EFFECT OF NEAR-INFRARED LIGHT THERAPY ON TIME TO TASK FAILURE AND THE MOTOR OUTPUT OF YOUNG AND OLDER ADULTS

By

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To my husband Chris, thank you for being a constant source of encouragement and support. Without you, success would never be as sweet.

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LIST OF ABBREVIATIONS

ANOVA	Analysis of Variance
ATPase	Adenosine Triphosphatase
ATP	Adenosine Triphosphate
BL	Blood Lactate
BP	Blood Pressure
Ca^{++}/Ca^{2+}	Calcium
cAMP	Cyclic Adenosine Monophosphate
cGMP	Cyclic Guanosine Monophosphate
CK	Creatine Kinase
COX	Cytochrome C Oxidase
CRP	C-Reactive Protein
DNA	Dioxyribonucleic Acid
EMG	Electromyography
EPR	Electron Paramagnetic Resonance
FDA	Food and Drug Administration
FDI	First Dorsal Interosseus
GaAlAs	Gallium Aluminum Arsenide
GaAs	Gallium Arsenide
HeNe	Helium Neon
HIF-1α	Hypoxia-inducible Factor One Alpha
HO	Hydroxyl Radical
H_2O_2	Hydrogen Peroxide
HR	Heart Rate
Hz	Hertz, Cycles per second

ICF	Informed Consent Form
J/cm^2	Joules per centimeter squared
K^+	Potassium
LASER	Light Amplification by the Stimulation Emission of Radiation
LDH	Lactate Dehydrogenase
LED	Light Emitting Diode
LEDT	Light Emitting Diode Therapy
LH	Left Hand
MAP	Mean Arterial Pressure
MCP	Metacarpophalangeal
MHQ	Medical Health Questionnaire
mm	millimeter
MRS	Magnetic Resonance Spectroscopy
MVC	Maximal Voluntary Contraction
mW	Milli Watt
Na^+	Sodium
NF-kβ	Nuclear Factor Kappa Beta
NIR	Near-Infrared
NO	Nitric Oxide
NOS	Nitric Oxides Synthase
O_2-	Superoxide Anion
PBM	Photobiomodulation
PDGF	Platelet Derived Growth Factor
pO_2	Oxygen partial pressure (tension)
RH	Right Hand

RMSE	Root Mean Square Error
RMS	Root Mean Square
RNA	Ribonucleic Acid
ROS	Reactive Oxygen Species
RPE	Rate of Perceived Exertion
SD	Standard Deviation
SOD	Superoxide Dismutase
SPI	Second Palmar Interosseus
TMD	Temporomandibular disorder
TTF	Time to Task Failure
VEGF	Vascular Endothelial Growth Factor
W	Watts
1RM	One Repetition Maximum

Abstract of Dissertation Presented to the Graduate School
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Near-infrared (NIR) light therapy has been implicated as an effective ergogenic aid to enhance muscle function and delay the onset of fatigue. The most common manifestation of fatigue is an impairment in muscle function and an inability to perform work. However, no studies have directly examined the dose-response properties of phototherapy and its ability to delay the onset of fatigue and prevent muscular strength loss. More specifically, no studies have previously examined the ability of NIR light therapy to offset the age- associated deleterious effects of normal aging on muscle function. Therefore, the purpose of Experiment 1 was to determine the most effective dose of NIR light therapy to delay the onset of task failure. Each subject received 3 different doses of phototherapy treatment (sham, 240 Joules, 480 Joules) over their first dorsal interosseus (FDI) during three separate testing sessions. The 240 Joule treatment demonstrated a significant increase in time to task failure compared with sham. Therefore, the 240 Joule treatment delayed the onset of fatigue by allowing subjects to sustain the submaximal isometric task 26% longer than when they received the sham. The purpose of Experiment 2 was to evaluate the use of NIR light therapy to enhance muscle function in older adults. Per Experiment 1, we used the 240 Joule

dose. Near-infrared light therapy enhanced time to task failure only in older adults. Enhanced time to task failure was explained by increases modulation of the motor neuron pool. More specifically, increases in motor unit synchronization (power from 13-30 Hz of the EMG). In addition, treatment with NIR light therapy improved absolute strength in older adults. Fine motor dexterity, as measured by the Purdue pegboard was also improved when the FDI was treated with NIR light therapy. Treatment with NIR light therapy ineffective in young adults. These results may be a result of inadequate dosing and subthreshold stimulation of the physiological processes that enhance muscle function. Therefore, results from these studies support the use of NIR light therapy as an effective ergogenic for older adults when applied prior to fatiguing exercise.

CHAPTER 1
INTRODUCTION

Since the seminal work of Endre Mester, the use of light as a therapeutic modality has expanded and become a novel tool in the field of complementary and alternative medicine. Therapeutic Light is defined as the medicinal use of light to treat various pathological states and enhance physiological function.[1] Light therapy has been shown to improve muscle function through a process known as photobiomodulation. Photobiomodulation involves the use of organized light to stimulate biochemical changes in tissue as a means to enhance tissue healing and/or function. Previous research has noted enhancements in collagen synthesis, pain reduction, soft-tissue healing, tissue perfusion and wound healing. Phototherapy has been used extensively in many European countries, Australia and Canada, and is now just starting to take hold in the United States.

An important factor associated with the therapeutic effectiveness of laser is the dosing parameters.[2–4] The ability to achieve a therapeutic dose without under or over-stimulating the target tissue is often the most challenging component of clinical NIR light therapy practice.[3] The Arndt-Schultz Principle has been adopted from early toxicology studies of yeast culture to explain the optimal therapeutic dosage level of light therapy. This principle states that a therapeutic laser dosage or level of photo-stimulation must be attained. If not, “no reactions or changes will occur in body tissues if the amount of energy absorbed is insufficient to stimulate the absorbing tissues”.[5] Optimal doses have been Experimentally established in cell, animal and tissue cultures. However, the threshold level of photobiostimulation for many human tissues is not known. Previous research suggests that dose-dependency also exists when treating humans, however,

with the exception of superficial tissues and some dental applications, standard treatment doses for deep musculoskeletal tissues have yet to be firmly established in human clinical populations

The threshold level for photostimulation must be met in order for the photons being admitted to be absorbed by a photoacceptor or chromophore.[1] Photons that successfully penetrate the tissues will be absorbed by chromophores, in the blood, mitochondria and cellular membranes. Chromophores that absorb these photons will then assume a stimulated state and result in chemical reactions. Research has focused primarily on the terminal enzyme of the electron transport chain, cytochrome c oxidase (COX).[6] Cytochrome C oxidase is responsible for transferring electrons to molecular oxygen resulting in increased molecular energy production in the form of ATP. Photon absorption also induces increased activity of proton pumps that will lead to alterations in RNA and protein synthesis, oxygen consumption and membrane potentials in the irradiated area. Photostimulation also removes inhibitory attachment of nitric oxide (NO) at the mitochondrial level and from hemoglobin and myoglobin resulting in vasodilation in the surrounding vasculature and an overall increase in tissue blood flow and oxygenation.[7–10]

The majority of research conducted on NIR light therapy has focused on the ability of photostimulation to improve soft tissue healing. In addition to recovery from injury, exercise and one's ability to perform work can be greatly enhanced by the vascular effects of NIR light therapy as musculoskeletal fatigue is a process that may result from inadequate blood flow.[11] Previous studies have been able to provide evidence for the ability of light therapy to enhance blood circulation resulting in an increase in substrate

delivery and tissue oxygenation.[9,12,13] Increased circulation in the irradiated tissue can lead to increased oxygen and substrate delivery leading to an overall ability for a muscle to perform work. An increase in oxygen delivery to the working muscle will also lead to increased ATP production via aerobic respiration. As a result of an increase in ATP bioavailability, the local musculature is able to perform more work and resist fatigue more readily as it limits accumulation of metabolites and impairment of oxygen delivery. Enhancement of blood flow, oxygen delivery and ATP synthesis within working muscle provides a physiological basis of support for laser therapy as an ergogenic aid that can be used prior to exercise. As such, recent research has begun to focus on the ergogenic effects of light therapy to reduce or delay the onset of fatigue. Ergogenic effects relate to any external influence such as NIR light, that is able to enhance physical performance.[14] Furthermore, a complementary therapy administered to skeletal muscle prior to exercise and has the capability of producing an ergogenic effect would be beneficial in rehabilitation by enhancing the target muscle's capacity to perform work.

As a result of this recent focus it is necessary to fully understand the physiological processes that contribute to losses in muscle function. Muscle fatigue is described as a complex process which results from a multitude of physiological processes and is commonly caused by strenuous physical activity's' and exercise. [15] The most common manifestation of fatigue is impairment in muscle function and an inability to perform work. The decline in muscular force observed with fatigue is often due to impairment in the neural activation signal or the dysfunction of contractile proteins within the muscle. The decline in muscular strength and work that occurs with fatigue is often due to a lack of ATP bioavailability, blood flow and tissue oxygenation, as well as the accumulation of

metabolites, neuromuscular alterations and is often associated with increased indices of sympathetic activation (heart rate and mean arterial pressure).[15–17] Muscle fatigue and its associated functional impairments are dependent on age, sex, motivation and adaptation to specific tasks. [15,18]

Most recently, research has implicated light therapy as an effective tool for enhancing skeletal muscle contractile function and increasing the number of repetitions and time it takes to fatigue a muscle.[19–23] Leal-Junior et al.[23] were able to show a significant delay in the fatigue response to repeated electronically-evoked tetanic contractions in the tibialis anterior muscle of rats exposed to light therapy. In human studies muscles exposed to laser therapy demonstrated enhanced performance by maintaining contractile force output and delaying the onset of fatigue when exposed to resistance exercise.[19,22,24] Empirical evidence also shows that light therapy can limit exercise-induced muscle damage, thereby improving biochemical and functional recovery by reducing inflammation and oxidative stress.[19,21] Therefore, thorough research has been conducted on the benefits of NIR light therapy to prevent fatigue via modulation of circulation, energy production (ATP) and contractile function. However, very few studies have directly examined the “ergogenic” effects of phototherapeutic modalities for improving muscle function via neural modulation.

Another important area of research with respect to muscle fatigue is the changes that are associated with the physiological process of normal aging. Aging is typically accompanied by muscle atrophy, loss of motor neurons, decreases in capillary density, and impairment in neuromuscular propagation.[25–28] Older adults also typically display reduced rates of increase in most physiological variables associated with fatiguing

contractions when compared with young adults. Age associated changes in the neuromuscular system increase its susceptibility to exaggerated impairment with fatiguing contractions. As a result, older adults appear to be less fatigable than young adults when performing submaximal contractions, however, they also exhibit decreases in maximal force capacity. Older adults also display greater increases in motor output variability. Motor output variability has been defined previously as the involuntary inconsistency in the output of voluntary muscle contractions, and results in inaccuracy motor tasks.[27] The most commonly prescribed intervention to offset the deleterious effects of aging is high-intensity resistance exercise. However, this form of exercise is contraindicated in many populations, including older adults.[29] The limitations of high-intensity resistance exercise in older adults emphasizes the need of finding a non-invasive therapeutic intervention coupled with low-intensity exercise to help increase muscle strength, function and independence in older adults. Most importantly, age associated changes in neuromuscular control and the viability of a therapeutic intervention, such as NIR light therapy, for improvement in muscle function is yet to be thoroughly examined.

The previously discussed studies have been able to provide substantial evidence for the use of light therapy as an effective prophylaxis to exercising muscles via enhancement of circulation and substrate production. However, there is a lack of research with respect to the modulatory effects of near-infrared light therapy on the neuromuscular system and how these effects may delay the onset of fatigue. More importantly, the phenotypic changes associated with normal aging provide a physiological basis to hypothesize that the positive changes associated with NIR light

therapy may be exacerbated with treatment in older adults. Therefore, it is necessary to conduct research and disseminate the neuromuscular mechanisms involved with modulation of the musculoskeletal system so that a thorough understanding of the benefits of light therapy and proper application thereof exists. Therfore, this dissertation, will address the following research questions:

1. Does near-infrared light therapy extend time to task failure in young adults in a dose dependent manner?
2. Is the effect of near-infrared light therapy on time to task failure and motor output similar for young and older adults?
3. What are the neuromuscular and cardiovascular mechanisms that mediate the physiological response to near-infrared light therapy?

Hypothesis

Near-infrared light therapy will beneficially modulate the physiological processes involved with musculoskeletal function, enhance time to task failure and reduce motor output variability. These improvements will be greater in older adults compared with young.

Experiments

1. To determine the most effective dose of near-infrared light therapy to extend time to task failure with the hand in young adults. Plan: This Experiment will determine if neuromuscular modulation with near-infrared light therapy is dose-dependent. More specifically we will aim to determine the most effective dose of near-infrared light therapy to enhance time to task failure in young adults. Expectations: Near-infrared light therapy will enhance time to task failure in a dose-response manner when compared to sham treatment in young adults. Novelty: This Experiment would identify an effective dose and extend our present knowledge on near-infrared light therapy's ability to resist musculoskeletal fatigue.
2. To determine if the effect of NIR light therapy on time to task failure and motor output is similar for young and older adults. In addition, to determine what the neuromuscular and cardiovascular mechnisms are that mediate the effects observed. Plan: This Experiment will aim to determine the differences in neuromuscular control during dynamic and constant isometric tasks as a result of near-infrared light treatment between young and older adults. Changes in time to

task failure and motor output variability will be compared between young and older adults. Expectations: Near-infrared light therapy will enhance time to task failure and reduce motor output variability to a greater extent in older adults when compared with young. Novelty: The ability to provide a therapeutic modality treatment to older adults that may have compromised neuromuscular ability may enhance clinical treatment outcomes and result in improvements in strength and motor control tasks that are essential to the maintenance of independent living.

In summary, Experiment 1 will allow me to identify the most effective dose for extending time to task failure and answer my first research question which proposes to understand the neuromuscular changes that occur as a result of near-infrared light treatment. In addition, Experiment 2 will address my second question to identify the differences in the ability of near-infrared light therapy to enhance time to task failure and reduce motor output variability between young and older adults. Experiment 2 will also allow me to address my third research question and determine the neuromuscular and cardiovascular changes that are associated with the benefits of NIR light therapy. These aims have been formulated based on the results from our previous studies that have implicated near-infrared light therapy as an effective modality to increase blood flow and attenuate fatigue development. The outcomes of this project will have a significant impact on the use of near-infrared light therapy to clinically enhance musculoskeletal performance in those suffering from loss of function and poor motor control.

CHAPTER 2
REVIEW OF LITERATURE

History of Laser Therapy

Light therapy is one of the oldest therapeutic modalities used by human kind. Light therapy was initially used by the Egyptians and termed solar therapy. Later, the term transitioned to UV therapy by Nils Finsen, for which he won the Nobel Prize in 1904.[30] More recently, light therapy has gained popularity as a therapeutic modality in the treatment of athletic injuries, dermal burns, and superficial wounds. Therapeutic Light, also known as phototherapy, is defined as the medicinal use of light to treat various pathological states and enhance physiological function.[31] The most common form of light therapy is 'LASER'. The term 'LASER' is an acronym for "Light Amplification by the Stimulated Emission of Radiation". The first working laser modality was invented in 1967 by Endre Mester at Semmelweis University in Budapest, Hungary. Mester created the modality based on the Hypothesis that applying laser to mouse tissue would cause cancer. Mester shaved the hair on the dorsal surface of the mice, divided them into two groups, and administered laser treatments with a low powered ruby laser (694 nm) to one group. Surprisingly, the mice treated with laser did not develop cancer and actually grew their hair back more quickly. This is the first known demonstration of photobiomodulation (PBM) as a direct result of laser treatment.[32] Since 1967, numerous studies have been published investigating the effects of PBM on different disease states. Of these studies, positive outcomes have been observed with the following pathologies; osteoarthritis[33], tendonopathies[2], muscle fatigue[14,20,22,23,34–36], back pain[37], neck pain[38], diabetic microangiopathy[39,40], diabetic ulcers[41] and wounds.[41–43]

Properties of Laser

A therapeutic laser diode is a relatively small, tubular-shaped, hand-held device (Figure 1-1). The laser console consists of several components that work to produce a uniform beam of light. The diode uses a chamber or resonant cavity for housing and containing the lasing medium. The lasing medium is typically in liquid, gas, or solid-state form and contains atoms used for storing and releasing energy in the form of a photon. Common lasing media include gallium aluminum arsenide (GaAlAs), gallium arsenide (GaAs), and helium neon (HeNe). The various lasing media produce light that is emitted in different wavelengths; GaAlAs = 650, 805, 820-830nm, GaAs = 904-970nm, and HeNe = 632.8. An electric pump is used to excite the medium thereby stimulating atomic movement and activity inside the chamber. Stimulated atoms release photons within the chamber where they are amplified through the use of reflective mirrors. Photons are eventually expelled through a controlled semi-permeable filter once a specified level of energy is attained inside the chamber. A fiber optic cable is coupled with an emission diode to deliver light energy from the base unit to the target tissue.

Therapeutic lasers deliver energy to tissues via a photo-stimulatory effect (Table 2-1). [34,37,44] Photo-stimulation uses organized light within the visible red and/or near infrared electro-magnetic radiation spectrum. Typically light from an incandescent light bulb is delivered in a random and/or divergent pattern, whereas laser light is delivered in tight homogeneous beams and is able to focus its energy on a localized area. This is done to maximize beam intensity and limit scatter. There are three distinguishing features of visible red and near-infrared laser light: monochromaticity, coherence, and direction.[5] Monochromaticity of light indicates that the energy will all be of the same wavelength, and therefore, color. A coherent laser beam implies that all photon

emission is occurring in phase. Laser light is collimated, meaning minimal divergence, and travels in one direction towards the target tissue.

Types of Lasers

There are two different types of lasers used by health care providers: surgical and therapeutic. Surgical lasers use power ranges that are destructive and have the ability to cut, shrink and bind tissues. Therapeutic lasers use power ranges and wavelengths that facilitate tissue repair, accelerate healing, and improve circulation and musculoskeletal function. Therapeutic lasers include light-emitting diodes (LEDs) which have multiple diodes and emit photonic energy to large surface areas and semi-conductor or solid-state lasers which use a diode to emit a collimated and coherent beam of light. Light emission from LEDs is non-coherent and more divergent.

Light-Tissue Interaction

Photobiomodulation (PBM) is the term used to refer to any alteration in physiological function that is caused by the application of light to tissue. Photobiomodulation encompasses the positive and negative effects of laser therapy for the treatment of acute and chronic musculoskeletal disorders including pain control[45,46], improved tissue repair, and musculoskeletal function.[31,38] However, the underlying mechanisms and clinical effectiveness of laser therapy remains poorly understood. Laser light interacts with tissue in 4 different ways. Photons may be reflected by the cutaneous layer, absorbed by the skin or blood, diffusely scatter during penetration and/or transmit to deeper tissue layers. Wavelength plays an important role in the ability of light to penetrate soft tissue.[31,47] Each wavelength will allow for different depths of penetration in target tissue**.** When laser is applied topically to any area of the body, there will always be a fraction of the photonic energy that will be reflected and/or

attenuated by the superficial cutaneous layers. Tuner and Hode[48] indicate that between 50 to 90% of energy is absorbed or reflected by the skin and subcutaneous tissues with the remainder of light energy penetrating into deeper tissue layers (muscle, deep fascia, and ligament). Esnouf et al.[49] used a near-infrared (NIR) 850nm laser diode at 100mW output power to irradiate a 0.784mm thick section of human skin and found that 66% of the initial beam intensity was attenuated by the skin section. Similarly, Kolari et al.[50] exposed dermal tissue to both visible red and NIR laser and found that a significant amount of light energy was absorbed within 0.5mm after penetration. Research has indicated that diodes with wavelengths ranging from 820 – 904 nm are best suited for treating deep soft tissues such as muscle, tendon and ligaments.[31,51,52] While diodes with wavelengths in the 400-700 nm range have been found to be best suited for treating superficial wounds and various skin disorders.[51,53]

The first law of photobiology states that in order for light to have any effect on living tissue the photons must be absorbed by absorption bands belonging to some photoacceptor, or chromophore. Photons that successfully penetrate the skin will be transmitted to the deeper layers of tissue where they are absorbed by chromophores located in the blood (hemoglobin, myoglobin), cytosolic organelles (mitochondria), and cell membranes.[1] In order for photons to be absorbed they must be delivered within the tissues “optical window”, where penetration of the light into and through the tissue is optimized. Water is responisible for the absorption of the majority of light emitted at wavelengths greater than 1100nm and absorption bands for biological chromophores (hemoglobin and cytochrome c oxidase) are much shorter. Therefore, the use of laser therapy occurs exclusively in the red to near-infrared range of light (600-1100 nm).[1]

Chromophores and other intracellular proteins that absorb photons from irradiation will assume an electronically stimulated state and produce chemical reactions leading to measurable biological effects. Chromophores involved in such reactions include the following:

Cytochrome C Oxidase (COX)

Current research on PBM includes in-depth mechanistic studies that observe the effect phototherapy has on mitochondria. Mitochondria are often referred to as the cellular “energy power plants” and are responsible for converting substrates into chemical energy in the form of ATP via oxidative phosphorylation. More specifically, research has focused on cytochrome c oxidase (COX), the terminal enzyme in the electron transport chain. Cytochrome c oxidase (complex IV) is responsible for transferring electrons to molecular oxygen, which will drive the production of ATP and enhance aerobic respiration.

