Study Title: Effects of Exercise by Neuromuscular Stimulation in Dialysis Patients

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Effects of Exercise using Electrical Muscle Stimulation in End Stage Kidney Disease

Protocol EMS-HD-100
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A. Significance

We propose a pilot feasibility study to determine if exercise delivered by passive electrical muscle stimulation (EMS) in patients with end stage renal disease (ESRD) on hemodialysis improves physical fitness and insulin resistance, outcome markers associated with morbidity and mortality in this population.

Every year more than 105,000 people in the U.S. initiate hemodialysis for end stage renal disease (ESRD) facing the grim prognosis of a mortality rate that is 20% per year.[1] Mortality rates on hemodialysis have improved over the years. Nevertheless, compared to the general population, excess mortality for patients initiating dialysis is more than 50-fold higher for young women under 45 years of age and nearly 3-fold higher for patients over 75 years of age.[2] For age-matched patients, having ESRD requiring dialysis is associated with a significantly higher mortality than having cancer, diabetes, heart failure, stroke or an acute MI.[1] Nearly half of the deaths on hemodialysis are attributed to cardiovascular causes. Patients on hemodialysis average 1.9 hospital admissions per year, mostly for cardiovascular causes (volume overload) and infections. The need for re-hospitalization within 30 days of discharge is 36%, nearly double that of patients hospitalized without ESRD.

Despite the high mortality rate there are no interventions proven to reduce mortality in this population.[3] Large randomized trials have examined the effect of dialysis dose, normalization of anemia, use of statins, lowering phosphate with a non-calcium binder, reduction in homocysteine level, the antioxidant vitamin E, human growth hormone and cinacalcet with no improvement in overall mortality.[3] Frequent hemodialysis 6 times per week reduces left ventricular hypertrophy and may reduce morbidity and mortality but this is not a feasible option for the majority of patients who are on in-center maintenance hemodialysis 3 times per week.[4] Clearly new approaches to improve morbidity and mortality for in-center hemodialysis patients are needed.

Low physical capacity is a common, modifiable risk factor strongly associated with mortality and morbidity in ESRD patients that is ripe for intervention.[5-9] Cardiovascular fitness and muscle strength in ESRD patients is reduced to 50-80% that of untrained healthy controls.[10,11] Exercise training in patients with CKD can improve aerobic capacity, muscle strength, some nutritional measures and health-related quality of life and lower blood pressure.[12,13] In addition, exercise training has been reported to improve multiple intermediate outcomes associated with mortality in hemodialysis patients including: endothelial function[14], heart rate variability[15,16], depression[15] and insulin resistance[17].

Despite these benefits, there are no trials of exercise training on survival in patients with ESRD on hemodialysis. The effect of exercise training on survival has been reported in patients with systolic heart failure with mixed results.[18,19] A meta-analysis of 9 randomized controlled trials reported that exercise training significantly reduced mortality and death or admission to the hospital.[18] However, a subsequent large multicenter randomized controlled trial of 2331 patients with systolic heart failure (HF-ACTION trial) found a non-significant reduction in all-cause death or hospitalization (HR=0.93, P=0.13).[19] A major limitation of most exercise trials including the HF-ACTION trial is that compliance with exercise training wanes with time and does not achieve prescribed target levels, particularly when performed in a home-based, non-supervised setting.[12] In addition, it is hard to motivate older, more debilitated, sedentary or depressed individuals who might derive the most benefit from exercise training to participate.

To address these concerns, we propose a pilot feasibility study to test a novel approach using passive electrical muscle stimulation (EMS) given 3 times a week to both quadriceps muscle groups while on hemodialysis for 16 weeks to determine whether this improves physical fitness, insulin resistance and other outcome markers associated with well being, morbidity and mortality in this population. In patients without CKD, EMS has been reported to improve peak VO_2 and endothelial

function, increase muscle strength and 6 minute walk time, improve insulin sensitivity and decrease inflammatory cytokines (tumor necrosis factor-alpha, TNFα and interleukin-6, IL-6).[20-28] In preliminary studies of patients with metabolic syndrome, we also have evidence that 8 weeks of EMS can reduce inflammatory cytokines (IL-6 and TNFα) and improve insulin sensitivity (as assessed by the homeostatic model assessment, HOMA-IR, unpublished data). However, patients with ESRD may be slow to respond to exercise and generally at least 12 weeks of exercise is recommended to observe an effect.[12]

EMS appears safe and may be beneficial to perform during hemodialysis.[29] In one acute study of patients on hemodialysis, passive EMS was found to increase phosphate and urea removal while helping to maintain blood pressure.[29] The improved phosphate removal is attributed to increased muscle perfusion allowing better equilibration of phosphate from the intracellular compartment into the blood stream. Elevated serum phosphate is linked to poor outcomes for hemodialysis patients.[30] Control of serum phosphate is difficult for many patients on hemodialysis and administration of phosphate binders with meals is one of the major contributors to the excessively high pill burden for these patients.[31] Lowering this burden would likely improve a patient's quality of life. Overall, we expect EMS will be safe during hemodialysis and will lower the threshold for patients to initiate and sustain exercise training. Administration as part of the routine dialysis prescription will help promote long-term exercise compliance in a supervised setting. Improvement in phosphate control and blood pressure stabilization may be additional benefits.

Insulin resistance as measured by HOMA-IR and the leptin-adiponectin ratio (LAR) will be the primary outcome measure. Insulin resistance in muscles is a known consequence of chronic kidney disease[32] and independently associates with cardiovascular mortality.[33] HOMA-IR and LAR are known to correlate strongly with insulin resistance as measured by the gold standard, hyperinsulinemic euglycemic clamp (HEGC) technique in patients with ESRD.[34] HOMA-IR and LAR can be measured by a blood test and are technically more feasible for these intervention studies. Insulin resistance in CKD may be mediated in part by adipokines released from increased visceral fat stores.[35-37] Exercise training can reduce visceral fat, which may be one mechanism leading to improvement in insulin resistance.[38,39] Hence, estimation of changes in visceral fat (by DXA and anthropometric measurements) will be important secondary outcomes for this clinical trial.

Elevations in inflammatory cytokines such as IL-6 are strongly associated with mortality in ESRD [40-42] and the effect of EMS on these cytokines will be measured. Other secondary outcomes of importance include measurement of change in aerobic capacity, quadriceps strength, mobility, nutrition and malnutrition-inflammation score (MIS), serum markers of oxidative stress and effect on depression and quality of life.

