Study Protocol and Statistical Analysis Plan

Project Title:	**Version Date:**
Alternative exercise program to improve skeletal muscle function and fatigue in cancer survivors	1-15-19

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Lay Summary:

Exercise training is effective at mitigating the negative health effects of cancer and its treatment and improving long-term prognosis, but there are substantial hurdles to disseminating exercise to the large, and rapidly growing cancer survivor population (~18 million by 2022). Clinical populations have traditionally participated in exercise primarily through facility-based programs (eg, cardiac and pulmonary rehabilitation). However, very few of these exist for oncology patients and such programs may not be feasible for both practical and clinical reasons; in particular, during chemotherapy, where logistical and clinical hurdles to participation are high, but when muscle adaptations likely evolve. Moreover, access to exercise facilities may be limited, particularly for patients living in rural communities (20% of US population), such as Vermont. Moreover, fatigue and other functional morbidities arising from cancer and its treatment deter participation. As positive results from randomized, controlled trials move exercise closer to standard of care in cancer survivors, a critical barrier to its broad dissemination will be the development of effective exercise modalities for patients who are underserved by facility-based programs.

Our long-term goal is to develop a clinically useful, exercise intervention that can serve as an alternative to facility-based programs, with the objectives of alleviating fatigue, forestalling disability and improving prognosis. Our objective in this application is to determine whether exercise training via neuromuscular electrical stimulation (NMES) has beneficial effects on skeletal muscle size and function in cancer patients. We hypothesize that NMES will improve skeletal muscle size, contractile function and oxidative capacity, based on our seminal observations in cancer patients that functional disability derives, in part, from deficits in skeletal muscle contractile protein and mitochondrial content and function, as well as our data showing that NMES counters such deficits. Our rationale underlying this proposal is that establishing proof-of-principle, mechanistic evidence for the utility of NMES to remediate these skeletal muscle deficits in cancer patients receiving treatment will provide a solid foundation upon which to support more definitive trials of the utility of NMES in the cancer survivor population.

We will use data from cancer patients studied prior to and 8 weeks following completion of NMES or control intervention on lower extremity musculature, with muscle structure and function being measured across a range of anatomic levels. We focus on testing our hypotheses with cellular and organellar level measurements because they permit a more rigorous evaluation of the effects of NMES on muscle biology by circumventing limitations in the detection of exercise effects at the whole body or whole muscle level due to methodological shortcomings, subjective motivation and, specific to NMES, effects on neural activation. Determination of the utility of NMES to influence the quantity and functionality of contractile proteins and mitochondria is important, as detrimental adaptations in these parameters leads to muscle atrophy, weakness and increased fatigability, skeletal muscle phenotypes that are likely at the root of cancer-related fatigue and disability and, in turn, reductions in physical activity that negatively affect long-term prognosis.

Successful completion of the proposed studies will provide mechanistic evidence for the utility of NMES to improve skeletal muscle size and function in cancer patients during treatment, which will support larger scale, randomized, control trials of its feasibility and efficacy. Specific to our investigative team, this will facilitate application of NMES to rural cancer survivors, a target population for this modality and one we are well-positioned to study. More broadly, development of NMES as an exercise modality in cancer patients could positively impact the oncology exercise rehabilitation field by providing a new, low-cost intervention that could extend the benefits of exercise to cancer patients underserved by facility-based training programs and during treatment, a time when facility-based programs are less feasible, but when detrimental muscle adaptations likely evolve. The history of NMES use within orthopedic and neurologic rehabilitation settings, its low-cost and the availability of FDA-approved devices for NMES and clinicians trained in its application (physical and occupational therapists), reduces logistical, financial and regulatory barriers to its broader repurposing for the cancer survivor population.

Purpose:

Exercise in cancer survivors is safe and has numerous health benefits (1-4). Less clear is how to disseminate the benefits of exercise to the large and growing number of cancer survivors (5, 6). In cardiac and pulmonary disease populations, this occurs primarily through facility-based programs.

However, very few of these programs currently exist for cancer survivors and participation rates in other conditions are quite low (eg, 10-20% for cardiac rehab (7)). Participation may be further hindered in cancer survivors because of limited access to exercise facilities, particularly in rural populations (8), and disease/treatment-related side-effects, such as fatigue (9). Thus, alternatives to facility-based exercise programs are needed that effectively promote an exercise training response, integrate easily into outpatient and home environments, are easy to use and require minimal costs and equipment.

Neuromuscular electrical stimulation (NMES) fulfills these criteria. An inexpensive (~$500), hand-held NMES unit permits extracorporeal initiation of muscle contractions that can mimic resistance- or aerobic-type exercise (10), producing a training response similar to these modalities (11) with near comparable effects (12, 13). The non-volitional nature of NMES is also attractive, as cancer-related fatigue may deter motivation towards volitional exercise (9). NMES improves muscle size, strength and performance in older adults with chronic disease (14) and counteracts muscle atrophy coincident with disuse (15) and catabolic clinical conditions (16-18). Despite these beneficial effects, NMES has received almost no attention in cancer patients, where factors related to the disease (19, 20) and/or its treatment (21) may hinder exercise adaptations, although preliminary results from a case report (22) and small clinical trial (23) are encouraging.

