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Neuromuscular Electrical Stimulation: A Novel Treatment Intervention for Improving Metabolic Health in an Overweight/Obese Population

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NEUROMUSCULAR ELECTRICAL STIMULATION: A NOVEL
TREATMENT INTERVENTION FOR IMPROVING
METABOLIC HEALTH IN AN
OVERWEIGHT/OBESE
POPULATION

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by

Michelle Galvan

2019

Dedication

I dedicate my thesis work to my family and friends; whose support was unwavering. To my parents, thank you for always providing me with a home to come to, a place where I can focus on my studies, and providing me with unconditional love and support.

To my father who never stopped believing in me and always told me he was proud of me every chance he got. I am saddened by his passing just before my last semester and wish deeply he could witness my accomplishment. Although something tells me he already knew may you rest in peace.

To my siblings for always keeping me in line and staying by my side in support.

To Eric, for always being there no matter what. Staying by my side at the UTEP library for hours on end. Thank you for supporting me, believing in me and never letting me give up.

To Dr. Bajpeyi, for providing countless feedback and making my thesis a priority.

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by

MICHELLE JOSIE GALVAN, B.S.

THESIS

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Abstract

Background: Most U.S. adults (80%) do not meet minimum exercise recommendations by ACSM (CDC, 2015). Using an in vitro primary cell culture model, we and others have shown that muscle contraction induced by electrical stimulation results in increased glucose transporter 4 (GLUT4) protein, glucose uptake and mitochondrial content. Neuromuscular electrical stimulation (NMES) is a novel alternate strategy to induce muscle contraction, using electrical impulses. However, effectiveness of NMES induced muscle contraction to improve insulin sensitivity and energy expenditure is not clear. The purpose of this study was to investigate the effects of four weeks of NMES on insulin sensitivity in a sedentary overweight/obese population.

Methods: Sedentary overweight/obese participants (n=10; age: 36.8 ± 3.8 years; BMI= 32 ± 1.3 kg/m²) were randomized into either a control or NMES group. All participants received bilateral quadriceps stimulation (12 sessions; 30 minutes/session; 3 times/week) either using low intensity sensory level (control) or at high intensity neuromuscular level (NMES) for four weeks (50Hz and 300 μ s pulse width). Insulin sensitivity was assessed by three-hour oral glucose tolerance test (OGTT), substrate utilization was measured by indirect calorimetry and body composition was measured by dual X-ray absorptiometry at baseline and after four weeks of NMES intervention.

Results: Control and NMES group had comparable fasting blood glucose (p=0.42), glucose tolerance (p=0.49), substrate utilization (p=0.99), and muscle mass (p=0.86) at baseline. Four weeks of NMES resulted in a trend to improvement in insulin sensitivity measured by OGTT, whereas no change was observed in control group (Control 430.73 ± 20.23 to 494.68 ± 77.21 AU; p=0.76; NMES 455.55 ± 26.07 to 415.36 ± 25.89 AU; p=0.07). There was no change in substrate utilization in control (p=0.26) and NMES (p=0.85). In addition, there was no change in muscle mass in both control (p=0.14) and NMES (p=0.17) groups. **Conclusion:** NMES is a novel and effective strategy to improve insulin sensitivity in an at-risk overweight/obese sedentary population in the absence of substrate utilization and muscle mass improvement.

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Introduction

Literature Review

One-third (39.8%) of adults in the United States are considered obese and 9.4% of U.S adults have type 2 diabetes mellitus (T2DM). [1, 2] In addition, obesity rate in adults has increased from 21.7% to 34.8% (2000-present) in Texas [3] where 37.9% were Hispanics. [4] In 2018, 12.5% of adults in Texas were diagnosed with T2DM [3] where 13.1% were Hispanic. [5] Moreover, Hispanics have one of the highest prevalence of obesity (33.3%) compared to other races. [1] Of the 9.4% national diabetes average 12.5% are Hispanic adults diagnosed with T2DM, in addition 23.8% of people with diabetes in the nation are undiagnosed. [6, 7] In 2017, 34.9% of adults in El Paso, TX were considered obese where 36.1% were Hispanic. [8] Furthermore, 13.9% of adults in El Paso, TX have been diagnosed with diabetes. [9] Insulin resistance, glucose intolerance, and T2DM increases risk for developing cardiovascular disease, the leading cause of death in the U.S. [10, 11]

Exercise is highly effective in improving insulin sensitivity and metabolic health [12], however, majority of U.S. adults (79%) do not meet American College of Sports Medicine (ACSM) recommended 150 minutes/week of physical activity, leading to dramatic increase in obesity, insulin resistance and T2DM over the past few decades. [13] Moreover, in 2007-2010 only 36% of individuals diagnosed with T2DM achieved physical activity standards (defined as ≥ 150 minutes of moderate or ≥ 75 minutes of vigorous leisure-time or work-related physical activity per week). [14] Walking for 20-30 minutes may be challenging, uncomfortable, and/or painful for individuals with severe obesity, arthritis, physical disabilities, and/or diabetes complications. [15] Obese individuals face challenges during weight-bearing movement such as jogging or running and are at a greater risk for injury and pain-related intolerance. [16] A study found that a 1% increase in glycated hemoglobin (HbA1c) among participant's is associated with a 28% increase in the risk of death; independent of age, blood pressure, cholesterol, and body mass index. [17] In 2016, The American Diabetes Association (ADA) recommendation calls for three

or more minutes of light activity, such as walking, leg extensions or overhead arm stretches, every 30 minutes during prolonged sedentary activities for improved blood sugar management, particularly for people with T2DM. [12]

Therefore, additional strategies to increase adoption and adherence to physical activity are warranted. Electrical Stimulation (ES) is a practical, non-invasive, cost-effective and innovative method to promote an alternative mode of muscle contraction among individuals who are less likely to engage in conventional physical activity. Although ES is frequently used in clinical setting for improving neuromuscular function and strength in disused/immobilized limbs [18, 19, 20, 21, 22], there is a gap in our knowledge of how effective ES is in improving insulin sensitivity and metabolic health. Skeletal muscle is the largest, most metabolically active tissue and is the major site for lipid and glucose metabolism. Therefore, muscle is considered a primary target for studying the pathogenesis of insulin resistance, obesity and T2DM.

NEUROMUSCULAR ELECTRICAL STIMULATION BENEFITS

Neuromuscular Electrical Stimulation (NMES) is often used in the rehabilitation setting using electrical pulses to induce involuntary muscle contractions; it helps with re-education of disused limbs, pain management, reducing inflammation and swelling. [20, 19, 21, 18] This therapy makes use of random special recruitment, based on the proximity of intramuscular nerve branches to adhesive electrodes. [22, 23, 24] These surface electrodes induce muscle contractions similar to when an action potential travels down a motor neuron from the brain initiating the signaling cascade of muscle contraction. [25] During muscle contraction, motor units receive electrical signals from the brain to move myosin isoforms (thick filaments) forming cross bridges in preparation for the power stroke, whereas use of NMES directly stimulate muscle contraction bypassing the brains signal changing the surface membrane potential of axon terminals causing the release of calcium initiating the signaling cascade of muscle contraction.

Effects of NMES on insulin sensitivity: Prolonged periods of exercise training has been proven to improve insulin sensitivity in individuals with obesity and T2DM. [26, 27, 28, 29, 30,

31, 32] Studies on the effects of NMES on insulin sensitivity are very limited. Previous studies in our laboratory have established the effectiveness of electrical stimulation in increasing mitochondrial content, lipid content and glucose transporter 4 (GLUT4) proteins in human myotubes using an *in vitro* model. [33, 34] Electrical stimulation of skeletal muscle cells (*in vitro*) has been shown to have an additive effect on insulin stimulated glucose uptake. [35, 36] Nikolic et al. 2012, electrically stimulated human skeletal muscle cells either using high-frequency (100 Hz for 5-60 minutes) or low-frequency (1 Hz continuously for 24 or 48 hours). [37] The study reported an increase in glucose uptake and cell lactate content in the high-frequency group, while ATP and PCr content decreased. In the low-frequency group, oxidative capacity increased along with doubling of mitochondrial content. Glucose uptake by ES have been evaluated in several studies in humans muscle cells. [38, 39, 37, 40] ES increased insulin-stimulated glycogen synthesis [41] and glucose oxidation [39, 37, 41]. Fatty acid oxidation, on the other hand, were reported to be increased by ES in some but not all studies. This conflicting results in fatty acid oxidation were concluded to be possibly dependent on both the varied ES protocols used and donor characteristics. [39, 37, 41]

In vivo studies by ES, have shown that increase in blood flow in a muscle cell is important for increasing rate of glucose uptake during muscle contraction [42]. An increase in insulin response in patients with T2DM was reported after 2 weeks of NMES treatment (50Hz of quadriceps stimulation) without any change in lipid profile. [43] Furthermore, Catalogna et al. 2016 reported an improvement in blood glucose control in patients with T2DM after daily five minute stimulation for eight weeks at 16Hz on the lower extremity [44]. Overall, there was a decrease in fasting glucose, lower serum cortisol level, and mean HbA1c level. The study also showed decrease in postprandial blood glucose after breakfast after eight weeks. [44] Jabbour et al. 2015, performed two sessions of electrical stimulation at 8Hz up to tolerable intensity during the first hour of a standardized glucose tolerance (OGTT) test. After obtaining two blood samples through venipuncture, at minute 60 and 120 of OGTT testing, the study reported a significantly lower blood glucose after NMES treatment compared to the control sessions. This study also

showed a positive correlation between NMES stimulation intensity and degree of decrease in blood glucose levels in individuals with T2DM. [45].

Although the present literature indicates promising potential of using NMES to improve insulin sensitivity in a population with T2DM, comprehensive randomized controlled trial using gold standard hyperinsulinemic euglycemic clamp to determine the effects of NMES on insulin sensitivity and substrate utilization are limited. A pilot study by Joubert et al. 2015, performed four weeks of quadriceps stimulation at 35Hz, in a T2DM population, and reported an increase in insulin sensitivity measured by hyperinsulinemic euglycemic clamp. [46] Two small studies by Hamada et al. [47, 48] investigated the acute effects of NMES, in a healthy all-male population, during a single NMES session for 20 min at 20Hz. The findings reported oxygen uptake increasing twofold with the onset of ES. The study also reported an initial increase in both lactate accumulation and respiratory gas exchange ratio (RER) and then gradually declined towards the end of ES. The glucose disposal rate (GDR), measured by clamp, in this study increased during stimulation and remained increased for at least 90 minutes after cessation of NMES (recovery period). [47, 48] Mahoney et al. 2005, reported, after a 12-week combination NMES and resistance training (RT) intervention, an improvement in blood glucose in participants with spinal cord injury (SCI) (a population at higher risk of developing T2DM). [49] In 2018 Miyamoto et al evaluated lower limb electrical stimulation for eight weeks, 40 min/session, 5x/wk at 4 Hz, in T2DM population. [50] The study reported a significantly greater percent change in the fasting glucose concentration and significantly greater percent change in body fat in NMES than in control. To the best of our knowledge, it can be concluded that NMES is an effective method towards improving insulin sensitivity in T2DM population, yet no studies have evaluated the effectiveness of NMES to improve insulin sensitivity, substrate metabolism, and muscle mass in a high-risk population for developing T2DM.

EFFECTS OF NMES ON SUBSTRATE UTILIZATION

Metabolic diseases are physiological abnormalities associated with developing heart disease, stroke, insulin resistance, obesity, and T2DM (increase in blood pressure, blood sugar, body fat, and abnormal cholesterol levels). [51] Whole body substrate utilization is measured by calculating RER or respiratory quotient (RQ). RQ is the proportion of carbon dioxide produced to oxygen consumed ($RQ = VCO_2 / VO_2$). Theoretically RQ value range from 0.7 to 1.0 indicating approximately 100% fat or carbohydrate oxidation, respectively. [13] A defect in substrate utilization has been identified as one of the major metabolic defects associated with obesity and T2DM. Kelly et al 1999, reported obese individuals had no change in RQ from fasted values to insulin stimulated values at baseline. After a 4-month weight loss intervention, fasted RQ had no change pre to post-intervention, however, insulin stimulated values significantly increased leg RQ. [52] Metabolic flexibility is characterized by the ability to switch from one fuel source to the other in response to a meal or insulin (delta RQ). [53, 54] Individuals with obesity and T2DM are often metabolically inflexible, and therefore have a lesser reliance on fat oxidation during fasted state and inability to switch to carbohydrate oxidation in an insulin stimulated state compared to those who are metabolically flexible. [55] These defects have been reported to improve with exercise training.

