt in/out of testing at this time point. The primary purpose of testing at 2 weeks post-surgery is to obtain data at a mid-point between surgery and the 5 week time point to better define potential mechanisms contributing to muscle atrophy and contractile dysfunction. As with 5 week post-surgery testing, the contractile response to the NMES stimulus will be assessed during strength testing. The only omission will be contractile function measurements on single muscle fibers.

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SUBJECT CHARACTERISTICS, IDENTIFICATION AND RECRUITMENT

A single cohort of patients with knee OA will be recruited and tested prior to and following TKA to address both aims. We chose to study TKA recipients for a number of reasons. First, the primary antecedent morbidity leading to TKA, knee OA, is ***the*** leading cause of disability in older adults (1), making TKA recipients an important segment of the older adult disabled population. Second, because of its elective nature, TKA is unique among disabling clinical events in permitting pre- and post-surgical assessments. Although other orthopedic trauma/surgeries (eg, hip fracture) may provoke greater functional disability (2), their emergent nature and complex clinical background/course (eg, blunt force trauma, other fractures, etc) make them extremely difficult to study, with no pre-injury/pre-op testing possible, and would introduce numerous confounders. Moreover, TKA is profoundly disabling, as we have shown (3), making it a useful clinical condition in which to study muscle adaptations and remediative interventions. Finally, we chose primary knee OA patients because they comprise the majority of TKA recipients (4) and because antecedent pathologies for secondary knee OA (eg, rheumatoid arthritis) could confound the response to TKA/NMES (5).

We chose 2 and 5 weeks for the post-TKA assessments. Broadly speaking, we chose to study the 1st 5 wk post-TKA because this represents an important gap in rehabilitative care, as detailed above in the *Purpose and Objectives* section. Moreover, large structural and functional skeletal muscle changes occur, making it an optimal time to intervene with preventive modalities, such as NMES. The 2 wk time point was chosen in response to reviewer's requests for evaluation of additional potential mechanisms underlying atrophy and contractile dysfunction because this is the ***absolute closest point*** to the TKA surgery that our surgeons would allow muscle biopsies to be performed because patients are typically on anti-coagulant therapy for the first ~7-10 d post-TKA, a contraindication for muscle biopsy. The fact that our preliminary data suggests that atrophy is continual throughout the 5 wk time period argues that mechanisms contributing to atrophy and dysfunction are likely operative at the 2 wk time point.

We chose to focus on knee extensors because we and others have shown that they experience the

greatest functional decline (-42 to -60%;(6, 7)), 2- to ~3-fold greater than dorsi/plantarflexors (6), with atrophy of comparable magnitude to functional deficits. Moreover, the greatest functional declines occur in activities of daily living (ADL) with strong reliance on the extensor mechanism (eg, SCT, TUG; (3)), making knee extensor function an important determinant of TKA-related disability. Knowledge of the adaptations to TKA in all lower extremity muscles, as well as their response to NMES, is needed, but in lieu of practical limitations in studying this population (eg, # of muscles studied/biopsied) and available technologies for NMES, we have focused on the knee extensors because of their profound impact on physical function.

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Inclusion/Exclusion Criteria:

Patients (50-75 yrs) with symptomatic, primary knee OA being evaluated for TKA will be recruited. Exclusion criteria include: 1) knee OA secondary to inflammatory/autoimmune disease; 2) untreated/uncontrolled hypertension, diabetes or thyroid disease; 3) chronic heart failure, actively-treated malignancy, exercise-limiting peripheral vascular disease, stroke or neuromuscular disease; or 4) body mass index $\geq$38 kg/m^2. Note that many of these exclusions are contraindications for TKA (eg, untreated hypertension/diabetes, actively treated malignancy). Thus, such patients would not be part of the pool of prospective patients, and we cite them for completeness to show that we are excluding potentially confounding comorbidities for muscle size and function. Finally, we will exclude patients with a lower extremity blood clot, known coagulopathies or an implanted pacemaker/ICD, contraindications for NMES and the muscle biopsy, as detailed above. These inclusion/exclusion criteria are similar to those used to generate our preliminary data. Of the ~300 TKAs/yr that meet initial procedural eligibility at our center, 53% (n=159/yr or 636 for 4 yrs of recruitment) would meet the above inclusion/exclusion criteria, suggesting a more than adequate patient base.