The absorption spectrum of cytochrome c oxidase shows that it is the primary photoacceptor for the red-NIR range in mammalian cells.[1] A study conducted by Pastore et al.[6] found that irradiating cells increased oxidation by COX and increased electron transfer allowing for an increase in energy production. Subsequent increases in electron transfer then result in increased production of ATP.[54] The effects of illumination at the mitochondrial level observed are not limited to those seen in COX. Photon absorption also induces an increase in the electrochemical potential of the mitochondrial membrane leading to increased activity of the proton pumps; Na^+/H^+, Ca^{2+}/Na^+, Na^+/K^+, ATPase and Ca^{2+}. An increase in the activity of these protons pumps will also lead to a subsequent increase in ATP synthesis[6], increased RNA and protein synthesis[55] , and

increases in oxygen consumption, membrane potential and accelerated synthesis of NADH and ATP.

Nitric Oxide (NO)

An additional effect of PBM at the mitochondria involves the removal of inhibition caused by nitric oxide (NO). Nitric oxide present in the mitochondria causes inhibition by binding to COX and competitively displacing oxygen, especially in stressed or hypoxic cells, for example during exercise.[7] When cells are irradiated by laser a photo-dissociation occurs between COX and NO releasing the inhibition and allowing COX to transfer electrons. A photonic dissociation of COX from NO allows an increase in enzymatic activity, thus allowing an influx of oxygen and the subsequent resumption of respiratory chain activity and generation of reactive oxygen species (ROS).[1,56] Research has found that ROS concentrations below cytotoxic levels create bio-stimulatory effects for the cell.[57] Therefore, the benefits of this dissociation are three-fold as it allows an increase in ATP synthesis, ROS synthesis and allows NO to be released and take effect in surrounding tissues.[31,35]

In addition to NO being dissociated from COX it is also photo-released from other intracellular sources. Nitric oxide (NO) signaling has been implicated as playing an integral role in the process of light-induced changes to vascular structures. Light mediated vasodilation was first discovered in 1968 by R.F. Furchgott in his research for which he earned a Nobel Prize in 1998. Nitric oxide sources within soft tissues include activated macrophages[58], vascular endothelial cells[59], and protein complexes such as hemoglobin, myoglobin and cytochrome c oxidase (COX).[10,60] Activated macrophages and endothelial cells up regulate the synthesis of NO[58,59], while hemoglobin, myoglobin and COX, once photonically stimulated, release NO via photolysis.[7,60] Nitric oxide's

ability to cause vasodilation of local vasculature occurs through its effect on cyclic guanine monophosphate (cGMP). These effects may last for hours to days following laser treatment mainly because the displaced NO cannot easily return to the mitochondria and inhibit COX. Nitric oxide removed from COX will also lead to a sharp drop in the pO_2 of the cell which will lead to hypoxic signaling by HIF-1α. This hypoxic signaling is one of the main factors leading to the synthesis of vascular endothelial growth factor (VEGF) and its consequent increase in angiogenesis.[61]

Reactive Oxygen Species

In addition to these cellular effects, laser therapy also has the ability to increase the production of reactive oxygen species (ROS). With exercise, stress and injury, cells respire and a small amount of oxygen is reduced, creating ROS such as superoxide anion (O2-), hydroxyl radical (HO) and hydrogen peroxide (H202). If ROS levels become too high they can become detrimental to the cell by causing lipid peroxidation, oxidation of amino acids, production of protein-protein cross-links, protein fragmentation, and DNA damage.[61] However, small amounts of ROS, produced as a result of increased electron transfer, as with irradiation, can act as cellular scavengers and protect the cell.[62] A slight elevation of ROS within the cell is referred to as oxidative stress. In response to elevated ROS, the cell will up-regulate its production of antioxidants, induce new gene expression, modify proteins and initiate a multitude of signal transduction pathways.

Transcription factors are a series of proteins that can translocate to the nucleus of the cell and bind to specific sequences of DNA. The most important transcription factor is nuclear factor Kappa-B (NF-ƙB) which regulates cellular processes associated with inflammation, stress and apoptosis. Photo-activation of NF-ƙB can be beneficial to the

state of the cell by preventing apoptosis, acting as an anti-oxidant, and inducing proliferation of growth factors, adhesion molecules, immunity genes, cytokines and chemokines.[1,30,61,63] The effects of NF-ƙB may explain the following results discovered in early laser research: Activation of anti-apoptic genes explains attenuation of cell death from noxious stimuli. Activation of pro-proliferation genes explains lasers ability to promote wound/tissue healing. Activation of fibroblasts explains the ability of laser to enhance collagen synthesis and activation of adhesion molecules explains lasers ability to enhance wound healing.

Dose-Response and the Therapeutic Window

An vital component associated with the therapeutic effectiveness of laser is dose.[3,4,38] Achieving an appropriate therapeutic dose is often the most difficult component of clinical laser therapy practice.[3] The concept of dose response is referred to as “biphasic” because a 2 phase curve can be used to explain the expected dose-response to laser therapy at the subcellular, cellular, tissue and clinical level. The biphasic curve illustrates the 2 phases of stimulation and inhibition when a laser is applied to tissue, suggesting an optimal “therapeutic window” for dose. As such, the Arndt-Schultz Principle has been adopted from early toxicology studies of yeast culture to explain the optimum therapeutic dosage level of laser (Figure 1-2). Optimal doses have been Experimentally established in multiple studies involving cell and tissue cultures. This therapeutic laser dosage or level of photo-stimulation must be attained; otherwise; “no reactions or changes can occur in body tissues if the amount of energy absorbed is insufficient to stimulate the absorbing tissues”.[5] The stimulatory threshold of laser biostimulation for many human tissues is not known. However, results from cell and animal studies suggest that the therapeutic effects of laser are dose-dependent and

are most pronounced during the acute and post-acute phase of injury. This initial therapeutic window may be within the first hours to days after soft tissue injury.[4] These studies suggest that dose-dependency also exists when treating humans, however, with the exception of superficial tissues and some dental applications, standard treatment doses for deep musculoskeletal tissues have yet to be firmly established in human clinical populations. Calculating an appropriate dose range for humans from studies using cell and animal models is challenging. Since there is no known algorithmic transformation that can be used to calculate a comparable dose-range in humans from those used in cell and animal models. The majority of studies have based dosage calculations on a 2-dimensional measurement (Joules/cm^2) of surface area exposure for laser. The actual dosage for deep tissues, however, must take into consideration the 3-dimensional irregular Gaussian distribution of the scattered and absorbed laser beam. This complex calculation can only be estimated with an adequate understanding of laser wavelength interactions with tissue chromophores, pigments, and tissue turbidity. These factors have a dynamic influence on the 3-dimensional pattern of absorption, scatter, reflectance or transmittance of the laser beam in deep tissues.

Low-Level Laser Therapy vs. High Power Laser Therapy

Lasers are classified by power level and their ability produce eye injury. The power and beam characteristic ratings are established by the American National Standards Institute and the International Electrotechnical Commission. The majority of therapeutic lasers available for use in clinical practice are classified as 3B or 4. Class 3b lasers emit an output power of greater than 5mW up to 500 mW, while class 4 lasers emit power outputs greater than 500 mW. Class 3b level emitting lasers are known as “low level”, “low intensity” or “cold” lasers because no significant thermal effect is generated in the

superficial tissue during irradiation. Class 4 lasers are known as “high power” or “hot” lasers because they have the capability of producing rapid increases in superficial tissue temperatures when maximum exposure limits are exceeded. More recently, the study of laser therapy has shown a preference for class 4 lasers in patient care settings.[64] Class 4 lasers can emit greater photonic energy in a shorter period of time than class 3b lasers without producing an appreciable rise in tissue temperature under normal treatment protocols.[5] This higher power density becomes important when treating injuries to deeper tissues such as ligament, muscle, tendon and cartilage.

Light emitting diodes (LEDs) and semi-conductor or solid-state diode lasers are used clinically and in current research for the treatment of musculoskeletal disorders.[24,35] Light emitting diodes are beneficial as they are less expensive and can treat a greater surface area in a shorter amount of time. Multiple emitting diodes are used in LEDs in the red to infrared range at powers that fall within the range of class 3 lasers. Therefore, LED’s ability to penetrate tissue is limited to a depth of approximately 10 mm and is commonly used for the treatment of superficial wounds, dermatological pathology and scars. Semi-conductor or solid-state laser diodes contain a lasing medium that stimulates photon emission in a more collimated and coherent manner when compared to LEDs. Both LEDs and semi-conductor or solid-state diodes have proven to be safe and effective treatment modalities.[24,35,21]

The majority of published clinical studies using laser therapy to treat musculoskeletal injuries have used class 3b “low-power” lasers. Several published reports have questioned the ability of “low-power” lasers to effectively transmit energy beyond the skin into deep musculoskeletal tissues.[49,50] Excessive beam scattering and

attenuation within the skin limit the potential biostimulative effects of laser in the deeper target tissues. This can be attributed to several factors related to dosimetry, such as sub-threshold optical power, insufficient treatment durations, and varied treatment frequencies.[65,66] Therefore, it is relevant and timely to study the dosimetric responses of specific infrared wavelengths of “high-power” class 4 lasers and their ability to modulate the physiological effects that are conducive to the healing process and improved function.

Physiologic and Therapeutic Effects

This portion of the review will focus predominately on laser’s physiological effects with respect to modulation of the micro-circulatory system and fatigue response to exercise.

Circulation

Improved blood circulation is considered to be one of phototherapy’s greatest contributions to healing following soft tissue injury and related pathology.[9,13,39,59] Phototherapeutic induced changes in blood flow and microcirculation have proven to be beneficial to healing by controlling ischemia, hypoxia, and edema post-injury; thereby creating a more favorable environment for limiting the zone of secondary tissue damage.[59,67,68] Phototherapy has been prescribed as a treatment for ischemic diseases, such as diabetic microangiopathy, a common complication of diabetes mellitus.[39,40,69] Three clinical trials were conducted that evaluated the effects of phototherapy on microcirculatory changes in both healthy individuals and individuals afflicted with diabetic microangiopathy. [9,39,40] All blood flow changes were monitored with Doppler ultrasound. Outcomes for all three trials showed significant increases in blood flow during and after phototherapy treatment in the targeted microcirculatory network as well

as systemically. Samoilova et al.[9] found that phototherapy enhanced microcirculation in healthy volunteers by as much as 47% in diabetic patients and by as much as 45% from baseline measures in the targeted tissue. Similarly, Schindl et al.[70] observed significant increases in athermal skin microcirculation in diabetic microangiopathy patients when treated with phototherapy. No change in skin microcirculation was observed in these patients when a sham phototherapy treatment was applied to the same area. An important consideration when comparing these studies is their respective treatment dose. Samoilova et al.[9] delivered a phototherapeutic dose of 12 J/cm^2, while Schindl et al. delivered a much higher dose of 30 J/cm^2. While appropriate dosing parameters have not been established in the literature, it appears from these studies that proper dosing of the targeted tissue will result in improvements in physiological function. Previous studies may have failed to find positive circulatory effects due to sub-threshold treatment doses which were too weak to stimulate the target tissue.

Long term effects of PBM also include the formation of new capillary networks during the healing process which help to facilitate cellular proliferation and repair. New Capillaries are formed by a process of budding from pre-existing capillaries after injury via the collateral circulation.[59] The development of new capillaries in damaged tissue can lead to enhanced tissue perfusion and eventual healing. If new capillaries are unable to develop the healing process will be altered and recovery from injury/disease states delayed.[59] In order for new capillaries to be formed circulatory flow in the existing capillaries must be maintained. Therefore, the ability for phototherapy to maintain or enhance local circulation is beneficial to the acceleration of healing by maintaining current blood flow and improving angiogenesis. In support of this, Mirsky et al.[71]

observed that the application of laser therapy in the regeneration zone of skeletal muscle enhanced formation of new blood vessels two-fold. Results of this study also observed an increase in the rate of proliferation of endothelial cells and vascular budding leading to enhancement of regeneration in skeletal muscle.[71] Likewise, Ihsan was able to demonstrate an increase in capillary density in rabbit tissue treated with phototherapy via the activation of ATP, ATPase, NO and VEGF.[59] Thus, the success of healing is largely dependent on the maintenance of microcirculation and the creation of new circulatory networks through the process of angiogenesis during healing. The ability of phototherapy to enhance these processes then provides the ability to accelerate the healing process and re-vascularize otherwise ischemic tissue.

Another area of interest in relation to microcirculation is the ability of phototherapy to modulate ischemic pain via improvements in blood flow and tissue perfusion. Research has been conducted on temporomandibular disorder (TMD), a pathological condition associated with decreased muscle blood flow resulting in local myalgia.[72] Local myalgia is one of the most reported symptoms related to TMD. Mechanisms of myalgia are thought to be due to overloading the temporomandibular muscles resulting in ischemia and pain.[73,74]

Tullberg et al.[72] sought to investigate the immediate local effects of low level laser therapy on blood flow within the masseter muscle in patients with chronic orofacial pain. Tullberg et al. found an increase in blood flow within the masseter muscle in healthy participants treated with phototherapy when compared to a sham phototherapy treatment. Results also confirmed that low microcirculatory blood flow is directly related to increases in muscle and joint pain experienced by individuals suffering from ischemic

conditions. Therefore, we can conclude that the benefits phototherapy provides in enhancing microcirculatory blood flow are not only related to reperfusion of ischemic tissue, but also in modulating pain responses related to musculoskeletal pathology.

Larkin et al.[75] recently conducted a dose-response study using phototherapy to study the effects on blood flow of the human forearm. Four treatment doses were administered; 1 Watt, 3 Watt, 6 Watt and 0 Watt. The doses were applied in a random sequence on four separate testing days without the participants' knowledge of dose. Results showed a biphasic dose-response effect when using class 4 laser therapy in our clinical model similar to what has been observed in animal models. Specifically, the use of a 3 watt dose, along with a 50% duty cycle, applied to the biceps brachii proved to be the most beneficial for increasing blood flow to the distal tissues of the forearm. Following the tenets of the Arndt-Shultz principle, this study exhibited a weakened vasodilatory response with the 1 watt dose, and a suppressed, or inhibited, response with the 6 watt dose. This finding suggests that a properly designed laser treatment protocol with appropriate dosing parameters may be a viable therapy to increase limb blood flow clinically.

Animal and Cell Studies

Since Furchgott's discovery of NO, and the receipt of the Nobel prize in 1998, substantive evidence has been compiled from animal and cell research that further supports the findings that vasodilation can be mediated by phototherapy. [76] In vivo studies have shown that vascularized tissues exposed to phototherapy respond with enhanced arteriolar dilation and increased microcirculation.[59] Maegawa et al.[13] and Chertok et al.[77] investigated the effect of phototherapy on rat mesenteric microcirculation. Phototherapy was shown to vastly improve vasomotor relaxation and

arterial blood flow. Interestingly, these same effects were completely abolished with prior inhibition of nitric oxide synthase (NOS) activity.[13,77]

Samoilova et al.[9] was able to show that human skin exposed to visible light at varying wavelengths enhanced microcirculation in a NOS-dependent mechanism. Nitric oxide present in the mitochondria can play an inhibitory role on COX activity. Cytochrome c oxidase is the terminal enzyme of the respiratory chain and functions to mediate the transfer of electrons to molecular oxygen.[58,78] Activation of COX leads to increased cellular respiration via conversion of light energy to biochemical energy resulting in an increased rate of ATP synthesis.[42] When phototherapy is administered to tissue and photons are absorbed by the mitochondria, COX enzymes will subsequently release NO into the cytosol. Photolysis of NO from COX sources increases the number of active binding sites on the electron transport chain resulting in enhanced cycling thereby facilitating cellular respiration. Additionally, activation of COX enzymes and the subsequent release of NO from the mitochondria will allow free NO to act as a local and transient vasodilator by signaling the local smooth muscle in the vessel to dilate. The enhancement of such processes is beneficial along with the activation of ATP synthesis as ATPase stimulates the conversion of cAMP to NO or vascular endothelial growth factor (VEGF).[79] Increases in NO and VEGF induce local vasodilation and tissue perfusion, as well as stimulating angiogenesis. Therefore, with repeated phototherapeutic treatments the absorption of photons by COX will play an essential role in contributing to long term circulatory changes in under perfused or damaged tissues.

Phototherapy studies have also observed a significant absorption of light from hemoglobin and myoglobin in the wavelength range of 760-1000nm.[10]Additionally, in the oxygen deficient ischemic state such as with injury and disease, it has been observed that deoxyhemoglobin and deoxymyoglobin increase light absorption.[80] Experimentally, the photosensitivity of hemoglobin and COX has been demonstrated using spectroscopy and electron paramagnetic resonance (EPR). Using these methods Zhang et al. observed that the signal for NO-hemoglobin is decreased during photo-irradiation.[10] Zhang's observation provides evidence that NO is released from hemoglobin during tissue photo-irradiation leading to further vasodilatory effects.

Fatigue and Muscle Function

Muscle fatigue is a complex process which results from a variety of physiological processes and is commonly caused by continuous physical activity.[15] Fatigue is associated with a progressive decline in muscular strength resulting from a combination of inadequacies in substrate availability, blood flow and neuromuscular activity.[17] The most common manifestation of fatigue is impairment in muscle function and an inability to sustain a task. It has been suggested that this decline in maximal force capacity is due to impairment in either the activation signal or function of the contractile proteins involved.[81] Such impairments are dependent on age, sex, motivation and specific adaptations to a specific task. Critical variables that contribute to loss of muscular force are the following; muscle group involved, task being performed, type of load, intensity of the task, and the physical environment in which the task is being performed.[15] Therefore, skeletal muscle fatigue is a process that involves physiological, biomechanical, psychological and environmental elements.[15,82]

During fatiguing exercise with submaximal isometric contractions an individual's baseline strength capabilities impact their fatigability, or time to task failure, greatly. An increase in muscular strength leads to greater intramuscular pressure, increased vascular resistance and an overall limitation in blood flow and oxygen delivery.[83] Therefore, stronger individuals are more fatigable and will reach task failure sooner. In combination with intramuscular pressure, decreased blood flow also stimulates the pressor response which will lead to quicker time to task failure.[83] The pressor response, as measured by mean arterial pressure, significantly contributes to the time to task failure. An increase in mean arterial pressure (MAP) is due to enhancement of the central command from the central nervous system as well as the build-up of metabolites in the periphery causing a metabo-reflex, via stimulation of group III and IV afferents.[16] Mean arterial pressure will increase rapidly during sustained contractions to compensate for the progressive lack of blood flow during sustained contraction.[84] As such, increases in MAP over the course of submaximal isometric contractions can be a strong predictor of task failure. As such, the ability of an external source to influence the onset of the pressor response and modulate blood flow may be a significant benefit and enhance time to task failure.

Recent research has begun to focus on the ergogenic effects of laser therapy to reduce or delay the onset of fatigue. The progressive decline in muscular strength that occurs as a result of fatigue is often due to a lack of ATP bioavailability, lack of blood flow and tissue oxygenation, and neuromuscular alterations.[14,15,24,85] Previously discussed studies have mentioned the ability of laser to enhance circulation leading to increased substrate delivery and tissue oxygenation. Research has also implicated

laser as an effective tool for enhancing contractile function and increasing the number of repetitions and time it takes to fatigue a muscle. In addition, researchers have been able to demonstrate a decrease in the levels of blood biomarkers related to exercise-induced muscle damage, inflammation, and oxidative stress.[19,20,22,24,35] Such studies have been able to provide substantial evidence for the use of laser therapy as an effective prophylaxis to exercising muscles.