B. Innovation

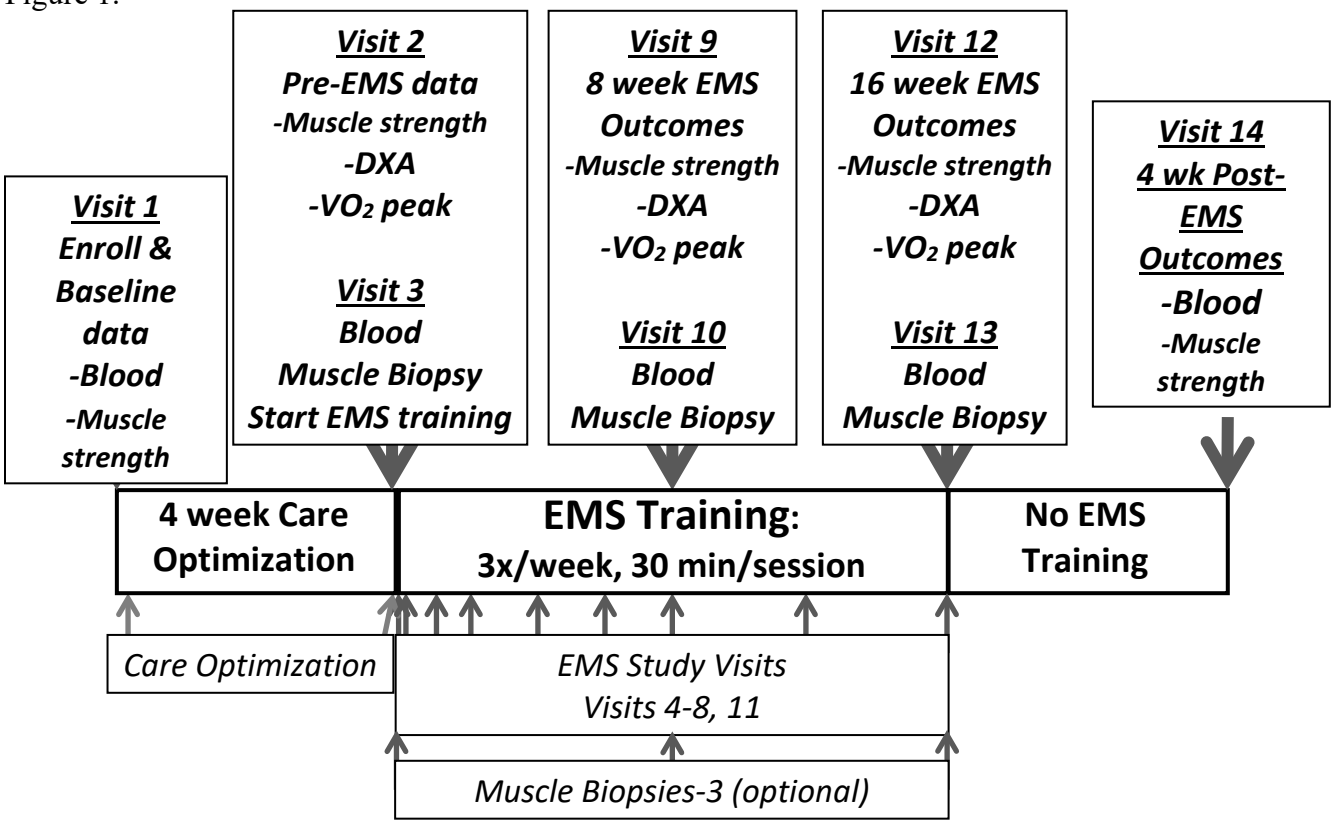
Passive exercise provided by EMS 3 times a week is an innovation that could finally bring the established benefits of regular exercise to hemodialysis patients. The benefits of passive EMS are similar to traditional exercise programs (vida supra). However, it addresses many of the barriers that limit implementation of traditional exercise programs in this population. Barriers to exercise include physiological, behavioral, psychological, and social/financial factors, including medical co-morbidities, patient motivation, inanition, depression, and the availability of suitable tools to overcome physical limitations (e.g. inability to walk due to a diabetic foot ulcer). Passive EMS which can be provided in the hemodialysis unit addresses each of these barriers. While it requires consent (like hemodialysis), passive EMS recommended by the dialysis provider can overcome lack of motivation, inanition and depression. By reducing depression, improving mobility and enhancing quality of life,

passive EMS may reinforce its own use. Passive EMS should be safe and effective to use in most all patients on hemodialysis, with the exception of patients with pacemakers. Administration during the dialysis session addresses patient concerns regarding lack of time for exercise and access to suitable tools to exercise. It will also improve intradialytic clearance of phosphate and may stabilize intradialytic blood pressure that could have additional benefits in this population. Moreover, exercise training achieves the best results when it is supervised. Provision within the dialysis unit as part of the dialysis prescription provides the external motivation and social support of dialysis unit staff and other patients to help sustain this treatment. Finally, if passive EMS were shown to improve outcomes and lower annual cost per patient for in-center hemodialysis it would be readily adopted by dialysis providers and third-party payers.

C. Approach

1. Overview. We propose a clinical study to test the feasibility and efficacy of 16 weeks of passive exercise induced by electrical muscle stimulation (EMS) to improve markers of insulin resistance associated with morbidity and mortality in hemodialysis patients (Figure 1). EMS is provided sequentially for 15 minutes to both the right and left quadriceps muscle groups three times a week. The primary outcome measure will be improvement in insulin resistance measured using the homeostatic model assessment (HOMA-IR) and leptin-adiponectin ratio (LAR).

Figure 1.



The efficacy of the exercise training will be assessed by change in aerobic capacity measured by peak VO_2 and quadriceps muscle strength. Additional measures will include measurement of fat and

muscle content by dual energy X-ray absorptiometry (DXA) and anthropometry, measures of physical activity, nutritional status, depression, quality of life, anemia, mineral metabolism, dialysis blood pressure and serum levels of inflammatory cytokines and markers of oxidative stress.

Following enrollment, baseline data and selected outcome markers will be assessed. Then all patients will undergo a 4-week care optimization phase during which time we will review the patient's dialysis care and make adjustments (if necessary) to meet standard of care guidelines as defined by CMS, KDOQI and KDIGO (Table 1). Outcome markers will be reassessed again after completion of the 4-week care optimization phase. At this time all patients will also undergo a baseline exercise test to measure peak VO_2 and a DXA scan for body composition analysis before starting EMS training.

Table 1	
Parameter	**Optimized care goals**
Vascular access	Discontinuation of central venous catheter and use of arteriovenous access
Fluid removal	Optimize dry weight. Limit UF rate to ≤13 ml/kg/hr
Solute removal	Minimum dialysis time ≥ 3.5 hours. Goal 4 hours. kT/V ≥ 1.4
Acid-base	Pre-dialysis HCO3 between 22-27 mEq/L
Anemia	Hemoglobin 9.5-11 g/L, if on erythropoietin; Iron saturation ≥20%
Ca, PO4, PTH	Calcium, PO4 within normal levels; PTH ≤600 pg/ml

EMS training will be done while the patient is on hemodialysis. At the first EMS training session, optimal EMS settings will be established and patients will be taught how to apply the electrodes and use the muscle stimulator. For patients who agree we will also do a muscle biopsy of the quadriceps (vastus lateralis) muscle at this first training visit before starting EMS training. Patients will be seen at the next dialysis session and again after 7 days to confirm that they are doing the EMS training properly. Patients are then seen at Day 14, 28, 42, 56, 84 and 112 after starting EMS training to assess for any adverse events, monitor progress with EMS training and adjust the EMS settings to make sure they are at the optimal tolerated intensity of muscle stimulation. The EMS training will continue for 16 weeks. At Day 56 and Day 112 (8 and 16 weeks) of EMS-training all the primary and secondary outcome variables including exercise stress test for peak VO2 and DXA scan will be performed. For patients who agree we will also perform a muscle biopsy at a dialysis visit around Day 56 and Day 112 (not the same day as the exercise stress test). One month after stopping EMS training the patient will be seen and the outcome variables will be repeated except for the DXA scan and the exercise stress test.