Resistance exercise training has been shown to improve insulin sensitivity as evidenced by enhanced insulin action in skeletal muscle, improved glucose tolerance, and a decrease in HbA1c levels. [56, 57, 26, 58, 59, 60] Jamurtas et al. 2004, has shown that a single bout of RT for 60 minutes increased fat oxidation (decrease in RQ), and elevated resting energy expenditure following 10 and 24 hours exercise bout. [61] Talanian et al. 2007, showed an increase in fat oxidation and maximal mitochondrial enzyme activity after two weeks of high-intensity aerobic interval training in healthy recreationally active women. [62] Ghanassia et al. 2006, demonstrated greater carbohydrate use (increase in RQ) with increasing exercise intensity during a 30 minutes of cycle ergometer test with increasing intensity in T2DM population. [63]

Studies investigating the chronic effects of NMES on substrate utilization and energy expenditure are limited. Hultmans and Spriet (1986) reported an increase in oxidative metabolism, measured by muscle biopsy, after an acute session, 45 minutes, at 20Hz quadriceps stimulation in a healthy population. [24] RQ measured during a single bout of stimulation session at 75Hz in healthy males showed an increase in RQ, the authors speculate that rapid elevation of RQ with electrical stimulation would indicate anaerobic breakdown and utilization of intramuscular glycogen in the activated muscle fibers. [64] Kemmler et al. 2012, investigated the effects of whole-body electrical stimulation (16 muscle regions; e.g., upper legs, upper arms, gluteals, abdomen, chest, lower back, upper back, and shoulder) at 85Hz on energy expenditure during low-intensity resistance exercise training on 19 moderately trained men. [65] The authors found a significantly higher energy expenditure during whole-body electrical stimulation with exercise training than without whole-body stimulation during exercise. [65] One of the only studies utilizing obese participants, examined the acute effects of one-hour low frequency stimulation (5Hz) to the quadriceps and hamstring muscles, found an increased oxygen uptake, RER, energy expenditure, lactate, heart rate and carbohydrate oxidation. [66] Hamada et al. 2004 compared the effects of 20 minutes of stimulation at 20Hz to the lower leg muscles (tibialis anterior and triceps surae) and the thigh (quadriceps and hamstrings) to a single bout of 20 minutes of cycling. [47] Authors found a greater carbohydrate use and an increase of oxygen uptake during stimulation as well as an increased requirement for glucose during stimulation and recovery. [47] The study also found similarities to the study done by Grosset et al. 2013 such as an increase in oxygen uptake, heart rate, lactate and RER at the onset of stimulation but not with exercise. [66, 47] These findings suggest an increased energy requirement during NMES, alongside increased glucose utilization during stimulation. Miyamoto et al. [67, 68] investigated the acute effect of lower limb NMES for 30 minutes at 4Hz 30 minutes after a standard meal on T2DM population. Both studies found blood glucose concentration in the NMES group was significantly lower compared to the control group at minute 60, 90, and 120 after the meal. C-peptide concentration was also found to be significantly smaller compared to the control group at minute 120. Oxygen uptake, RQ and lactate

concentration were significantly higher in the NMES group compared to control. Given most of the studies were evaluating acute effect performed in healthy and or physically active population, it is unknown whether chronic NMES use can improve substrate utilization and energy expenditure of those with metabolic diseases such as obesity.

EFFECTS OF NMES ON MUSCLE MASS

Skeletal muscle is one of the largest, most metabolically active tissues and is the major site for lipid and glucose metabolism. Therefore, muscle is considered a primary target for studying the pathogenesis of insulin resistance, obesity and T2DM. Increase in muscle mass with RT has been suggested to be the main regulator of increased glucose uptake (i.e. improvement in insulin sensitivity). [69, 70]. In a four-month strength training study, 3x/wk, showed an increase in maximum strength of all muscle groups including an improvement in long-term glycemic control in an obese and T2DM population. [71] Several studies report an improvement in muscle mass, muscle strength and improvement in insulin sensitivity with varied exercise does and duration using RT. [72, 73, 74, 75, 76, 77, 78, 26, 79] RT indicates an effective method towards improving overall health in obese and T2DM population.

Information is limited on effects of NMES (or NMES combined with RT) on muscle mass in an obese and T2DM populations. Limited number of studies that reported the NMES effects on muscle strength and muscle mass were conducted in healthy sedentary population, trained cyclists, and patients with SCI. Therefore, no direct conclusions can be drawn regarding effects of NMES on muscle mass and strength in population with metabolic diseases such as obese and T2DM. Edwards et al. 1997, reported that NMES at a frequency of 50Hz produces 60% of maximal muscle contraction. [80] Griffin et al. 2009, showed an increase in muscle power and work done, and a 4% increase in lean muscle mass using cycling functional electrical stimulation (used for paralyzed body limbs) for 10 weeks in a SCI population. [81] Chilibeck et al. 1999, reported an increase in two glucose transporter isoforms (GLUT1 and GLUT4) and an improvement in insulin sensitivity measured by OGTT, after 8 weeks of combined NMES and exercise training. [82] Increases in

muscle fiber cross sectional area (CSA) after four [83] and eight weeks [18] of NMES has been reported in healthy volunteers. Additionally, an increase in isokinetic strength after four weeks (16 min/session 3x/wk at 100 Hz) [84] and increases in abdominal strength after 6 weeks (30 min/session 5x/wk) [85] of NMES have also been reported in healthy individuals. Martin et al. [86] reported no change in muscle CSA in healthy individuals after four weeks of NMES, which could have been attributed to short session time (10min) compared to others (ranging from 16-34min) [83, 84, 85, 87]. Neder et al. 2002 found increases in peak torque and a trend to improve isometric mean force after 6 weeks of electrical stimulation in participants with chronic obstructive pulmonary disease (COPD) [88]. Two studies, in participants with chronic heart failure, found an increase in gastrocnemius muscle mass after five weeks [89] and an increase in CSA after eight weeks with NMES (50Hz) [90]. After 6 weeks of NMES (50Hz) in patients with end-stage osteoarthritis also found increases in CSA [91]. However, two other studies reported no change in CSA in patients with lower limb or knee joint injury/surgery, with two frequency groups of intensities in each, 20 and 80Hz for 12 weeks [92] and 50 and 100Hz for four weeks [93]. Since the mechanisms of improvements, or lack thereof, are still unclear with this type of therapy, it is possible that these studies were in a population more concerned about muscle re-education rather than muscle gain. Finally, two studies in patients with SCI found an increase in total work output for 30 min/session, 3x/wk, for eight weeks at 30Hz [94] and increases in other variables of power, fiber area, and capillarization for 2-3x/week for ten weeks at 50Hz [81]. It can be theorized from previous studies finding improvements in work output, [94] resistance to fatigue, [21] muscle mass and maximal voluntary strength, [18] that some hypertrophic/strength changes occur with NMES. However, to our knowledge there has been no studies that has measured the effectiveness of using NMES to increase muscle mass in a sedentary overweight/obese population, a population at risk of developing T2DM.

Taken together, current evidence indicates promising potential in using NMES to improve metabolic health. NMES therapy has reported a reduction in fasting blood glucose [95, 44, 43, 45] and improvements in HbA1c levels in obese and T2DM suggesting metabolic benefits [41, 96].

Acute NMES use seem to be effective in increasing glucose uptake, energy expenditure, and oxygen utilization. [45, 47, 48, 24, 64, 66, 67, 68] The use of NMES in preventing muscle loss during immobilization and increase in muscle strength and power have mainly been investigated in non-obese healthy [97], patients with COPD [98], SCI [94], and surgical populations [99]. Most of the studies that evaluated effects of NMES on glucose uptake are in muscle cells (*in vitro*), patients with T2DM or SCI, or investigated acute effects of NMES in healthy males. There is a lack of literature on effects of NMES on energy metabolism in general. Only few studies using NMES are in healthy active males or a single bout in T2DM. Effect of NMES on muscle mass have been evaluated generally in patients with SCI, COPD, T2DM, or healthy individuals. Therefor there is a gap in literature to understand the effects of chronic NMES with comprehensive understanding on insulin sensitivity, substrate metabolism and muscle mass/strength in sedentary overweight/obese individuals.

MUSCLE STRUCTURE AND FUNCTION

The structural organization of a skeletal muscle is shown in **Figure 1** taken from Powers

Figure 1.

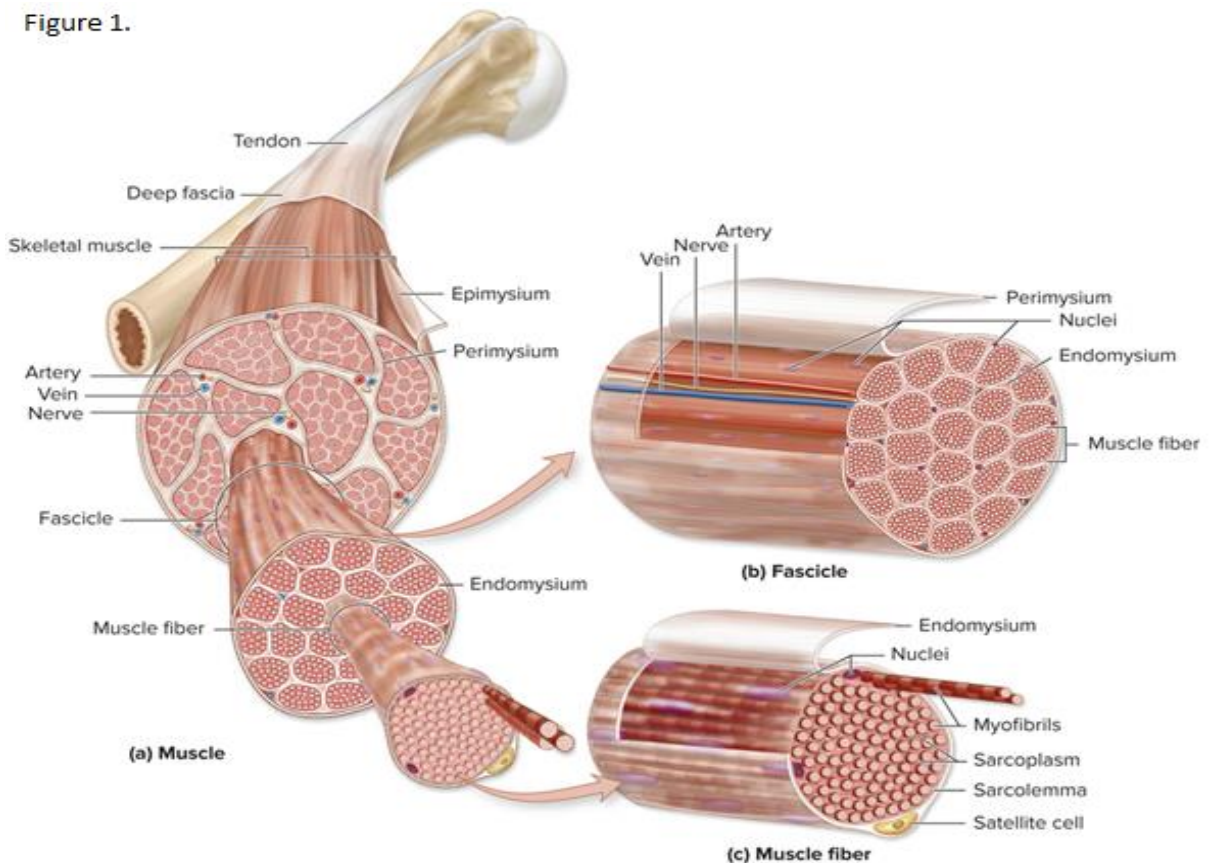


Figure 1. Skeletal muscle structure

SK and Howley ET. [100] Epimysium is a layer of connective tissue that surrounds an individual muscle. Perimysium is a layer of connective tissue that surrounds a group of fibers within that muscle that are arranged into bundles. Sarcolemma or cell membrane surrounds a single muscle fiber. Within each muscle fiber contains numerous myofibrils and contains even more of myofilaments. Myofilaments are formed by connected sarcomeres, which are the contractile units

of a skeletal muscle. The structure of an individual skeletal muscle fiber is shown in **Figure 2** taken

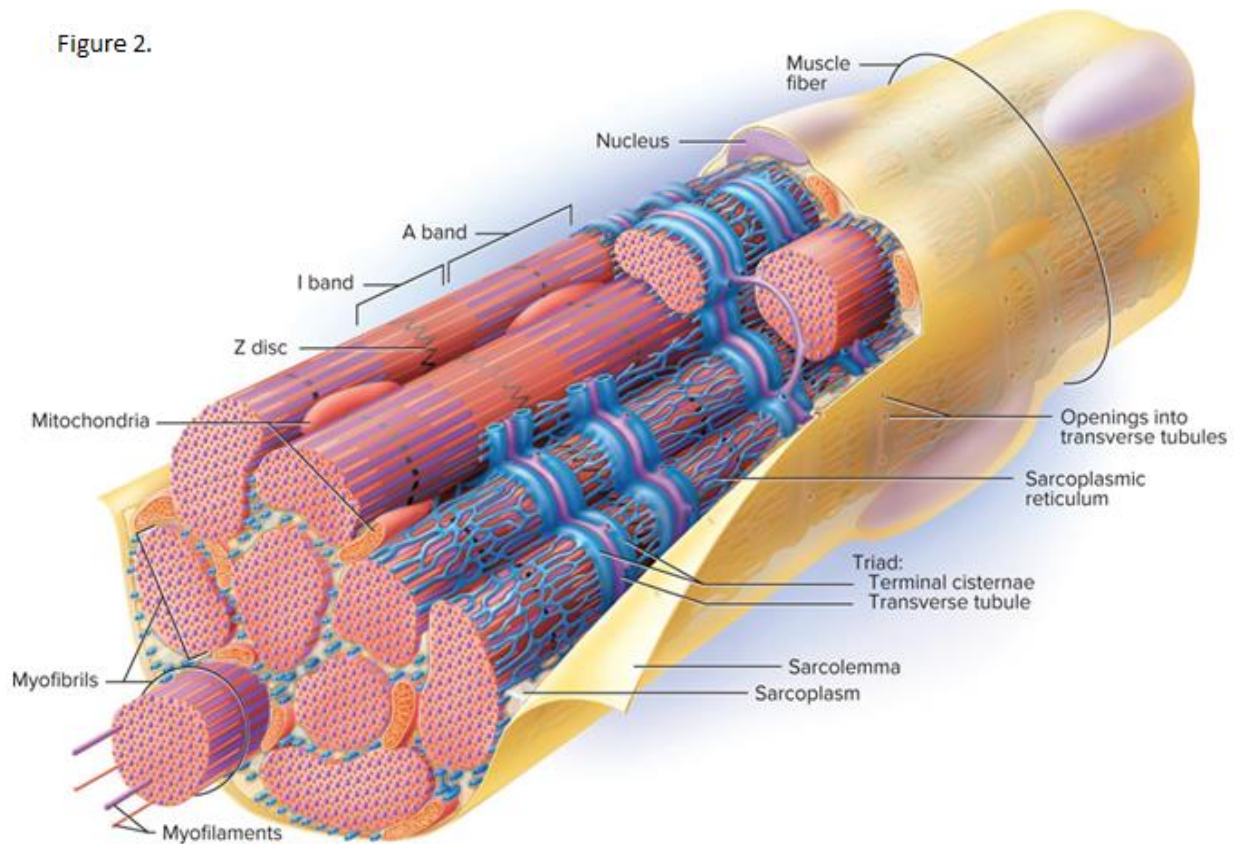


Figure 2. A single skeletal muscle fiber of dark and light bands

from Powers SK and Howley ET. Sarcomeres are basic unit of striated muscle tissue containing dark and light bands known as A band (dark) and I band (light). Each A band has a lighter region in its midsection called the H zone; each H zone is bisected vertically by a dark line called M line. Each I band also has a midline interruption, a darker area called the Z disc. A single fiber contains ~70-80% of total protein known as actin and myosin (myofilament proteins).