A decrease in biomarker level that typically results from exercise and inflammation has been one of the areas of interest associated with laser therapy. Blood lactate (BL) is produced in exercising muscle by lactate dehydrogenase (LDH) and is a by-product of working muscles. Blood lactate accumulates within the tissue because the speed of production is greater than the capacity for removal. Likewise, creatine kinase (CK) is produced as a direct result of cell damage and the high level of ATP consumption during strenuous exercise. Baroni et al.[19] delivered an 180 J dose of laser therapy before resistance exercise and evaluated muscle fatigue and functional recovery of the quadriceps femoris muscle after eccentric contractions. Following laser therapy and exercise an increase in LDH was observed 48 hours following exercise along with increases in CK at 24 hours and 48 hours following exercise.[19] Similarly, Leal Junior et al. conducted multiple studies assessing changes in inflammatory biomarker levels following strenuous physical exercise. In two studies Leal Junior et al.[14,22] treated 20 volleyball and soccer players with light therapy or placebo-control and had them perform Wingate testing with subsequent blood testing of BL and CK. Results from these studies indicated a decrease in the production of CK in volleyball players and a decrease in BL in soccer players suggesting a decrease in muscle damage and inflammation along with

an enhanced post-exercise recovery. In addition to this study, Leal Junior et al.[35] conducted a similar study using 10 volleyball players in which he irradiated the biceps brachii prior to arm exercise. Subjects completed bicep contractions at 75% of their maximal voluntary contraction until volitional fatigue. Following completion of exercise BL and CK were measured along with C-reactive protein (CRP), a marker of inflammation resulting from physical exercise. All biomarkers, BL, CK and CRP decreased significantly with active laser therapy when compared to placebo laser therapy.[35]

Two studies conducted in a rat model, also evaluated laser therapy's effect on biomarkers after exercise. These studies also focused on the dose-response effect laser therapy can have on post-exercise recovery and the biochemical reactions associated with strenuous resistance exercise. Leal Junior et al. assessed skeletal muscle fatigue and its resultant biochemical marker production in exercising rats.[23] Four doses of laser therapy, 0.1J, 0.3J, 1.0J and 3.0J, were used to assess a dose-dependent response of the biomarkers CK and BL. Electrical stimulation was used to induce tetanic tibialis anterior contractions on thirty male Wistar rats. Following 30 muscle contractions, blood lactate levels were significantly lower in all dose groups treated with laser therapy. However, all laser irradiated groups, except the 3.0J group showed a significant decrease in CK activity when compared to the control group displaying a dose-response of laser therapy when used ergogenically prior to exercise. Liu et al.[86] conducted a research study using 72 Sprague-Dawley rats and downhill running to induce muscle injury in the gastrocnemius muscle. Three doses, 12, 28 and 43 J/cm^2, were used prior to the exercise protocol and measures of CK, muscle

superoxide dismutase (SOD), and malondialdehyde (MDA), a marker of oxidative stress, were analyzed at 24 and 48hrs. The 43 J/cm^2 dose was effective in reducing CK, MDA and increasing SOD following downhill running, while the two lower doses were ineffective, again displaying a dose-response effect of laser therapy.[86]

Functional measures have also been used in current research to evaluate the effects of laser therapy. These measures include the number of repetitions of muscular contractions and the time that it takes such fatiguing exercise to be completed. Leal Junior et al. performed a number of research studies evaluating functional outcomes of muscular performance following the ergogenic properties of laser therapy. A study involving 10 professional volleyball players undergoing phototherapeutic treatment (830nm, 20J) to their biceps brachii prior to exercise, resulted in an average increase of 4.5 repetitions to fatigue.[14] Leal Junior et al. conducted a similar study irradiating the biceps brachii of 12 professional volleyball players with a laser with a wavelength of 655 nm and a dose of 500 J/cm^2. Irradiation occurred prior to voluntary biceps contractions with a load of 75% of the athlete's 1 repetition maximum. Participants were instructed to perform biceps contractions until volitional fatigue. The irradiated group in this study significantly increased the total repetitions to volitional fatigue by 8.5 repetitions (SD ±1.09) when compared to the placebo control.[20] Leal Junior et al. also conducted a study evaluating the effect of irradiating the biceps brachii of 10 professional volleyball players, using light emitting diode therapy (LEDT, 660 nm, 850 nm). In this study, this application of laser therapy was able to yield an increase in the time it took to fatigue the biceps brachii muscle by 11.6%, along with an increase in number of contractions completed by 12.9%, when compared to the placebo group.[35]

Measures of strength were also evaluated in the studies discussed previously by measuring individual participant's maximal voluntary contractions. In a study conducted by Baroni et al. using 36 healthy male participants, laser therapy was performed on the quadriceps femoris.[19] Maximal voluntary contractions were measured before (baseline), immediately following exercise, and 24 hours and 48 hours post-exercise. At each time point of measurement a significant difference was observed between the laser therapy group and the placebo control group. Strength loss was attenuated following exercise in the laser group by 35 newton/meters immediately, 44 newton/meters and 51 newton/meters at 24 hours and 48 hours post-exercise respectively. In a similar study conducted by Baroni et al.[24] using LEDT, laser therapy was able to significantly attenuate strength loss by a difference of 12 newton/meters when compared to the placebo group. Leal Junior et al.[23] was also able to observe an attenuation of strength loss when studying its ergogenic effects in rats. In this dose-response study the two highest doses of 1.0 Joules and 3.0 Joules were able to attenuate strength loss by 151.27% and 144.84% when compared to baseline MVC measures and the placebo control group.

A weakness in the existing literature is that there is a lack of evidence on the effects of laser on neuromuscular control in the recruited musculature. Neural excitability is an important factor with respect to musculoskeletal fatigue and has yet to be examined in laser research as a possible mechanism for the attenuation of strength, loss and prevention of impairment measures associated with fatigue. Improvements in neural signaling could explain changes in motor control and improvements in task performance. As a result, we are uncertain if lasers ability to provide fatigue resistance

is driven by metabolic (increased ATP synthesis), circulatory (increased blood flow) or neural (increased motor neuron excitability, nerve conduction velocity) modulation. Therefore, future studies are needed to determine if modulatory effects of laser therapy are a result of changes in neuromuscular control.

To date, there has only been one research study conducted on the modulatory effects of light therapy on muscle activity. Kelencz et al.[4] conducted a clinical study to examine the muscle activity of the masseter muscle with constant isometric contractions following treatment with light. Significant increases were observed with muscle activity as represented by the amplitude of the EMG signal following the application of the light treatment. Additionally, this research group was able to show attenuation of strength loss following contractions and a significant increase in time to task failure. Therefore, neural excitability is an important factor with respect to musculoskeletal fatigue and has yet to be examined in light therapy research as a possible mechanism for the attenuation of strength loss and prevention of impairment measures associated with fatigue. Improvements in neural signaling could explain changes in motor control and improvements in task performance.

A more complex component of the study of muscle fatigue is the effect that normal aging has on the neuromuscular processes involved with the progressive decline in force capability of exercising muscle. The phenotypic changes associated with aging predispose aged muscle to being more susceptible to the effects of NIR light therapy. Changes associated with aging are typically muscle atrophy, loss of motor neurons, decreased motor unit discharge rate, decreases in capillary density, and impairment in neuromuscular propagation. Aged muscle also displays a more oxidative profile of the

muscle tissue due to selective atrophy of type II muscle fibers. As a result, aged muscle relies predominately on aerobic respiration, as an energy source for work, has slower contractile properties and a decreased overall fatigability. [87] Older adults also typically display reduced rates of increase in most physiological variables associated with fatiguing contractions when compared with young adults. Such physiological variables include; EMG activity, motor output variability, motor unit recruitment, heart rate and mean arterial pressure.

Although older adults are more endurant than young adults they also exhibit decreased force capacity. Losses in relative strength are detrimental to older adults as they try to perform activities of daily living, i.e. stair climbing. More importantly, it is necessary to assess the potential benefits of therapeutic interventions like NIR light therapy, during constant isometric tasks. Furthermore, it is critical to try to improve older adult's performance with constant position tasks because tasks that require stabilization of a load (i.e. Holding a glass of water) are critical to a person's independence and level of daily function. In addition to the decline in static force capabilities in older adults, aging is also associated with greater motor output variability. Motor output variability can be defined as the involuntary inconsistency in the output of muscle contraction and is associated with greater noise in the descending input to the motor neuron pool.[88] The increased variability in muscular contraction with normal aging leads to detrimental performance, increased time to perform a given task and an overall increase in muscular work. It is also well established that older adults exhibit greater motor output variability during isometric contractions, especially with lower loads. Therefore, it is important to study the ability of NIR light therapy to enhance muscle function in older

adults as it may be a promising, non-invasive, ergogenic and therapeutic intervention to attenuate the functional declines in aged muscle.

Clinical Implications

Several studies have been able to demonstrate the enhancement of vasodilation and/or proliferation of the microvasculature leading to an increase in content of oxygen within the photonically treated tissue. As a consequence ischemia, oxidative stress, secondary tissue death, and necrosis were all greatly reduced, thereby improving the local environment for tissue repair, healing and respiratory function. The effects of phototherapy in the studies discussed previously have provided strong evidence for the use of phototherapeutic devices as an effective non-invasive modality to re-perfuse ischemic tissues and deliver oxygen and other substrates necessary to facilitate skeletal muscle function. Furthermore, both in vivo and in vitro studies have been able to identify post-injury recovery mechanisms such as enhanced growth factor release,[59] angiogenesis[71], enhanced fibroblastic and cellular proliferation[89], enhanced myofibrillar regeneration and decreased fibrosis[90] when exposed to phototherapy. Maintenance of angiogenesis throughout processes such as wound healing, inflammation and ischemia is essential to functional and structural integrity.[71] Therefore, the use of phototherapy during the recovery (injury-repair) process is strongly supported by the literature as it is essential that new capillaries are formed, and re-perfused, within damaged tissue. If new capillaries are not formed during the repair process, capillary density will be reduced and the healing process hindered. In addition, new capillaries are formed only if the muscle maintains a normal blood supply, which comes from the collateral circulation. Muscle repair depends upon the presence of angiogenic factors such as growth hormone, PDGF and VEGF. Therefore, it is essential to maintain circulation to

injured tissue to re-establish capillary beds and enhance healing. The use of phototherapy in stimulating these growth factors and improving circulation and thus tissue repair has been widely accepted as an important benefit of phototherapy.[91]

In addition to recovery from injury, exercise and one's ability to perform work can be greatly enhanced by the vascular effects of laser as musculoskeletal fatigue is a process that may result from inadequate blood flow.[85] Increased circulation in the irradiated tissue will lead to increased oxygen and substrate delivery leading to an overall ability for a muscle to perform work. An increase in oxygen delivery to the working muscle will also lead to increased ATP production via aerobic respiration. As a result of an increase in ATP bioavailability, the local musculature is able to perform more work and resist fatigue more readily as it would limit accumulation of metabolites and impairment of oxygen delivery. Enhancement of blood flow, oxygen delivery and ATP synthesis within working muscle provides a physiological basis of support for laser therapy as an ergogenic aid that can be used prior to exercise.

Recent research has supported the positive outcomes of laser therapy as an ergogenic and prophylactic aid for reducing skeletal muscle fatigue, improving functional recovery and protecting muscle from exercise-induced damage. Therefore, laser therapy is a beneficial modality for those suffering from conditions associated with muscular impairments, neural inhibition and low blood flow. A therapist's ability to enhance muscle performance and functional recovery in a clinical setting is of great benefit to patients during rehabilitation when inflammation, atrophy, ischemia and impaired muscle function are some of the greatest limitations to recovery.

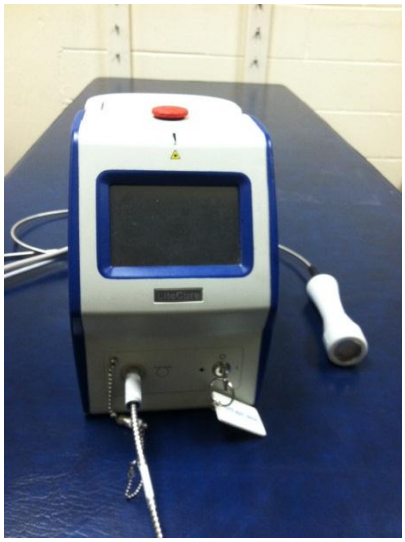


Figure 2-1. Hand-held class IV laser device (LiteCure™, LLC., Newark, DE)

Table 2-1. Laser treatment parameters

Irradiation Parameter	Units	Comment
Wavelength	nm	Light is electromagnetic energy which travels in discrete packets. Light is visible in the wavelength of 400-700 nm.
Intensity	W/cm^2	Power density= Power (W)/Area (cm^2)
Pulse Structure	Power (W) Freq (Hz) Width (s) Duty Cycle (%)	If the beam is pulsed then the Power should be average power Avg Power= Power (W) x width (s) x frequency (Hz)
Energy	Joules (J)	Calculated as: Energy (J)=Power (W) x time (s)
Energy Density	J/cm^2	Also known as, dose

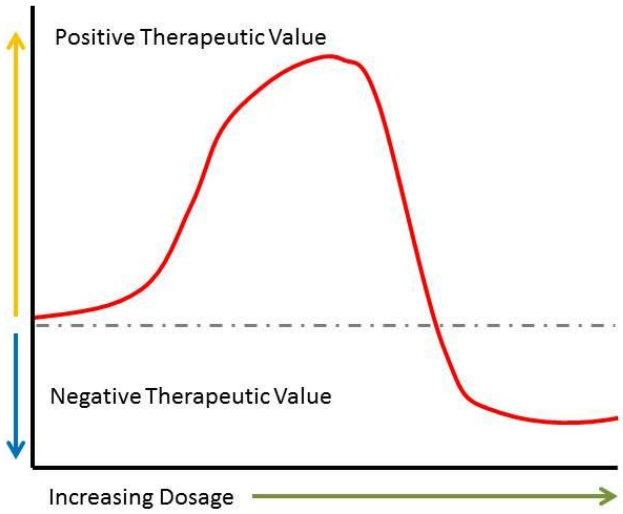


Figure 2-2. An illustration of the physiological response to NIR light therapy as followed by the Arndt-Schulz Principle.

CHAPTER 3
RESEARCH DESIGN AND METHODS

Subject Identification and Selection

Participants (men/women) were recruited from the university community. All subjects were physically healthy and right-handed (Edinburgh Handedness Inventory; (Oldfield 1971)). Participants were excluded from participation if they had suffered any injury to the neck or upper extremity (shoulder, elbow, wrist or hand) in the past six months that would prevent them from performing the tasks. Participants were also excluded if they had a tattoo in the near-infrared light treatment area on their left hand. Individuals who performed activities that involved skilled use of their fingers (e.g., musicians) were excluded. Participants' demographic data including age, gender, height (cm), and weight (kg) were taken prior to testing. These healthy volunteers were recruited from the local community via advertisements posted around the University of Florida campus and the city of Gainesville, FL. Written informed consent was obtained from all participants prior to the initiation of the Experiments.

Sample Size and Power Analysis

Power analysis uses hypotheses about the expected results of new research based on scientific experience, along with certain statistical assumptions, to obtain the probability for detecting as statistically significant the expected results when using a given sample size. A software package, G*Power 3 for Windows, was used to determine the sample size based on power which would detect statistically significant treatment-time interactions. The treatment-time interaction is the critical result for determining differences between treatment dosages. The sample size calculations were based on an $\alpha = 0.05$ and a minimum $\beta = 0.80$ and we used the effect size of the time to

task failure variable from our pilot study (Experiment 1) performed in our lab. The previous data for time to task failure revealed an effect size of 0.628. This task failure data suggests that a total of 18 subjects (6/group) would need to be recruited to detect a significant treatment-time interaction, resulting in an actual power of 0.86. Therefore, we recruited a total of 7 young and 7 older adults for each of the groups, (control, Experimental 1 and Experimental 2) for a total of 42 subjects. It was our thought that increasing our sample size beyond that suggested by power analysis would lead to more robust changes and increase the significance of our findings in Experiment 2.

Experimental Design

The subjects were tested using a single joint movement with their index finger. The movement of the index finger in abduction-adduction is controlled by the first dorsal interossei (FDI)(agonist) and the second palmar interossei (SPI)(antagonist). This movement was selected because: a) it is a single-joint movement that is primarily controlled by a single agonist muscle (first dorsal interosseus) and a single antagonist muscle (second palmar interosseus).[92,93] In addition, using this model allows our findings can be compared across studies which have used the index finger to study fatigue and aging differences.[94,95]

Experimental Setup

All subjects were seated upright with their arm slightly abducted, elbow flexed at 90 degrees and left forearm pronated and secured in a custom made hand device. Their thumb, middle, ring and fifth digit of their left hand were abducted and restrained by a metal plate. The index finger of the left hand was placed in an orthosis to maintain extension of the middle and distal interphalangeal joints. Only the left index finger was free to move.

Near-Infrared Light Therapy

A commercially available FDA approved near-infrared light device (LiteCure™, LLC., Newark, DE) was used for all phototherapy applications. The LiteCure™ Laser is a class IV laser that emits near-infrared light using a double wavelength of 810nm and 980nm. Near-infrared light is a therapeutic modality used to deliver light to biologic tissue in a non-invasive manner. Near-infrared light treatments were performed using a direct contact technique following a set pattern covering the first dorsal interosseus of the left hand. The cumulative dose to the muscle group was 240 or 480 J. These doses were selected based on their effectiveness in previous studies in our lab. Treatment time was equal to 2 minutes. Prior to each near-infrared light application the surface area to be treated was cleaned with alcohol. The near-infrared light therapy unit was tested and calibrated regularly using a standard power meter. The dosage used for Experiment 2 will be dependent on the outcomes from Experiment 1.

1 Repetition Maximum (1RM)

A 1RM test was performed following phototherapeutic treatment at each visit to determine the strength of each participant prior to the Experimental testing. Subjects were instructed to abduct their left index finger while lifting a weighted bag. The 1RM test was repeated with successive increases in mass until the subject could no longer complete the full range of 10 degrees of abduction.

Sinusoidal Task

The sinusoidal task was performed first because it is a non-fatiguing task. This task required subjects to match a position-time sinusoidal trace at amplitude of 10^{o} and frequency of 0.6 Hz with index finger. The index finger abduction task involved movement of the index finger about the second metacarpophalangeal (MCP) joint from

5^o (0^o is neutral position) to 15^o of abduction. Each sinusoidal movement trial lasted for 35 seconds and subjects repeated the sinusoidal task 5 times per visit. A light load was used for all Experiments and was 10% of the 1 repetition maximum (1 RM).

Constant Position Task

Subjects abducted their left index finger to a 5^o range of motion and maintained the position of the testing limb throughout. The subjects were instructed to match a red target line corresponding to 5^o range of motion (abduction for the index finger) as accurately and smoothly as they could. The trials lasted until volitional fatigue. A light load was used for all Experiments and was 30% of the 1 repetition maximum (1 RM).

Purdue Pegboard

Evaluation with the Purdue Pegboard (Lafayette Instrument, Lafayette, IN) was conducted at the end of each Experimental testing session. The Purdue Pegboard is a standardized and reliable tool that is commonly used to evaluate fine dexterity of the hand and finger.[96] Subjects were given standardized directions from the Purdue Pegboard Test manual for administering the three-trial test.[97] The test board consists of a board with four cups across the top, with two vertical rows below the cups comprised of 25 small holes. The two outermost cups at the top left and right hand corners of the board hold 25 pegs each. Subjects were asked to start the first trial with their left hand (LH). Directions were given to use the LH to retrieve pegs from the cup and place as many pegs as possible down the respective row within 30 seconds. The score for each trial was the number of pegs placed successfully in the time allowed. A subsequent trial was repeated with the right hand (RH). Two additional trials were completed, alternating between the left and right hand. The three scores with each hand were averaged to yield a final score with the LH and the RH.

Experimental Setup and Measurements

Subjects were comfortably seated upright and facing a 32-inch screen (2 m away) for the Experimental sessions. The left arm was slightly abducted and the elbow was flexed at a 90 degree angle. The custom-made hand device isolated abduction/adduction of the index finger while fully supporting the palm of the hand and preventing any flexion or extension of the index finger.

Potentiometer

Positional variability was quantified as the standard deviation of the position signal. The position and movement of the second MCP joint was detected with a low-friction potentiometer under the axis of rotation of the second MCP joint (SP22GS, Precision Sales Inc., Newtown Square PA).The output from the potentiometer was sampled at 1000 Hz.