2. Intervention. EMS is delivered to the quadriceps muscle groups using a neuromuscular electrical stimulator (using either the EMPI 300PV or its replacement, the Continuum device, EMPI, Inc). The device generates electrical impulses that are delivered to nerves stimulating the muscles through electrodes applied to the skin surface. Patients are taught how to place the adherent electrode pads over the quadriceps muscles on the upper thigh a few inches below the anterior iliac crest and just above the patella bilaterally. The electrodes will be applied at home or after coming to the dialysis unit just prior to each dialysis session. The patient's legs are extended straight on the reclining dialysis chair. The device is set to deliver biphasic electrical stimulation at 1-20 Hz and a pulse duration of 400 μS applied for 1-12 seconds on and 1-12 seconds off. The stimulus intensity (typically 10-100 mA) is gradually increased to the maximum tolerable level for muscle contraction. The patient will feel contraction of the muscles and may feel mild shortness of breath and tingling of the skin but otherwise is expected to tolerate the stimulation well. The stimulation is delivered to one side for 15 minutes and then to the

opposite side for 15 minutes. This will be repeated at each dialysis session 3x/week. The first intervention will be observed through the entire treatment. If the patient feels excessively fatigued or other untoward signs the stimulator will be turned off until they feel better and then restarted to achieve the full treatment time.

At each study visit the study coordinator will confirm that the settings are correct and readjust the maximal tolerated stimulus intensity as needed to account for training. Patients will also be encouraged to be active and exercise as much as possible outside of the dialysis unit.

The total duration of the study for any patient is expected to be 6 months. We plan to complete the study of all patients within 2 years.

3. Study population.

Patients on hemodialysis three times a week at participating study sites within the UIHC dialysis program will be eligible to participate.

3.1 Inclusion & exclusion criteria.

Inclusion criteria include: age over 18 year old, able to provide informed consent, on maintenance hemodialysis for end stage renal disease and expected to stay in a participating dialysis unit for at least 6 months.

Exclusion criteria include: presence of a cardiac pacemaker or presence of any other implanted electrical stimulation device, uncontrolled hypertension (SBP>170 mmHg), unstable angina, heart attack within the last month, expected survival <6 months, unsuitable for participation based on physician assessment, unwilling or unable to give informed consent.

4. Outcome measures.

4.1 Primary Outcome.

The primary outcome measure will be the change in insulin resistance as determined by either HOMA-IR or LAR after 8 and 16 weeks of EMS training. The analysis will also be stratified by whether patients achieved optimum dialysis care goals at randomization to see if optimization of care influenced the response to EMS. The correlation between change in HOMA-IR or LAR with change in aerobic capacity (peak VO_2) and quadriceps strength after 16 weeks training and with maximal EMS training intensity (in mV) achieved will be assessed to determine if change in insulin resistance depends on intensity of training.

4.2. Secondary outcomes measures.

4.2.1. Aerobic capacity and quadriceps strength. The effect of EMS on quadriceps muscle strength will be assessed 5 times (Visits 1, 2, 9, 12 and 14 in Figure 1) : at baseline, pre-EMS training, at 8 and 16 weeks of EMS training and again 4 weeks after stopping EMS training. Quadriceps muscle strength will be determined using a belt-stabilized portable dynamometer.[43] Change in peak VO_2 will be measured 3 times (Visits 2, 9 & 12): pre-EMS training and after 8 and 16 weeks of EMS training. Peak VO_2 will be determined using graded exercise treadmill test using a Balke protocol. Cycle ergometry will be used to determine peak VO_2 in patients who can't walk on a treadmill but can do cycling. This will determine if EMS produced an increase in quadriceps strength or improvement in aerobic capacity after 8 and 16 weeks of training. It will also determine how rapidly the effect on muscle strength is lost after stopping EMS training.

4.2.2. Body and leg composition changes. The effect of EMS on total body fat and lean mass, visceral fat mass and regional thigh fat and lean mass will be determined by DXA scanning. DXA scanning will be done 3 times: pre-EMS training and after 8 and 16 weeks of EMS training. The DXA results will be supplemented by measuring the effect of EMS on anthropometric measures including: body mass index (BMI), waist (minimal circumference) to hip (maximal gluteal protuberance) ratio, anterior-posterior diameter at the umbilicus and thigh circumference (1/2 way between the anterior iliac crest and upper border of the patella, bilaterally). The anthropomorphic studies will be performed 5 times: at baseline, pre-EMS, after 8 and 16 weeks of EMS training and 4-weeks after stopping training. Collectively these studies will determine whether EMS had an effect on body fat or muscle composition. Of particular interest is change in visceral/abdominal fat and change in quadriceps muscle mass after 8 and 16 weeks of EMS training.

4.2.3. Measures of physical activity. Changes in physical activity will be assessed at 5 time points: baseline, pre-EMS, after 8 and 16 weeks of EMS training and 4 weeks after stopping EMS training using a standardized 6-minute walk test and by monitoring 48 hour activity level between dialysis sessions using a wristwatch activity monitor. The 6-minute walk test is determine by measuring the distance a patient can walk on a standardized track within 6 minutes at whatever pace they choose to walk.

4.2.4. Malnutrition-inflammation (MIS) assessment. The MIS is a 10 item test which includes assessment of change in patient dry weight over 3-6 months, dietary intake, gastrointestinal symptoms, functional capacity, major co-morbidities, decreased fat mass, signs of muscle wasting, BMI, serum albumin and serum TIBC.[44] It has been validated as a predictor of mortality in hemodialysis patients.[44,45] MIS will be assessed at 5 time points: baseline, pre-EMS, after 8 and 16 weeks of EMS training and 4-weeks after completion of EMS training.