Sarcoplasmic reticulum (SR) and transverse tubules (T tubules) are the skeletal muscle fibers intracellular tubules that help regulate muscle contraction. SR surrounds each myofibril longitudinally to allow communication with each other at the H zone. The SR stores and regulates intracellular levels of calcium ions (used during muscle contraction). At each A band-I band

junction, the sarcolemma of the muscle cell runs deep into the cell interior, forming a tube called the T tubule.

An electrical impulse that is sent down from the brain to the targeted muscle results in muscle movement, this is known as voluntary muscle contraction. Once the brain sends the signal it is transported to the muscle through the motor neurons. In a nerve a neural message is generated when a stimulus of enough strength reaches the neuron membrane (shown in **figure 3** taken from

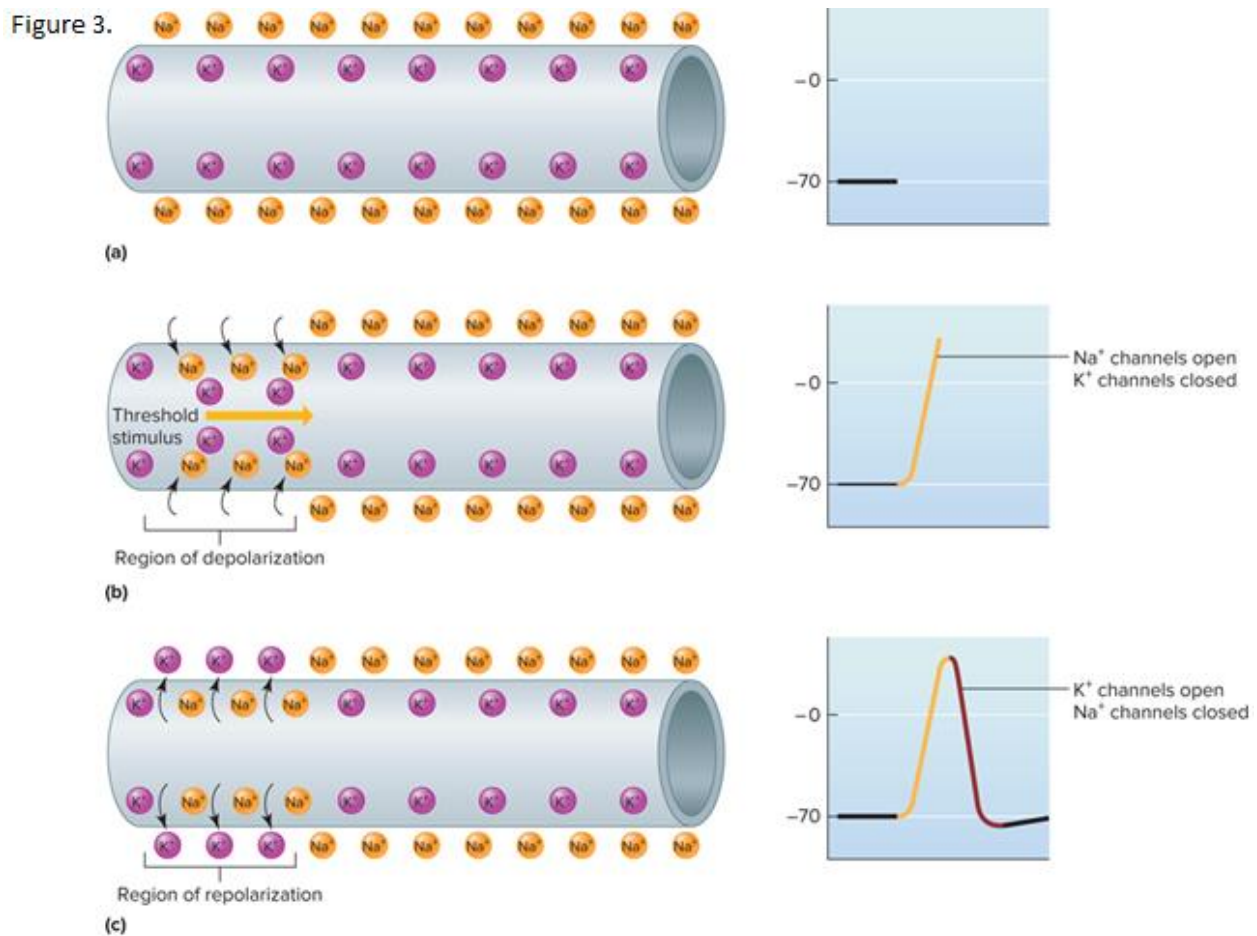


Figure 3. Electrical impulse in a nerve

Powers SK and Howley ET) and opens sodium gates, allowing sodium ions to enter into the neuron, making the inside of the cell positively charged (depolarizing the cell). When depolarization reaches a “threshold,” more sodium gates open and an action potential is formed. After an action potential has been generated, a sequence of ionic exchanges (sodium channels

open) occurs along the axon to generate the nerve impulse. This ionic exchange along the neuron occurs in a sequential fashion at the nodes of Ranvier.

Repolarization occurs immediately following depolarization, resulting in a return of the resting membrane potential (making inside the cell negative) causing the nerve ready to be stimulated again. This occurs when potassium channels open to allow potassium ions to leave the cell making the voltage potential negative. The sodium-potassium pump causes sodium (three sodium ions) to pump outside of the cell and potassium (two potassium ions) to pump inside the cell causing the voltage potential to return to resting voltage. The “all-or-none” law of action potentials refers to the development of a nerve impulse. Meaning once an impulse is initiated the voltage travels the entire length of the nerve without a decrease.

Communication between neurons and muscles occurs through a process called synaptic transmission. Synaptic transmission occurs when sufficient amounts of chemical messengers known as neurotransmitter are released from synaptic vesicles contained in the presynaptic neuron. An impulse causes synaptic vesicles to release stored neurotransmitter into the space between the presynaptic neuron and postsynaptic membrane called the synaptic cleft. After neurotransmitters are released into the synaptic cleft, these bind to a “receptor” on the target membrane.

The axon of a motor neuron innervates skeletal muscle fibers from the spinal cord. At the muscle the axon splits into collateral branches allowing for each to innervate a single muscle fiber. A motor unit consists of motor neuron and all the muscle fibers that it innervates. Calcium (Ca^{2+}) release occurs when an action potential travels down t-tubules changing the membrane potential and the release of sodium (Na^+), opening voltage gated channels on the sarcoplasmic reticulum releasing Ca^{2+} ions into cytoplasm, thus muscle contraction begins. [25]

EFFECTS OF EXERCISE ON GLUCOSE TRANSPORTER 4

GLUT4 is one of 13 facilitative glucose transport proteins and is expressed most abundantly in adipose tissue, cardiac and skeletal muscle. [101] GLUT4 is a major mediator of glucose removal from the circulation and a key regulator of whole-body glucose homeostasis.

[102] GLUT4 translocation increases muscle glucose transport during exercise from intracellular sites to the sarcolemma and T-tubules. [101] GLUT4 translocation is a process that alters the subcellular distribution of GLUT4 from intracellular stores to the plasma membrane due to insulin stimulated glucose uptake. [103] During submaximal exercise an inverse relationship between skeletal muscle GLUT4 protein content and glucose disappearance was observed. [104] Increased skeletal muscle GLUT4 expression has also been shown to enable post-exercise glucose uptake and glycogen storage. [105] During a six-week lower body strength training program, individuals with T2DM showed a 40% increase of GLUT4 density in the muscle. [57] It has been shown that a single bout of exercise increases the translocation of GLUT4 to the skeletal muscle plasma membrane in participants with and without T2DM. [106]

EFFECTS OF EXERCISE ON INSULIN SENSITIVITY

It has been well documented that exercise training improves insulin sensitivity. Short term (2 – 6 weeks) and long term exercise training reported improvements in fasting plasma glucose [107, 108, 109, 110], fasting insulin levels [111, 107, 112, 113, 114], glucose tolerance [108, 115, 116] and insulin sensitivity measured by hyperinsulinemic euglycemic clamp. [111, 112, 117] These improvement in insulin sensitivity have been documented in all populations including healthy sedentary [118], insulin resistant [119], obese [110, 120, 121], and individuals with T2DM. [107, 108, 109, 116]

Skeletal muscle is the most metabolically active tissue and is the major site for glucose disposal. Therefore, increases in muscle mass plays an important role in improvement in insulin sensitivity, obesity, and T2DM. During increasing exercise intensity, blood flow and circulating glucose concentration increases resulting in an increase of glucose delivery to the working skeletal muscles [122]. Exercise intensity and duration are primary determinants of skeletal muscle glucose uptake, accounting for up to 40% of oxidative metabolism during exercise. [101] During exercise blood flow to skeletal muscle can increase up to 20-fold from rest to intense dynamic exercise. [101] The increase in blood flow is the larger contributor to the exercise-induced increase in

muscle glucose uptake. [101] Therefore, the increase in muscle perfusion can lead to an increase in glucose supply and delivery [123]. Likewise, increased skeletal muscle glucose uptake has been reported during exercise [123, 124], even when insulin levels are clamped (kept at a specified level). In population with metabolic disease (obesity and T2DM) impairment of insulin signaling leading to low glycogen synthesis and oxidative glucose disposal has been reported. [125] Exercise-stimulated molecular mechanism resulting in increased skeletal muscle glucose uptake increases GDR even in states of insulin resistance [126]. A single resistance exercise session (consisting of 16 sets of leg-exercise) improves whole-body insulin sensitivity by as much as 13% when measured 24 hours after exercise. [127] Therefore, it can provide a relevant alternative pathway for individuals with obesity and T2DM.

Insulin resistance (i.e. decreased insulin sensitivity) is the inefficiency of insulin to uptake glucose, which has been shown to be associated with physical inactivity, obesity, and T2DM. [128, 129, 130] The pathogenic nature of insulin resistance in skeletal muscle is characterized by the inability of insulin to effectively signal glucose uptake. [131, 132, 133] Elevated levels of lipid metabolites such as long chain fatty acyl-CoA (LCFA-CoA), diacylglycerols (DAGs) and ceramides in skeletal muscle have been reported in insulin resistant population (obesity and T2DM). [134, 135, 136, 137, 138, 139, 140, 141, 142] Human and animal studies have shown that these lipid metabolites (LCFA-CoA, DAGs, and ceramides) to disrupt the insulin signaling cascade at several levels [134, 139, 143, 144, 145, 146, 147, 148], leading to a decrease in GLUT4 protein (the main isoforms expressed in skeletal muscle) content and translocation, resulting in a reduction in glucose uptake. [131, 132, 133, 149, 150, 151, 152, 153, 154, 155] It has been shown skeletal muscle contraction is effective in increasing glucose uptake in individuals with insulin resistance. [43, 44, 45, 46]

Martin et al. 1995, showed a decline in blood glucose during exercise in nonobese individuals with non-insulin dependent diabetics by enhanced peripheral glucose utilization. [156]

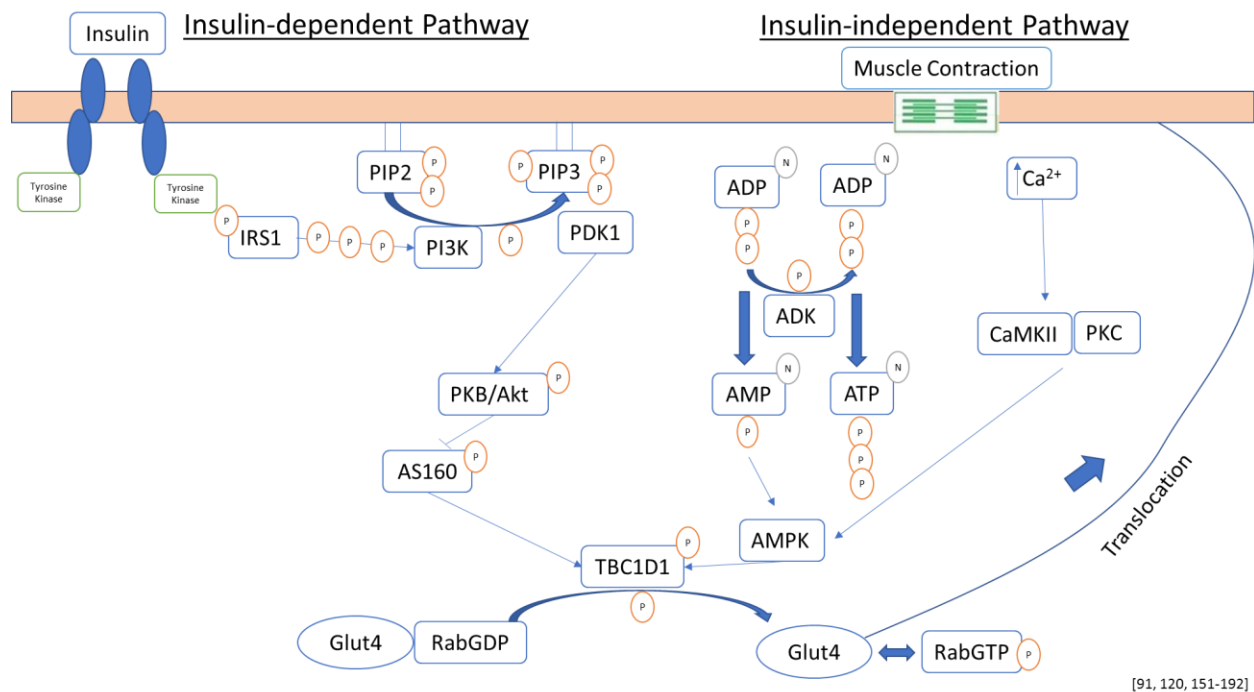


Figure 4. Insulin Dependent and Insulin Independent GLUT4 Translocation

Figure 4 (adapted from [91,120, 151-192]) shows a generalized overview of the molecular mechanisms of glucose uptake pathways (insulin dependent and insulin independent/muscle contraction) [157]. Non-insulin mediated [157] exercise induced glucose uptake is of high importance for those with insulin resistance (e.g, obese and individuals with type 2 diabetes), due to defects in glucose uptake through insulin dependent pathway.