Recruitment:

Research coordinator, who is an UVMMC employee in the Dept of Medicine, will screen surgical schedule for physicians in the Dept of Orthopaedics and Rehabilitation for patients that meet basic procedural eligibility (receiving a unilateral total knee arthroplasty procedure) and eligibility criteria (age, BMI, absence of exclusionary comorbid conditions, etc) to identify prospective participants. The coordinator will notify care providers in the Dept of Orthopedics and Rehabilitation of potentially eligible patients. An Ortho care provider will notify the patient that they may qualify for a research study and provide the patient with an IRB-approved trifold information pamphlet or provide a brief verbal summary of the study if contact is made by phone. The care provider will ask the patient if they would like to receive more information from research study staff.

Risks/Benefits:

Potential risks: Below we have highlighted those procedures/measurements that have anything greater than negligible risk to the volunteers' health for each phase of the study.

Pre-surgery and 2 and 5 week Post-surgery Testing: Risks associated with the muscle biopsy procedure include excessive bleeding, persistent numbness, an allergic reaction to the antiseptic used to clean the thigh or infection. In general, all of these risks are well below 1% (1). Regarding bleeding risk, any volunteer with known coagulopathies will be excluded from participation. The majority of our TKA recipients take NSAIDS for joint pain pre-surgery and aspirin for deep vein thrombosis (DVT) prophylaxis post-surgery, although some with greater risk for DVT are placed on low MW heparin. For pre-surgical testing, the NSAIDS therapy will be stopped 5 d prior to the muscle biopsy, with prior approval of the prescribing physician, to limit bleeding during the biopsy. For the 2 wk post-surgery testing, none of the physicians will allow stoppage of aspirin or low MW heparin therapy. For those patients on low MW heparin, who are at greater risk for DVT, they will not be allowed to undergo 2 wk post-surgery testing. For patients on NSAIDs for pain at 2 wks post-surgery, For those patients on aspirin, this will not be stopped for the 2 wk post-surgery visit. Instead, pressure will be held longer following the biopsy to minimize bleeding. Historically, our laboratory's practice for patients on aspirin was to not take them off of aspirin prior to the biopsy, a practice that was inherited from working with Dr. Ades as the study physician for cardiac disease patients. This practice had been long-standing in his studies that utilized muscle biopsies in cardiac disease populations and was only changed in Dr. Toth's studies when one volunteer who was on aspirin developed a hematoma following her biopsy. However, the anti-thrombotic effects of aspirin will not dissipate with discontinuation of 5 d. For this reason, and because of the necessity of having TKA recipients on aspirin for DVT prophylaxis at the 2 wk post-surgery time point, we will not stop this medication. At 5 wk post-surgery, most patients will be off of anti-thrombotic therapy (aspirin or low MW heparin), but, if not, the same rules as for the 2 wk visit will apply. Finally, for any patient taking NSAIDs for pain at the 2 wk or 5 wk visit, they will only be included with prior approval from the prescribing physician to stop the NSAIDs 5 d pre-biopsy, as well as the patient in accordance with their pain control. Our laboratory has never had an adverse event related to the muscle biopsy procedure and it will be performed by trained, qualified clinicians in a closely-controlled, medical environment. There is risk for muscle soreness or injury resulting from whole body and whole muscle functional testing. Appropriate warm up exercises will be performed to prevent muscle soreness/injury. Finally, each DEXA scan exposes the volunteers to x-rays, although the total exposure is equivalent to approximately 1 d of normal daily background radiation and each CT scan of the mid-thigh exposes volunteers to the equivalent of 110 d of normal background radiation. Importantly, the exposure from the CT is focused on a single slice at mid-thigh. Any woman volunteer who is premenopausal will undergo a urine pregnancy test prior to either procedure to assure that she is not pregnant.