4.2.5. Depression assessment. Assessment of depression will be performed using the Patient Health Questionaire-9 (PHQ-9) at 5 time points: baseline, pre-EMS, after 8 and 16 weeks of EMS training and 4-weeks after completion of EMS training. The PHQ-9 has been validated as a screening tool for detection of depression in ESRD patients with a score ≥ 10 having both a sensitivity and specificity of 92% for detecting depression compared to the Structured Clinical Interview for Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition as the gold standard.[46] PHQ-9 has been used as a screening tool for depression in the ACCORD trial and shown to associate with overall mortality. [47]

4.2.6. Quality of life will be assessed using the Kidney Disease Quality of Life-36 (KDQOL-36) form at 5 time points: baseline, pre-EMS, after 8 and 16 weeks of EMS training and 4-weeks after completion of EMS training. The KDQOL-36 is a validated standardized form used to assess quality of life in hemodialysis patients that is associated with morbidity and mortality.[48,49]

4.2.7. Inflammatory cytokines. The level of inflammatory cytokines, particularly IL-6 and the inflammatory marker, C-reactive protein (CRP) are strongly associated with mortality in dialysis patients [50,51] and can be modulated by exercise[52]. To determine if EMS can decrease the levels of these inflammatory cytokines, we will measure IL-6, TNFα and CRP at 5 time points: baseline, pre-EMS, after 8 and 16 weeks of EMS training and 4-weeks after completion of EMS training.

4.2.8. Oxidative stress markers. Markers of oxidative stress are increased in patients with ESRD [53-55] and associated with increased mortality.[56,57] Treatment with the antioxidant alpha tocopherol has been reported in a small study to decrease mortality.[58] Chronic exercise training can reduce oxidant stress and may be one mechanism whereby exercise improves cardiovascular outcomes.[59,60] To determine whether EMS alters markers of oxidative stress we will measure serum F2-isoprostane (a measure of lipid oxidation), protein thiols (a measure of protein oxidation) and the ratio of reduced to oxidized glutathione (GSH/GSSG) in erythrocytes.[61-63] To assess the effect of EMS on antioxidant levels we will measure erythrocyte glutathione peroxidase (GPx), superoxide dismutase (SOD) and catalase (CAT) activity.[64] These markers will be measured at 5 time points: baseline, pre-EMS, after 8 and 16 weeks of EMS training and 4-weeks after completion of EMS training.

4.2.9. Anemia is a marker of inflammation[65] and associated with morbidity and mortality in dialysis patients.[66,67] The blood levels of hemoglobin, iron saturation and ferritin, as well as the dose of erythropoietin and iron administered are tracked monthly as routine standard of care for hemodialysis patients. We will collect each of these variables from the patient chart starting with the labs drawn just before enrollment and continuing through labs drawn 4 weeks after completing the EMS training. We will plot this data to see whether any change occurred during treatment with EMS.

4.2.10. Serum phosphorus is associated with mortality for hemodialysis patients.[68] Control of phosphorus levels using phosphate binders taken 3 times a day with meals is one of the major causes of excessive pill burden for these patients.[31] EMS has been shown to increase intra-dialytic phosphate removal and may help lower serum phosphate or reduce pill burden for these patients.[29] Serum phosphorus, calcium, and PTH as well as dose of phosphate binders prescribed are recorded monthly for hemodialysis patients as part of standard of care. We will collect each of these variables from the patient chart starting with the labs drawn just before enrollment and continuing through labs drawn 1 month after completing the EMS training. We will plot this data to see whether any change occurred during treatment with EMS.

4.2.11. Blood pressure and dialysis hypotension. Maintenance of blood pressure on dialysis can be a challenge for some patients, particularly those with large intra-dialytic fluid gains, and is associated with poor outcomes.[69] A prior study of EMS during hemodialysis found that EMS produced a statistically significant increase in BP and fewer hypotensive episodes compared to no EMS.[29] For the first EMS treatment in each patient we will directly record BP and pulse at 15-minute intervals before, during and after treatment with EMS. Thereafter, we will collect the BP and pulse data that is recorded (approximately every 15-30 minutes) as standard of care for every hemodialysis session. We will compare the average hemodialysis BP and pulse during the 4-week "care optimization" phase to that obtained during the EMS treatment and the 4-week post-EMS treatment phases to determine if EMS was associated with any change in BP or pulse. We will also analyze the frequency of dialysis sessions with a hypotensive episode (defined as SBP<90 mmHg or need for IV saline for hypotensive symptoms) during EMS compared to non-EMS treatment.

4.2.12. Muscle gene expression. Gene expression profiling of mRNA extracted from a skeletal muscle biopsy will be performed to give insight into the biological pathways activated by EMS both acutely and with chronic training in hemodialysis patients. Patients will be allowed to opt out of any of the muscle biopsy samples and still participate in the rest of the study. For these studies a muscle biopsy will be obtained as previously described[70] from the vastus lateralis muscle prior to the first EMS

treatment and after 8 and 16 weeks of EMS training to determine the chronic effect of EMS training on muscle gene expression. Gene expression analysis will be done using Affymetrix Human Exon 1.0 ST arrays as previously described.[70] We will first examine specific genes that are highly overexpressed or under-expressed after acute and chronic exercise compared to the baseline (pre-EMS). We will also look for biological pathways activated or suppressed by acute and chronic exercise specifically looking at pathways relating to muscle glucose metabolism and insulin response, mitochondrial biogenesis and function, muscle phenotype, growth and energy metabolism, oxidant stress and inflammation. A future goal of this research is to compare the gene expression response to EMS in this study with that seen in patients with normal renal function and varying levels of CKD to see if the gene response profile is qualitatively different in patients with varying levels of kidney function. We are also interested how the gene expression profiles seen with EMS compare to that seen with traditional volitional aerobic exercise to determine if EMS activates different genes or biological pathways than volitional exercise.

5. Visits & Assessments.

Patients will be identified from the population of patients on regular maintenance hemodialysis at one of the University of Iowa dialysis units. Potential subjects will be approached at a dialysis visit and informed of the study.
5.1. Visit 1. If they indicate an interest in participating we will proceed with obtaining written informed consent and confirm that they meet all the inclusion criteria and none of the exclusion criteria.
- Obtain informed consent to participate and consent to access additional appropriate medical records
- Review inclusion and exclusion criteria

Baseline data collection (Day -35 to -28). Performed at dialysis unit or CRU as appropriate. Up to 4 hours time.
- Record demographics and baseline disease characteristics
- Record past medical history and current review of symptoms
- Record current dialysis orders
- Record episodes of dialysis hypotension (SBP<90 or symptoms needing saline, last 6 HD sessions)
- Record baseline exercise status (minutes of exercise per week)
- Record concomitant medications
- Malnutrition-inflammation (MIS) assessment
- Kidney Disease Quality of Life-36 (KDQOL-36) assessment
- Patient Health Questionaire-9 (PHQ-9)
- Record sitting blood pressure and pulse
- Physical examination by a physician
- 6-minute walk test
- Record height, weight, waist circumference, hip circumference
- Thigh circumference bilaterally
- Record most recent standard dialysis monthly lab tests.
    - Monthly labs (predialysis): albumin, alkaline phosphatase, bicarbonate, blood urea nitrogen, calcium, chloride, complete blood count, creatinine, iron studies, ferritin, hemoglobin A1c (if available), phosphorus, potassium, sodium, total protein, 25 hydroxy vitamin D. eGFR calculation by modified MDRD equation from enzymatic creatinine determination)
    - Monthly labs (postdialysis): blood urea nitrogen. Calculated urea reduction ratio from pre and

post dialysis BUN.