INSULIN-DEPENDENT GLUCOSE UPTAKE PATHWAY

The insulin receptor (IR) is a heterotetrameric membrane glycoprotein composed of two α -subunits and two β -subunits. [158] Insulin binds to the extracellular α -subunits, and this leads to activation of the transmembrane β -subunits and auto-phosphorylation of the receptor. [158] The phosphorylation sites on the β -subunit of IR play important functional roles in promoting receptor

kinase activity and facilitating the interaction between the receptor and intracellular substrates. [158] Insulin signaling pathways are not necessarily linear, as there is a high degree of cross communication between the signal transducers. [158]

Briefly, GLUT4 are localized intracellularly, however an increase of blood glucose levels causes pancreatic beta-cells to secrete insulin, which binds to IR on skeletal muscle cell membranes. Insulin receptor substrate isoform (IRS-1) link the initial event of insulin receptor signaling cascade to downstream events. [158] IRS molecules contain multiple tyrosine phosphorylation sites that become phosphorylated after insulin stimulation [158, 159], allowing phosphoinositide 3-kinase (PI3-K) to bind. PI3-K promotes phosphatidylinositol-triphosphate (PIP3) production by catalyzing phosphatidylinositol-diphosphate (PIP2) into PIP3 [160, 158], which serves as an allosteric regulator of phosphoinositide-dependent protein kinase (PDK). [161] PIP3 mediates glucose transport via signaling to protein kinase B (PKB) (also known as Akt) [162] and/or protein kinase C (PKC) [163], Akt2 plays an important role in glucose metabolism by directing GLUT4 to the plasma membrane and assist in anabolic processes [164, 165]. Activated Akt phosphorylates Akt substrate 160 (AS160) and Tre-2/BUB2/cdc 1 domain family member 1 (TBC1D1) [165, 166]. AS160 and TBC1D1 is proposed to inhibit Ras homologous from brain (Rab)-GTPase-activating protein (GAP) activity toward particular Rab isoforms, thus inhibition of GAP promotes conversion of less active GDP-loaded Rab (Rab-GDP) to more active GTP-loaded Rab (Rab-GTP). [165] Conversion of Rab-GDP to Rab-GTP results in the release of GLUT4 vesicles to move to and fuse with the plasma membrane allowing glucose uptake into the cell [166, 165].

INSULIN-INDEPENDENT GLUCOSE UPTAKE PATHWAY: MUSCLE CONTRACTION MEDIATED

Skeletal muscle contraction, increases glucose uptake independent of the insulin activated PI3-K, IRS-1, AS160 [167, 168] (AS160 – facilitates GLUT4 translocation [167, 169]), and Akt/PKB pathways [170, 171, 172, 173, 174]. Exercise mediated muscle contraction activates insulin independent adenosine monophosphate (AMP)-activated protein kinase (AMPK)

(increased AMP/adenosine triphosphate (ATP) ratio) and calcium calmodulin-dependent protein kinase (CAMK) (increased Ca^{2+} secretion and activation of calmodulin [175]) pathways, but also upregulates AS160 [168, 176] increasing GLUT4 translocation and as a result improved glucose uptake within skeletal muscle [168, 177].

Adenylate kinase (ADK) plays a key role in catalyzing an exchange reaction known as the nucleotide phosphoryl: two adenosine diphosphate (ADP) \leftrightarrow ATP + AMP [178, 179]. During skeletal muscle contraction there is a depletion of ATP thus a fall in ATP:ADP ratio and ADK will run from left to right, leading to a large increase in AMP as ATP falls. [178] AMPK has been implicated as an important mediator of muscle contraction-induced glucose transport [180]. AMPK has been shown to positively regulate glucose and fatty acid uptake through glycolysis and oxidation [181]. AMPK is a heterotrimeric protein, composed of one α -subunit and two non-catalytic (β and γ) subunits [180] and is activated by cellular stress associated with ATP depletion. [182] The protein is activated in response to an increase in the ratio of AMP:ATP within the cell. [183] AMPK phosphorylates TBC1D1 and AS160 [184] and inactivating GTPase causing Rab-GTP to increase and accelerates GLUT 4 translocation [181]. AMPK phosphorylates the molecules in the muscle contraction pathway and Akt phosphorylates the molecules in the insulin pathway [181]. Intracellular Ca^{2+} levels are related to motor nerve activity [126]. Original *in vitro* animal studies utilizing frog sartorius muscle incubated in caffeine resulted in the release of calcium from the sarcoplasmic reticulum increasing glucose transport [185, 186]. But other studies have shown no effect of Ca^{2+} with an impairment in insulin stimulated glucose transport [187, 188]. These differences are difficult to explain, but Lee et al. 1995 [188] hypothesizes these are due to magnitude and duration of cytosolic Ca^{2+} increase.

Calcium/calmodulin-dependent protein kinase kinases (CAMKKs) is hypothesized to increase glucose uptake by direct AMPK activation independent of energy depletion (AMP/ATP ratio) [101]. This leads to AS160 phosphorylation allowing GLUT4 storage vesicles to move to and fuse with the plasma membrane [165]. Ca^{2+} activates CAMK, calcineurin, and PKC. Calcineurin is a Ca^{2+} -dependent serine/threonine phosphatase, activated by calmodulin, related to

muscle hypertrophy [189, 190] and muscle fiber type transition [191, 192]. CAMK is a serine/threonine protein kinase where CAMKK is of particular interest in skeletal muscle. CAMKK is thought to decode the frequency of Ca^{2+} spikes into graded amounts of kinase activity [193] enhancing glucose transport. The overexpression of a CAMKK inhibitor, decreased glucose transport by skeletal muscle contraction independent of AMPK phosphorylation, suggesting CAMKK playing a critical role in glucose uptake [194]. PKC are activated in response to increases in Ca^{2+} , which in turn may mediate glucose uptake by extracellular signal-related kinase (ERK) [195, 196]. ERK signaling is highly dependent to intensity of exercise [197] where it has been shown that when one leg was exercised, activation of ERK was only detected in the exercised muscle, suggesting a local response to muscle contraction [198, 199]. However, Ca^{2+} increase is hypothesized to not exactly be the cause for the increase skeletal muscle glucose uptake, but rather via the activation CAMKK from direct AMPK activation due to increased ATP need from skeletal muscle contraction from Ca^{2+} release [101].

Musi et al. 2001, reported after a single bout of exercise on a cycle ergometer for 45 minutes showed an increase in AMPK activity and decrease in blood glucose concentration in a T2DM population. [200] Hutber et al. 1997, used electrical stimulation for 30 minutes at 1Hz *in vitro* resulting in a significant increase in the ratio of AMP to ATP and AMPK activation at minute 20 of stimulation. [201] An *in vitro* study by Atherton et al. 2005, applied electrical stimulation of 10Hz to skeletal muscle cells resulting in an increase of AMPK phosphorylation. [202] Literature provided has shown that muscle contraction (voluntary or involuntary) can initiate the insulin-independent signaling pathway increasing glucose uptake in individuals with insulin resistance.

Purpose

The primary purpose of this study is to determine the effects of four weeks of Neuromuscular Electrical Stimulation induced muscle contractions on metabolic health and muscle mass in a sedentary population with metabolic impairments.

Specific Aims

Specific Aim 1. To determine the effects of four weeks of NMES on insulin sensitivity by oral glucose tolerance test and metabolic markers (e.g. glucose and lipid profiles) by blood samples in a sedentary overweight and obese population.

Specific Aim 2. To determine the effects of four weeks of NMES on energy metabolism (e.g. resting metabolic rate [RMR] and whole-body fat oxidation capacity) by indirect calorimetry, and lactate accumulation by frequent blood sampling in a sedentary overweight and obese population.

Specific Aim 3. To determine the effects of four weeks of NMES on body composition measured by dual energy x-ray absorptiometry and muscle strength measured by isokinetic dynamometer in a sedentary overweight and obese population.

Methods

Ten overweight and obese participants between the ages of 18 and 54 were recruited to this study and were randomized into two groups (n=5/group) (Figure 5 top portion). Study protocol was approved by the University of Texas at El Paso Institutional Review Board and each participant signed a written informed consent form. All participants were sedentary as defined by a daily physical activity levels of less than 60 minutes per week.

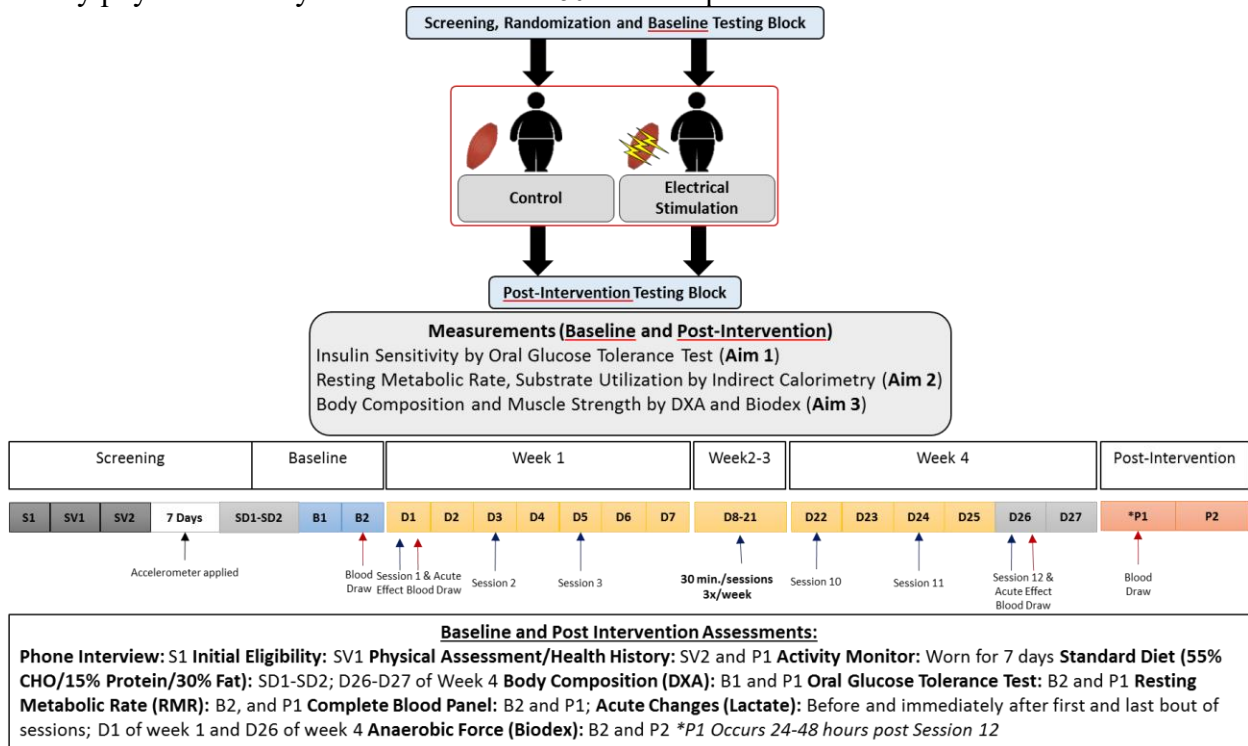


Figure 5. Study Design

Figure 5 provides the overview of the study design. Interested study participants were screened over the phone using a standardized questionnaire to determine eligibility. If the participants met basic inclusion criteria through self-reported age, height, and weight status, a meeting was scheduled to obtain signed informed consent form and following screening measurements (Table 2) to determine any exclusion criteria (Table 1). If no exclusion criteria were met, participants were issued an accelerometer to determine physical activity level over a period of 7 days. If participant met the sedentary physical activity level criteria, defined as less than 60

minutes spent in moderate to vigorous physical activity per week, [203] the participants were then instructed to return for assessment of strength, insulin sensitivity, substrate utilization, and body composition. Two days prior to baseline measurement of insulin sensitivity by OGTT, all participants were provided with a standard diet (~55% carbohydrate, ~15% protein, ~30% fat) to control for dietary effects on primary outcome measures after reporting any food allergies. Substrate utilization were assessed using indirect calorimetry during the fasted/resting state prior to the ingestion of a 75g glucose drink and again 15 minutes after ingestions. Fasting blood samples were collected to determine complete blood count (CBC), complete metabolic panel (CMP), Thyroid profile, plasma lipids, and plasma insulin.

Table 1 Inclusion/Exclusion Criteria

Inclusion Criteria	Exclusion Criteria
<ul style="list-style-type: none"> • 18 ≤ Age ≤ 54 years • 25 ≤ BMI kg/m² • Sedentary/Moderately Active Lifestyle (PAL <1.4) <ul style="list-style-type: none"> ○ less than 150 minutes per week of voluntary exercise 	<ul style="list-style-type: none"> • Anyone taking anti-hypertensive, lipid-lowering, or insulin sensitizing medications • Smoking • Excessive drinking • Pregnant Women • Unwilling to adhere to the study intervention

Table 2 Screening Measurements

Measurement	Method
Physical Activity Level	Physical Activity Questionnaire & PAL (Accelerometer data)
Fasting Glucose	Blood Sample via lancet prick/ Analysis via Contour Next Blood Glucose Meter
Lipid Profile (HDL, LDL, Cholesterol)	Laboratory Corporation of America ©
Blood Pressure	Automated Blood Pressure Device
Resting Heart Rate	External Heart Rate Monitor
Body Mass Index (BMI)	Height and Weight Measurements
Medical History	Questionnaire

Table 3 Study Variables

Variable	Method of Measuring
Body Composition	Dual Energy X-ray Absorptiometry (DXA)

Strength	Isokinetic Dynamometer
Resting Metabolic Rate	Indirect Calorimetry
Substrate Utilization	Indirect Calorimetry - respiratory quotient
Lactate Accumulation	Blood Lactate Measurements
Insulin Sensitivity	Oral Glucose Tolerance Test (OGTT)
Plasma Lipids	Laboratory Corporation of America ©
Fasting Insulin (C-Peptides)	Laboratory Corporation of America ©
Complete Blood Count w/Differentials	Laboratory Corporation of America ©
Complete Metabolic Panel	Laboratory Corporation of America ©
Thyroid Profile II	Laboratory Corporation of America ©

Physical Activity Level

After confirming eligibility, participants were issued an activity monitor to confirm a sedentary physical activity level to measure the time spent in moderate to vigorous activity. The ActiGraph GT3XP-BTLE 2GB activity monitor (Pensacola, FL) was attached to an elastic belt which the subject wore on the level of the anterior superior iliac crest and wore for 7 consecutive days (5 week-days and 2 weekend-days) including during sleep times. After the activity monitors were returned, a compliance time of >90% total wear time was confirmed and physical activity level was quantified to ensure sedentariness.