NMES intervention: NMES is a generally safe procedure, delivered in the proposed study by an FDA approved device. Although evidence is limited, some have suggested that NMES could increase the risk of DVT, which may have serious health consequences. However, several published reports show that NMES significantly reduces the risk of developing DVTs (2) and we will actively exclude any individual with a known coagulopathy. Because of the location of the stimulating electrodes (upper leg), the risk of NMES dislodging a DVT is likely minimal. We will also exclude any prospective volunteers that currently have an implanted cardiac defibrillator or pacemaker, as this is contraindicated for NMES use (2). Patients may experience some painful muscle contractions as they first adjust the stimulus to a tolerable level, this will be quickly mitigated by reducing the stimulation intensity. After treatment, muscles soreness may occur. The level of fatigue and/or soreness, however, will be similar to that which occurs following a standard exercise training session and should dissipate over time as the volunteer's muscles become accustomed to the electrically-stimulated contractions (ie, they become trained). In studies of the use of NMES in the post TKA population, our laboratories (including our co-I, Dr. Stevens-Lapsley, who has conducted NMES in NIH-funded RCTs; (3)) have never experienced an adverse event related to the use of NMES.

Leg movement with Biostamp sensors: Wearing the sensors causes little to no discomfort in most individuals, similar to wearing a Band-Aid. The BioStamp nPoint sensors are not intended for use on patients with known allergies or hypersensitivities to adhesives or hydro-gel. In absence of a known

allergy or hypersensitivity, it is possible the sensor and/or adhesive may still cause mild discomfort, skin irritation, redness, rash or contact dermatitis in some individuals. The BioStamp nPoint sensors are not intended for use on patients who have implanted pacemakers, defibrillators, or other active implantable devices. As volunteers with these implanted devices are already excluded from study, this risk is obviated. There is a small risk that a participant's electronic information could be accessed thereby affecting their confidentiality.

Benefits: The direct benefit of the research to volunteers is minimal. NMES may improve skeletal muscle structure or function and, in turn, improve physical functional capacity, as our collaborator's RCT demonstrated (3). Because of this, patients may experience improved physiological capacity, which could reduce disability post-surgery. If NMES is shown to have beneficial effects on skeletal muscle, further research and application of the technique to TKA recipients may assist in the development of more effective post-TKA care to mitigate long-term functional morbidity in these patients in the future, as well as provide compelling evidence to extend this intervention into other post-surgical populations to improve functional recovery.

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Data Safety and Monitoring:

Our study is a clinical trial based on the recently-revised NIH definition (NOT-OD-15-015), but not a phase III clinical trial. Thus, pursuant to NIH regulations, we had originally established a formal Data Safety Monitoring Plan, which would provide additional oversights to further protect against risk to safety and confidentiality. The Clinical Trial Branch of the NIA has reviewed our protocol and made the determination that our study could be monitored by an outside Safety Officer through a standard DSMP format.

DSMP: The PI (Toth) and Dr. Nelms will monitor the safety of the research procedures for this study. Dr. Toth will be available on-site for all major experimental procedures (eg, muscle biopsy) that might reasonably be expected to pose safety concerns for volunteers. In this context, the PI will be readily available to monitor volunteer safety throughout the study. If an event occurs that affects participant safety, the PI and/or Dr. Nelms will alert the Safety Officer (Dr. Richard Wilson, M.D.; Director of Inpatient Rehabilitation and Stroke Rehabilitation; MetroHealth Medical Center; Cleveland OH), who will adjudicate the event with respect to its severity, expectedness and relatedness to participation in the study according to the aforementioned criteria. A formal copy of the DSMP that has been approved by the Clinical Trial Branch of the NIA is now included with this Protocol.