- Fasting blood sample (30 ml) for:
  - Insulin and glucose
  - Interleukin-6 (IL-6), tumor necrosis factor alpha (TNFα), C-reactive protein
  - Glutathione (reduced and oxidized forms), glutathione peroxidase (GPx), superoxide dismutase (SOD), catalase (CAT), F2 isoprostane and protein thiols.

- Measure quadriceps muscle strength (bilaterally)
- 48 hour activity monitoring (using wrist watch monitor between dialysis sessions).

5.2. Visit 2. Pre-EMS training data collection (Day -7 to 0). Performed at dialysis unit or CRU where specified. Time required up to 4 hours.

- Record any new medical conditions, symptoms or complaints
- Record current dialysis orders
- Record episodes of dialysis hypotension (SBP<90 or symptoms needing saline, last 6 HD sessions)
- Record current exercise status (minutes of exercise per week)
- Record concomitant medications
- Malnutrition-inflammation (MIS) assessment
- Kidney Disease Quality of Life-36 (KDQOL-36) assessment
- Patient Health Questionaire-9 (PHQ-9)
- Record vital sign measurements (predialysis or non-dialysis day, same timing as baseline measures)
- 6-minute walk test
- Record weight, waist circumference, hip circumference
- Thigh circumference bilaterally
- Record most recent dialysis monthly lab tests. (Drawn no more than 7 days prior to optimization visit)
  - Monthly labs (predialysis): albumin, alkaline phosphatase, bicarbonate, blood urea nitrogen, calcium, chloride, complete blood count, creatinine, iron studies, ferritin, hemoglobin A1c (if available), phosphorus, potassium, sodium, total protein, 25 hydroxy vitamin D. eGFR calculation by modified MDRD equation from enzymatic creatinine determination)
  - Monthly labs (postdialysis): blood urea nitrogen. Calculated urea reduction ratio from pre and post dialysis BUN.

- Fasting blood sample (30 ml) for:
  - Insulin and glucose
  - Interleukin-6 (IL-6), tumor necrosis factor alpha (TNFα), C-reactive protein
  - Glutathione (reduced and oxidized forms), glutathione peroxidase (GPx), superoxide dismutase (SOD), catalase (CAT), F2 isoprostane and protein thiols.

- Measure quadriceps muscle strength (bilaterally)
- 48 hour activity monitoring (using wrist watch monitor between dialysis sessions).

- DXA scan (performed in CRU)

- Perform exercise stress test to determine peak VO_2 (performed in CRU)

5.3 Visit 3. Initiate EMS training (Day 0). Performed in dialysis unit and CRU. 60-75 minutes. 30 minutes extra for optional muscle biopsy, if performed.

- Perform pre-EMS muscle biopsy before starting dialysis (optional)
- EMS training is done while on hemodialysis starting approximately 30 min after dialysis initiation.
  - Record pre-EMS blood pressure and pulse
  - Initiate EMS training on quadriceps muscle of leg not used for muscle biopsy for 15 minutes while on dialysis
  - Measure and record BP and pulse before and just prior to completion of EMS training.
  - Record any symptoms related to EMS
  - Train patient how to use EMS device.

5.4 . Visits 4-8, 11. EMS training visits (Day 2/3 and 7). Performed in dialysis unit. Time required up to 45 minutes.

- Record any new medical conditions, symptoms or complaints
  - Observe patient initiate and perform EMS training.
  - Adjust stimulus intensity to maximum tolerated by patient for both legs
- Record EMS setting used.
- Record pre-dialysis sitting BP and pulse
- Record post-dialysis sitting BP and pulse

5.5 Visits 9 &12. Outcome assessment visits (Day 56 & 112 ± 7 days). Performed in dialysis unit or CRU where specified. Time required up to 4 hours.

- Record any new medical conditions, symptoms or complaints
- Record current dialysis orders
- Record episodes of dialysis hypotension (SBP<90 or symptoms needing saline, last 6 HD sessions)
- Record current exercise status (minutes of exercise per week)
- Record concomitant medications
- Malnutrition-inflammation (MIS) assessment
- Kidney Disease Quality of Life-36 (KDQOL-36) assessment
- Patient Health Questionaire-9 (PHQ-9)
- Record vital sign measurements (predialysis or non-dialysis day, same timing as baseline measures)
- 6-minute walk test
- Record weight, waist circumference, hip circumference
- Record thigh circumference bilaterally
- Record most recent dialysis monthly lab tests.
  - Monthly labs (predialysis): albumin, alkaline phosphatase, bicarbonate, blood urea nitrogen, calcium, chloride, complete blood count, creatinine, iron studies, ferritin, hemoglobin A1c (if available), phosphorus, potassium, sodium, total protein, 25 hydroxy vitamin D. eGFR calculation by modified MDRD equation from enzymatic creatinine determination)

- Monthly labs (postdialysis): blood urea nitrogen. Calculated urea reduction ratio from pre and post dialysis BUN.

- DXA scan (performed in CRU)
- Measure quadriceps muscle strength (bilaterally)
- Perform exercise stress test to determine peak VO_2 (performed in CRU)
- 48 hour activity monitoring (using wrist watch monitor between dialysis sessions).

5. 6 Visits 10 & 13 Outcome assessment visits (Day 56 & 112 ± 7 days).
- Fasting blood sample (30 ml) for:
  - Insulin and glucose
  - Interleukin-6 (IL-6), tumor necrosis factor alpha (TNFα), C-reactive protein
  - Glutathione (reduced and oxidized forms), glutathione peroxidase (GPx), superoxide dismutase (SOD), catalase (CAT), F2 isoprostane, protein thiols.
- Muscle biopsy (optional)

5.7. Visit 14. Post-EMS visit (Day 142 - 149). Performed in dialysis unit. Time required 2-3 hours.
- Record any new medical conditions, symptoms or complaints
- Record current dialysis orders
- Record episodes of dialysis hypotension (SBP<90 or symptoms needing saline, last 6 HD sessions)
- Record current exercise status (minutes of exercise per week)
- Record concomitant medications
- Malnutrition-inflammation (MIS) assessment
- Kidney Disease Quality of Life-36 (KDQOL-36) assessment
- Patient Health Questionaire-9 (PHQ-9)
- Record vital sign measurements (predialysis or non-dialysis day, same timing as baseline measures)
- 6-minute walk test
- Record weight, waist circumference, hip circumference
- Record thigh circumference bilaterally
- Record most recent dialysis monthly lab tests. (Drawn no more than 7 days prior to optimization visit)
  - Monthly labs (predialysis): albumin, alkaline phosphatase, bicarbonate, blood urea nitrogen, calcium, chloride, complete blood count, creatinine, iron studies, ferritin, hemoglobin A1c (if available), phosphorus, potassium, sodium, total protein, 25-hydroxy vitamin D. eGFR calculation by modified MDRD equation from enzymatic creatinine determination)
  - Monthly labs (postdialysis): blood urea nitrogen. Calculated urea reduction ratio from pre and post dialysis BUN.