Body Composition

ANTHROPOMETRIC MEASUREMENTS

Height (cm) and weight (kg) measurements were obtained to verify a body mass index (BMI, Kg/m²) classified as overweight (25-29.9 kg/m²) or obese (≥ 30 kg/m²). [13] Furthermore, circumference measures of the hips, waist, and mid-thigh were obtained. As recommended by ACSM Guidelines hip circumference (cm) were taken at the largest part of the hip at midway of the inguinal crease; waist circumference (cm) were taken with the participant standing, arms at sides, feet together, and abdomen relaxed at the narrowest part of the torso (above the umbilicus and below the xiphoid process). As recommended by ACSM Guidelines circumference of the mid-thigh (cm) were taken with the subject standing on one foot on a bench/chair with the knee

flexed at 90 degrees and the measure taken midway between the inguinal crease and the proximal border of the patella (midway measure will be ensured by measuring the distance between the patella and the inguinal crease and dividing by 2). Waist to hip ratio (WHR) were calculated by dividing the measure of the waist by the hip. [13]

DUAL ENERGY X-RAY ABSORPTIOMETRY (DXA)

Participants were asked to lie down supine on the scanner table of a GE Medical Systems, Lunar iDXA Dual Energy X-ray Absorptiometer (Madison, WI). Participants were instructed to keep their arms close to the body, inside the marked regions with thumbs facing the ceiling and remain as still as possible. Knees and ankles were fastened together to prevent movement and standardize participant positioning. A scanner bar moved in the direction from head to toe of the participant, taking approximately 7-10 minutes depending on the participant's body size. Measurements of total lean mass, total fat mass, bone mineral density (BMD), percent body fat, percent android fat, percent gynoid fat, legs percent fat, legs percent lean, leg fat mass/total fat mass ratio, and visceral adipose tissue volume and mass were obtained.

Strength

ISOKINETIC DYNAMOMETER

An Isokinetic Dynamometer Biodex System 3 Pro (Shirley, NY) was used to measure lower limb strength. The participant was seated in the chair, stabilized with cross-body shoulder straps, a waist strap, and thigh straps. The participant's knee was aligned appropriately with the dynamometer shaft and secured to the knee attachment proximal to the medial malleoli. The participant was instructed to fully extend/contract their leg to set the maximum range of motion (ROM) in both directions. Furthermore, participants were instructed to fully extend their legs again and the knee attachment was locked into place to weigh the leg. The participant was then instructed to perform a series of maximal flexions and extensions of the dominant limb (at 60 degrees per second).

Dietary Control

Participants were provided with all food for two days prior to insulin sensitivity testing, to control for dietary effects on insulin sensitivity and blood profile. Meals were designed to comply with the USDA 2015-2020 Dietary Guidelines for Americans and individualized to participant preferences/allergies. The standardized diet consisted of macronutrient energy contents of ~55% carbohydrates, ~15% protein, and ~30% fat (<10% of total fat consisting of saturated fat). The Mifflin St. Jour equation was utilized to match participants to their estimated energy requirements. For the duration of the intervention, participants were encouraged to follow the USDA Dietary Guidelines for Americans (detailed above) and consume an energy balanced diet.

Insulin Sensitivity

ORAL GLUCOSE TOLERANCE TEST (OGTT)

Participants were instructed to avoid drinking alcohol, smoking, and strenuous exercise 24-48 hours prior to test days. Following a (12-h) overnight fast, participants arrived at the UTEP Health Sciences Building and were instructed to lie down for 5 minutes prior to obtaining fasting blood glucose sample. The participant was then asked to orally ingest a drink containing 75-grams of glucose as quickly as possible. Blood samples were then collected at timed intervals of 15, 30, 60, 90, 120, 150, and 180 minutes following glucose ingestion using CONTOUR® NEXT One, Ascensia Diabetes Care handheld glucose monitoring system (Parsippany, NJ). Insulin sensitivity were assessed from calculating glucose area under the curve over the 3-hour test.

Substrate Utilization

RESTING METABOLIC RATE AND SUBSTRATE UTILIZATION

Resting substrate utilization were measured prior to conducting an OGTT test, on the same day of the OGTT using indirect calorimetry. Participants were placed into a semi-recumbent position with a hood canopy over their head to obtain measurements of oxygen utilized and carbon dioxide produced; estimating RMR and RQ using Parvomedics TrueOne 2400 metabolic cart (Salt

Lake City, UT). Participants arrived after an overnight fast for this procedure and were instructed to breathe normally throughout the duration of this test.

ACUTE METABOLIC EFFECT

Acute effects of NMES on energy expenditure and substrate utilization were measured during the first and last NMES sessions. A fasting RMR and RQ were obtained prior to application of the stimulation and then monitored throughout the duration of stimulation (30 minutes). Given small sample size, data from session 1 and session 12 were combined for both groups to evaluate the effects of control and NMES.

LACTATE ACCUMULATION

Lactate production was measured during the first/last NMES intervention. Lactic acid was measured by whole blood samples using a hand-held Lactate Plus blood lactate meter (Nova Biomedical, Waltham, MA). A resting/fasted (~3-hr fast) blood sample was obtained using a lancet prick. Samples from a fingertip were collected prior to stimulation, in intervals of 5 minutes during the 30 minutes of stimulation. Lactate accumulation over the 30 min of stimulation was assessed by calculating lactate AUC. Given small sample size, data from session 1 and session 12 were combined for both groups to evaluate the effects of control and NMES on lactate concentration.

Assay Samples

COMPLETE BLOOD COUNT WITH DIFFERENTIAL & PLATELET COUNT

On the same day of the OGTT, a fasting blood sample was obtained via antecubital venipuncture for analysis of hematocrit, hemoglobin, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), red cell distribution width (RDW), platelet count (PC), white blood cell count (WBC), and red blood cell count (RBC). Blood samples were evaluated by Laboratory Corporation of America (Burlington, NC).

COMPREHENSIVE METABOLIC PANEL

On the same day of the OGTT, a fasting blood sample was obtained via antecubital venipuncture for analysis of alkaline phosphatase, aspartate aminotransferase (AST), alanine aminotransferase (ALT), albumin, blood urea nitrogen (BUN), creatinine, calcium, sodium, potassium, chloride, CO₂, glucose, total bilirubin, total protein, and total globulin. Blood samples were evaluated by Laboratory Corporation of America (Burlington, NC).

THYROID PROFILE II

On the same day of the OGTT, a fasting blood sample was obtained via antecubital venipuncture for analysis of free thyroxine index (FTI), T3 uptake (THBR), thyroid-stimulating hormone (TSH), thyroxine (T4), and tri-iodothyronine (T3). Blood samples were evaluated by Laboratory Corporation of America (Burlington, NC).

LIPID PANEL

On the same day of the OGTT, a fasting blood sample was obtained via antecubital venipuncture for analysis of low-density lipoprotein (LDL), high-density lipoprotein (HDL), triglycerides (TG), total cholesterol (TC) and very low-density lipoprotein (VLDL). Blood samples were evaluated by Laboratory Corporation of America (Burlington, NC).

FASTING INSULIN (C-PEPTIDE)

On the same day of the OGTT, a blood sample was obtained via antecubital venipuncture for analysis of c-peptide. Blood samples were evaluated by Laboratory Corporation of America (Burlington, NC).

NEUROMUSCULAR ELECTRICAL STIMULATION PROTOCOL

All participants received NMES intervention at the UTEP Metabolism, Nutrition, & Exercise Research (MiNER) Laboratory, with the QuadStar® II Digital Multi-Modality Combo Device (TENS-INF-NMS) (BioMedical Life Systems, Vista, CA) and eight 2" x 2" square electrodes (BioMedical Life Systems, Vista, CA). Electrodes were placed, shown in **Figure 6**,

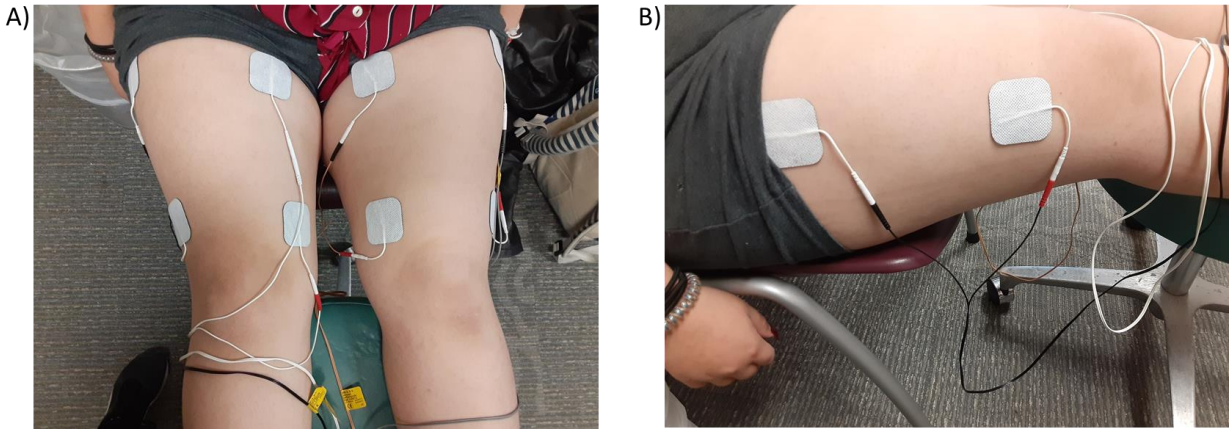


Figure 6. Electrode Placement. A) Frontal view. B) Lateral view

bilaterally in the proximal location of the quadriceps motor point using anatomical reference points. [204, 205, 206] The stimulation device was set to cycled biphasic waveform with pulse duration of 300 μ s and frequency set to 50Hz. Participants assigned to the NMES group received stimulation up to maximum tolerable levels and those assigned to Control received stimulation on the lowest possible setting (sensory level: described previously as a tingling sensation. [43]

Statistical Analysis

Statistical analyses were conducted using GraphPad Prism, version 7.0 (GraphPad Software, La Jolla, CA). Two-way ANOVA with repeated measures was used to compare groups (control, and NMES), time (before and after), and group by time effects. For all comparisons, a $p < 0.05$ was considered significant, and values are means \pm SEM. Considering this is a proof-of-concept/pilot study with very small sample size, we have also performed additional analyses using paired t-test to understand the effectiveness of NMES on primary outcome measures of the study.

RESULTS

Table 4 summarizes the participant’s baseline characteristics and outcomes following four weeks of NMES. At baseline, age, weight, BMI, body composition, fasting glucose, lipid profile, RMR, and strength were not significantly different between control and NMES groups.