The proposed studies are designed to determine the effect of NMES on skeletal muscle in cancer patients receiving chemotherapy, with the expectation that our results will demonstrate salutary effects on muscle morphology and function. These findings would advance the oncology rehabilitation field by providing a solid, mechanistic evidence base to support the utility of NMES as a surrogate exercise modality during cancer treatment. Such evidence would reduce the risk for failure of this modality in more definitive, randomized, controlled trials in the broader cancer population.

The proposed studies are innovative in seeking to develop effective exercise modalities for cancer survivors who are underserved by facility-based programs. They build on our seminal work describing fundamental cellular and sub-cellular deficits in skeletal muscle structure and function that contribute to disability in cancer survivors (24) by evaluating the utility of NMES to improve these same parameters. In this context, our experimental approach is also innovative in seeking to provide mechanistic evidence for the beneficial effects of NMES on skeletal muscle. We believe that this approach can economize the initial exploratory phase of developing new exercise modalities by testing the efficacy of an intervention to affect those skeletal muscle properties that are typically improved in response to exercise: size, contractility and oxidative function.

Background, Preliminary Results and Hypothesis for Aim 1

Cancer survivors often experience skeletal muscle weakness (25), due, in part, to atrophy resulting from cancer and its treatments (26). However, strength deficits exceed the loss of muscle (24, 27), due to intrinsic muscle contractile dysfunction, as our seminal work (24) showed that cancer (mostly lung) was associated with reductions in both slow-twitch, myosin heavy chain (MHC) I and fast-twitch, MHC II fiber contractility, which scaled to the whole muscle level. Whole muscle weakness, in turn, contributed to real-world disability, as it was associated with diminished walking performance (24). Our preliminary data show that functional deficits in MHC I and IIA fibers are apparent in women with breast cancer tested during chemotherapy similar to women with lung cancer from our prior work (24) and scale to the whole muscle level. In fact, contractile deficits tended to be greater in breast vs. lung cancer patients when compared to women without cancer. Our new data further show that muscle fiber atrophy and dysfunction develop during chemotherapy in lung cancer patients. Collectively, these results suggest both atrophy and intrinsic contractile deficiencies as determinants of reduced muscle functionality in breast cancer patients and identify treatment as a time when these phenotypes may evolve, emphasizing the need to identify interventions that counter muscle adaptations at this time.

NMES improves muscle size and contractile function at the whole muscle level in other diseases (14). That NMES has effects in cancer patients is supported by one trial showing a trend toward increased whole muscle strength after 4 wks of stimulation in lung cancer patients (23), although it is unclear whether these improvements were due to enhanced neural activation (28) or durable changes in muscle fiber size and/or contractility. This is an important distinction, as the former would diminish following cessation of NMES, whereas the latter would provide more lasting functional benefits. Our new data suggest that NMES directly regulates muscle fiber size and function, as it counters both single fiber atrophy and functional deterioration in older adults following total knee arthroplasty, a period

characterized by profound atrophy and dysfunction. These effects of NMES to prevent atrophy in MHC I fibers and cut atrophy in ~½ in MHC II fibers is remarkably similar to our data on the effects of classical training to prevent atrophy in lung cancer patients receiving treatment, suggesting NMES may function as a surrogate of classical training programs. Collectively, these data support the hypothesis that *NMES will increase single skeletal muscle fiber contractile function and size in breast cancer patients compared to patients randomized to control intervention.* Aim 1 will test this hypothesis.

Background, Preliminary Results and Hypothesis for Aim 2

Fatigue is a common side effect of cancer and its treatment, affecting a large majority of patients (29). It is multi-factorial in nature, comprising both central neural and periperal physiological adaptations (30). One peripheral muscle adaptation that may promote fatigue is reduced oxidative capacity (31). We recently showed, for the first time in humans, that cancer and its treatment are associated with mitochondrial rarefaction (24), which we have subsequently observed in breast cancer patients studied during chemotherapy. Further compounding reductions in mitochondrial content, cancer (32, 33) and chemotherapeutic agents used in breast cancer (34) have been shown to impair mitochondrial function in pre-clinical models. Mitochondrial loss and dysfunction during cancer treatment, therefore, may contribute to cancer-related fatigue and functional disability.