- Fasting blood sample (30 ml) for:
  - Insulin and glucose
  - Interleukin-6 (IL-6), tumor necrosis factor alpha (TNFα), C-reactive protein
  - Glutathione (reduced and oxidized forms), glutathione peroxidase (GPx), superoxide dismutase (SOD), catalase (CAT), F2 isoprostane, protein thiols.

- Measure quadriceps muscle strength (bilaterally)
- 48 hour activity monitoring (using wrist watch monitor between dialysis sessions).

6. Patient risks.

6.1. Risk attributed to use of EMS device:
6.1.1. Muscle stimulation. Most patients will feel muscle contractions and perhaps some tingling of the skin but this should not be painful or harmful. There may be some muscle soreness initially as expected with new exercise but this should abate in a few days. The risks are minimal.

6.1.2 Muscular tetany. If EMS is misused it could cause muscular tetany leading to muscle pain and muscle breakdown (rhabdomyolysis). The stimulation intensity will be monitored so the risk will be minimal. Patients will have control of the EMS device to turn it off or decrease the stimulation intensity if it is causing pain. They will be monitored clinically for signs of excessive muscle pain and told to decrease the stimulus intensity or cut down the length of training as needed to prevent this complication.

6.1.3. Pacemaker or electrical stimulator complications. The electrical impulses generated by the EMS device might interfere with a cardiac pacemaker or other CNS/nerve stimulators. Patients with any implanted electrical device will be excluded from the study.

6.1.4. Electric shock. If the device is not properly maintained or defective then it could deliver an electric shock. This risk will be minimized by maintaining and using the device per manufacturer's instructions and have biomedical engineering check the device before use.

6.1.5. Skin burns. This is theoretically possible if the electrodes are kept in place and stimulated for prolonged periods of time. The risk is minimal in the current study since skin contact time is limited.

6.1.6. Skin bruising. Skin blood vessels may be damaged by the electrical stimulation and bruising could develop. The risk is minimal and will be monitored. EMS treatment parameters will be adjusted if this is clinically significant.

6.2. Risks attributable to increased exercise from EMS or exercise stress tests for VO_2 peak:
Potential risks seen with the exercise stress testing for VO_2 peak or EMS training are listed below along with the proposed management. All patients will have a formal exercise stress test to determine VO_2 peak in the CRU before starting EMS. A physician will be immediately available for these tests. Problems seen with exercise will be detected during that initial testing and addressed before proceeding with EMS training. The cardiopulmonary demand of EMS will be less than the maximal exercise stress test.

6.2.1. Angina or ischemic EKG changes. Exercise will be terminated and the patient monitored until the angina or EKG changes resolve. The study physician will be contacted. If the chest pain or EKG changes persist the patient will be transported to the ER for evaluation. Additional physician evaluation will be needed before the patient is allowed to continue in the study.

6.2.2. Arrhythmia. Complex arrhythmias or second or third degree heart blocks will lead to immediate termination of the exercise stress test. The study physician will be contacted. The patient will be

monitored until the arrhythmia resolves and the primary physician will be notified to follow-up on the results. Additional cardiology evaluation will be required before the patient can proceed in the study.

6.2.3. Hypertension. Systolic BP >250 mmHg or diastolic > 120 mmHg will be grounds to terminate the exercise stress test and contact the study physician. The patient will be monitored until the blood pressure returns to baseline. The patient's blood pressure management will be reviewed before proceeding into the EMS training phase of the study. An EMS session will be terminated for a systolic BP>200 or diastolic BP>110.

6.2.4. Hypotension. Systolic BP dropping more than 20 mmHg during the exercise stress test will be grounds for terminating the test. The study physician will consult with the primary care physician about further cardiology evaluation before proceeding with the EMS training. An EMS session will be terminated for a systolic BP<90.

A prior study of EMS during hemodialysis found that EMS produced a statistically significant increase in BP (SBP increased by 8 mmHg and DBP increased by 3 mmHg) while the device was turned on compared to when it was turned off.[29] Overall the average BP on dialysis was higher when people used EMS for 15 minutes out of every hour while on dialysis compared to no EMS (average BP = 125/66 with intermittent EMS compared to 121/64 when no EMS was performed). Fewer hypotensive episodes during a dialysis session were seen when patients used intermittent EMS compared to no EMS.

6.2.5. Fracture. Stress testing or EMS could lead to a bone fracture particularly in a patient with severe osteoporosis but this is considered unlikely to occur. EMS is also being studied as a mechanism to prevent osteoporosis.

6.2.5. Dizziness or confusion. Dizziness or confusion occurring during the exercise stress test or during an EMS training session is grounds for terminating the exercise and monitoring the patient until the symptoms resolve. The study physician will evaluate the circumstances of the event to decide whether further evaluation is needed and whether the patient can continue in the study.

6.2.6. Hypoglycemia. Low blood sugar with exercise might occur particularly in patients who are diabetic. If symptoms of hypoglycemia occur then the exercise will be terminated and sugar administered to the patient. A blood sugar will be measured and the study physician notified. This is unlikely to occur during EMS as the patient will be on hemodialysis, which provides glucose in the dialysate.

6.3. Risk of phlebotomy.

Phlebotomy may cause discomfort due to needle puncture, hematoma formation, blood loss, infection or syncope. It will be performed at the time of dialysis from your dialysis needle by trained personnel to limit risk.

6.4. Risk of radiation from a DXA scan.

A DXA scan delivers a dose of radiation that is approximately one tenth that of a standard chest X-ray and equivalent to the dose of radiation received during one-day exposure to natural background radiation.

6.5. Risk of taking health and depression assessment questionnaires.

Questions about personal health or depression might raise anxiety or concerns about personal health. If this occurs these will be addressed with the patient by a member of the study team or referred to their primary provider or social worker as appropriate.

6.6. Risk of a muscle biopsy under ultrasound guidance.

The muscle biopsy may cause transient pain from the injection of lidocaine, infection or hematoma at the biopsy site. There may be soreness or a small bump at the biopsy site for a few days that will resolve. Risks are limited by having the procedure done by trained investigators under aseptic technique using an automated biopsy gun (Temno) under ultrasound guidance.

6.7. Risk of gene expression analysis.

No risks are expected to occur from gene expression analysis. Gene expression analysis looks at mRNA expression and not DNA sequence. We will not be doing DNA analysis and will not investigate a patient's genetic mutations or susceptibility to disease.

6.7. Risk of loss of confidentiality.

Data collected for this study will be labeled with a unique patient study number that does not identify the patient. The data will be stored in study binders in a secure locked room and electronically in folders on a network server that is password protected. The link between patient study number and patient identification information such as name or hospital number will be stored separately. Access to the study data will be limited to members of the study team who require access and regulatory bodies such as the IRB. Blood or tissue samples will be labeled and stored only with the de-identified patient study number.