Accelerometer

Limb acceleration quantified as the standard deviation of the acceleration signal. The acceleration of the index finger was measured with a piezoresistive accelerometer (model 7265A-HS, Endevco). The output from the accelerometer was sampled at 1000 Hz.

Electromyographic Activity

The EMG activity from the first dorsal interosseus, was recorded using silver-silver chloride surface electrodes (model E220N-LS, In Vivo Metric, Healdsburg, CA, USA) and taped on the skin distally to the innervation zone (Homma and Sakai 1991). The EMG signals were amplified (2,000 times) and band pass filtered at 3-500 Hz(Grass Model 15LT system; Grass Technologies, West Warwick, RI, USA). The EMG was sampled at 2 kHz with a power 1401 A/D board, stored and analyzed off-line using

Spike2 data analysis system (Cambridge Electronics Design, Cambridge, UK) and custom-designed software (Matlab 6.1, The Math works Inc., Natick, MA, USA).

Dependent Variables

Sinusoidal Task

- Motor-output variability**:** Motor-output variability was quantified as the standard deviation (SD) of the movement trajectory, and the SD of the limb acceleration as done previously.

- Accuracy: Accuracy was quantified as the root mean square error (RMSE) between the target and the position-time sinusoidal created by the subject

- Electromyographic activity: The FDI muscle activity was measured as the amplitude of the EMG signal (RMS of the interference signal). In addition, the power spectrum density of the EMG signal was analyzed from 5-60 Hz. We chose to analyze the power spectra because it lends valuable insight into modulation of the motor neuron pool. We organized our analysis into the following three frequency bands;(1) 5-13 Hz, because it is indicative of changes in the motor unit discharge rate, (2) 13-30 Hz, known as the beta band, because it reflects changes in motor unit synchronization and (3) 30-60 Hz, known as the piper band, because it is associated with strong voluntary effort.[88,98–100]

Constant Task

- Motor-output variability: The SD of the position trajectory for the constant task was used to quantify potential variability similar to previous studies.

- Accuracy: For the constant task, accuracy was quantified as the root mean squared error (RMSE) of the performance trajectory from the targeted position.

- Electromyographic activity: The FDI muscle activity was measured as the amplitude of the EMG signal (RMS of the interference signal). In addition, the power spectrum density of the EMG signal was analyzed from 5-60 Hz. We chose to analyze the power spectra because it lends valuable insight into modulation of the motor neuron pool. We organized our analysis into the following three frequency bands;(1) 5-13 Hz, because it is indicative of changes in the motor unit discharge rate, (2) 13-30 Hz, known as the beta band, because it reflects changes in motor unit synchronization and (3) 30-60 Hz, known as the piper band, because it is associated with strong voluntary effort. [88,98–100]

- Net impulse: The net impulse of the performance was quantified by multiplying the time to task failure (s) with the force the subject exerted throughout the constant position task (N). Net impulse of the performance is expressed as newton seconds (N-s).

- Time to task failure (TTF): Time to task failure was quantified as the time to volitional fatigue in seconds.

- Heart rate (HR) and Blood Pressure (BP): Heart rate and blood pressure were measured throughout the constant position task in 30 second intervals using an automated blood pressure monitor (Omron Healthcare Inc., Bannockburn, IL). Mean arterial pressure (MAP) was also calculated using the results of the blood pressure analysis (MAP=DBP+1/3(SBP-DBP).

- Rate of perceived exertion (RPE): An index of perceived exertion (RPE) was assessed with the modified Borg Scale. Ratings of perceived exertion are a reflection of how heavy and strenuous the exercise feels to an individual. It is based on physical sensations an individual experiences during exertion or exercise. These sensations include heart rate, breathing rate, sweating, burning pain and fatigue. Although it is a subjective rating, an individual's exertion rating provides a fairly reliable and valid estimate of the actual heart rate during physical activity. The scale ranges from 6 to 20, where 6 indicates "no exertion at all" and 20 indicates "maximal exertion".

Experimental Protocols 1

Experiment 1

Nine young subjects (age=18-35 years) were recruited for the study. Subjects were randomly assigned to one of 3 treatment orders (Table 3-1). The Experimental setup and measurements were described previously. Each subject received their assigned near-infrared light therapy treatment (sham, 240 Joules or 480 Joules) prior to exercise. Each subject then performed a 1-RM test followed by 5 sinusoidal tasks with 10% 1-RM as a warm-up before the fatiguing constant task was performed. The sinusoidal task lasted for 35 seconds at a frequency of 0.6 Hz with an amplitude of 10^{o}. The constant position task was conducted with a target force of 5 N and 30% 1-RM and maintained until volitional fatigue. Ratings of perceived exertion were assessed at 30 second intervals throughout the constant task. Heart rate and blood pressure were assessed every 30 seconds throughout the constant task. Following completion of the constant task 1-RM was assessed to quantify fatigue. Subjects returned to the

laboratory for 2 additional visits to receive the remaining doses of near-infrared light therapy.

Statistical Analysis

The dependent variables for this Experiment included: net impulse, time to task failure (TTF), strength, accuracy as the root mean square error (RMSE), movement/positional variability as the standard deviation (SD) of the acceleration signal, neural activation as the RMS amplitude of the EMG signals and the three frequency bands of the power spectrum density. The independent variables included the 3 doses of near-infrared light therapy; sham, 240 joules and 480 joules.

A repeated-measures ANOVA was used to assess TTF, RMSE and SD of movement, RMS of EMG amplitude and the frequency bands of the power spectrum with repeated measure on the 3 near-infrared treatments. A two-way repeated measure ANOVA (2 time points x 3 treatments) was used to assess 1-RM (pre- and post-task failure) with repeated measures on the 3 near-infrared light therapy treatments. The alpha level for all statistical tests (SPSS 17) was set at 0.05, unless corrected and all significant interactions were followed by appropriate post hoc analyses. For example a between treatments interaction/main effect will be further analyzed with Bonferroni post-hoc analyses.

Experimental Protocols 2

Experiment 2

Twenty one young subjects (age=18-35 years) and 21 older adults (age= 60-90 years) were recruited for this Experiment. Subjects were randomly assigned to one of 2 treatment orders or the control group(Table 3-2). The Experimental setup and measurements were described previously. Each subject received their assigned near-

infrared light therapy treatment (control, sham, or Joules= 240 J) prior to exercise. Each subject then performed a 1-RM test followed by 5 sinusoidal tasks with 10% 1-RM as a warm-up before the fatiguing constant task was performed. The sinusoidal task lasted for 35 seconds at a frequency of 0.6 Hz with an amplitude of 10^{o}. The constant task was conducted with a force of 30% 1RM and maintained at 5^{o} of abduction with the index finger until volitional fatigue. Ratings of perceived exertion were assessed at 30 second intervals throughout the constant position task. Heart rate and blood pressure were assessed every 30 seconds throughout the constant position task. Following volitional fatigue 1-RM was assessed to quantify fatigue.

Statistical Analysis

The dependent variables for this Experiment included: net impulse, time to task failure (TTF), accuracy as the root mean square error (RMSE), movement/positional variability as the standard deviation (SD) of the acceleration signal, neural activation as the RMS amplitude of the EMG signals, the three frequency bands of the power spectrum density, ratings of perceived exertion, heart rate and blood pressure. The independent variables include the condition (control, sham, 240J) and the age of the subject (young or older).

A two-way repeated-measures (2 age groups x 3 conditions) ANOVA was used to assess net impulse, TTF, RMSE and SD of movement and RMS of EMG amplitude, the three frequency bands of the power spectrum, RPE, HR and BP with repeated measure on the 3 conditions. A three-way ANOVA (2 age groups x 2 time points x 3 conditions) was used to asses 1-RM (pre and post task failure) changes with repeated measures on the 2 near-infrared light therapy treatments. The alpha level for all statistical tests (SPSS 17) was set at 0.05, unless corrected and all significant interactions will be

followed by appropriate post hoc analyses. For example a between treatments interaction/main effect will be further analyzed with Bonferroni post-hoc analyses. Multiple regression analysis was also performed to determine the associated changes in time to task failure, strength and power spectrum of EMG from 5-60 Hz.

Conclusion

In conclusion, Experiment 1 addressed the first research question which proposes to determine whether near-infrared light therapy can effectively enhance time to task failure when performing novel motor tasks with the hand. Second, this Experiment determined if these effects occur in a dose-dependent manner. Experiment 2 allowed us to determine the full extent to which near-infrared light therapy modulates neuromuscular control. Furthermore, Experiment 2 allowed us to compare the activation of muscle and modulation of motor control in young and older adults following the near-infrared light treatment. These Experiments have been formulated based on previous findings from our lab, which suggest near-infrared light therapy is effective at increasing blood circulation and attenuating the onset of muscle fatigue. The outcomes of this project have significant impact on the clinical application of near-infrared light therapy in both young and older adults.

Table 3-1. Study Design of Experiment #1

Session 1 (Day O)	Group	Experimental (Day 1)	Experimental (Day 3)	Experimental (Day 5)
-ICF -MHQ -Baseline Measures -Group Assignment	A (n=3)	Sham	240 Joules	480 Joules
	B (n=3)	480 Joules	Sham	240 Joules
	C (n=3)	240 Joules	480 Joules	Sham

Table 3-2. Study Design of Experiment #2

Session 1 (Day O)	Group Assignment	Experimental (Day 1)	Experimental (Day 7)
-ICF -MHQ -Group Assignment	Experimental 1 (Young, n=7) (Old, n=7)	Sham	240 Joules
	Experimental 2 (Young, n=7) (Old, n=7)	240 Joules	Sham
	Control (Young, n=7) (Old, n=7)	Control	Control

CHAPTER 4
RESULTS

Experiment # 1

Demographics

All subjects read and signed the university approved informed consent prior to participation in this investigation. A total of 9 subjects were recruited from the University of Florida campus (n=9). No adverse events were reported for any of the Experimental visits. Demographic data for all subjects in Experiment #1 are summarized in Table 4-1.

Baseline Strength

All participants performed a baseline 1 repetition maximum (1RM) strength test to determine their strength prior to Experimental testing. Baseline 1RM testing was not different on any of the Experimental testing days (1.92 ± 0.33 kg, p=0.850). Baseline strength measures were used to calculate the 10% 1RM weight used during the sinusoidal task and the 30% 1RM weight used during the constant position task (Table 4-2).

Sinusoidal Task

Motor output variability: We quantified motor output variability by assessing the standard deviation (SD) of the limb acceleration and the SD of the movement trajectory. No difference in SD of limb acceleration was found for any of the Experimental treatments, $F_{2, 24}$=0.518, p=0.602 (Figure 4-1). In addition, no difference in SD of movement trajectory, as measured by the potentiometer, was found for any of the Experimental treatments, $F_{2, 24}$=0.296, p=0.746 (Figure 4-2).

Accuracy: We quantified accuracy as the root mean square error (RMSE) between the target and the position-time sinusoidal created by the subject. No

difference in RMSE of position was detected between any of the Experimental treatments ($F_{2, 24}$=1.020, p=0.376 ; Figure 4-3).

Electromyographic (EMG) activity: Electromyographic activity from the first dorsal interossei (FDI) was first quantified as the root mean square (RMS) amplitude of the EMG signal. There were no differences between the Experimental treatments, with respect to RMS amplitude ($F_{2, 24}$=0.445, p=0.646; Figure 4-4). The organization of the EMG during the sinusoidal task was evaluated as the power spectrum. Although there was a significant difference among frequency bands (frequency band main effect ($F_{2, 48}$=19.969, p<0.001; Figure 4-5), there were no significant interactions found between any of the Experimental treatments and the 3 frequency bands analyzed ($F_{4, 48}$=0.728, p=0.525).

Constant Position Task

Motor output variability: Motor output variability was quantified by assessing the standard deviation (SD) of the limb acceleration and the SD of the movement trajectory. The SD of limb acceleration was not affected by any of the Experimental treatments (p>0.05). However, the SD of limb acceleration significantly increased as the constant position task progressed ($F_{4, 64}$=3.536, p=0.046; Figure 4-6). The treatment x time interaction for the task was not significant (p>0.05). Similarly, the movement trajectory as measured by the SD of the potentiometer significantly increased as the constant position task progressed ($F_{4, 64}$=4.223, p=0.037; Figure 4-7). However, there were no significant differences detected between Experimental treatments. In addition, the treatment x time interaction for SD of movement trajectory throughout the constant position task was not significant (p>0.05).

Accuracy: Accuracy was quantified as the root mean square error (RMSE) between the target and the performance of the subject during the constant position task. As expected, the accuracy of the performance significantly decreased as the constant position task progressed ($F_{4, 64}$=7.498, p=0.005; Figure 4-8). However, there were no significant differences detected between the Experimental treatments at any time point throughout the constant position task (P>0.05).

Electromyographic (EMG) activity: Electromyographic activity from the first dorsal interossei (FDI) was first quantified as the root mean square (RMS) amplitude of the EMG signal. Although the RMS amplitude was greater with both active doses of near-infrared light therapy there were no significant differences between the Experimental treatments ($F_{2, 16}$=2.307, p=0.150; Figure 4-9(A)). RMS amplitude significantly increased as the constant position task progressed ($F_{4, 32}$=7.498, p=0.005; Figure 4-9(B)). However, there were no significant differences in RMS amplitude between any of the Experimental treatments at any time point. The modulation of the FDI during the constant position task was evaluated as the power spectrum of the FDI from 5-60 Hz. There were significant differences among frequency bands (frequency band main effect; $F_{5, 40}$ = 40.002, p<0.001; Figure 4-10). None the less, the interaction of treatment x frequency bands was not significant ($F_{40, 320}$=1.119, p=0.354).

Time to task failure (TTF): We quantified time to task failure as the time in seconds that each participant was able to sustain 30% of their 1RM with their left index finger at 5 degrees of abduction. A significant main effect for treatment was observed between the three Experimental treatments (sham= 245.11 ± 85.183, 240J= 332.56 ± 108.393, and 480J= 310.56 ± 102.949; $F_{2, 16}$= 3.990, p=0.039). Post-hoc analysis

indicated that the 240 J dose of near-infrared light therapy was able to significantly extend time to task failure by 26% compared with the sham condition ($p<0.05$; Figure 4-11).

Impulse: We quantified the net impulse of performance as the area under the curve for the constant position task. To achieve this we multiplied the load lifted (N) by the duration of the task (s). The net impulse significantly increased when participants received the 240 Joule dose compared with the sham condition (16694.61 ± 611.35 N-s vs. 12563.23 ± 499.46 N-s; $p<0.01$). The net impulse of performance was also significantly greater when participants received the 480 Joule dose of NIR light therapy (15952.68 ± 731.80 N-s; $p<0.05$; Figure 4-12).

Fatigue-index: We quantified the fatigue-index as the percent change in 1RM from baseline to post-task failure. No differences between the fatigue-index for any Experimental treatments were detected ($F_{2, 24}=0.087$, $p=0.916$; Figure 4-13). Our results indicate that despite participants being able to sustain the constant position task longer they did not fatigue to a greater extent. An improvement from sham, with respect to time to task failure with the 240J dose was strongly associated with a conservation of strength ($R^2=0.54$, $p=0.024$; Figure 4-14)

Experiment # 2

Demographics

All subjects read and signed the university approved informed consent prior to participation in this investigation. A total of 42 subjects were recruited from the University of Florida campus and surrounding Gainesville community (n=42). No adverse events were reported for any of the Experimental visits. Demographic data for all subjects in Experiment #1 are summarized in Table 4-3.

Baseline Strength

All participants performed a 1 repetition maximum (1RM) strength test to determine their strength prior to Experimental testing. Baseline strength analysis indicated that the young adults were significantly stronger than older adults (1.540 ± 0.048 vs. 1.122 ± 0.058, $F_{1, 48}$=30.465, p<0.001; Figure 4-15). Analysis of treatment also revealed a main effect that baseline strength was greater in older adults after they received the 240J treatment when compared with the sham treatment (p<0.05; Figure 4-16). The increase in strength with NIR light therapy (from sham to 240 J) was explained by decreased power from 5-13 Hz of the EMG signal (R^2=0.59, p=0.016; Figure 4-17). We used the baseline strength measures to calculate the 10% 1RM weight we used during the sinusoidal task and the 30% 1RM weight we used during the constant position task (Table 4-4).

Sinusoidal Task

Motor output variability: Motor output variability was quantified by assessing the standard deviation (SD) of the limb acceleration and the SD of the movement trajectory. Older adults had a more variable movement trajectory when performing the sinusoidal task (age main effect, $F_{1, 64}$=4.859, p=0.031; Figure 4-18). We found no other main effects or interactions (p>0.05).

Accuracy: Accuracy was quantified as the root mean square error (RMSE) between the target and the position-time sinusoidal created by the subject. Older adults were significantly less accurate when performing the sinusoidal task when compared to the young adults (2.986 ± 0.160 degrees vs 2.395 ± 0.160 degrees, $F_{1, 64}$=6.786, p=0.011; Figure 4-19). We found no other main effects or interactions (p>0.05).

Electromyographic (EMG) activity: We quantified the electromyographic activity from the first dorsal interosseus (FDI) as the root mean square (RMS) amplitude of the EMG signal. Older adults had greater RMS amplitude compared with young adults (0.273 ± 0.021 and 0.220 ± 0.015; $F_{1,62}$=4.509, p=0.038). However, there were no differences between the Experimental conditions, p>0.05. In addition, we quantified the FDI activation by examining the modulation from 5-60 Hz. There were significant differences among the frequency bands (frequency band main effect; $F_{2, 48}$=19.969, p<0.001), However, we found no significant interactions between any of the Experimental treatments and the 3 frequency bands analyzed ($F_{4, 48}$=0.728, p=0.525).

Post-sinusoidal task strength testing: An additional 1RM test was performed following the sinusoidal testing to ensure muscle fatigue did not occur due to the sinusoidal task. There were no differences in 1RM following the sinusoidal task for any of the conditions (p>0.05). Therefore, the sinusoidal task was not responsible for inducing any fatiguing effects to the subjects.

Constant Position Task

Time to task failure (TTF): We quantified time to task failure as the time in seconds that each participant sustained 30% of their 1RM with their left index finger at 5 degrees of abduction. There was a significant age by condition interaction ($F_{2, 48}$= 4.123, p=0.022; Figure 4-20 (A) and (B)). Post-hoc analysis revealed that TTF significantly increased in older adults when comparing laser with sham (810.833 ± 92.833 s and 586.250 ± 92.936 s), and laser with control (810.833 ± 92.833 s and 454.900 ± 109.963 s; p<0.05; Figure 4-21). In contrast, TTF did not change across the 3 conditions for younger adults.

Electromyographic (EMG) activity: We quantified the electromyographic activity from the first dorsal interosseus (FDI) as the root mean square (RMS) amplitude of the EMG signal. RMS amplitude significantly increased throughout the constant position task ($F_{4, 172}$=75.071, p<0.001; Figure 4-22). Young adults displayed greater RMS amplitude throughout the constant position task compared with older adults ($F_{1, 43}$=15.124, p<0.001; Figure 4-23). In addition, a condition by time interaction was detected ($F_{8, 172}$=2.909, p=0.044). Post-hoc analysis indicated that the NIR condition elicited greater RMS amplitude throughout the entire task. However, this difference was only significant during the 60% time segment (p=0.05; Figure 4-24).

We evaluated the modulation of the FDI during the constant position as the power spectrum of the FDI from 5-60 Hz. Multiple regression analysis (stepwise) was used to examine the contribution of the change in TTF at each frequency band. The change in power in the 5-13 Hz frequency significantly explained the enhanced time to task failure in the older adults when treated with NIR light therapy (R^2=0.586, p=0.016; Figure 4-25). Likewise, the increase in power in the 13-30 Hz frequency band explained the enhanced time to task failure in older adults when they received the 240 Joules of NIR light therapy (R^2=0.689, p=0.006; Figure 4-26). Changes in the ratio between 13-30 Hz and 30-60 Hz indicated that older adults were able to sustain the constant position task longer when they had increases in power from 13-30Hz and decreased power from 30-60 Hz following NIR light therapy treatment (R^2=0.836, p=0.001; Figure 4-27).