NMES improves skeletal muscle oxidative function, with effects that rival those found with classic aerobic-type exercise training (12, 13). In fact, our preliminary data show that NMES can activate mitochondrial biogenesis and improve mitochondrial function. NMES use in patients for 5 wks following TKA not only prevented mitochondrial loss, but increased fractional content, and mitigated the effect of TKA to reduce mitochondrial function. Our new preliminary data show that reductions in ADP-stimulated OCR during treatment in lung cancer patients are attenuated by resistance training, suggesting that NMES may function similar to classical training to prevent mitochondrial functional decline in cancer patients during treatment. Collectively, these results support the hypothesis that: *Skeletal muscle mitochondrial content and function will be increased by NMES compared to breast cancer patients randomized to control intervention.* This hypothesis will be tested in Aim 2 of these studies.

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Objectives:

Our ***objective*** of this study is to determine whether exercise training via neuromuscular electrical stimulation (NMES) has beneficial effects on skeletal muscle size and function in cancer patients. We ***hypothesize*** that NMES will improve skeletal muscle size, contractile function and oxidative capacity, based on our seminal observations in cancer patients that functional disability derives, in part, from deficits in skeletal muscle contractile protein and mitochondrial content and function, as well as our preliminary data showing that NMES counters such deficits. To test this hypothesis, we propose two specific aims:

***Aim 1:* Determine the effect of NMES on skeletal muscle fiber size and contractile function.**

We *hypothesize* that skeletal muscle fiber size and contractile function will be greater in cancer patients receiving treatment who are randomized to NMES compared to controls.

***Aim 2:* Determine the effect of NMES on skeletal muscle mitochondrial content and function.**

We *hypothesize* that skeletal muscle mitochondrial content and function will be greater in cancer patients receiving treatment randomized to NMES compared to controls.

Study Design:

We will use a prospective, randomized controlled design, in which recently-diagnosed, breast cancer patients (40 to 75 yrs) will be recruited (see Appendices I-VI for recruitment materials/data collection sheets) and undergo *Baseline Testing* during the 1st cycle of neoadjuvant or adjuvant chemotherapy. Patients will then be randomized (1:1) to 8 wks of *NMES* or *Attention control* intervention (n=12/group; *Treatment Phase*), with stratification for age and adjuvant or neoadjuvant chemotherapy. Following completion of the intervention, they will undergo *Post-intervention Testing*.

Baseline testing will include assessments of single muscle fiber size and mechanical function and mitochondrial content and function, as well as whole muscle size and contractile function and walking performance. Detailed descriptions of these assessments are provided below.

Treatment Phase Volunteers randomized to both *NMES* and *attention control* groups will receive instruction to maintain or increase their physical activity according to the clinics usual standard of care. Both interventions are described below in detail.

Post-Intervention Testing will occur in patients who complete the NMES program and controls and will include measurements identical to Baseline Testing, with post-training biopsies performed ≥3 d following NMES to eliminate any residual effects, per our usual protocol in other training studies that have been IRB-approved. Post-intervention testing will occur within ±14 d from the 8 week intervention point

New methodology: Our study employs cellular and organellar level measurements of skeletal muscle structure and function. Although the methodology used in these studies is not new per se, it has not been used in the setting of exercise intervention trials in cancer patients. We feel that our approach is innovative in circumventing limitations in the detection of exercise effects at the whole body or whole muscle level due to methodological shortcomings, subjective motivation and, specific to NMES, effects on neural activation. This approach can economize the initial exploratory phase of developing new exercise modalities by more accurately characterizing the effects of such programs on those properties typically improved with exercise; namely, muscle size, contractility and oxidative function.

Procedures:

All of the procedures and interventions in this study are carried out solely for research purposes, as none are part of standard therapy/care in breast cancer patients.

Primary outcomes include: skeletal muscle fiber cross-sectional area (CSA) and function and mitochondrial content, morphology and function. *Secondary outcomes* include: whole muscle size/function and 6 min walk performance and indices of chemotherapy-induced peripheral neuropathy (CIPN). Procedures that will be performed on volunteers to obtain these primary and secondary outcome measures are described below.

Biopsy of the vastus lateralis (VL) will be performed, as used by our research group in a range of patient populations, including cancer patients (1), with the study leg chosen as the stronger leg in ½ of patients and the weaker leg in the other ½ so as not to bias adaptations based on minor functional asymmetries. Tissue will be proportioned for mechanical, immunohistochemical (IHC), mitochondrial respiratory 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 (2) and its structural and functional deficiencies in cancer patients previously described by our laboratories (1).

Single fiber function, including tension, velocity and power, will be measured on chemically-skinned, muscle fiber segments (n≈25/subject/biopsy) using isotonic load clamps, as described by us (3), with data available on MHC I and IIA fibers, the two most prevalent fiber types in human muscle (1, 4).

Single fiber cross-sectional area (CSA) will be measured by IHC, as we have described (1, 3), with minor modifications to permit assessment of all MHC isoforms, as described (5).