7. Safety monitoring plan

An independent physician from the University of Iowa Department of Medicine who is not an investigator in this research study and has no conflicts of interest with the proposed research will be asked to periodically review patient safety data and provide written feedback on whether the study can continue as planned. The reviews will occur every 3 months starting after the first patient is enrolled. The Independent Safety Monitor (ISM) will be provided a copy of all the adverse events that we have recorded for patients in the study. If an unexpected SAE occurs that is possibly or probably related to the study procedures or intervention then the ISM (and IRB) will be notified of this as soon as the investigators become aware of the event. The ISM will be asked to provide written documentation of whether the study can continue as planned, should be modified or should be terminated. The IRB will be notified expeditiously if the ISM feels the study should be modified or terminated. If the ISM feels the study can continue as planned then this information will be provided to the IRB at the time of annual continuing reviews.

8. Statistical analysis/Power calculations.

This is a pilot feasibility and preliminary efficacy study to determine whether EMS can be delivered to this population and to assess the magnitude of the change and variability in the primary and secondary outcome measures. Data derived from this study will allow planning of a larger randomized controlled study. We plan to recruit up to 15 patients into the trial and complete the trial within 1 year. There will be 5 measurements of the primary and most of the secondary outcome variables in each patient. These will be performed at enrollment, after 4 weeks of "dialysis care

optimization" before starting the EMS intervention, then at 8 and 16 weeks after initiation of the EMS intervention and finally 4 weeks after stopping EMS. The DXA scan, measurement of peak VO_2 will only be performed three times (before starting EMS and then again at 8 and 16 weeks of EMS training). The optional muscle biopsy for gene expression analysis will be performed up to 4 times in patients who agree to this analysis (before and after the first EMS treatment, at 8 and 16 weeks of EMS training). Summary statistics will be used to describe and analyze the data. Changes over time in the outcome variables will be analyzed using the Friedman analysis of variance by ranks to determine if there was a statistically significant effect of EMS. A $P<0.05$ will be considered statistically significant. Regression analysis will be used to determine if the change in HOMA-IR or LAR is correlated with the magnitude of the observed exercise training effect (i.e. the increase in peak VO_2 or quadriceps muscle strength at 4 months) or maximum intensity of EMS stimulation used during training.

Based on an estimated baseline mean (± SD) HOMA-IR score of 4.8 (± 1.7) mg/dl·μU/ml, a sample size of 15 patients will provide 80% power to detect a 28% decrease in the HOMA-IR score (by a paired t-test analysis) at the P=0.05 level. This decrease in HOMA-IR score would be predicted to be associated with a clinically significant reduction in mortality.[33]

9. Selected methods

9.1. HOMA-IR and leptin-adiponectin ratio (LAR). HOMA-IR and LAR are both separate measures of insulin resistance. Patients will fast for 12 hours and withhold taking their morning insulin or oral antidiabetic agents prior to the test. Venous blood (10 ml) will be drawn into a red top tube (serum separator) placed on ice and centrifuged at 3,000 rpm at 4°C to separate serum. The serum is aliquoted and stored at -80°C until assay. Fasting serum insulin (μU/ml) is measured using an electrochemiluminescent assay and fasting serum glucose (mg/dl) by a hexokinase assay in the UIHC clinical chemistry lab. HOMA-IR score is calculated using the homeostatic model assessment equation (HOMA-IR = fasting glucose (mg/dl) x fasting Insulin (μU/mL) / 405). Serum leptin (ng/ml) and adiponectin (ng/ml) are measured by RIA using Linco kits (Linco, St. Charles, MO) in Dr Murry's lab at Iowa.

9.2. Exercise stress test for peak VO_2. A supervised graded symptom-limited treadmill exercise test to determine peak VO_2 will be performed before starting EMS training and again after 8 and 16 weeks of EMS training. This will establish baseline aerobic exercise capacity (peak VO_2) and determine if there are any cardiopulmonary problems with performing exercise. The testing will be performed in the CRU using a Balke protocol. Briefly for the Balke protocol patients walk on a treadmill at a set speed (3.3 mph for men and 3.0 mph for women) with a stating grade of 0%. For men the grade is increased to 2% at 1 minute and 1% per minute thereafter. For women the grade is increased by 2.5% every 3 minutes. The study ends when the patient is exhausted and asks to stop. The total duration of exercise time is recorded. The study is performed with continuous EKG monitoring, continuous measurement of respiratory O2 uptake and CO2 excretion using a metabolic cart and blood pressure monitoring each minute. The monitoring starts at rest and is continued until the symptoms and vital signs return to near baseline levels. The total study time is expected to be 30-45 minutes. The study is terminated early for angina, excessive shortness of breath or fatigue, dizziness or confusion, complex arrhythmias, second or third degree heart block, ECG changes suggestive of ischemia, excessive hypertension (systolic BP > 250 mmHg or diastolic BP > 120 mmHg) or hypotension (a sudden drop in systolic BP > 20 mmHg). The report will include the peak work rate achieved in METs and measured peak VO_2, peak heart rate and blood pressure, and any abnormal signs or symptoms that occurred during or after the test. The

ECG report will note any abnormalities in the EKG at rest, with exercise, and after return to baseline including the occurrence of any arrhythmias. If the EKG demonstrates ischemia changes, the time and double product at which the changes initially occurred will be specified. The time required for the exercise test is approximately 45 minutes.

For patients who cannot walk on a treadmill we also can offer bicycle ergometry as an option.

9.3. Quadriceps muscle strength. Quadriceps muscle strength will be measured in both legs five times: at baseline, after care optimization, at 8 and 16 weeks of EMS training and finally 4 weeks after stopping EMS training to determine the effect of EMS training on muscle strength. Muscle strength will be measured using a belt-stabilized hand held dynamometer (BSHHD) using a validated technique.[43] Briefly, isometric knee extension strength (torque) will be measured with patients seated on an elevated chair and their knees bent at about 90^{o} of flexion (i.e., the legs hanging vertical). Patients are stabilized to the chair with straps around proximal thighs and waist. A dynamometer-stabilizing belt is passed around a bar secured behind the back legs of the chair and over a calibrated hand-held dynamometer that was placed against the anterior leg of the patient just proximal to the malleoli. The distance from the estimated knee axis of rotation (lateral epicondyle of femur) to the midpoint of dynamometer is measured and recorded for calculation of torque. The patient is asked to perform three leg extensions separated by 30 seconds of rest. The first effort is submaximal to get the patient accustom to the procedure. Then two full strength efforts are performed and used for calculation of average peak force (N) and peak torque (N·m) generated. The test is performed sequentially on both legs at each measurement session. The total test time is approximately 15 minutes.