Motor output variability: We quantified the motor output variability by assessing the standard deviation (SD) of the limb acceleration and the SD of the movement trajectory. The SD of limb acceleration significantly increased as the constant position

task progressed for all treatment conditions ($F_{3, 162}$=12.563, p<0.001). Although the SD of limb acceleration increased following treatment with the NIR therapy compared with sham, it did not reach statistical significance (p=0.067; Figure 4-28). However, the increase in SD of limb acceleration explained the increase in TTF observed with the 240 Joule treatment of NIR light therapy (R^2= 0.769, p= 0.0048; Figure 4-29). Similarly, the movement trajectory as measured by the SD of the potentiometer, significantly increased as the constant position task progressed ($F_{3, 162}$=13.540, p<0.001; Figure 4-30). We detected an age main effect, which indicated that older adults exhibited greater movement variability compared with young adults (0.601 ± 0.042 vs. 0.405 ± 0.038; $F_{1, 54}$= 11.922, p=0.001; Figure 4-31). No significant differences were detected between Experimental treatments (p>0.05).

Accuracy: Accuracy was quantified as the root mean square error (RMSE) between the target and the performance of the subject during the constant position task. The accuracy of the performance significantly decreased as the constant position task progressed, ($F_{3, 162}$=26.376, p<0.001). An age main effect revealed that older adults displayed less accurate movements compared with young adults (0.738 ± 0.047 vs. 0.521 ± 0.042; $F_{1, 54}$= 11.671, p=0.001; Figure 4-32).However, there were no significant differences detected between the Experimental treatments at any time point throughout the constant position task (p>0.05).

Heart rate: Heart rate was monitored throughout the constant position task. Older adults exhibited a lower heart rate throughout the task compared with younger adults (66.3 ± 1.583 vs. 80.968 ± 1.4; $F_{1, 54}$=48.678, p<0.001). Heart rate had a significant time by age interaction ($F_{2, 108}$=16.172, p<0.001). Post-hoc analyses revealed significant

differences between young and older adults throughout the entirety of the constant position task. Older adults did not increase their HR throughout the task, however, young adults significantly increased their HR as the task progressed ($p<0.05$; Figure 4-33). There was no condition effect on heart rate for any of the Experimental treatments ($p>0.05$).

Blood pressure: We monitored blood pressure throughout the constant position task. Older adults exhibited higher systolic and diastolic blood pressure (141.78 ± 85.667 and 85.667 ± 2.029) throughout the task compared with the younger adults (123.321 ± 3.636 and 81.583 ± 1.795; $F_{1, 54}=16.483$, $p<0.001$). Blood pressure also had a significant time x age interaction ($F_{1, 54}=11.757$, $p=0.001$). Older adults displayed higher BP measures throughout the task compared with younger adults ($p<0.05$; Figure 4-34). There was no condition effect on blood pressure for any of the Experimental treatments ($p>0.05$). However, a decreased rate of change in MAP throughout the constant position task with NIR light therapy treatment significantly predicted the time to task failure difference in older adults ($R^2=0.581$, $p=0.016$; Figure 4-35).

Rate of perceived exertion: We evaluated perceived exertion throughout the constant position task. All participants increased their RPE score progressively until task failure ($F_{3, 162}=300.175$, $p<0.001$; Figure 4-36). However, there were no differences in the rate of change in RPE for between age and condition.

Post 1RM: We assessed strength immediately following task failure with the constant position task. Strength decreased significantly from baseline 1RM measurement ($F_{1, 48}=317.27$, $p<0.001$). In addition, a significant treatment effect was observed ($F_{2, 48}=3.765$, $p=0.030$; Figure 4-37). Post-hoc comparison of the three

treatment conditions revealed a significant conservation of strength post-failure when participants were treated with the 240J dose of near-infrared light therapy when compared with sham (1.457 ± 0.07 and 1.214 ± 0.07 respectively). This conservation of strength explained the ability of older adults to delay the onset of task failure by approximately 44% (R^2=0.439, p=0.05; Figure 4-38). However, a treatment effect on young adults did not exist.

Impulse: We quantified the net impulse of performance as the area under the curve for the constant position task. To achieve this we multiplied the load lifted by the participant (N) by the duration of the task (s) (Table 4-5). Although young adults had greater net impulse than older adults (2823 ± 202.1 Ns vs. 2153 ± 236.9 Ns; age main effect $F_{1, 52}$=4.624, p=0.036; Figure 4-39 (A)), the effects of NIR therapy influenced only the older adults (age x treatment: $F_{2, 52}$=5.569, p=0.006, Figure 4-39 (B)). Specifically, older adults exhibited greater net impulse when they received the 240 J dose (3053 ± 401.1Ns) compared with sham (1597 ± 401.1 Ns) and the control groups (1810 ± 428.7 Ns).

Purdue Pegboard

Purdue pegboard scores were quantified as an average of the three scores obtained with each hand. Young adults scored better on the task when compared with older adults, 15.595 ± 0.313 and 11.801 ± 0.313 respectively (age main effect, $F_{1, 70}$=73.220, p<0.001). Participants also performed better on the Purdue pegboard task when they received the NIR treatment compared with the control treatment, 14.524 ± 0.333 and 13.071 ± 0.470 respectively (condition main effect, $F_{2, 64}$= 3.970, p=0.024; Figure 4-40). There was also a significant hand x condition interaction ($F_{2, 64}$=6.151, p=0.004). The Purdue pegboard score with the left hand was significantly increased

when the participant received the laser treatment (14.524 ± 0.33) compared with the sham (13.500 ± 0.33) and control conditions (13.071 ± 0.47). This observation is meaningful as the NIR treatment was able to enhance the score of the left hand (treated hand) to a level greater than the participants dominant (right) hand (14.524 ± 0.33 vs. 13.857 ± 0.35; $p<0.05$; Figure 4-41). Linear regression revealed that the improvements observed in pegboard score were associated with increased power from 5-13 Hz ($R^2=0.534$, $p=0.039$; Figure 4-42 (A)) and 13-30 Hz ($R^2=0.4926$, $p=0.052$; Figure 4-42 (B)).

Table 4-1. Descriptive Statistics for Experiment #1

Variable	Values	SD
Sex	Male= 4, Female= 5	
Age	24.3	4.97
Height (cm)	171.0	7.78
Weight (kg)	71.2	11.6

All values are means and SDs, unless otherwise indicated

Table 4-2. Mass used for individual tasks at each Experimental visit, Experiment #1

Task	Visit	Mean (kg)	SD
Sinusoidal (10% 1RM)	Sham	0.19	0.04
	240J	0.19	0.04
	480J	0.19	0.03
Constant Position (30% 1RM)	Sham	0.58	0.12
	240J	0.57	0.11
	480J	0.57	0.08

Table 4-3. Descriptive Statistics for Experiment #2

Age	Variable	Values	SD
Young	Sex	Male =11, Female=10	
	Age	25.24	3.37
	Height (cm)	170.17	8.83
	Weight (kg)	70.61	12.03
Old	Sex	Male =11, Female=10	
	Age	72.14	5.79
	Height (cm)	170.78	10.93
	Weight (kg)	73.64	16.70

All values are means and SDs, unless otherwise indicated

Table 4-4. Mass used for individual tasks at each Experimental visit, Experiment #2

Age	Task	Visit	Mean (kg)	SD
Young	Sinusoidal (10% 1RM)	Control	0.19	0.03
		Sham	0.18	0.05
		240J	0.18	0.05
	Constant Position (30% 1RM)	Control	0.57	0.08
		Sham	0.54	0.14
		240J	0.54	0.14
Old	Sinusoidal (10% 1RM)	Control	0.15	0.04
		Sham	0.12	0.03
		240J	0.12	0.04
	Constant Position (30% 1RM)	Control	0.45	0.12
		Sham	0.35	0.10
		240J	0.36	0.11

Table 4-5. Force, Time to task failure and Impulse for young and older adults

Age	Measure	Control	Sham	Laser
Young	Force (N)	5.59 ± 0.29	5.34 ± 0.39	5.34 ± 0.37
	TTF (s)	630.43 ± 63.22	473.00 ± 66.42	430.21 ± 42.08
	Impulse (N-s)	3620.87 ± 509.30	2506.87 ± 344.15	2341.33 ± 309.19
Old	Force (N)	4.34 ± 0.49	3.48 ± 0.33	4.74 ±0.50
	TTF (s)	427.86 ± 63.53	598.44 ± 84.74	781.22 ±148.01
	Impulse (N-s)	1810.10 ± 231.03	1861.86 ± 299.33	3274.01 ± 484.94

All values are reported as means and standard errors, unless otherwise indicated

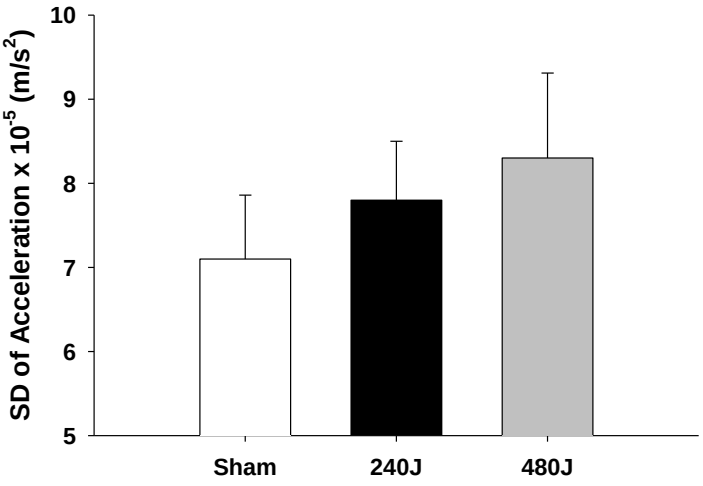


Figure 4-1. SD of acceleration for each Experimental treatment during the sinusoidal task was not different for any of the Experimental treatments.

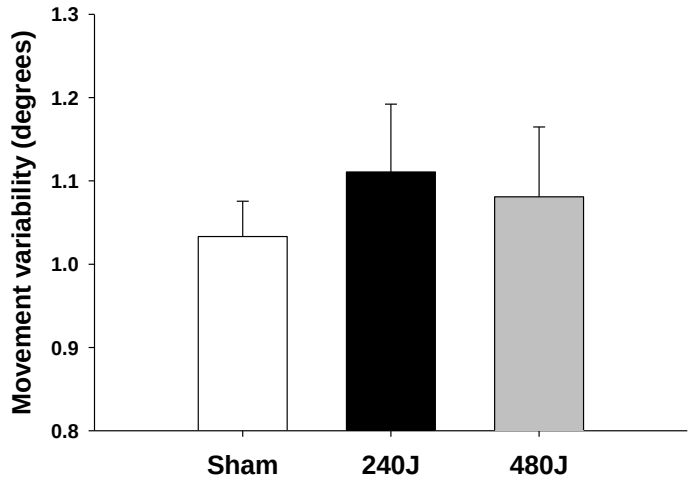


Figure 4-2. SD of movement trajectory for each Experimental treatment during the sinusoidal task was not different for any of the Experimental treatment

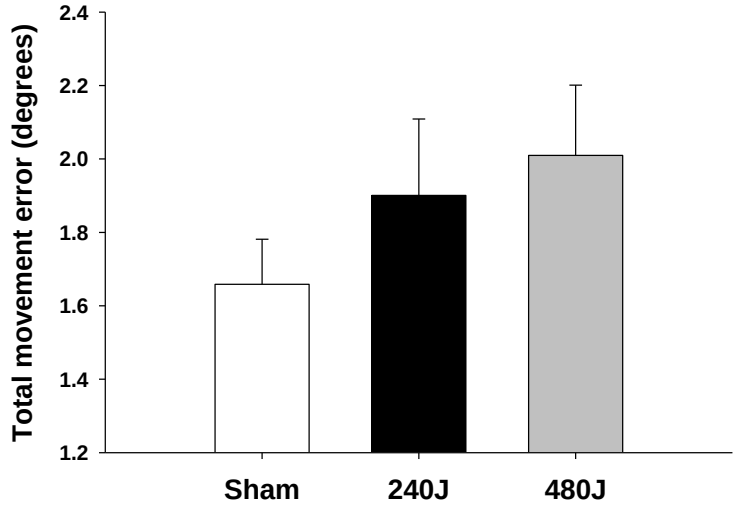


Figure 4-3. Accuracy, as measured by RMSE, for each Experimental treatment during the sinusoidal task was not different for any of the Experimental treatments.

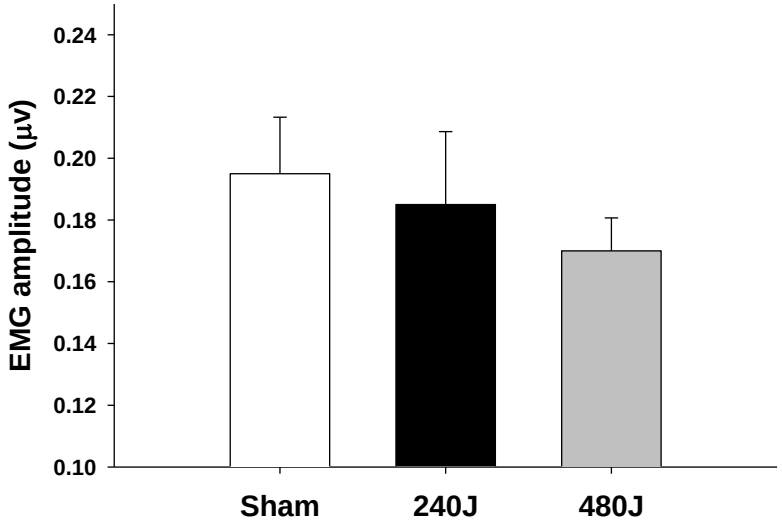


Figure 4-4. EMG amplitude as measured by RMS of the EMG signal, for each Experimental treatment during the sinusoidal task was not different for any of the Experimental treatments

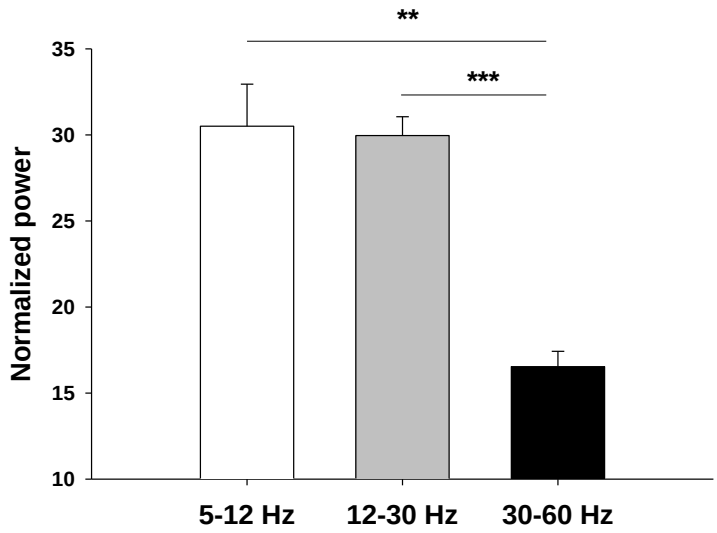


Figure 4-5. Power spectrum analysis by frequency band. ** denotes p<0.005, *** denotes p<0.001. The 5-13 Hz frequency band had significantly more power than the 30-60 Hz frequency band. The 13-30 Hz frequency band had significantly more power than the 30-60 Hz frequency band.

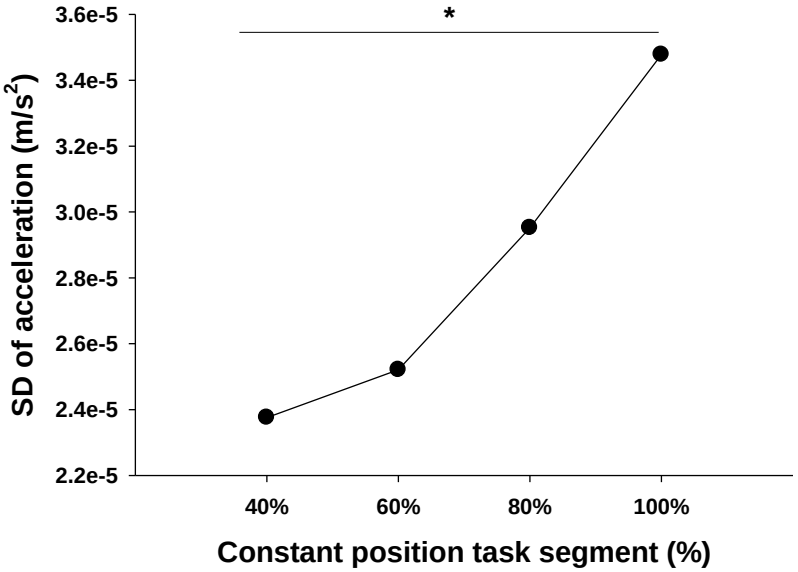


Figure 4-6. SD of acceleration for constant position task increased as the task progressed, * denotes p<0.05

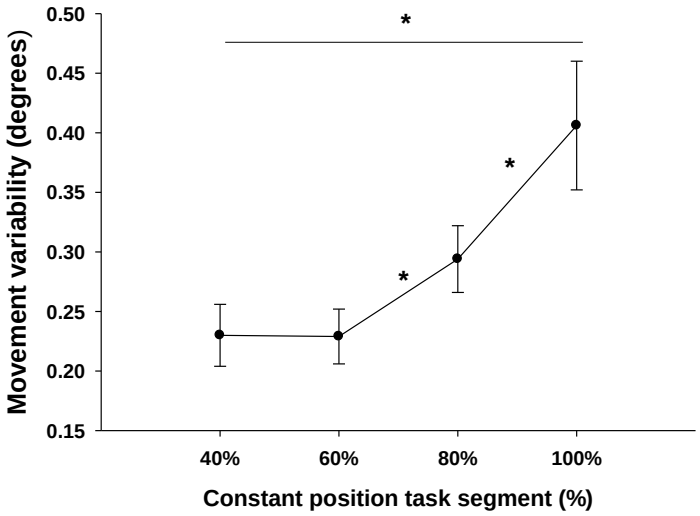


Figure 4-7. SD of movement trajectory for constant position task increased as the task progressed. * denotes p<0.05

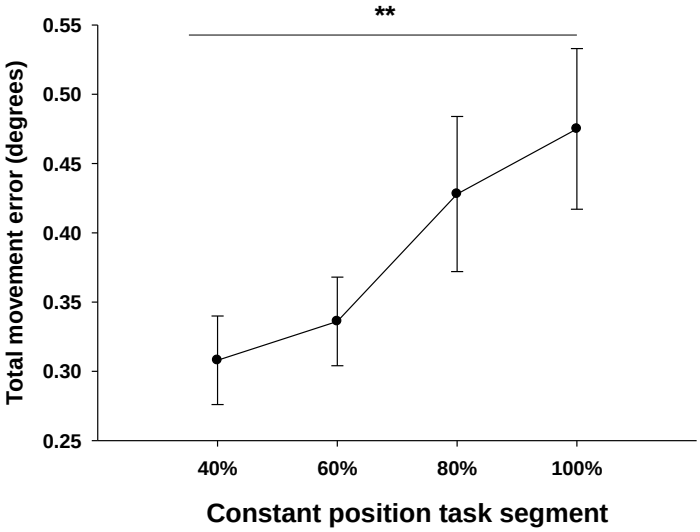
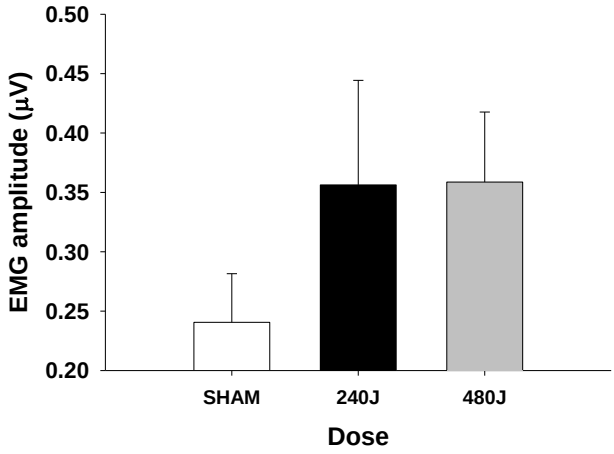


Figure 4-8. Accuracy, as measured by RMSE, for the constant position task increased as the task progressed. ** denotes p<0.005

A



B

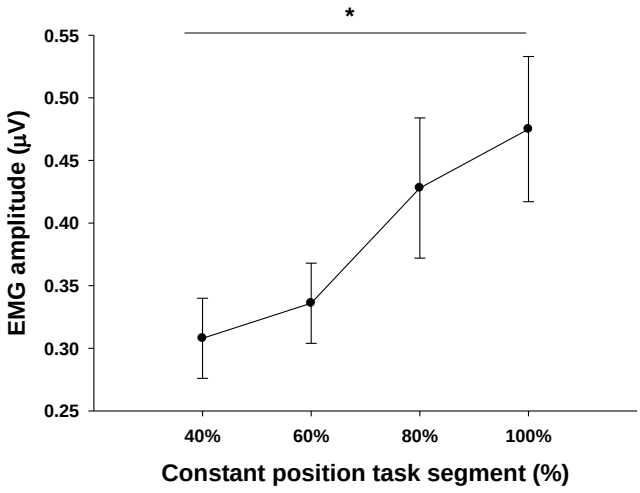


Figure 4-9. EMG activity during the constant position task (A) EMG amplitude as measured by RMS of the EMG signal by dose and (B) throughout the constant position task. * denotes $p<0.05$. RMS amplitude of the EMG signal was not significantly different between any of the Experimental conditions. However, RMS amplitude increased significantly as the task progressed.