Whole muscle contractile function will be measured, as described by us (6). Maximal peak isometric (70°, 90°) and isokinetic knee extensor torque (60 and 180°/sec) will be adjusted for thigh muscle CSA to account for variation in muscle size. Additionally, muscle fatigue will be assessed following repeated

(n=24) isometric contractions. Briefly, 24 maximal voluntary isometric contractions (2 min of intermittent contractions at 70º), following a duty ratio of 3 seconds of contraction and 2 seconds of rest. Fatigue will be defined as the average peak torque from the final 3 contractions divided by the highest peak torque achieved prior to initiation of the fatigue protocol (ie, Isometric knee extensor strength determination prior to the fatigue measure).

Thigh muscle size will be measured by dual energy x-ray absorptiometry, as described by us (1). Additionally, anthropometrics and body mass will be assessed. Height will be measured without shoes, using a stadiometer to the nearest 0.5cm. Weight will be obtained from the DEXA estimate. BMI will be calculated as weight (kg)/height(m^2).

6 min walk test will be performed, as described (1), to assess changes in physical functional capacity.

Single legged stance will be performed to test balance in each leg separately. The participants place their hands on their hips then raise the 1 foot off the floor. Stance is timed until the raised foot touches the floor, the stationary foot moves, the raised foot touches the stationary leg or the hands are taken from their starting position. It is repeated three times and the best value is used. If stance exceeds 30 seconds the test will be stopped at that time.

Accelerometry will be performed, as described (6), to monitor weight-bearing activity levels, which can be used to account for possible variation in muscle use between groups (Appendix VII).

Questionnaires (provided in Appendix VIII) will be provided to assess fatigue symptoms (Fatigue Symptom Index), physical activity level (Stanford Brief Physical Activity Questionnaire), general health/well-being (MOS SF-36) and CIPN (EORTC CIPN 20).

Nerve conductions studies (NCS) will include the sural, peroneal and tibial nerves bilaterally and are the best measure of large nerve fiber function that is affected by both exercise and chemotherapy. NCS take about ten minutes and record electrical signal after providing a very mild electrical stimulus to the skin. The amplitude, distal latency, and conduction velocity will be recorded for each nerve. Study of both legs require a total of 15-20 stimulus applications, some of which are too weak to be felt.

Clinical neuropathy assessment: The Utah Early Neuropathy Scale (UENS) is a lower extremity examination performed by a neurologist that tests different modalities of strength, sensation and reflexes to detect neuropathy. It examines great toe strength, pin sensation, allodynia, vibratory sensation and Achilles reflexes in both legs.

Treatment Phase

Volunteers randomized to both the NMES intervention and control groups will receive instruction to maintain or increase their physical activity according to the clinics usual standard of care.

NMES will be carried out bilaterally on the quadriceps using a portable stimulation device (EMPI Continuum or 300PV). Study personnel will teach volunteers to use the NMES device and provide detailed directions for its use (Appendix IX). Briefly, two adhesive electrodes will be affixed to anterior surface of each thigh: ~1 cm distal to the inguinal crease and ~5 cm proximal to the patella, with the leg maintained in isometric conditions by placing a bolster under the knees and using ankle weights. Symmetrical, biphasic pulses (400 μs duration at 50 Hz) will be used, with a duty cycle of 25% (10 s on, 30 s off) at stimulation intensities sufficient to cause visible muscle contractions below pain threshold. NMES sessions will occur 5 d/wk for 1 hr/d (two 30 min or one 60 min session) for 8 wks. This regimen (lower duty cycle and 60 min/d) differs slightly from that put forth in the grant proposal in lieu of reviewer's comments on the resubmission and consultation with out co-investigator (Dr. Stevens-Lapsley), as well as comments from volunteers in our VCC/LCCRO Pilot Project Grant, where volunteers suggested that we offer shorter sessions, as finding time for longer sessions was problematic for women with family and work commitments. We will monitor adherence to the NMES program covertly using the compliance monitoring feature built in to the NMES device software. Additionally, volunteers will self-report compliance, stimulation intensity and muscle soreness/side effects (Appendix VIII and X) and these reports will be reviewed during weekly phone contacts by study personnel. Finally, we will assess acceptability of the program using a questionnaire. These measures are taken to inform future RCTs if our hypotheses are supported.

Attention control patients will not receive an NMES device. They will receive weekly phone calls to discuss measurements of leg muscle soreness to standardize the amount of interaction with study personnel to the NMES group.

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Surveys/Questionnaires:

The Fatigue Symptom Inventory (FSI; Appendix VII) has been tested in several cancer patient populations and is a 14-item self-report of severity, frequency and pattern of fatigue. An 11 point scale is used (0=not at all fatigued; 10=as fatigued as I could be). Frequency is measured in numbers of days in the past week. Interference is measured on an 11 point scale (0=interference none of the day; 10=interference the entire day). A mean score of ≥ 3 across the three-item FSI severity composite has been established as a cutoff for clinically significant cancer-related fatigue.