9.4. Dual energy X-ray absorptiometry (DXA). Advanced body composition analysis will be performed by DXA scan to measure total body and regional fat and lean mass before starting EMS training and again after 8 and 16 weeks of EMS training. Specific areas of interest will be in determining whether EMS training reduces visceral fat mass and produces the expected changes in thigh fat and muscle mass. The test is performed in the CRU at the University of Iowa using a Discovery A DXA scanner with the latest software version package (Hologic Inc., Bedford, MA). The advanced body composition analysis software package measures total and regional body composition and visceral fat mass (cm^2) using a validated technique that produces comparable results to a CT scan with less cost and radiation exposure.[71] The total radiation dose is less than one tenth that of a standard CXR and equivalent to one-day exposure to natural background radiation. Patients will be asked to report to the CRU dressed metal-free (no zippers, jewelry, piercings, underwire bra, etc.). Each study will take approximately 30 minutes.

9.5. Circulating markers of inflammation (serum cytokines & CRP). Serum levels of the pro-inflammatory cytokines, interleukin-6 (IL-6) and tumor necrosis factor alpha (TNFα) and the acute phase inflammation marker C-reactive protein (CRP) will be measured five times: at baseline, after care optimization, at 8 and 16 weeks of EMS training and finally 4 weeks after stopping EMS training to determine the effect of EMS training on inflammation. Venous blood (10 ml) will be drawn into a red top tube (serum separator) placed on ice and centrifuged at 3,000 rpm at 4^{o}C to separate serum. The serum is aliquoted and stored at -80^{o}C until assay. The assays are performed in Dr Daryl Murry's lab at Iowa. Circulating IL-6 and TNFα will be analyzed using ELISA (Biosource International). C-reactive protein will be quantified via immunoturbidimetric assay (Raichem).

9.6. Oxidative stress markers. Markers of oxidative stress and erythrocyte antioxidant levels will be

measured five times: at baseline, after care optimization, at 8 and 16 weeks of EMS training and finally 4 weeks after stopping EMS training to determine the effect of EMS training on oxidative stress. Markers of oxidative stress include plasma F2 isoprostane (isomers of the cyclooxygenase-derived prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$), plasma protein thiols and the ratio of reduced to oxidized glutathione (GSH/GSSG) in red blood cells. Venous blood is drawn into 10 ml tubes containing anticoagulant (EDTA), placed on ice until being centrifuged at 3,000 rpm at 4ºC to separate plasma and RBCs. The plasma is aliquoted and stored at -80ºC until assay. F2 isoprostanes are measured using liquid chromatography/mass spectroscopy (LC/MS) as described.[72] Protein thiols are measured using Ellman's reagent (5'5' dithio-bis (2-nitrobenzoic acid)) that reacts with free thiol groups to cleave the disulfide linkage ultimately releasing 2-nitro-5-thiobenzoate (NTB^{-2}) which can be quantitated spectrophotometrically by its absorbance at 412 nm.[61] The RBC's separated from plasma are used to measure the content of reduced (GSH) and oxidized (GSSG) glutathione by HPLC in Dr Beuttner's lab at Iowa. Determination of the RBC cell count and size (MCV) allows determination of GSH and GSSG content per RBC. RBC's separated from plasma are also used to measure the activity GPx, CAT and SOD by standard techniques used in Dr Beuttner's lab.[73]

9.7. Muscle biopsy. Muscle biopsies are done for the extraction of mRNA to analyze gene expression at 3 time points: immediately before and after 8 and 16 weeks of EMS training to determine the chronic effect of EMS training on gene expression. The muscle biopsies will be optional and the patient can participate in the study without agreeing to have the muscle biopsies. Percutaneous muscle biopsies are taken from the vastus lateralis muscle using a Temno biopsy needle (T1420; Cardinal Health) under ultrasound guidance as previously performed here.[70] Up to 4 biopsies are performed to obtain a total of ~100-200 mg of muscle tissue which is sufficient for ~100-400 ng of total RNA. Muscle tissue is immediately placed into RNAlater (Ambion) and stored at -80ºC until use.

9.8. Gene expression profiling. Gene expression profiling is done using the Human Exon 1.0 ST arrays (Affymetrix, Inc) which measures >17,000 different mRNA transcripts with about 4 probes per exon and 40 probes per gene. This recognizes both the gene being expressed and differences in alternative splice variants of that gene. To perform gene expression analysis total RNA must be extracted, converted to cDNA, the cDNA amplified, sense target cDNA prepared and then fragmented and labeled for detection prior to applying to the gene expression arrays.

As previously described[70], total RNA is extracted using TRIzol solution (Invitrogen) according to manufacturer's instruction. Microarray hybridization will be performed at the University of Iowa DNA facility.[70] Briefly, 50 ng total RNA is converted by Single Primer Isothermal Amplification (SPIA) to cDNA using the whole transcriptome (WT)-Ovation Pico RNA Amplification System (NuGEN Technologies, San Carlos, California). The amplified cDNA product is purified through a Qiagen MinElute Reaction Cleanup Column (Qiagen). A 4-µg quantity of amplified cDNA was used to generate sense target (ST)-cDNA using the WT-Ovation Exon Module (NuGEN Technologies), and again cleaned up with the Qiagen column. A 5-µg quantity of this product is fragmented (50-100 bases) and biotin labeled using the NuGEN FL-Ovation cDNA Biotin Module (NuGEN Technologies) prior to use for gene expression profiling.

For gene expression analysis the resulting biotin-labeled cDNA is mixed with eukaryotic hybridization buffer (Affymetrix, Inc., Santa Clara, California), placed onto Human Exon 1.0 ST arrays and incubated at 45ºC for 18 h with 60-rpm rotation in a GeneChip hybridization oven (Model 640; Affymetrix). After hybridization, the arrays are washed, stained with streptavidin–phycoerythrin (Molecular Probes, Inc., Eugene, Oregon), and signal amplified with anti-streptavidin antibody (Vector

Laboratories, Inc., Burlingame, California) using a fluidics station (Model 450; Affymetrix). Arrays are scanned (Model 3000 7G scanner; Affymetrix) and data collected using the using GeneChip operating software (GCOS), version 1.4.

In Affymetrix Human Exon 1.0 ST arrays, the log2 hybridization signal reflects the mean signal intensity of all exon probes specific for an individual mRNA, and is proportional to the level of that mRNA. We will perform two main comparisons: 1) the effect of acute exercise comparing the gene expression levels 4 hours after the first EMS training to baseline before training and 2) the effect of chronic EMS training (8 and 16 weeks) compared to the Pre-EMS training sample. We will restrict the analysis to mRNA's that have a mean log2 hybridization signal of >5 in either the pre-EMS sample or the 8 or 16 week EMS treatment conditions. We will use paired t-tests to determine which of the remaining mRNA's are present at a significantly different level (adjusting for the false discovery rate inherent in multiple testing) in the EMS treatment compared to the baseline sample. The list of differentially expressed genes will be examined for important biological pathways relating to muscle glucose metabolism and insulin response, mitochondrial biogenesis and function, muscle phenotype, growth and energy metabolism, oxidant stress and inflammation. We will also use bioinformatics tools such as Onto-Express to help translate the list of differentially expressed genes into functional categories such as biological process or cellular role based on the annotation using Gene Ontology terms. All microarray data will be deposited in the GEO database.

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