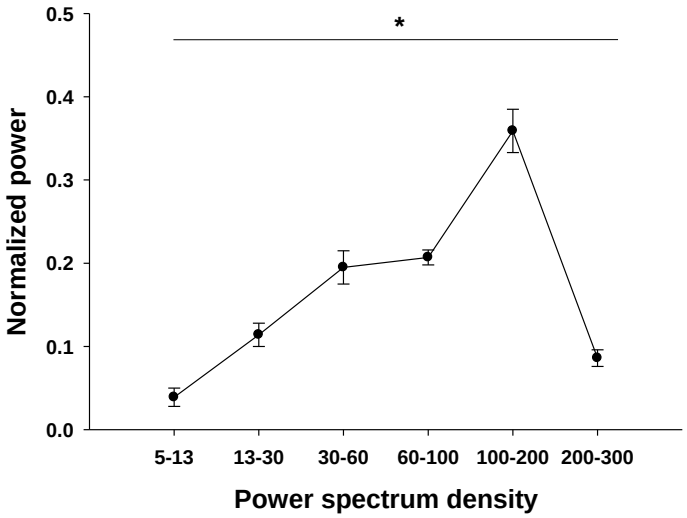


Figure 4-10. Power spectrum analysis by frequency band for the constant position task. *** denotes p<0.001.

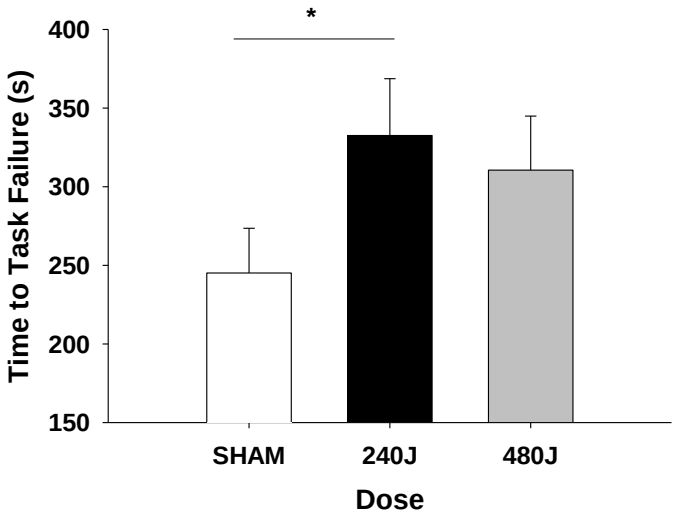


Figure 4-11. Time to task failure during the constant position task for each Experimental treatment dose. The 240 Joule dose elicited a significantly longer time to task failure when compared with sham. Although 480 Joules elicited and increase it did not reach statistical significance. * denotes p<0.05

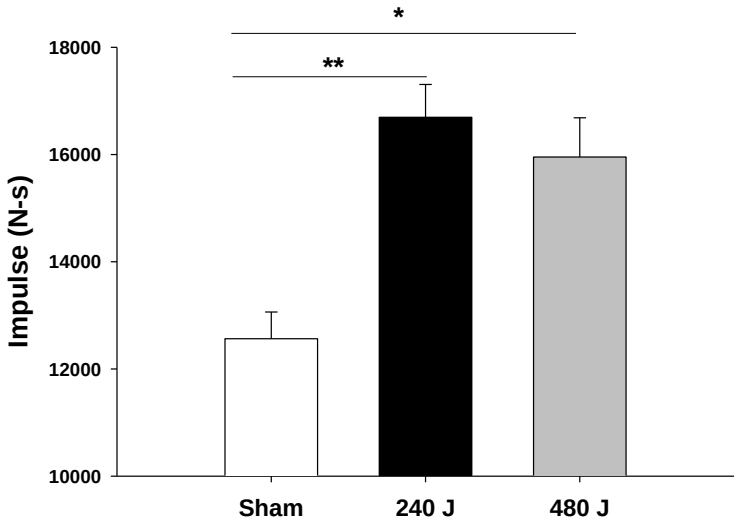


Figure 4-12. Net impulse of performance of constant position task for each Experimental dose. Both doses of NIR light therapy elicited an increase in net impulse of performance. ** denotes p<0.005 and * denotes p<0.05

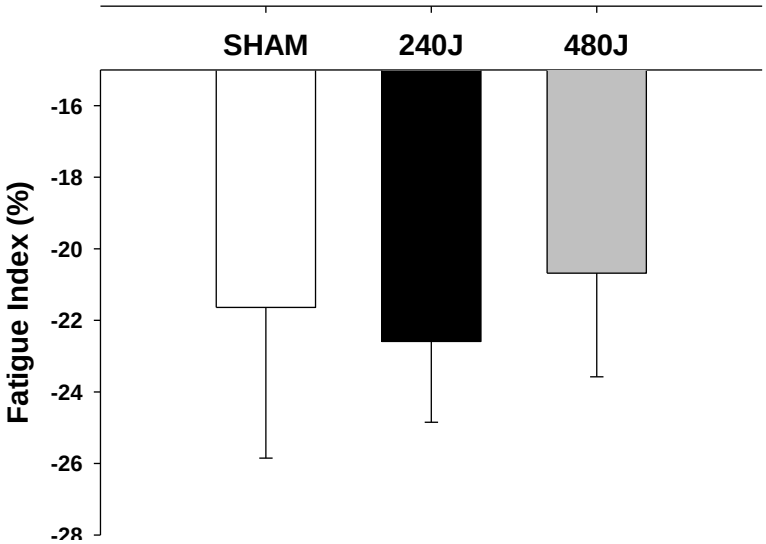


Figure 4-13. Fatigue-index following constant position task was not different for any of the Experimental conditions

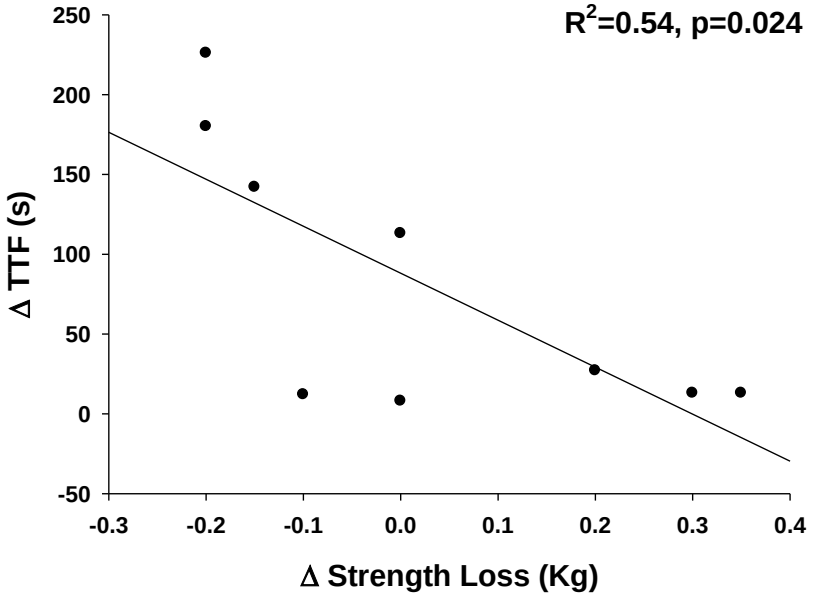


Figure 4-14. Regression explaining the difference in time to task failure between the 240J dose and sham with changes in strength

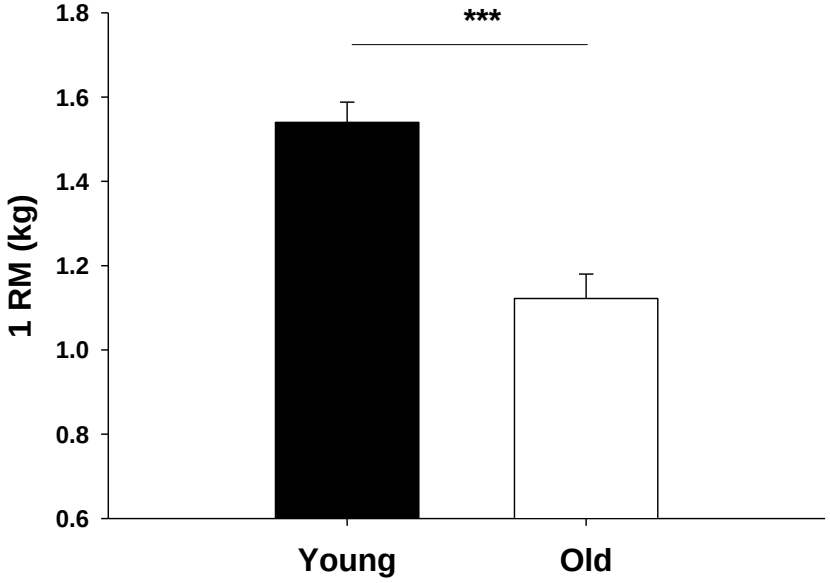


Figure 4-15. Young adults are stronger at baseline than older adults. *** denotes p<0.001

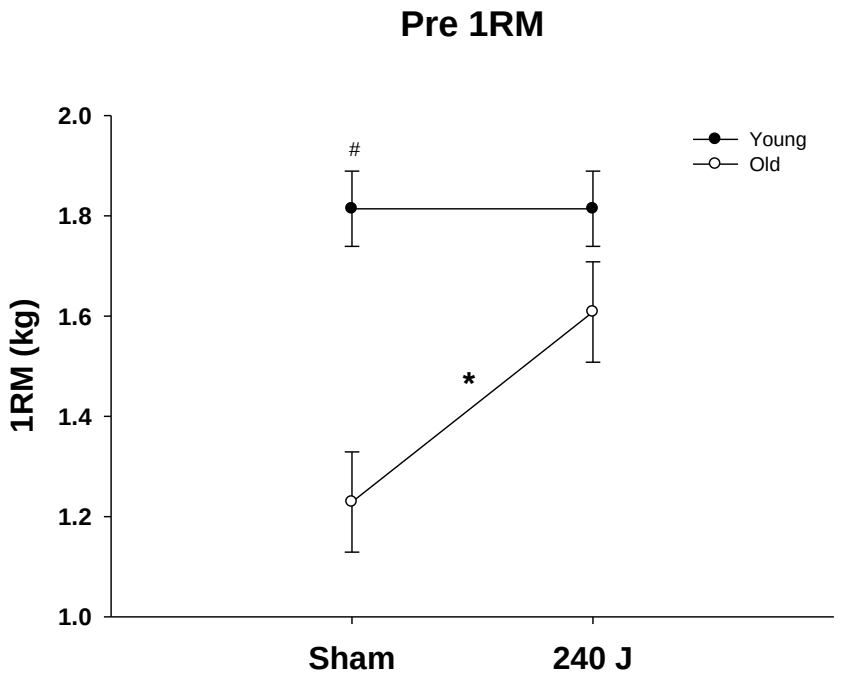


Figure 4-16. Young adults are stronger at baseline than older adults. Older adults were stronger when they received the 240 Joule dose of NIR light therapy than when they received the sham condition. # denotes a between age effect of p< 0.001, * denotes p<0.05

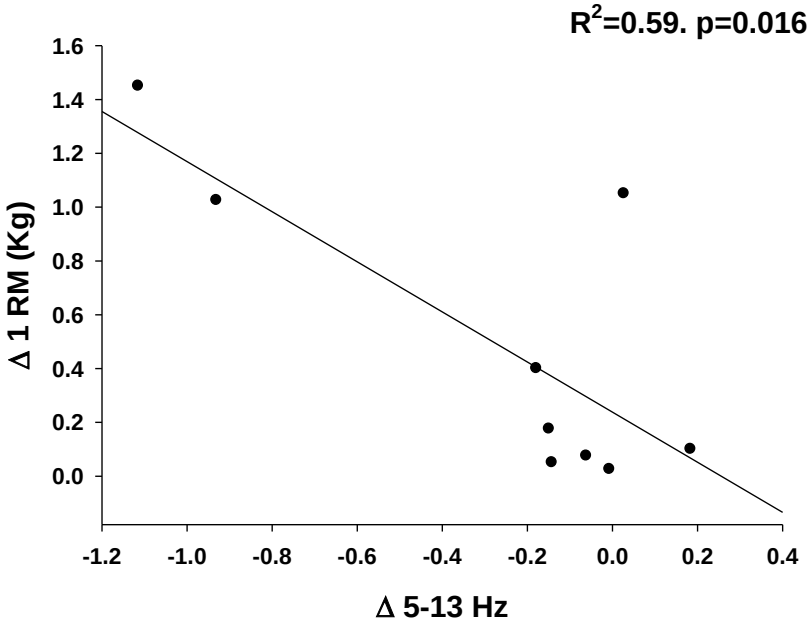


Figure 4-17. Enhanced strength in older adults is explained by decreased power from 5-13 Hz.

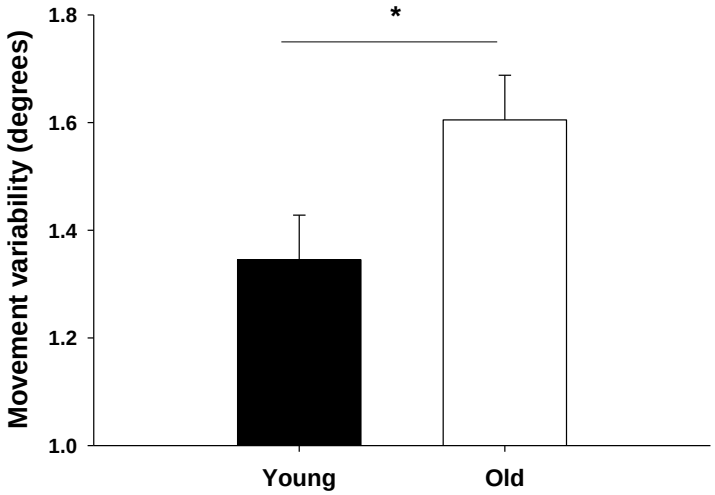


Figure 4-18. SD of movement trajectory for each age during the sinusoidal task. Older adults were significantly more variable in their movements when compared with young. * denotes p<0.05

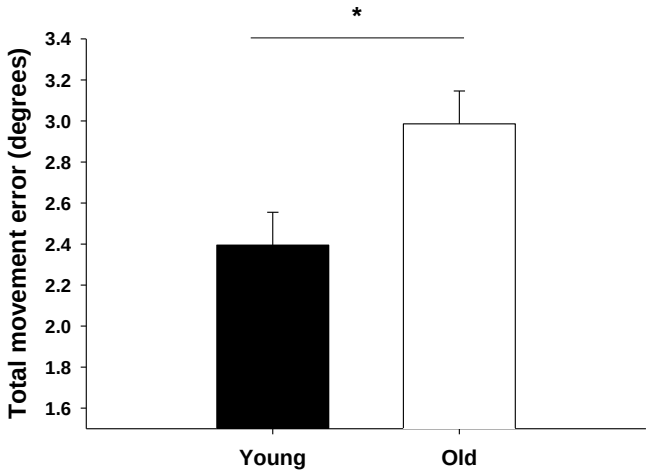
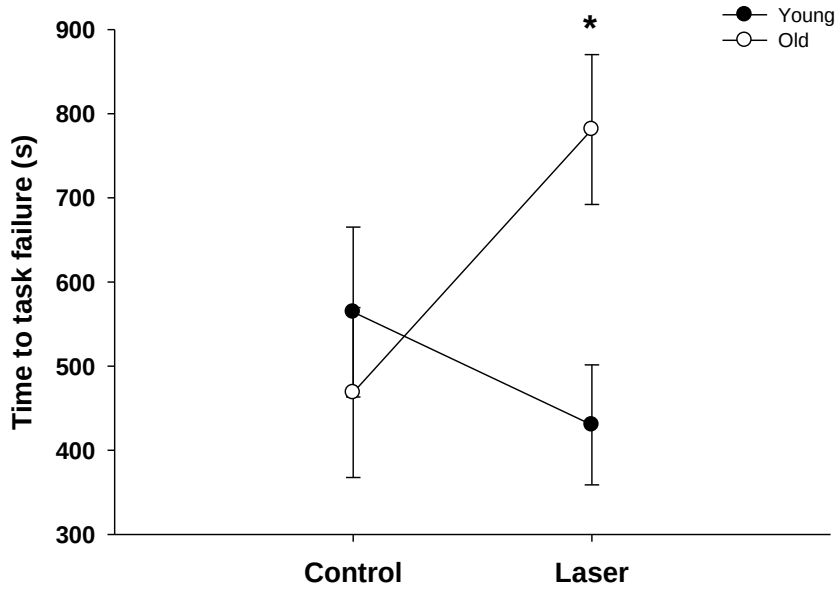


Figure 4-19. Age comparison of accuracy, as measured by RMSE, for the sinusoidal task. Older adults were less accurate than young adults during the sinusoidal task * denotes p<0.05

A



B

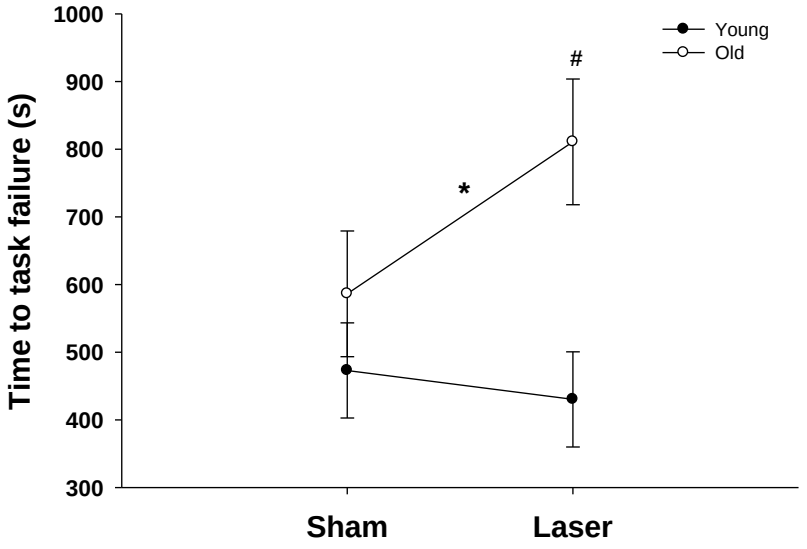


Figure 4-20. Age comparison for time to task failure across Experimental conditions. Older adults had greater TTF when they received the 240 Joule dose of NIR light therapy when compared to control (A) and when compared to sham (B) * denotes $p<0.05$ and # denotes between age difference $p<0.05$

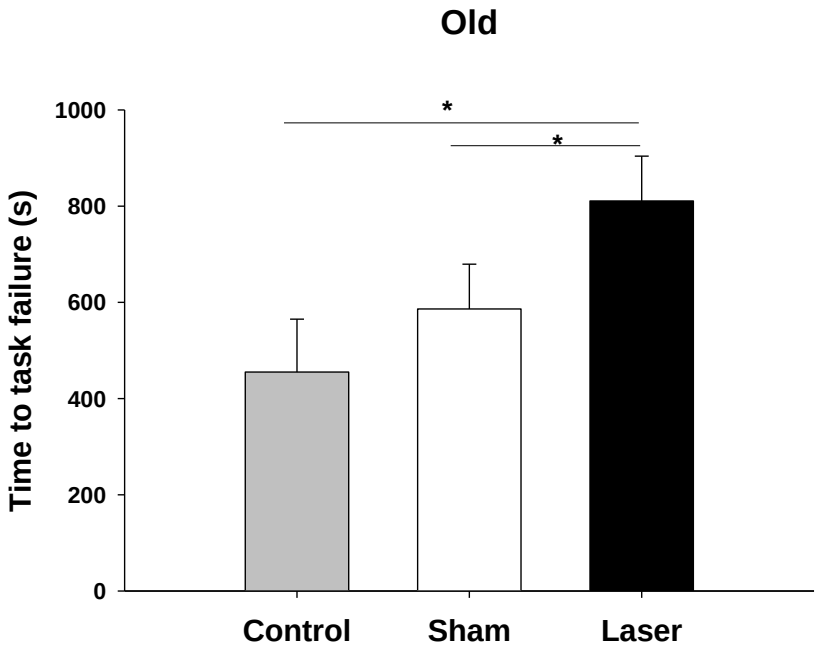


Figure 4-21. Time to task failure across Experimental conditions for older adults. Older adults sustained the constant position task longer when they received the 240 Joule dose of NIR light therapy when compared with the sham condition and the control group. * denotes p<0.05.