Statistical Analysis Plan:

Our primary goal is to assess the effect of NMES on: single fiber tension (force/CSA), velocity and CSA and mitochondrial content and ADP-stimulated oxygen consumption rate. A general linear mixed model will be used, with treatment group as between-subject and time as within-subject effects. A random effect will be included to account for clustering of observations within subjects (ie, fibers within each subject), as described (1), with the model conceptualized as a multi-level analysis with single fiber/organellar-level outcomes comprising level 1 variables and person-level covariates comprising level 2 variables (2). Covariates (eg, stage, chemotherapeutics, and activity level) can be included in the model as level 2 variables to control for potential confounders that differ by group. Whole muscle size and function and 6 min walk performance will be used in secondary analysis to evaluate whether changes in myofilament or mitochondrial content or function scale to variation in tissue-level or whole body function or size, as we have previously observed that molecular- and organellar-level function scale to the tissue and whole-body level in cancer (3), aging (4) and chronic disease (5).

For Aim 1, based on whole muscle results in cancer patients (3, 6), other diseased populations (7, 8) and our preliminary data, we anticipate that volunteers randomized to NMES will have greater single muscle fiber tension (20%) and CSA (20%) versus controls. We focused on these variables because they represent the two most disabling effects of cancer on muscle contractility: weakness and atrophy (3, 9). We would need n=12/group to detect these differences between groups (power=90% and significance of 5%). Of note, we will likely have greater improvements in contractile velocity, per our preliminary data, which should further increase aggregate muscle power output and functionality and is readily detectable with this sample size. For Aim 2, based on our preliminary data showing the ability of NMES to improve mitochondrial content and function in the context of a profoundly atrophyic stimulit (ie, knee replacement surgery), we expect that patients randomized to NMES will have greater mitochondrial content (50%) and function (40%). Our sample size is driven by detecting changes in mitochondrial content, which would require n=12/group with a power of 90% and a significance of 5%. Using this sample size, our minimal detectable differences for changes in mitochondrial function would be 14%. We have not performed sample size estimates on secondary outcomes because of the exploratory nature of these studies, but also because it is likely that we will either not be able to detect effects at this anatomic level because of confounding factors or we will detect effects at this level that are unrelated to the effects of NMES to improve skeletal muscle size and/or function (eg, enhanced neural activation related to NMES training improves whole muscle strength). This was a primary impetus

for our studies to evaluate the effects of NMES at the cellular/organellar level. However, we will evaluate these outcomes nonetheless, as they will provide important preliminary data for future trials if our hypotheses are supported by our results.

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Risks/Benefits:

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

Baseline/Post-intervention Testing: There is a minimal risk for hematoma or infection from the muscle biopsy. All equipment used will be sterile and every precaution will be taken to reduce the possibility of infection. Risks associated with the muscle biopsy procedure include excessive bleeding, persistent numbness or infection. Regarding bleeding risk, we will exclude any patients taking anti-coagulant medication or with known coagulopathies. All of these risks are well below 1% (81) and our laboratory has never had an adverse event related to the muscle biopsy procedure. Although evidence is limited, there is some evidence that the rhythmic muscle contraction induced by the use of NMES could increase the risk of dislodging a lower extremity blood clot. However, several published reports show that NMES reduces the risk of developing such clots. Thus, the risk of NMES causing or dislodging such a clot is minimal, but difficult to measure exactly. There is a risk for having an allergic reaction to the antiseptic that we use to clean the site of the biopsy. The risk for such a reaction is relatively low (1% or less). Finally, the adhesive strips use to close the biopsy incision could cause irritation to the skin in individuals with sensitive skin, which could cause a rash or blister. The muscle biopsy will be performed by trained, qualified clinicians in a closely controlled, medical environment. There is risk for muscle soreness or damage from whole muscle functional testing. Appropriate warm up exercises will be performed to prevent muscle injury. The DEXA scan exposes the volunteers to x-rays, although the total exposure is equivalent to approximately 1 d of normal daily background radiation.

Anti-coagulant therapy/muscle biopsy: Stage 3 and 4 cancer patients being treated at University of Vermont Medical Center typically receive prophylactic anti-coagulant therapy to prevent deep vein thrombosis. Accordingly, patients will have to discontinue this therapy just prior to the biopsy to limit the possibility of forming a hematoma or excessive bleeding during the biopsy. The following schemes detail the types of possible medications that could be taken and the discontinuation and resumption procedures for each (note: these schemes have been approved by Dr. Chris Holmes, who oversees this treatment program for patients in the Division of Hematology/Oncology):

Apixiban (Eliquis)
Prophylactic dose: 2.5 mg BID
Treatment dose: 10 mg BID for 7 days then 5mg BID