Adverse Event and Unanticipated Problem (UAP) Reporting: .

The PI (Toth), the study coordinator and Dr. Nelms will monitor the safety of the research procedures/interventions for this study. Dr. Toth will be available on-site for all major experimental procedures (eg, muscle biopsy) that might reasonably be expected to pose safety concerns for volunteers. In this context, study personnel will be readily available to monitor volunteer safety throughout the study. If an event occurs that affects participant safety, the PI will alert Dr. Nelms and the Safety Officer, who will adjudicate the event with respect to its severity, expectedness and relatedness to participation in the study. Because numerous studies in our laboratories and others have demonstrated the safety of this regimen of testing in patients with a broad range of clinical backgrounds (e.g., heart failure patients, cancer patients, advanced-stage, knee OA patients; healthy elderly; see PI's *Biosketch*), as well as the safety of NMES, we expect minimal problems related to testing or the NMES intervention. The research coordinator and Dr. Nelms will remain unblinded during the studies, as they will not specifically participate in testing, and to enable them to respond directly to any adverse event/unanticipated problem that may arise in either treatment group. Considering the low risk nature of

these studies, we have not incorporated "stoppage criteria" for the overall study. Instead, the Safety Officer will decide whether an individual participant should continue with the study following occurrence of any adverse events or unanticipated problems, taking into consideration what is in the best interest of each individual patient.

Adverse events will be reported by one of 3 mechanisms. First, the joint University of Vermont/University of Vermont Medical Center (UVMC) Committee for Human Subject Research Adverse Event Reporting Document. These reports will be forwarded to the office of the Committee for Human Research in the Medical Science (CHRMS) within 5 days of the event. Reporting any adverse events will be the responsibility of the PI. The CHRMS will make a determination as to whether additional reporting requirements are indicated. Second, the UVMC Patient Safety Reporting system (SAFE), which may be initiated by health care center staff or study personnel. These forms will be forwarded within 3 days to the PI, UVMC Risk Management Office, CHRMS and other appropriate agencies, as indicated by the nature of the report. Finally, the UVMC Medication/IV Event Report Form, with distribution and timing as noted above. This latter mechanism might be used with events related to blood sampling. Reviews of protocol specific adverse events will be performed no less than annually. Additionally, any adverse event that occurs will be forwarded to the PI for reporting to the Human Subject Research Protection Office within 1 week of occurrence. Of note, these protections against risk include both physical risks to the volunteers, as well as risks associated with any breach in confidentiality.

On an annual basis, Drs. Toth, Nelms and the Safety Officer will assess data being gathered and safety of volunteers to assess the pattern or frequency of events to identify occurrence of any event or problem that significantly alters the safety profile of the procedures being performed, unless occurrence of a serious adverse event or unanticipated problem necessitates re-evaluation of the expected risk of the study procedures at an earlier time point. Additionally, they will evaluate data collection and storage to ensure the confidentiality of data and quality. Each of these evaluations will be followed by reports of study progress and patient safety to the University of Vermont CHRMS via yearly progress reports and to the appropriate NIH program officer. The report will include information regarding study status and safety of volunteers (occurrence of AEs/SAEs. This report will also be forwarded to the NIH Program Officer for his review.

Withdrawal Procedures: *Define the precise criteria for withdrawing subjects from the study. Include a description of study requirements for when a subject withdraws him or herself from the study (if applicable).*

Volunteers will be withdrawn if the research team, clinician and/or safety officer feels that further participation in the study or performance of any procedure associated with this study would, in any way, put the volunteer at undue risk or not be in their best interest. Moreover, volunteers may be withdrawn if s/he fails to attend scheduled visits or do not comply with instructions from research staff.