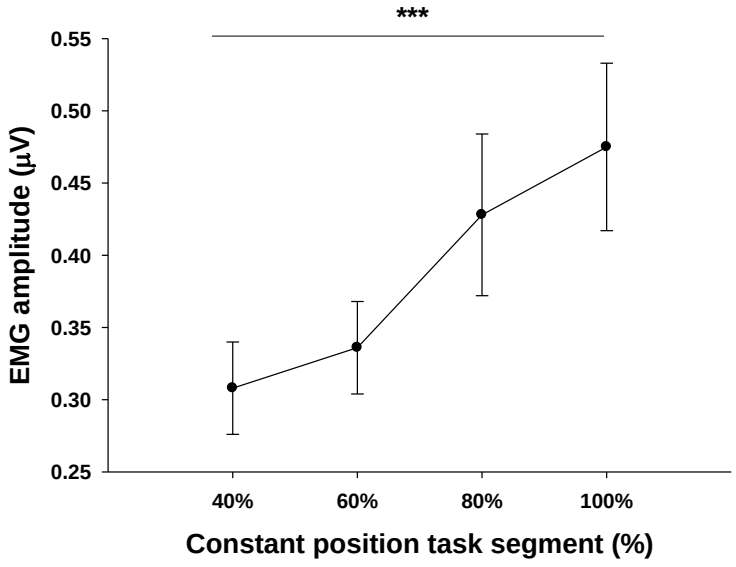


Figure 4-22. RMS amplitude of the EMG signal significantly increased as the constant position task progressed. *** denotes p<0.001

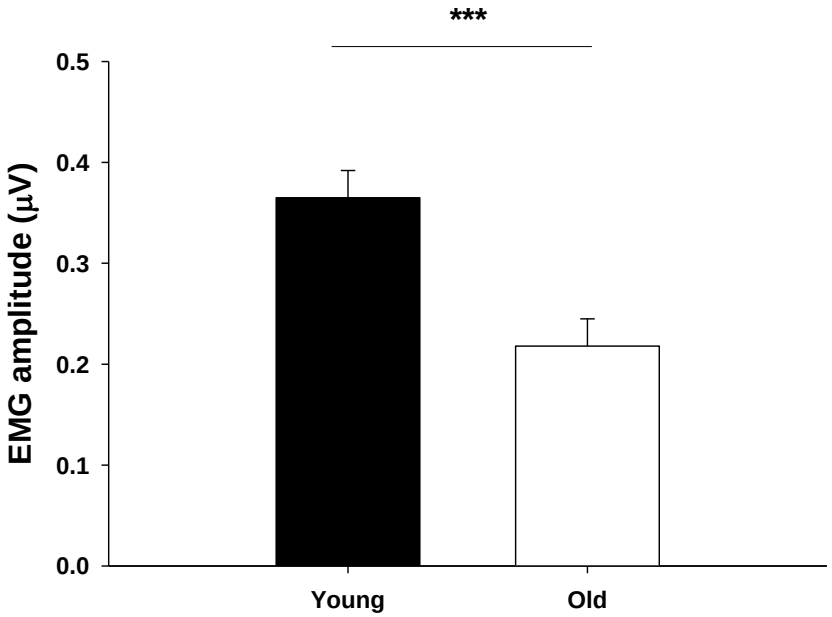


Figure 4-23. RMS amplitude of the EMG signal was significantly greater in young adults compared to older adults. *** denotes p<0.001

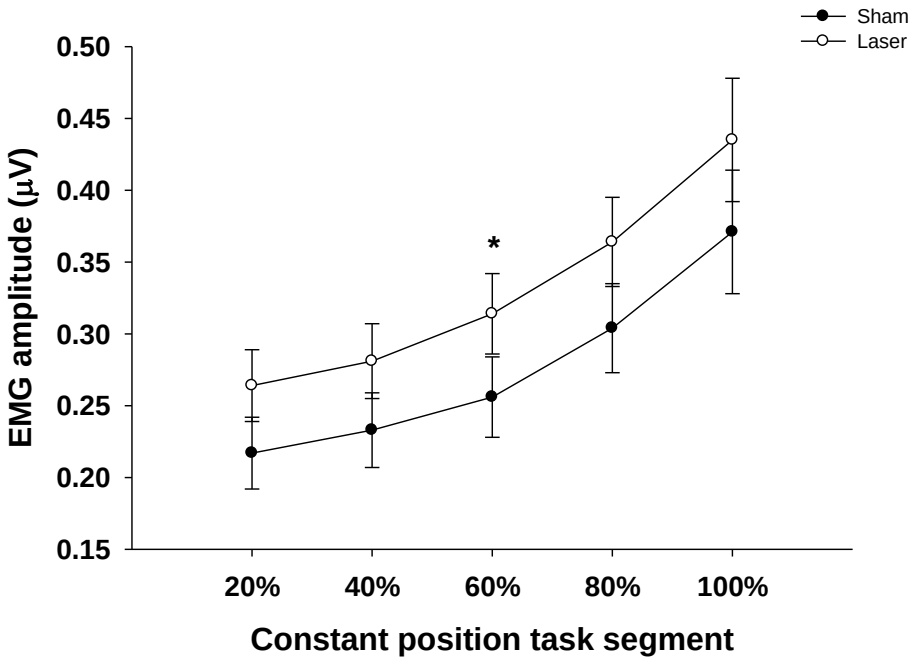


Figure 4-24. RMS amplitude of the EMG signal significantly increased as the constant position task progressed and was different between sham and NIR light therapy. * denotes p<0.05

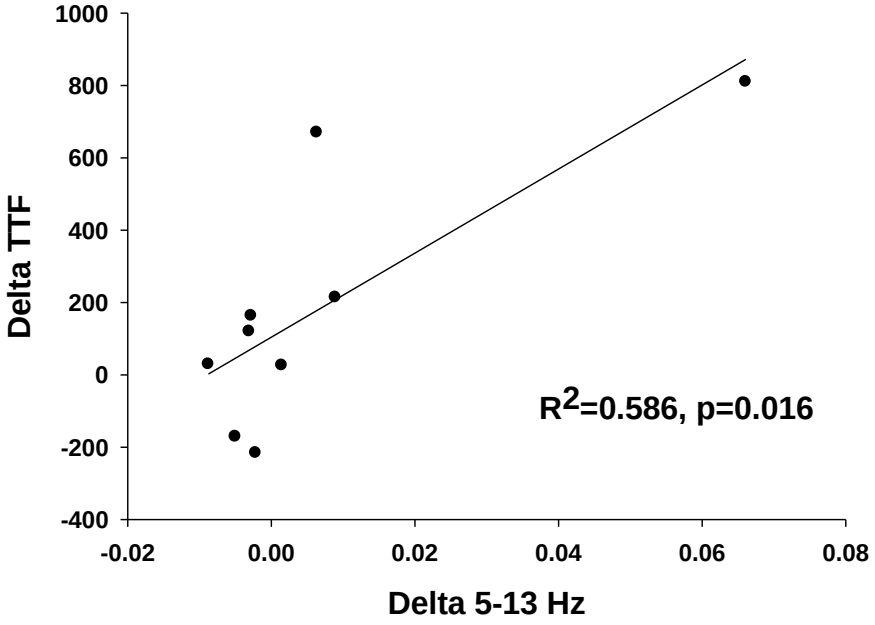


Figure 4-25. Enhanced time to task failure was explained by increased power from 5-13 Hz for older adults.

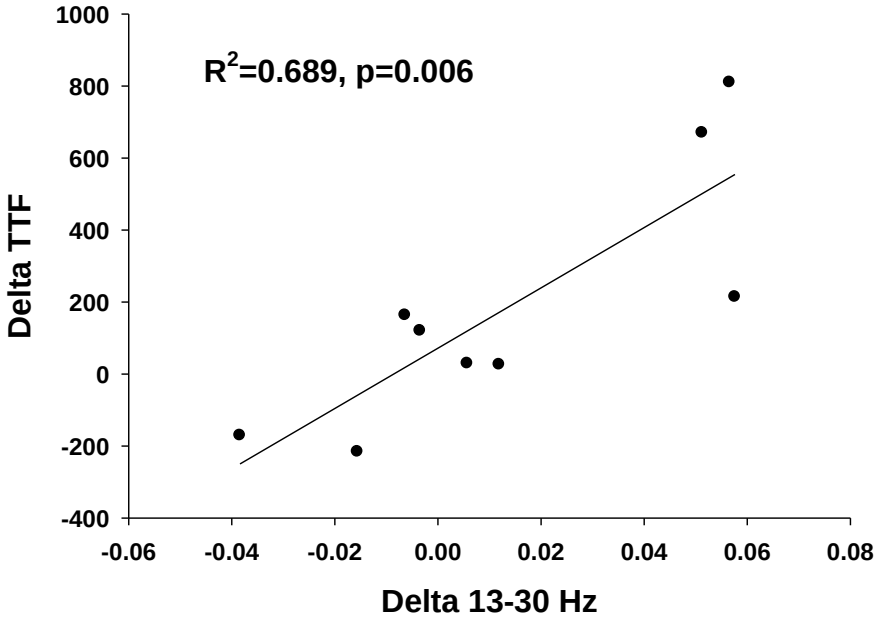


Figure 4-26. Enhanced time to task failure was associated with increased power from 13-30 Hz

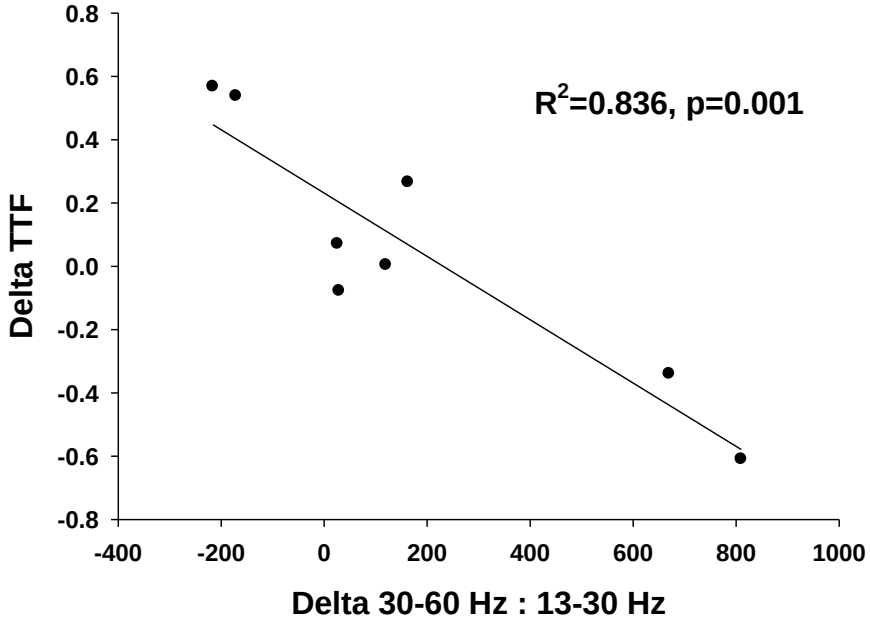


Figure 4-27. Enhanced time to task failure was explained by increased power from 13-30 Hz and decreased power from 30-60 Hz

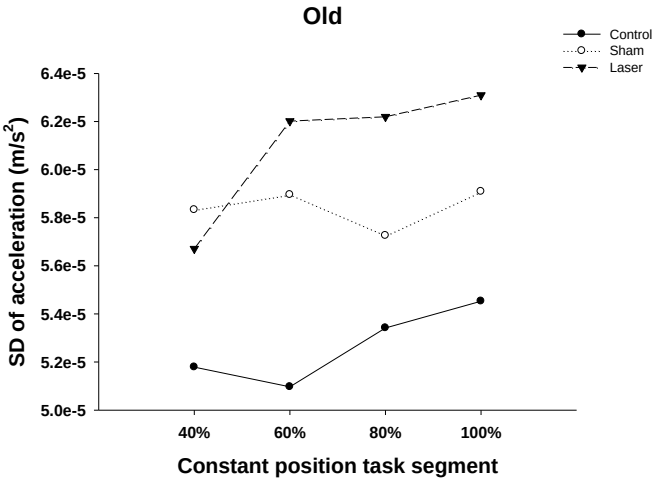


Figure 4-28. SD of limb acceleration for older adults throughout the constant position task.

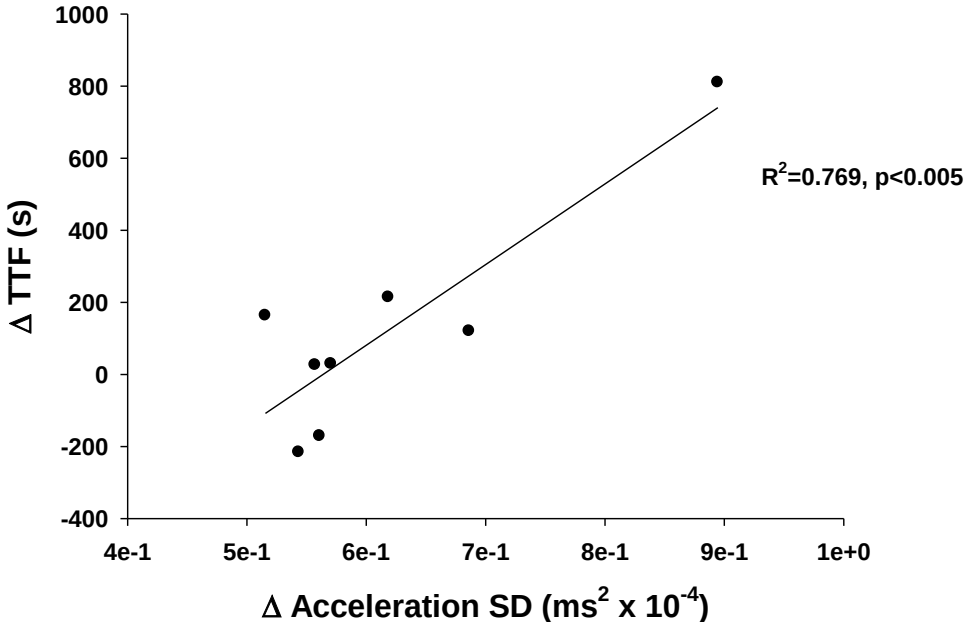


Figure 4-29. SD of limb acceleration for older adults was associated with the enhancements in time to task failure when older adults were treated with 240 Joules of NIR light therapy.

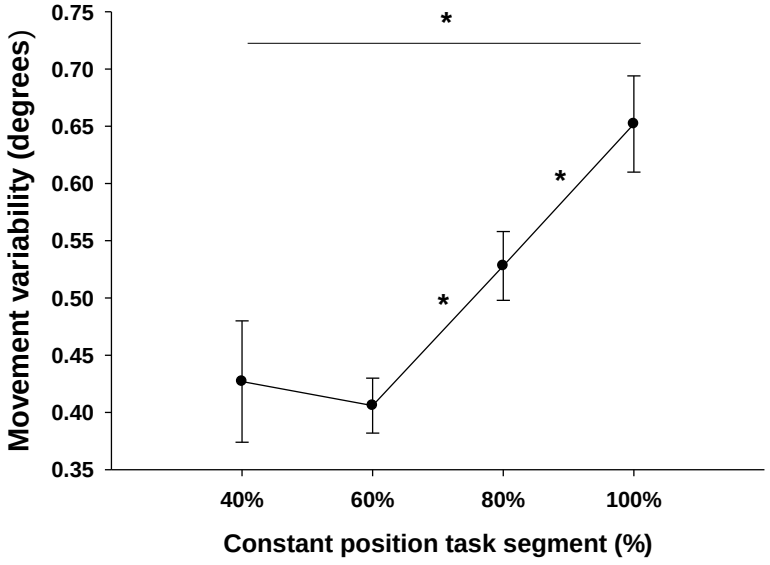


Figure 4-30. SD of movement trajectory increased as the constant position task progressed. * denotes p<0.05

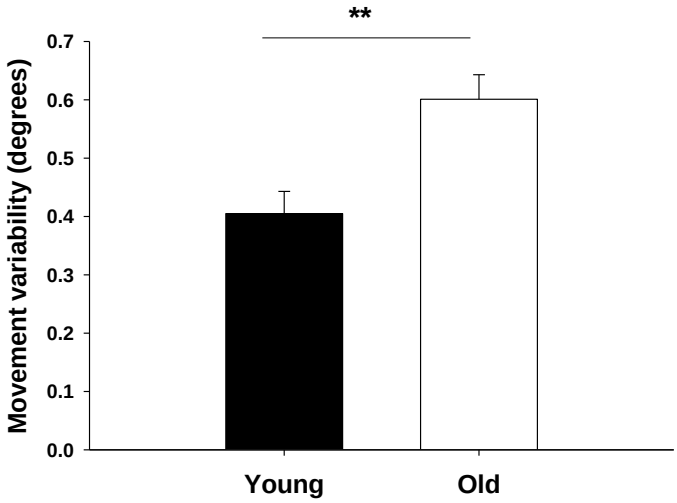


Figure 4-31. Age comparison for SD of movement trajectory for constant position task. Older adults had greater movement variability when compared with young.** denotes p<0.005

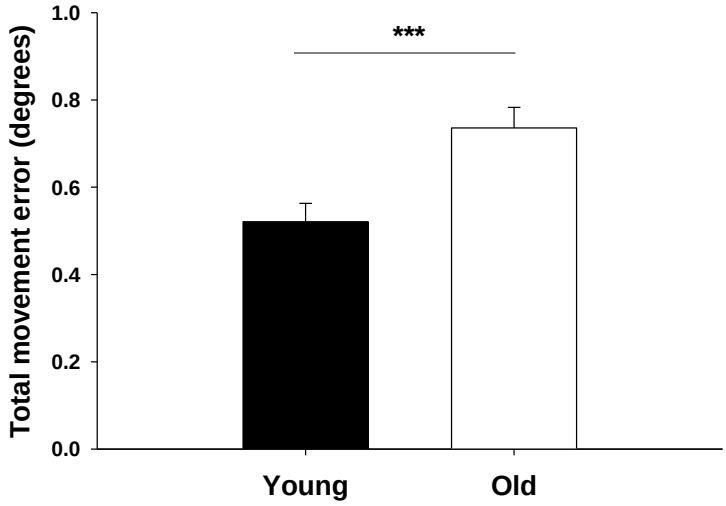


Figure 4-32. Accuracy, as measured by RMSE, for the constant position task. Older adults were less accurate than young adults throughout the entirety of the task. *** denotes p<0.001

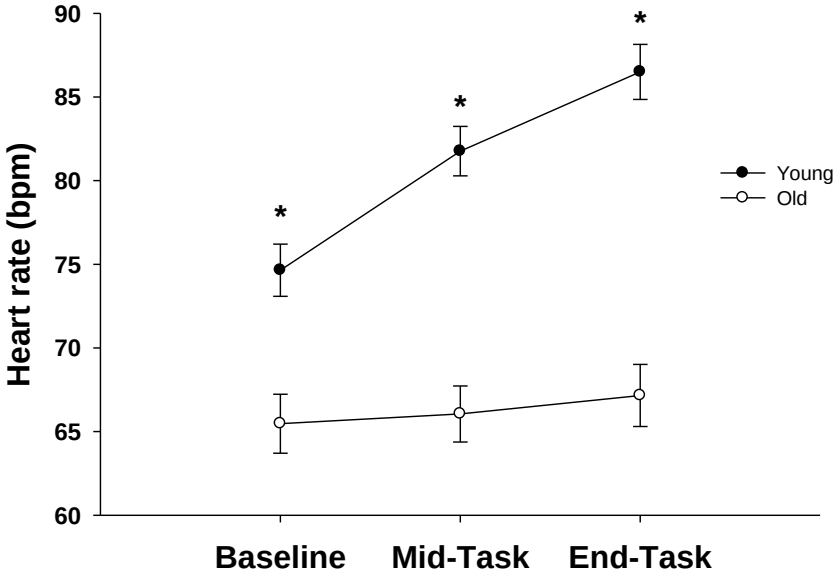


Figure 4-33. Heart rate monitored throughout the constant position task. Heart rate was greater for young adults throughout the task when compared with older adults. * denotes p<0.05