Discontinue: 48 hours prior to biopsy

Resumption: Day following the biopsy

Rivaroxaban (Xarelto)
Prophylactic dose: 10mg
Treatment dose: 15mg BID x 21 days than 20 mg daily

Discontinue: 48 hours prior to biopsy
Resumption: Day following the biopsy

Enoxaparin (Lovenox)
Prophylactic dose: 40 mg subcutaneous daily

Discontinue: If taken in the morning do not take am dose day of biopsy
If taken in the evening, hold evening dose
Resumption: Day following the biopsy if taken in the am
Evening after the muscle biopsy if taken in the evening

Treatment dose: 1mg/kg BID

Discontinue: Hold dose evening prior to biopsy
Resumption: Evening after the muscle biopsy

Dalteparin (Fragmin)
Prophylactic dose: 5,000 units subcutaneous daily
Treatment dose: 200 units/kg daily first month than 150 units/kg daily

Discontinue: If taken in the morning do not take am dose day of biopsy
If taken in the evening, hold evening dose
Resumption: Day following the biopsy if taken in the am;
Evening after the muscle biopsy if taken in the evening

UF Heparin
Prophylactic dose: 5000 units subcutaneous TID
Treatment dose: Variable

Discontinue: Day prior to biopsy
Resumption: Day following the biopsy

Aspirin or NSAIDs – Discontinue 5 days prior
Resume day following biopsy

NMES intervention: NMES is a generally safe procedure, delivered in this study by an FDA-approved device. Although evidence is limited, some groups have suggested that NMES could increase the risk of dislodging a deep vein thrombosis (DVT), which may have serious health consequences. However, several published reports show that NMES significantly reduces the risk of developing DVTs 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. Electrical stimulations associated with NMES of the upper leg may be sensed by cardiac defibrillators as an arrhythmia, causing the device to discharge inappropriately. Thus, consistent with current clinical practice guidelines, we will exclude any volunteers that currently have an implanted cardiac defibrillator or pacemaker.

Patients will be encouraged to adjust stimulation to a tolerable level and will have full control over the stimulator to continue to make adjustments as necessary. During the first couple of NMES sessions, muscle soreness may occur. The level of fatigue and/or soreness will be similar to that which occurs following a standard exercise training session and will dissipate over time as the volunteer's muscles become accustomed to the electrically-stimulated contractions (ie, they become trained). Nonetheless,

in response to concerns of the NIH reviewers, we have instituted a regimen of assessing muscle pain each week using a hand-held pressure algometer, with results being reviewed with volunteers during weekly phone contacts. Volunteers will be instructed on how to assess muscle soreness manually at standardized points along their quadriceps. Briefly, the volunteer will provide 2 kg/cm^2 of pressure using the algometer at each site, which will provide mild pressure over 1 cm^2-size of rubber algometer probe that will be below pain threshold. If muscle soreness related to the NMES is present, there may be mild soreness/pain at the site of algometer testing and the volunteer will note her level of discomfort using the Wong pain scale (0-10). Any level of discomfort greater than 6, or if the patient reports muscle soreness that hinders simple activities of daily living, such as walking, the research coordinator will contact the PI for referral to the Safety Officer. The Safety Officer will then follow up with the patient by phone (or in person, if deemed necessary) to assess the volunteer's level of discomfort and whether she should continue in the study. In our past experience with exercise training and NMES, there is some level of discomfort expected as the muscle is trained. However, in all cases, muscle soreness has not been sufficient that volunteers have chosen to discontinue the study or that it substantially hinders daily activities.

Benefits: The direct benefit of the research to volunteers is not immediately known because of the paucity of studies that have evaluate NMES in cancer survivors. NMES may improve skeletal muscle structure or function and, in turn, improve physical functional capacity. Because of this, patients may experience improved physiological capacity, which could reduce fatigue and disability. If NMES is shown to have beneficial effects on skeletal muscle, further research and application of the technique to cancer patients may help to extend the benefits of exercise to cancer survivors not able to participate in facility-based exercise training programs.

More broadly, the results of these studies may impact supportive care regimens for cancer patients in the future. Our studies will provide seminal information regarding the effects of NMES on skeletal muscle at the cellular and organellar levels. If successful, our results will provide a solid, mechanistic evidence base on which clinical trials designed to further examine the utility of NMES in cancer patients can be launched. Because of the mechanistic nature of our data, our findings may also be used to guide the development and refinement of clinical rehabilitation interventions designed to counter detrimental skeletal muscle adaptations in cancer patients, as guidelines for oncology rehabilitation are still in the early stage of development. Additionally, because we will be obtaining evidence on the mechanisms whereby NMES is affecting skeletal muscle structure and function at the fundamental molecular/cellular level, our data could stimulate further basic science research into the mechanisms of action of NMES and how stimulation of these signaling pathways counteracts the effects of cancer and its treatment. Thus, our work may identify the mechanisms whereby cancer and its treatment impact skeletal muscle size and function.