Table 4. Descriptive Characteristics

	Control group (n=5) (M/F: 1/4)				NMES (n=5) (M/F: 1/4)				C vs N Baseline	Group p-value	Time p-value	Interaction p-value
	Baseline Mean	SEM	Post-Intervention Mean	p-value SEM	Baseline Mean	SEM	Post-Intervention Mean	p-value SEM				
Age (years)	42.20 ± 4.953786				30.33 ± 4.499383							
Blood Pressure												
Systolic (mmHg)	104.40 ± 0.509902		103.60 ± 2.158703	0.9794	112.00 ± 6.284903		102.40 ± 3.854867	0.1071	0.2625315	0.4997	0.1239	0.1839
Diastolic (mmHg)	69.60 ± 2.657066		68.60 ± 1.749286	0.96	79.60 ± 6.087693		68.00 ± 4.266146	0.032	0.1706126	0.3783	0.0479	0.0853
Body Composition												
Height (cm)	162.84 ± 5.502196				163.09 ± 2.092271							
Body weight (kg)	87.18 ± 5.851957		86.66 ± 6.224757	0.6578	87.22 ± 7.121475		86.76 ± 7.313248	0.7179	0.9966438	0.9942	0.2851	0.9458
BMI (kg/m ²)	32.84 ± 1.449375		32.65 ± 1.511019	0.7092	32.70 ± 2.294233		32.47 ± 2.380828	0.5224	0.9599886	0.9571	0.2246	0.8311
Waist circumference	101.05 ± 3.338989		98.80 ± 4.278304	0.3418	96.10 ± 4.733333		95.63 ± 5.543615	0.9485	0.4179548	0.5395	0.2547	0.4441
Hip circumference	114.99 ± 2.864966		112.70 ± 1.856221	0.1301	115.80 ± 4.571166		115.27 ± 4.607542	0.8676	0.8850271	0.7508	0.1025	0.2841
Waist-to-hip ratio (WHR)	0.88 ± 0.015142		0.88 ± 0.030947	0.9871	0.85 ± 0.022497		0.83 ± 0.029837	0.9985	0.1096567	0.1965	0.8923	0.9468
Lean mass (kg)	48.55 ± 5.540353		47.98 ± 5.565273	0.1432	47.30 ± 4.291417		46.77 ± 4.241068	0.1773	0.8630198	0.8651	0.0232	0.9213
Fat mass (kg)	35.73 ± 2.189951		35.61 ± 1.991273	0.9723	37.02 ± 3.543103		37.21 ± 3.937885	0.9285	0.7641366	0.7441	0.9254	0.6969
Body fat (%)	42.94 ± 3.005761		43.20 ± 2.831784	0.7848	43.82 ± 1.950743		44.12 ± 2.290502	0.7266	0.8121872	0.809	0.3537	0.9457
Androd % fat	51.32 ± 2.737408		52.16 ± 2.392196	0.421	48.72 ± 2.972776		50.04 ± 3.407873	0.1548	0.5379771	0.5785	0.0494	0.6211
Gynoid % fat	42.22 ± 3.104416		42.78 ± 2.943026	0.1357	47.58 ± 3.019834		47.06 ± 3.110723	0.1694	0.2509491	0.2951	0.9186	0.0216
A/G ratio	1.23 ± 0.045343		1.23 ± 0.043012	0.962	1.04 ± 0.077756		1.07 ± 0.077679	0.0991	0.0677835	0.0855	0.109	0.1871
Lean leg mass (kg)	16.61 ± 1.886488		16.29 ± 1.905105	0.1239	16.58 ± 1.913354		16.29 ± 1.914953	0.1592	0.991247	0.9948	0.0193	0.9085
Lipid Panel												
Total Cholesterol (mmol/L)	188.40 ± 11.77115		199.40 ± 10.28397	0.1365	183.80 ± 17.35339		191.20 ± 18.70936	0.3578	0.8318546	0.7665	0.0391	0.6426
Triglycerides (mmol/L)	169.00 ± 31.72538		144.80 ± 9.866104	0.5069	162.20 ± 37.08558		191.20 ± 26.05264	0.9995	0.8926312	0.8823	0.4646	0.443
HDL cholesterol (mmol/L)	42.40 ± 5.095096		45.20 ± 6.748333	0.4533	45.80 ± 2.332381		45.20 ± 1.019804	0.9607	0.5608332	0.785	0.5202	0.329
VLDL cholesterol (mmol/L)	33.80 ± 6.374951		29.00 ± 2.04939	0.5256	32.40 ± 7.44043		32.40 ± 5.221111	0.9999	0.8899128	0.895	0.4666	0.4666
LDL cholesterol (mmol/L)	112.20 ± 7.927168		125.20 ± 8.033679	0.0307	105.60 ± 11.28982		113.60 ± 14.07338	0.1829	0.6451411	0.5543	0.0008	0.4288
Substrate Utilization												
Resting metabolic rate (Kcal)	1801.27 ± 153.2034		1902.08 ± 130.4259	0.5372	2050.82 ± 214.2476		1927.74 ± 204.3599	0.4091	0.3711417	0.5884	0.8724	0.1342
Fasting substrate utilization (RQ)	0.78 ± 0.017837		0.73 ± 0.022258	0.2609	0.78 ± 0.017382		0.78 ± 0.015483	0.8538	0.9953947	0.0996	0.4522	0.1659
Strength Parameters												
Peak TQ/BW Right Leg (%)	182.87 ± 20.70344		176.79 ± 22.47135	0.6507	163.18 ± 17.85136		160.77 ± 20.77052	0.9321	0.4917916	0.5497	0.415	0.7192
Peak TQ/BW Left Leg (%)	167.43 ± 21.96707		173.47 ± 18.55206	0.3934	163.37 ± 17.07677		155.56 ± 16.65439	0.234	0.8877791	0.6863	0.7909	0.0637
Work to Fatigue Right Leg (%)	0.66 ± 4.727196		-3.18 ± 3.511704	0.9989	-123.40 ± 130.1917		10.39 ± 2.31734	0.3249	0.3688387	0.4282	0.3406	0.3145
Work to Fatigue Left Leg (%)	9.11 ± 1.279731		8.50 ± 6.082004	0.9938	7.29 ± 3.895017		2.81 ± 5.370313	0.7213	0.6699917	0.4624	0.5615	0.6568
Insulin Sensitivity												
Fasting*	89.75 ± 6.66302		104.80 ± 13.2567	0.6967	96.00 ± 3.962323		93.00 ± 2.48998	0.4688	0.42494	0.5611	0.8479	0.2061
2 Hour Mark*	151.75 ± 6.142407		173.20 ± 34.17404	0.2827	150.90 ± 7.588478		138.20 ± 7.606905	0.1895	0.9356423	0.9055	0.0435	0.9496
AUC*	430.73 ± 20.22866		494.68 ± 77.21001	0.7624	455.55 ± 26.0663		415.36 ± 25.89178	0.0798	0.495091	0.7363	0.0653	0.2857
Lactate Accumulation												
Lactate AUC	0.83 ± 0.090602		0.90 ± 0.139489	0.8086	1.15 ± 0.16		1.29 ± 0.254093	0.6158	0.115067	0.1401	0.31	0.8243

Data are presented as mean ± SEM, * indicates n = 4 for control group; bolded text represent statistical significance p < 0.05.

Improvement in Insulin Sensitivity after 4 weeks of NMES

Fasting blood glucose, glucose level 15, 30, 60, 90, 120, 150, and 180 minutes after glucose ingestion during an OGTT, glucose AUC, and C-peptide were similar between groups at baseline (p > 0.05). While no change in fasting glucose levels were observed (Figure 7A), there was a trend to decrease glucose AUC (p = 0.07; Figure 7D) following four weeks of NMES (Table 4). Given the current study is a pilot/proof-of-concept study with small sample size and lacks the power to

detect changes between two groups, we also analyzed the data within the NMES group (paired t-test) to understand the clinical relevance of the effectiveness of NMES. There was a significant decrease in blood glucose level at minute 30 ($p=0.03$) and 120 ($p=0.02$; Figure 7C) and a trend to decrease at minute 15 ($p=0.09$) and 60 ($p=0.08$) (Figure 7B); the two-hour mark is used for clinical diagnosis of T2DM using OGTT. [207] Finally, there was a significant decrease in glucose AUC in the NMES group ($p=0.029$) following the four-week intervention. There was no change in C-peptide in the control and NMES groups following four weeks of NMES (Table 4).

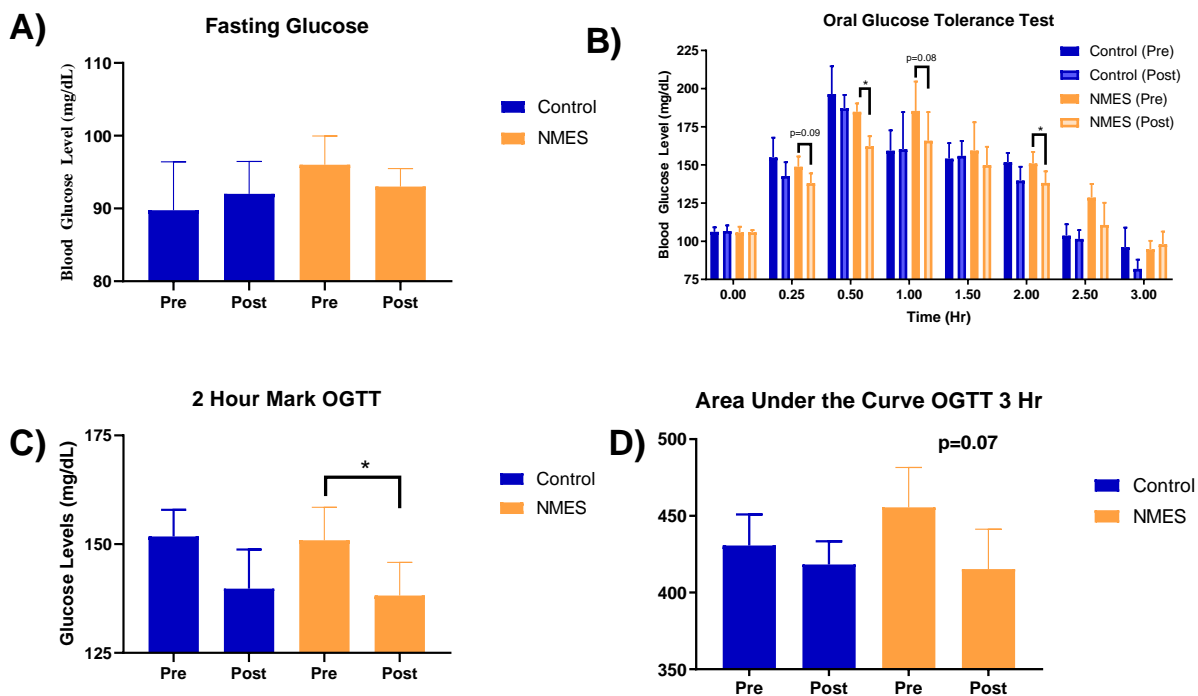


Figure 7. Improvement in insulin sensitivity after 4 weeks of NMES, measured by OGTT. Pre and post intervention fasting blood glucose (A), 3 hour OGTT at minutes 0, 15, 30, 60, 120, 150, and 180 (B), glucose level after 2 hours of OGTT (C), and glucose area under the curve (D) are shown above. (Control $n=4$, NMES $n=5$).

* Significantly difference pre vs. post intervention.

Acute and chronic effects of NMES on Energy Expenditure and Substrate utilization

There was no significant difference between control and NMES groups in resting energy expenditure and substrate utilization measured by RQ (Figure 8).

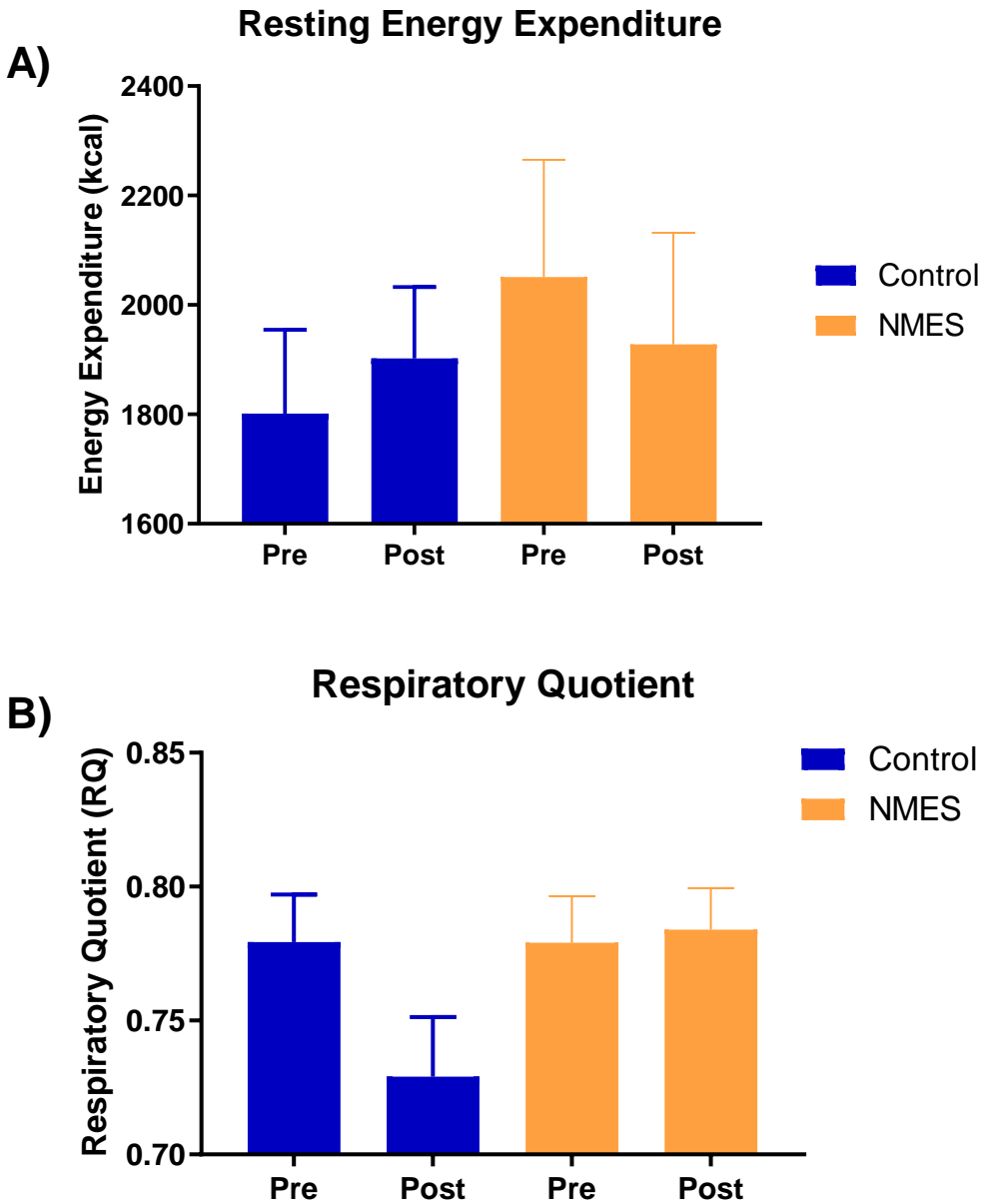


Figure 8. No change in resting energy expenditure (A) and substrate utilization (B) measured by indirect calorimetry, after 4 weeks of NMES.

There was no significant change in energy expenditure and RQ during NMES compared to BL (Figure 9A and 9B). However, when compared within only NMES group, average energy expenditure during NMES was significantly greater during session 12 compared to session 1 ($p=0.04$; Figure 10).