Sources of Materials: *Identify sources of research material obtained from individually identifiable human subjects in the form of specimens, records or data. Indicate whether the material or data will be obtained specifically for research purposes or whether use will be made of existing specimens, records or data.*

An individual research record will be kept on each volunteer in compliance with HIPAA standards. This record will contain identifying data, demographic information and results from all clinical research measurements and evaluations. The results of all testing will be kept confidential. In addition, skeletal muscle tissue samples will be taken and will be used for measurement of muscle size, structure and function. All materials gathered in conjunction with the proposed studies will be used for research purposes only and will be available only to research personnel working on these studies, who have obtained proper training in human subjects research and privacy protection.

DRUG AND DEVICE INFORMATION

Drug (s) [X] ***Not applicable***
Device (s) [ ] ***Not applicable***

Device name and indications

The interventional device used in this study: EMPI Continuum complete electrotherapy system has received FDA approval (501K: K093324) for retarding disuse-related atrophy, which we believe is one of the primary mechanisms whereby muscle adaptations evolve in TKA recipients in the early, post-surgical period. That is, muscle disuse secondary to pain, limited range of motion and muscle weakness

(the latter being due to neural inhibition caused by joint pain/swelling) causes skeletal muscle fiber atrophy and weakness and mitochondrial rarefaction and dysfunction.

Is it FDA approved:

Yes. It is approved to mitigate muscle atrophy/dysfunction associated with muscle disuse (501K: K093324) (see Appendix 3).

Risk assessment (non-significant/significant risk)

The device (and similar devices) has been used extensively in the orthopedic and neural rehabilitation settings by physical and occupational therapists and in numerous disease states (heart failure, chronic obstructive pulmonary disease, knee replacement) to improve muscle size and function in clinical trial settings. Thus, NMES is generally a safe modality, with a long safety record. Although evidence is limited, some have suggested that NMES could increase the risk of dislodging a deep vein thrombosis (DVT) because of the rhythmic muscle contractions induced by the electrical stimulation. However, several published reports show that NMES significantly reduces the risk of developing DVTs. In fact, the device we are using is FDA-approved for prevention of DVT of the calf muscles immediately following surgery, as it would function similar to intermittent pneumatic compression. Moreover, we will actively exclude any individual with a known coagulopathy or DVT. Because of the location of the stimulating electrodes (upper leg), the risk of NMES dislodging a DVT is likely minimal. There are also several case reports that NMES may be sensed by cardiac defibrillators as an arrhythmia, causing the device to discharge inappropriately. However, interference with ICDs mostly involved low frequency stimulation of the upper or lower back. In contrast, a more recent study has shown that higher frequency stimulation of the leg muscles does not cause electromagnetic interference with the device (1). Regarless, consistent with current clinical practice guidelines (2), we will exclude any volunteers that currently have an implanted cardiac defibrillator or pacemaker. Finally, during the first couple of NMES sessions, muscle soreness may occur, but this is comparable to what might occur with classical exercise training and dissipates over time.

Literature cited

1. Kamiya, K., Satoh, A., Niwano, S., Tanaka, S., Miida, K., Hamazaki, N., Maekawa, E., Matsuzawa, R., Nozaki, K., Masuda, T., and Ako, J. Safety of neuromuscular electrical stimulation in patients implanted with cardioverter defibrillators. *Journal of Electrocardiology* **49**, 99-101
2. Houghton, P. E., Nussbaum, E. L., and Hoens, A. M. (2010) Electrophysical agents. Contraindications and precautions: an evidence-based approach to clinical decision making in physical therapy. *Physiother Can* **62**, 1-80

Inclusion of Minorities and Women: .

Inclusion of Women

This study will include equal numbers of men and women.

Inclusion of Minorities

Every effort will be made to recruit minorities for the proposed studies. The contribution of minorities to the total population of Vermont is 3.2%, with a similar minority profile in Chittenden County (3.6%), where the University of Vermont (UVM) is located.

Inclusion of Children:.

The proposed studies will not include children. The rationale for this decision is based on the fact that total knee replacement is confined to the adult/ older adult population.