Overall, the risks to general health and well-being of the volunteers are quite minimal and, based on the utility of NMES in other diseased populations to increase muscle size and functionality, the likelihood that NMES will improve physiological function in patients is quite high. For these reasons, and together with the advances in knowledge to be gained from these studies, we believe that the risks of this study are reasonable given the potential gains in functionality for the patients and knowledge to improve exercise interventions for future cancer survivors.

Therapeutic Alternatives:

NMES seeks to provide an exercise training stimulus to volunteers to help maintain skeletal muscle size and functionality. During treatment, there is generally not an alternative exercise program available, as our institutional oncology rehabilitation program does not typically begin until after treatment is complete. This lack of therapeutic alternatives during treatment was a primary impetus for undertaking these studies. Volunteers can chose to exercise on their own, however, and not participate in the current study.

Data Safety and Monitoring:

Our study is a clinical trial, but not a phase III clinical trial. Thus, pursuant to NIH regulation, we will establish a formal Data Safety Monitoring Plan, which will provide additional oversights to further protect against risk to safety and confidentiality.

The PI (Toth) and Dr. Dittus 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. Dittus will alert the Safety Officer (Dr. Michelle Sowden), who will adjudicate the event with respect to its severity, expectedness and relatedness to participation in the study according to the aforementioned criteria. Because numerous clinical trials in our laboratory have demonstrated the safety of this regimen of testing in patients from a broad range of clinical backgrounds (eg, heart failure patients, cancer survivors, healthy elderly), we expect minimal problems related to testing. NMES is generally a very safe intervention, having been used in a variety of acute and chronic conditions, from heart failure patients awaiting transplant to ICU patients. Additionally, we will actively monitor volunteers at regular intervals (weekly phone calls) to assure that no problems develop. Thus, we do not feel that it is reasonable to incorporate "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 that individual, including instances of undue muscle soreness related to the NMES device.

Adverse Event and Unanticipated Problem (UAP) Reporting:

Adverse events will be reported by one of 3 mechanisms. First, the UVM/UVM Medical Center Committee for Human Subject Research Adverse Event Reporting Form. Report of any event will be forwarded to the office of the Committee for Human Research in the Medical Science (CHRMS) within 2 days of the PI learning about the event. Reporting adverse events will be the responsibility of the PI. The CHRMS will determine whether additional reporting requirements are indicated. Second, the Patient Safety Reporting system (SAFE) through UVM Medical Center can also be used, which may be initiated by clinical research center staff or study personnel. These forms will be forwarded within 3 days to the PI, UVM Medical Center Risk Management Office, CHRMS and other appropriate agencies, as indicated by the nature of the report. Finally, the UVM Medical Center Medication/IV Event Report Form, with distribution and timing as noted above. This latter mechanism might be used with events related to blood sampling or other invasive procedure, such as the biopsy. 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 and Dittus 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, Dr. Toth 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 Committee on Human Research in the Medical Sciences via yearly progress reports and to the appropriate NCI program officer. The report will include information regarding study status, participant information and safety information. This report will also be forwarded to the Safety Officer for his review.

Withdrawal Procedures:

Patients will be removed from study if they are found to have cancer progression during the study, if they experience a hospitalization greater than 48 hrs, if they require dexamethasone therapy for longer than 5 d, if they are non-compliant with research personnel instruction or if continued participation would not be in the best interest of the volunteer’s health.

Sources of Materials:

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. Data will be gathered from the volunteer and her medical records after she freely gives consent. 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. All materials collected for this study will be used for research purposes only.

Device (s):

The interventional device used in this trial: EMPI Continuum complete electrotherapy systems have both received FDA approval for retarding disuse-related atrophy, which we believe is one of the primary mechanisms whereby muscle adaptations evolve in cancer survivors. That is, fatigue and weakness associated with chemotherapy cause patients to be less physically active, which, in turn, 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, which we hypothesize is one of the major underlying precipitants of muscle changes in cancer patients during treatment, as others have characterized profound reductions in muscle use with cancer treatment (1).

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Risk assessment:

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 a generally safe procedure 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. 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. Thus, consistent with current clinical practice guidelines, 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. Thus, we will actively monitor muscle soreness in volunteers to prevent undue discomfort.

Subject Selection:

We propose to study NMES in breast cancer patients receiving chemotherapy for several reasons. First, breast cancer is the most prevalent cancer in women and the second most prevalent cancer overall, making our results broadly relevant. Second, women show higher disability rates than men (1, 2), which may relate to their more detrimental skeletal muscle molecular and cellular functional adaptations that we have characterized with aging (3) and muscle disuse (4, 5). Third, breast cancer patients show structural and functional muscle changes similar to those we have found in other cancers that relate to disability (6). Finally, we include a broad age range of patients because we have observed phenotypic adaptations in younger patients (mitochondrial rarefaction) and prior reports suggest that cancer and its treatment cause the greatest functional deficits in younger patients (7), arguing that younger women may benefit from NMES. We exclude “old” and “oldest old” populations (>75 yrs) because of their higher rates of co-morbidities, which could affect the ability to detect NMES effects.