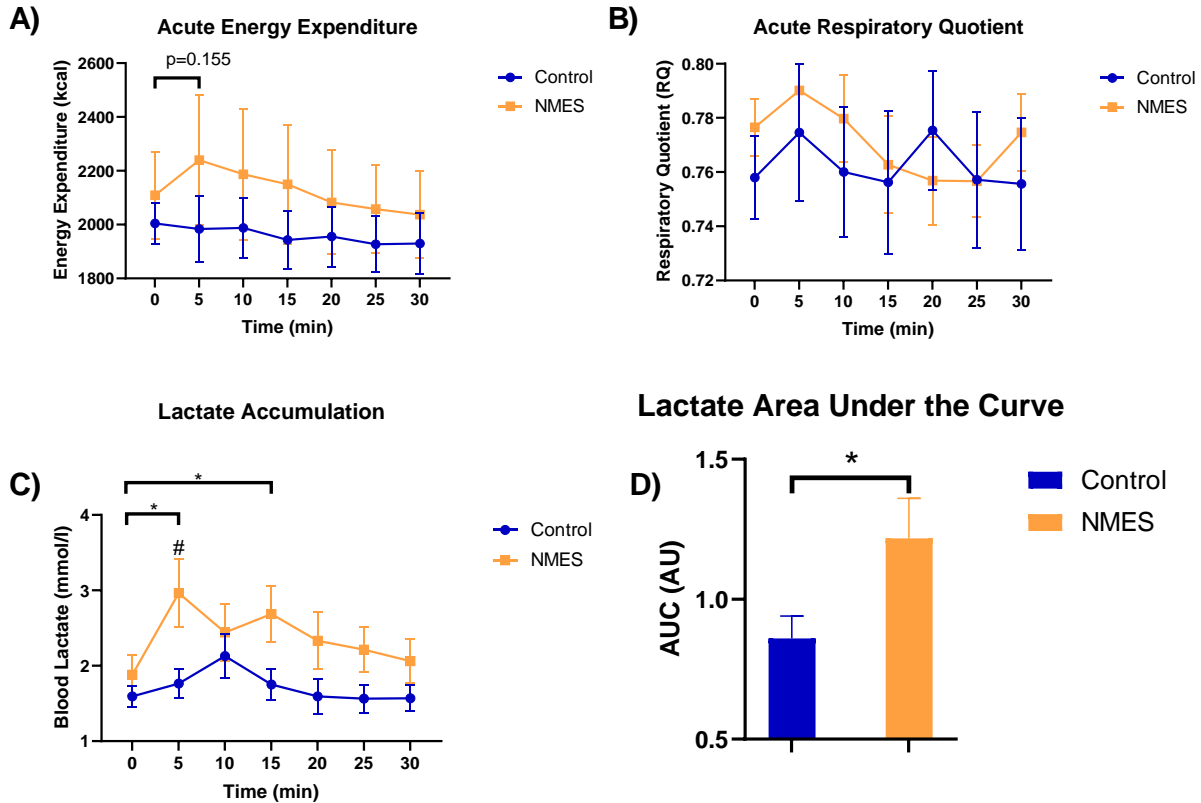


Figure 9. Acute effects of NMES on energy expenditure (A), respiratory exchange ratio (RER) (B) lactate concentration (C) and lactate AUC (D) measured during session 1 and session 12 of the intervention.

* Significantly different compared to baseline

Significantly different between control and NMES groups

Lactate concentration significantly increased during minute 5 ($p=0.002$) and minute 15 ($p=0.04$) in the NMES group compared to resting lactate level (Figure 9C). Moreover, lactate concentration after 5 min of NMES stimulation was also greater compared to the control group ($p=0.02$) (Figure 9C). Finally, lactate AUC assessed during 30 min of NMES was significantly greater compared to that of control group ($p<0.02$; Figure 9D).

Average Energy Expenditure/Body Weight

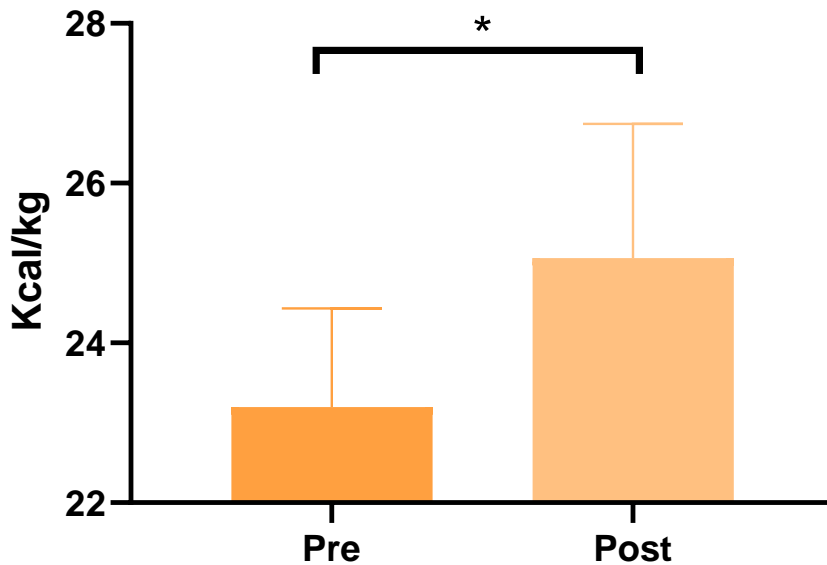


Figure 10. Increase in average energy expenditure measured during session 12 compared to session 1

* Significantly different session 1 vs session 12.

No Change in Body Composition after 4 weeks of NMES

Body weight, BMI, waist circumference, hip circumference, WHR, blood pressure, body mass, fat mass, percent body fat, lean mass, lean leg mass, android percent fat, gynoid percent fat, and android to gynoid (A/G) fat ratio were similar between groups at baseline (Table 4). There were no changes in these parameters following four weeks of NMES (Figure 11 and 12). There was a trend to decrease systolic blood pressure ($p=0.1$) and a significant decrease in diastolic pressure ($p=0.03$) in the NMES group following four weeks of intervention (Table 4). There were no changes in blood pressure in the control group. Peak torque per body weight (TQ/BW) and work to fatigue in both legs were similar between groups at baseline (Table 4.) There was no change in peak TQ/BW in both legs or work to fatigue in the left leg following four weeks of NMES. However, work to fatigue in the right leg significantly increased ($p=0.04$) following four weeks.

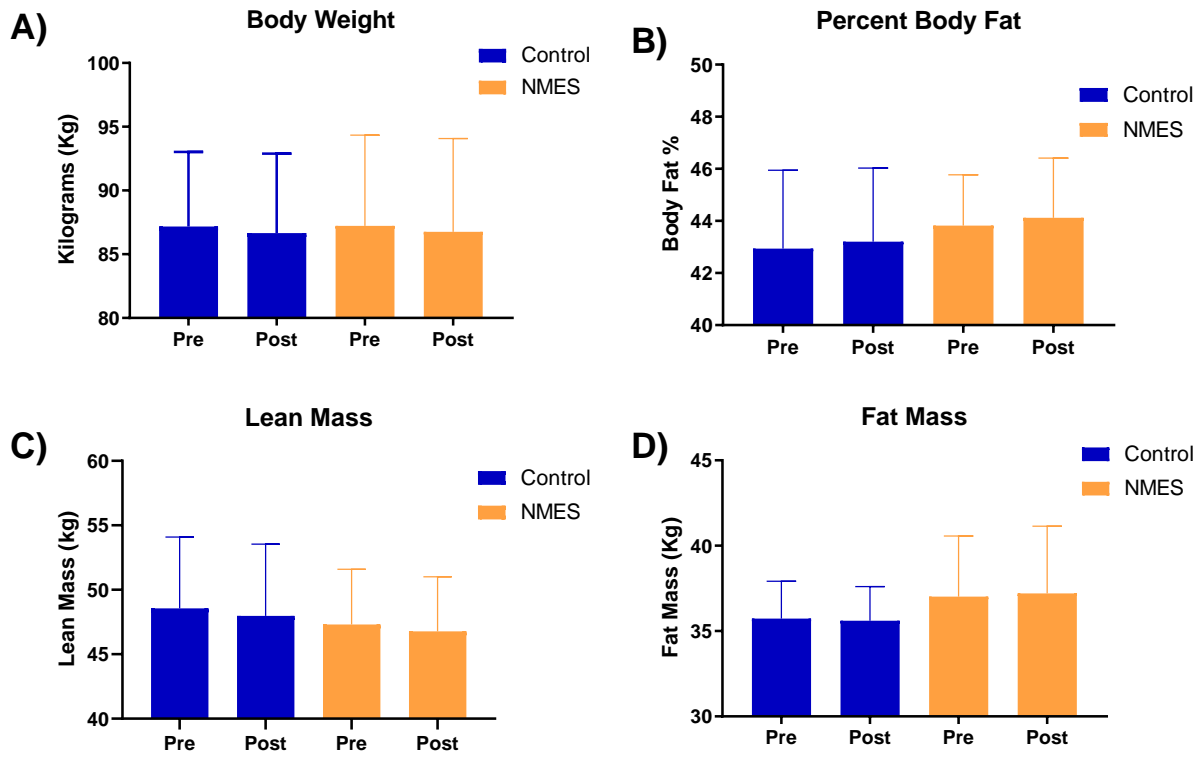


Figure 11. There was no change in body weight (A), percent body fat (B), lean mass (C) or fat mass (D) following 4 weeks of NMES.

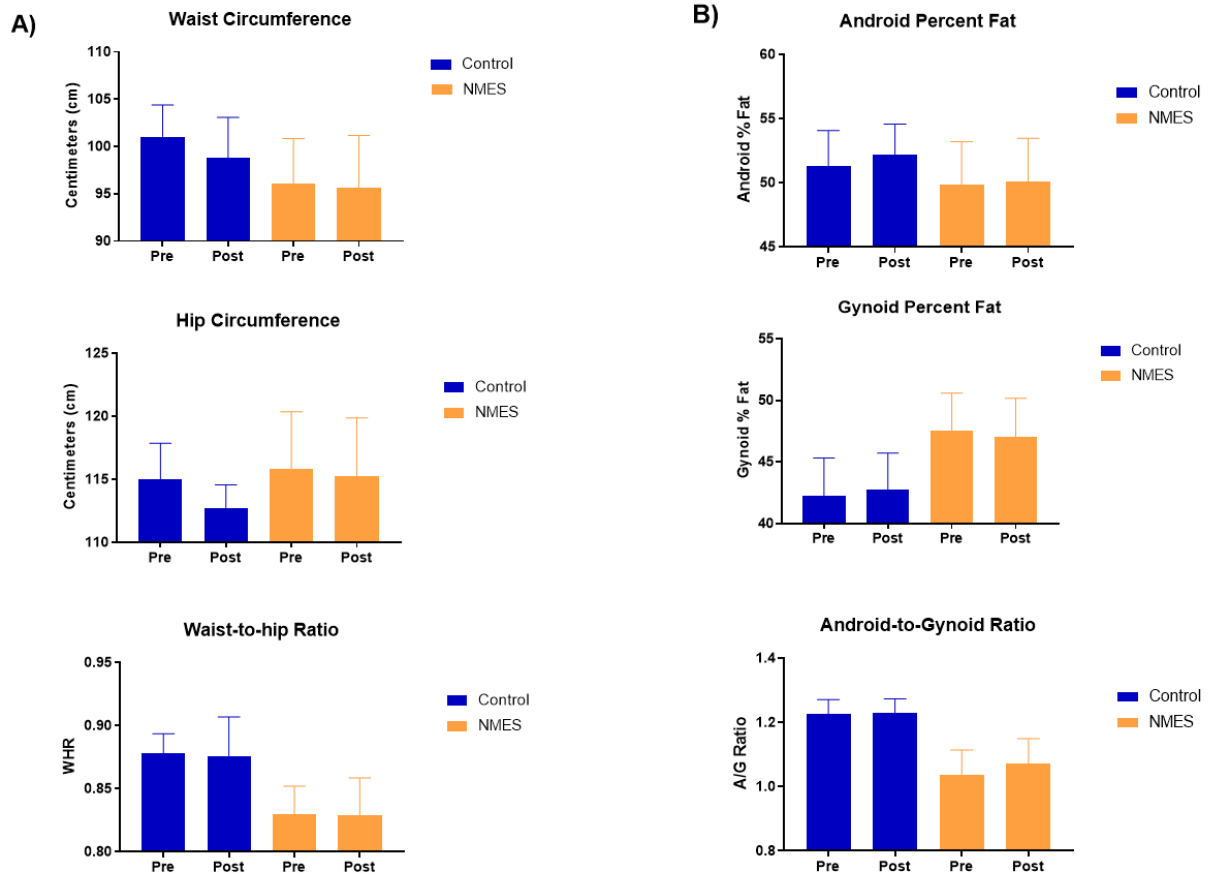


Figure 12. There was no change in waist, hip circumference and WHR (A) and android, gynoid percent fat, and A/G ratio (B) following 4 weeks of NMES

No Change in Lipid Profile after 4 weeks of NMES

LIPID PANEL

Total cholesterol, triglycerides, HDL, VLDL, and LDL showed no difference between groups at baseline and showed no change following four weeks of NMES (Table 4).

Discussion

The primary purpose of this pilot study was to determine the effects of chronic NMES on insulin sensitivity, substrate utilization and muscle mass in a sedentary overweight/obese population with metabolic impairments. Our data indicates that four weeks of NMES resulted in improvement in insulin sensitivity, without any effect on resting substrate utilization and muscle mass. Moreover, we demonstrate greater lactate accumulation during acute application of NMES compared to sensory level stimulation (control group). To our knowledge this is the first comprehensive longitudinal study using NMES in an at-risk Mexican American population

This pilot study is of high clinical significance demonstrating improvement in glucose tolerance after only four weeks of NMES treatment (30min/session, 3x/wk, at 50Hz) in a high-risk overweight and obese predominantly Mexican-American population. Two small studies by Hamada et al. [47, 48] investigated the acute effects of NMES, in a healthy all-male population, during a single NMES session for 20 min at 20Hz. The GDR, measured by clamp, in this study increased during stimulation and remained elevated for at least 90 minutes after cessation of NMES (recovery period). [47, 48] Jabbour et al. 2015, performed two sessions of electrical stimulation at 8Hz up to tolerable intensity during the first hour of OGTT. After obtaining two blood samples, at minute 60 and 120 of OGTT testing, the study reported a significantly lower blood glucose after NMES treatment compared to the control sessions. This study also showed a positive correlation between NMES stimulation intensity and degree of decrease in blood glucose levels in individuals with T2DM [45]. Few longitudinal studies that investigated the effects of NMES in patients with T2DM, reported improvement in insulin sensitivity. An increase in insulin response in patients with T2DM was reported after 2 weeks of NMES treatment (50Hz of quadriceps stimulation) without any change in lipid profile. [43] A pilot study by Joubert et al. 2015, performed four weeks of quadriceps stimulation at 35Hz, in a T2DM population, and reported an increase in insulin sensitivity measured by hyperinsulinemic euglycemic clamp. [46] Furthermore, Catalogna et al. 2016 reported an improvement in blood glucose control in patients

with T2DM after daily five minute stimulation for eight weeks at 16Hz on the lower extremity [44]. Overall, there was a decrease in fasting glucose, lower serum cortisol level, and mean HbA1c level. The study also showed decrease in postprandial blood glucose after breakfast after eight weeks. [44] In 2018 Miyamoto et al. evaluated lower limb electrical stimulation for eight weeks, 40 min/session, 5x/wk at 4 Hz, in T2DM population. [50] The study reported a significantly greater percent change in the fasting glucose concentration and significantly greater percent change in body fat in NMES than in control. Our results are in agreement with these studies conducted in a T2DM population indicating the effective method to improve insulin sensitivity. However, we demonstrate the effectiveness of NMES to improve insulin sensitivity in an at-risk predominantly Mexican-American overweight and obese population without T2DM. Moreover, we show a significant improvement in glucose tolerance 2 hours after the consumption of glucose drink during an OGTT test; supporting the use of NMES as a clinically relevant strategy to improve glucose metabolism in an overweight or obese population. These improvements were observed without any change in resting substrate utilization or body composition – suggesting possible role of muscle contraction induced insulin independent glucose uptake pathway. Future investigation should evaluate the mechanism of muscle contraction induced improvement in insulin sensitivity. Our study, for the first time shows improvement in insulin sensitivity using OGTT with as little as 4 weeks of NMES in an overweight and obese population without type 2 diabetes.