We chose to examine effects of NMES during chemotherapy for several reasons. This is a logical time for exercise intervention to counteract concomitant reductions in habitual physical activity (8-10) and their physiological sequelae (11). Support of muscular activity and maintenance of physiological reserve may be critical to improving long-term outcomes, as inactivity is associated with increased mortality in breast cancer patients (12, 13). Moreover, in light of our new preliminary data in cancer survivors, this period is characterized by reductions in skeletal muscle size and function and classical exercise training can counteract these adaptations, with the latter effects being similar to those observed with NMES in

other populations. In this context, our study promotes a more proactive, preventative approach to functional deterioration in cancer patients, which, by maintaining muscle size and function, may lead to greater functional and long-term health benefits (14).

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3. Miller, M. S., Bedrin, N. G., Callahan, D. M., Previs, M. J., Jennings, M. E., Ades, P. A., Maughan, D. W., Palmer, B. M., and Toth, M. J. (2013) Age-related slowing of myosin actin cross-bridge kinetics is sex specific and predicts decrements in whole skeletal muscle performance in humans. *J Appl Physiol* **115**, 1004-1014
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Number of Subjects:

We anticipate enrolling and completing testing on n=24 patients. We will recruit 28 volunteers with the anticipation of a dropout rate of ~15%. This is a reasonable dropout rate considering that, in our experience with other clinical populations (eg, heart failure patients, advanced stage knee osteoarthritis patients) we have had minimal dropouts during facility-based exercise interventions (<5%) that pose a greater burden on volunteers than the home-based NMES intervention in the current study.

Inclusion/Exclusion Criteria:

Patients will be considered for study if they are/have/will: 1) 40-75 years of age; 2) stage I, II or III breast cancer; 3) receive neoadjuvant or adjuvant chemotherapy and 4) a body mass index <38 kg/m^2. Patients will be excluded if they have/had: 1) a prior history of cancer that required chemotherapy, excluding non-melanoma skin cancer; 2) autoimmune, vascular or neuromuscular disease that could alter skeletal muscle (eg, rheumatoid arthritis, peripheral arterial disease); 3) prior knee or hip replacement; 4) an implanted cardiac defibrillator or pacemaker; 5) a known coagulopathy, current lower extremity DVT or are taking anti-coagulant therapy (patients taking prophylactic anti-coagulation therapy are eligible if they discontinue this therapy according to the schemes detailed in the Risks/Benefits section) or 6) oral corticosteroid medication for non-cancer indications, excluding inhaled or topical formulations. We will exclude women who report being highly active prior to diagnosis (Stanford Brief Activity Survey), as these individuals may be less responsive to NMES (ie, ceiling effect), though this is likely to be a small fraction (<10%) of the overall population.

Inclusion of Minorities and Women:.

Inclusion of Women

This study will include only women. The rationale for this decision is based on our choice to study women with breast cancer, as detailed above in the *Subject Selection* section. Briefly, this decision was based partially on the fact that breast cancer is the second most prevalent type of cancer in the US, but also on work by our laboratory showing marked skeletal muscle structural and functional deficits in female breast cancer patients and that healthy older women tend to suffer greater functional deterioration with aging and the development of chronic disease compared to men. Thus, we believe that women breast cancer patients would have more necessity for alternative exercise interventions, such as NMES. We recognize that, if our studies are successful, our work will have to be duplicated in other cancer populations, including male volunteers. For these proof-of-principle studies, however, we felt that it was prudent to limit our investigation.

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 breast cancer is confined to the adult population. There may be instances where the NMES exercise intervention being tested in the proposed studies may be useful in children with cancer. However, the Belmont Report (Part C, §3) stipulates that research that does not contain a therapeutic component, or by extrapolation to the proposed studies, where the utility of the therapeutic component is in question, those less burdened populations should be called upon first to accept the risks of research. Accordingly, this would mean that adults be studied before children. If our studies are successful, future work could determine whether this training modality would be useful in childhood cancers.

Recruitment:

Patients will be recruited from the Multi-disciplinary Breast Cancer Clinic of the Division of Hematology/Oncology, as well as local, private practices.

***Consent Procedures**:*

Someone with a treatment relationship will introduce the study to potential volunteers. If the patient expresses interest in the study and would like more information about the study, the research coordinator or PI will contact the volunteer, briefly explain the study and will provide a copy of the informed consent. The PI and/or the research coordinator will answer any questions by phone or in person. Moreover, the PI or the research coordinator will discuss the protocol with the volunteer at length and answers any remaining questions.