Acute NMES has generally been shown to increase energy expenditure and carbohydrate utilization in a variety of populations (healthy, recreationally active, obese adults and T2DM population) using varying stimulation frequencies and durations (5-75Hz, 20-60 minutes). [47, 48, 66, 64, 67, 68]. Hultmans and Spriet (1986) reported an increase in oxidative metabolism, measured by muscle fibers after an acute session for 45 minutes, at 20Hz on quadriceps in a healthy population. [24] RQ measured during a single bout of stimulation session at 75Hz in healthy males showed an increase in RQ. [64] Only study to our knowledge conducted in an obese population, examined the acute effects of one-hour low frequency stimulation (5Hz) to the quadriceps and hamstring muscles and reported an increased oxygen uptake, RER, energy expenditure, lactate,

heart rate and carbohydrate oxidation. [66] Hamada et al. 2004 compared the effects of 20 minutes of stimulation at 20Hz to the lower leg muscles (tibialis anterior and triceps surae) and the thigh (quadriceps and hamstrings) to a single bout of 20 minutes of cycling. [47] Authors reported a greater carbohydrate use, measured by elevated RER and lactate, and an increase in oxygen uptake during stimulation as well as an increased glucose uptake during stimulation and recovery. [47] The study also found similarities to the study done by Grosset et al. 2013 such as an increase in oxygen uptake, heart rate, lactate and RER at the onset of stimulation but not with exercise. [66, 47] These findings suggest an increased energy expenditure, alongside increased glucose utilization during stimulation. Miyamoto et al. [67, 68] investigated the acute effect of lower limb NMES for 30 minutes at 4Hz 30 minutes after a standard meal on T2DM population. Both studies found blood glucose concentration in the NMES group was significantly lower compared to the control group at minute 60, 90, and 120 after the meal. Oxygen uptake, RQ and lactate concentration were significantly higher in the NMES group compared to control. In our pilot study, NMES treatment increased energy expenditure and glucose utilization measured by increase in lactate accumulation during the duration of the stimulation. The increase in lactate accumulation compared to baseline indicating reliance on glucose utilizations is similar to two previous studies conducted by Hamada et al. [47, 48] in a healthy population. However, our study did not show an increase in RQ during the stimulation. Given the RQ represents the whole-body glucose utilization capacity, it is possible that our study is not appropriately powered to detect these changes in whole body substrate utilization within this small sample size. However, our study is the first, to our knowledge, that evaluated the long-term effect on substrate utilization in an overweight/obese population. Although we observed no improvement in resting energy expenditure and substrate utilization after 4 weeks of NMES, there was a significantly greater energy utilization during NMES during post intervention (12th session) compared to baseline (1st session) NMES stimulation, suggesting the role of stimulation intensity in increasing energy expenditure. NMES stimulation intensity was set to maximal tolerable level of the participants and was increased with

progressing number of NMES sessions. This result agrees with a study done by Grosset et al. 2013. [66]

Our study also shows no change in body composition and leg muscle mass using DXA. Previous studies indicated increase in quadricep muscle CSA measured by MRI [91, 89], muscle fiber CSA by muscle biopsy [18], CSA of the mid thigh by tomography [90], and MRS [89]. Only one study, to the best of our knowledge, measured whole body composition using DXA and reported an increase in muscle mass after 10 weeks of NMES in a SCI population. [81]. Majority of the studies reported an increase in muscle CSA/mass after NMES were conducted in populations with SCI, chronic heart failure, and end-stage osteoarthritis where habitual movement is impacted. Griffin et al. 2009, showed an increase in muscle power and work and a 4% increase in lean muscle mass by DXA using cycling functional electrical stimulation for 10 weeks in a SCI population. [81] Two other studies in patients with SCI found an increase in total work output for 30 min/session, 3x/wk, for eight weeks at 30Hz [94] and increases in other variables of power, muscle fiber area, and capillarization for 2-3x/week for ten weeks at 50Hz [81]. Two studies, in participants with chronic heart failure, found an increase in gastrocnemius muscle mass after five weeks [89] and an increase in CSA after eight weeks with NMES (50Hz) [90]. After 6 weeks of NMES (50Hz) in patients with end-stage osteoarthritis also found increases in muscle CSA [91]. Few studies that focused on evaluating the effect of NMES in a healthy population reported increases in muscle fiber CSA after four [83] and eight weeks [18] of NMES. However, Martin et al. [86] reported no change in muscle CSA in healthy individuals after four weeks of NMES, which could have been attributed to short session time (10min) compared to others (ranging from 16-34min) [83, 84, 85, 87]. Two other studies reported no change in CSA in patients with lower limb or knee joint injury/surgery, with two frequency groups of intensities in each, 20 and 80Hz for 12 weeks [92] and 50 and 100Hz for four weeks [93]. Our study evaluated the effects of NMES in relatively healthy population without habitual movement impairment. Here we have reported improvement in insulin sensitivity without these adaptations in substrate utilization or muscle mass. Given most of the studies that has reported an increase in muscle mass, measuring CSA by

MRI, muscle biopsy, tomography, and MRS, are either in a diseased population (SCI, chronic heart failure etc.) [81, 89, 90, 91] has used a longer duration (4 to 10 wks) [83, 86, 89, 91, 97, 90, 81] or frequency (10Hz to 120Hz) [89, 90, 91, 81, 86, 97, 83] of electrical stimulation. It is possible that NMES induced improvements is only effective in population at a greater risk for muscle atrophy. The primary outcome of our study is an improvement in insulin sensitivity in an at-risk overweight/obese population using an effective stimulation frequency and allowing the participant to use the tolerable intensity to ensure adherence. Therefore, it is possible that the study duration (4 weeks) and or the stimulation (50Hz) is not adequate to see changes in muscle mass within this small sample size. Our study is the first, to our knowledge, to measure android, gynoid fat, A/G ratio, and visceral adipose tissue. Visceral fat is strongly linked to metabolic disease and insulin resistance. [208] There was no change in android, gynoid fat, A/G ratio, and visceral fat, however, an improvement in insulin sensitivity in this population without a change in muscle mass or substrate utilization indicates possible local effects of muscle contraction induced signaling pathways to improve glucose uptake.

In vivo studies by ES, have shown that increase in blood flow in a muscle cell is important for increasing rate of glucose uptake during muscle contraction [42]. Our study indicates an improvement in insulin sensitivity in overweight or obese population after four weeks of NMES, without concurrent changes in substrate utilization or muscle mass. Furthermore, the pilot study shows a trend towards improvement in systolic blood pressure and a significant decrease in diastolic blood pressure. Although we did not measure the effects of NMES on blood flow in this study, based on the literature indicating increase in blood flow with NMES [42], we speculate increase in blood flow to stimulated muscles, may play a role in overall decrease in diastolic blood pressure in this population. Future studies should investigate the possible mechanism relevant to hemodynamics aspects of NMES.

In addition to traditional insulin dependent glucose uptake pathway, an alternate insulin independent glucose uptake pathway (muscle contraction) has been well established, (see **Figure 4**). [175, 168, 176, 177, 178, 179, 180, 181, 182, 183] Our laboratory and others have shown an

increase in GLUT 4 protein, AMPK and glucose uptake after electrical stimulation induced muscle contraction on human primary muscle cells (*in vitro*). [35, 36, 37, 38, 39, 40, 41, 33, 34] current study translates these findings in human subjects suggesting an improvement in glucose tolerance possibly due to local effect of muscle contraction without changes in metabolic markers and muscle mass. As overweight/obese Hispanic individuals are at a greater risk for developing T2DM compared with other races. [4, 1, 7, 8, 9, 6, 3], NMES holds the potential to provide an alternative strategy to improve insulin sensitivity for this population and possibly for obese individuals who are at high-risk for developing T2DM and are unable to perform traditional exercise.

Our study is limited by small sample size. However, this pilot/proof-of-concept study provides compelling evidence for considering NMES as an alternative mechanism to increase insulin sensitivity in an at-risk Mexican American population. Another limitation of this study is that fasting insulin was not obtained. Despite having these limitations, one of the strengths of this study is the use of OGTT to assess insulin sensitivity and in evaluating the chronic effect of NMES. In addition, this study was conducted in an overweight/obese Mexican-American population who are at high-risk for developing T2DM.

In summary, we have demonstrated that four weeks of NMES (12 sessions) improves glucose tolerance in a sedentary overweight and obese population. NMES led to acute increase in energy expenditure and glucose utilization, evident by increase in lactate concentration, during the NMES stimulation. However, this adaption in glucose tolerance was possible without any improvement in resting substrate utilization and muscle mass. Future studies should evaluate the mechanisms of muscle contraction induced improvement in insulin sensitivity to develop alternate strategies to improve metabolic health in a sedentary, at-risk population.

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Glossary

Adenosine diphosphate (ADP)
Adenosine monophosphate (AMP)
Adenosine triphosphate (ATP)
Adenylate kinase (ADK)
Akt substrate 160 (AS160)
Alanine aminotransferase (ALT)
American College of Sports Medicine (ACSM)
American Diabetes Association (ADA)
AMP-activated protein kinase (AMPK)
Android/Gynoid ratio (A/G ratio)
Aspartate aminotransferase (AST)
Blood urea nitrogen (BUN)
Bone mineral density (BMD)
Calcium calmodulin-dependent protein kinase (CAMK)
Calcium/calmodulin-dependent protein kinase kinases (CAMKKs)
Chronic obstructive pulmonary disease (COPD)
Complete blood count (CBC)
Complete metabolic panel (CMP)
Complete thyroid profile (CTP)
Diacylglycerols (DAG)
Electrical Stimulation (ES)
Free thyroxine index (FTI),
GDP-loaded Rab (Rab-GDP)
Glucose disposal rate (GDR)
Glucose transport 4 (GLUT4)
Glycated hemoglobin (HbA1c)
GTP-loaded Rab (Rab-GTP)
High-density lipoprotein (HDL)
Insulin receptor (IR)
Insulin receptor substrate isoform (IRS-1)
Long chain fatty acyl-CoA (LCFA-CoA)
Low-density lipoprotein (LDL),
Mean corpuscular hemoglobin (MCH)
Mean corpuscular hemoglobin concentration (MCHC)
Mean corpuscular volume (MCV)
Neuromuscular Electrical Stimulation (NMES)
Oral glucose tolerance test (OGTT)
Phosphatidylinositol-diphosphate (PIP2)
Phosphatidylinositol-triphosphate (PIP3)
Phosphoinositide-dependent protein kinase (PDK).
Phosphoinositide 3-kinase (PI3-K)
Platelet count (PC)
Protein kinase B (PKB)
Protein kinase C (PKC)
Rab-GTPase-activating protein (GAP)

Range of motion (ROM)
Ras homologous from brain (Rab)
Red blood cell count (RBC)
Red cell distribution width (RDW)
Resistance exercise training (RT)
Respiratory exchange ratio (RER),
Respiratory quotient (RQ),
Resting metabolic rate (RMR)
Sarcoplasmic reticulum (SR)
Spinal cord injury (SCI)
Thyroid-stimulating hormone (TSH)
Thyroxine (T4)
Total cholesterol (TC)
Transverse tubules (T tubules)
Tre-2/BUB2/cdc 1 domain family member 1 (TBC1D1)
Triglycerides (TG)
Tri-iodothyronine (T3)
Type 2 diabetes mellitus (T2DM).
T3 uptake (THBR)
Very low-density lipoprotein (VLDL)
Waist to hip ratio (WHR)
White blood cell count (WBC)

Vita

Michelle Galvan is a graduate student pursuing a career as an exercise physiologist in the cardiac rehabilitation field. Michelle earned a Bachelor of Science in Kinesiology from The University of Texas at El Paso (UTEP) and is earning her Master of Science in Kinesiology. During her time as a graduate student, Michelle worked as a Teaching Assistant for the Department of Kinesiology.

During her academic career Michelle received the 2018 Miners Helping Miners scholarship and the 2018 UTEP Grant for Graduates. As a research assistant under the supervision of Dr. Sudip Bajpeyi, in the Metabolic, Nutrition & Exercise Research (MiNER) Laboratory, Michelle earned several awards including the 2019 Dodson Research Grant, 2019 Sandy Tyler Endowed Fellowship, and 2019 College of Health Sciences Applied and Translational Research Fund. She was also selected as the graduate marshal of students for the College of Health Science at the 2019 Winter Commencement graduation ceremony.

Michelle plans to pursue a career in cardiac rehab to focus on providing the best exercise and education program; to help those improve their health and help recuperate from a cardiac event, heart diseases or surgery to treat heart disease.

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This thesis was typed by Michelle Josie Galvan.