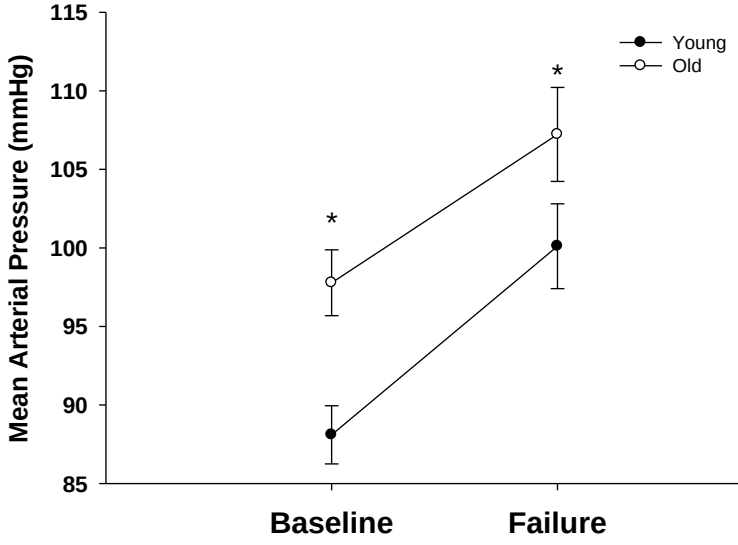


Figure 4-34. Blood Pressure monitored throughout the constant position task. Older adults had greater mean arterial pressure than young adults * denotes p<0.05

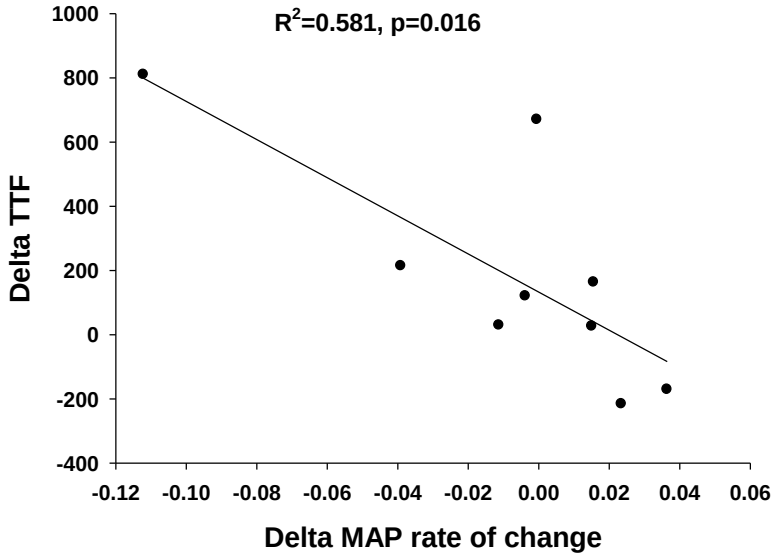


Figure 4-35. Difference in rate of change in MAP between laser and sham predicts difference in time to task failure in older adults.

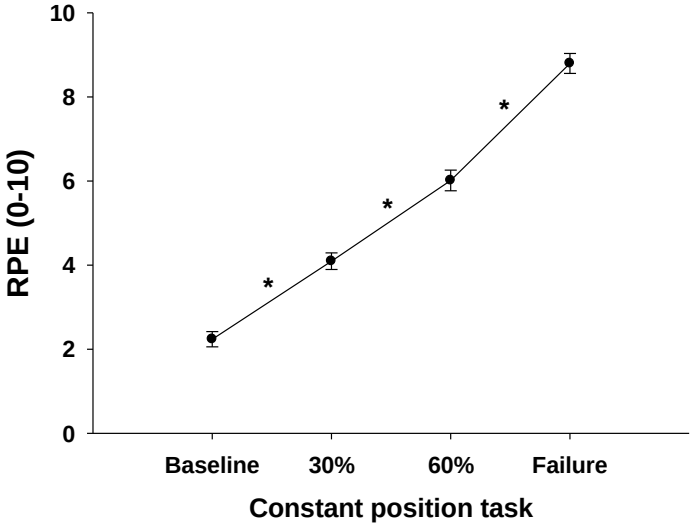


Figure 4-36. Rate of perceived exertion increased for all participants throughout the constant position task. * denotes $p<0.05$

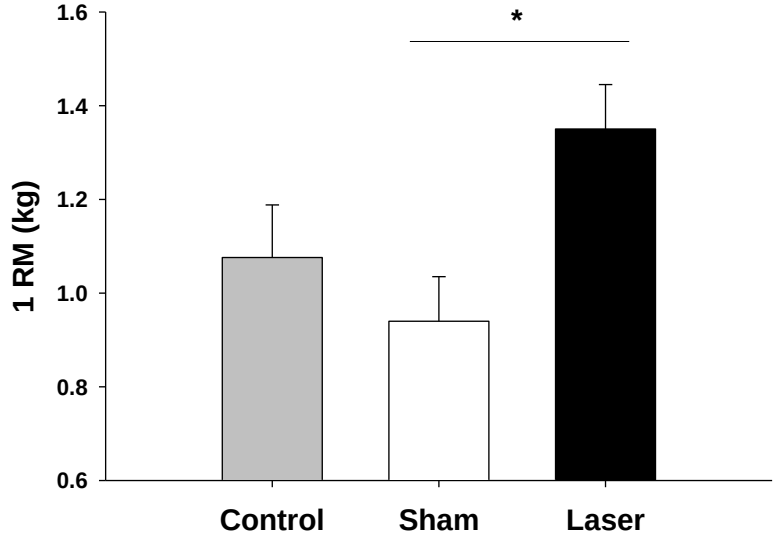


Figure 4-37. Post-task failure strength treatment effect. * denotes p<0.05

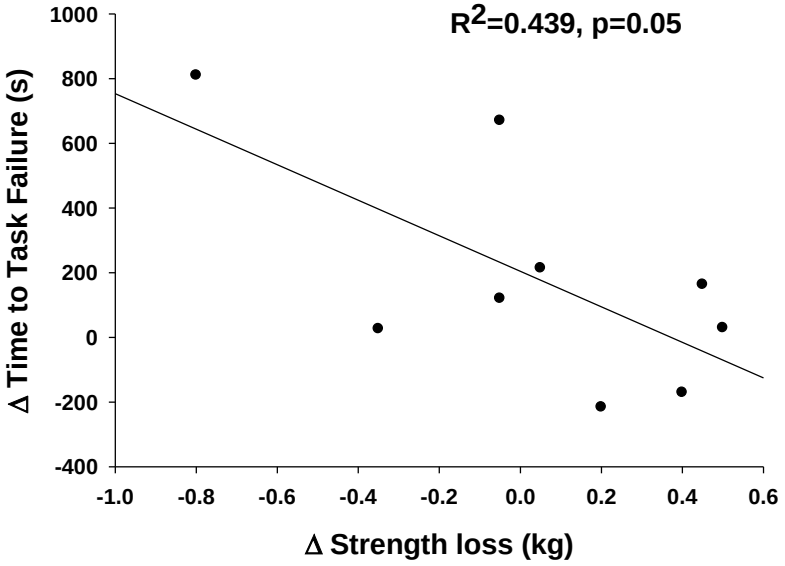
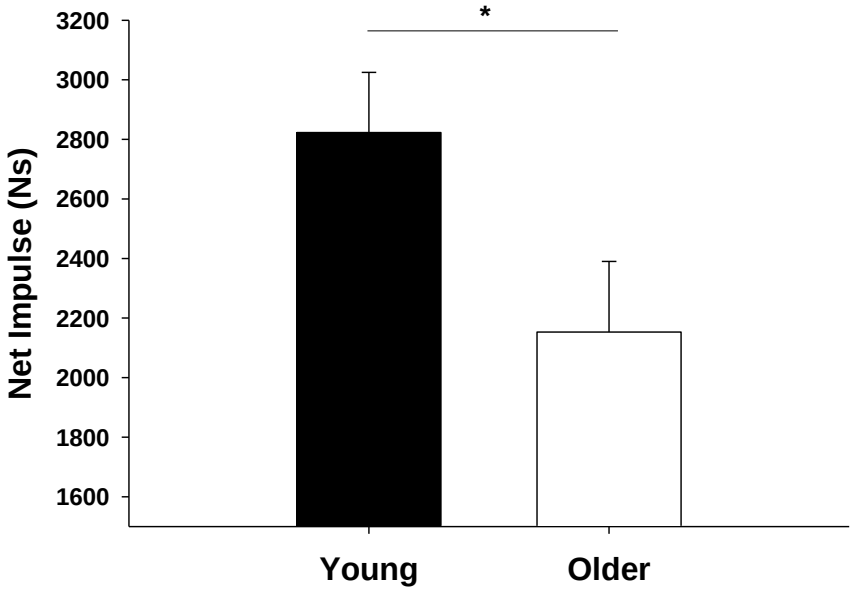


Figure 4-38 Enhanced time to task failure was explained by increased strength in older adults post-task failure

A



B

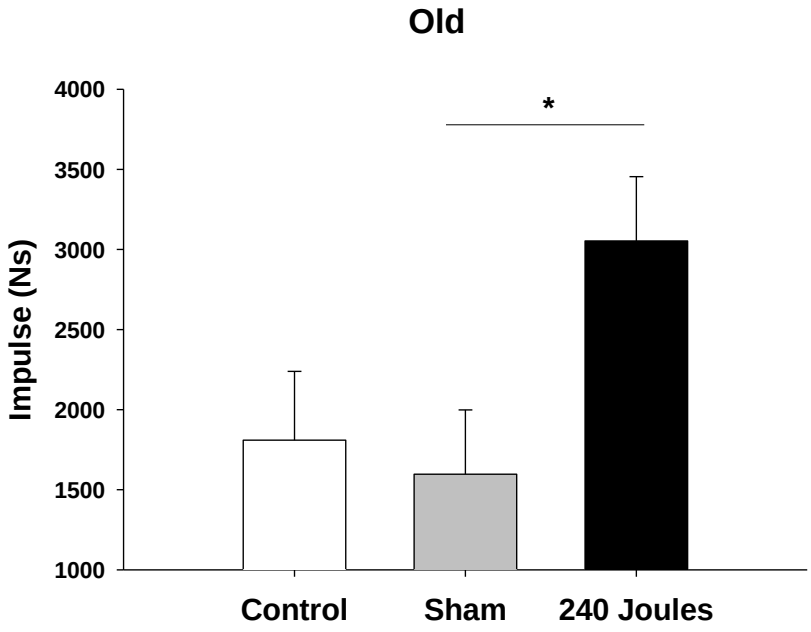


Figure 4-39. A significant age effect was detected for net impulse (A). Older adults increased the net impulse of their performance when they received 240 Joules of NIR light therapy (B). * denotes $p<0.05$.

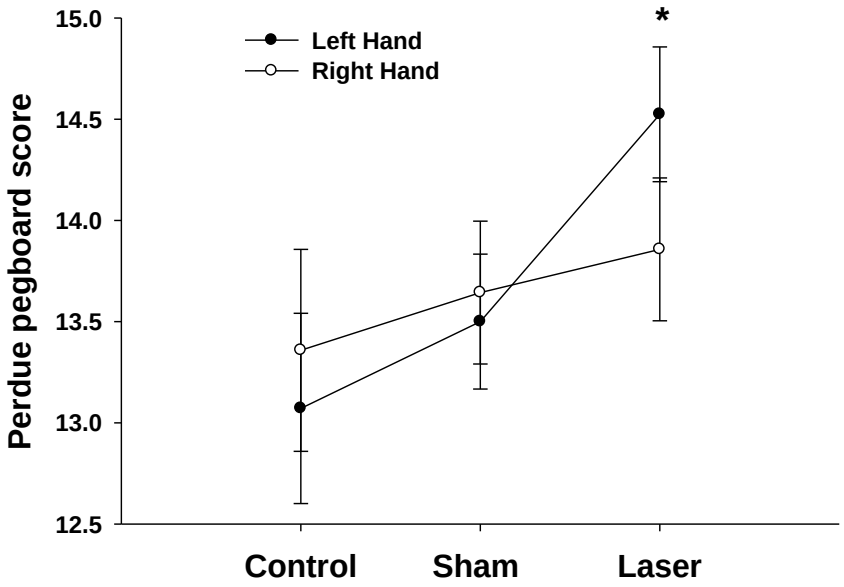


Figure 4-40. Purdue Pegboard scores for each Experimental condition by hand. The score on the Purdue pegboard improved with the left hand when treated with the NIR light therapy. * denotes p<0.05

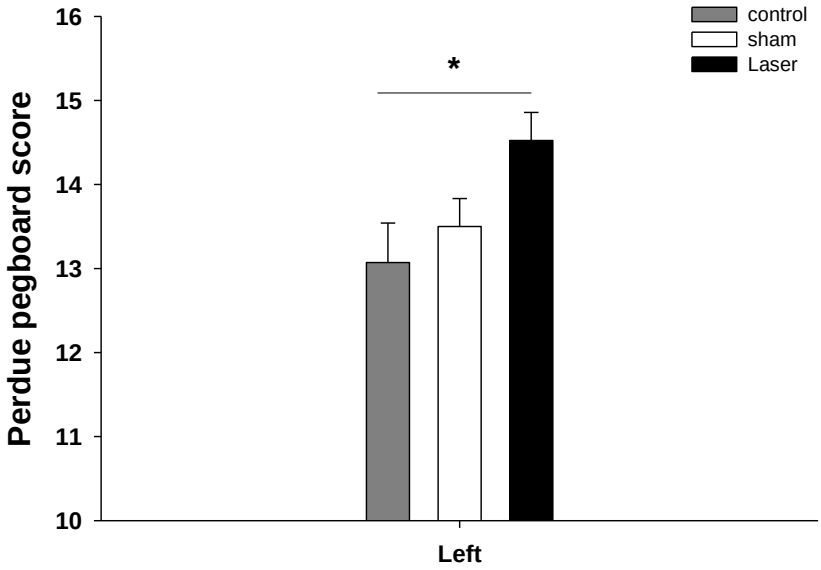
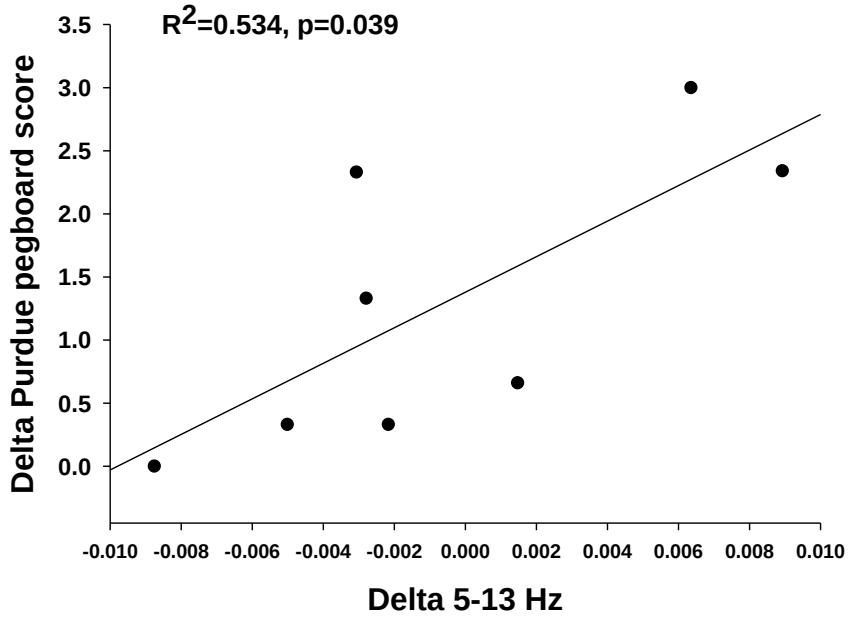


Figure 4-41. Purdue Pegboard scores for each Experimental condition with the left hand. The score on the Purdue pegboard improved with the left hand when treated with the NIR light therapy. * denotes p<0.05

A



B

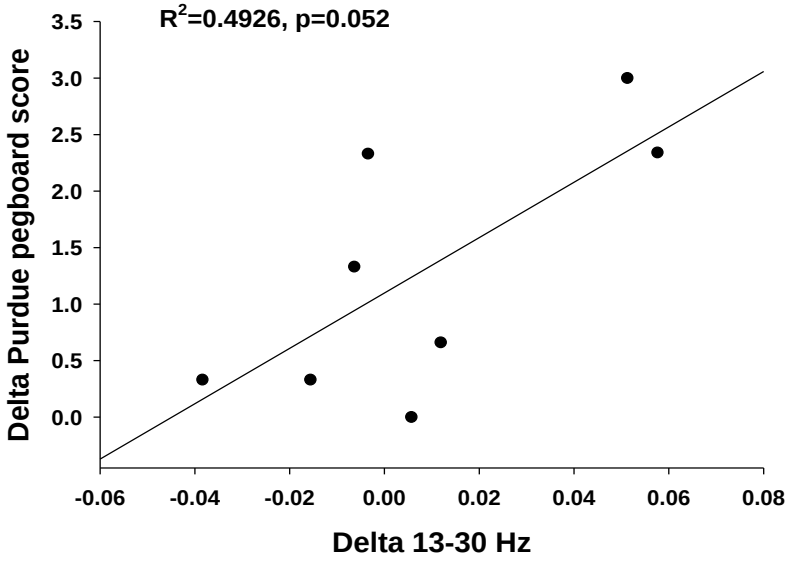


Figure 4-42. Improved Purdue pegboard were associated with increased power from (A) 5-13 Hz and (B) 13-30 Hz

CHAPTER 5
DISCUSSION

Experiment 1

Research on NIR light therapy has implicated phototherapy as an effective treatment modality to enhance muscle function, and increase the time it takes to fatigue a muscle.[20,22,23] However, a lack of research exists to identify appropriate dosing parameters for NIR light therapy and its ergogenic effects. Therefore, the purpose of this Experiment was to evaluate if there is at dose-response effect of phototherapy on musculoskeletal fatigue in young adults. The Experimental procedures utilized allowed us to identify the most effective dose of phototherapy to enhance time to task failure. A crossover, repeated measures study design was used in this study to limit the amount of variability between subjects. Baseline strength was similar on all Experimental visits, which demonstrates that the participants' performance was similar across all testing sessions. Since there were no differences observed in pre-exercise strength measures between treatments we can eliminate the possibility of a cross-over or unwanted learning effect between any of the Experimental treatment sessions.

Time to task failure. Muscular endurance was evaluated across the three different Experimental treatment sessions and quantified in seconds as time to task failure. Analysis revealed that a dose of 240 Joules, applied to the FDI of the non-dominant hand is effective at extending time to task failure by 26%. Increased time to task failure following treatment with NIR light may be a result of stimulation of the nervous system, circulatory network, intramuscular bioenergetic pathways, or a combination of the 3. This Experiment focused primarily of the association between increased time to task failure and modulation of the motor neuron pool.

Our results are consistent with the results of previous studies that have observed the positive effects of NIR light therapy when utilized as an ergogenic aid. These studies have also examined the time that it takes to fatigue a muscle, or muscular endurance following application of phototherapy. The previous studies that have assessed NIR light therapy's ergogenic effects have employed isokinetic and isotonic dynamic contractions, and have assessed the number of repetitions it took to fatigue a specific muscle group.[19,20,22,23] The investigators observed enhancements in the number of repetitions completed by 4.5 to 8.5 repetitions when comparing a placebo to an active dose of phototherapy. In addition, these studies observed a significant increase in the time it took to reach muscular fatigue by 11.6%.[35] In our work, we employed a constant isometric position task to assess muscular endurance. Although, these studies utilized vastly different tasks to assess NIR light therapy, we are still able to conclude from these works that there is strong evidence that this modality has positive ergogenic properties.

The ability for NIR light therapy to increase time to task failure is clinically significant as it allowed participants to enhance their muscular endurance. These results, aid in the potential development of the use of NIR light therapy as an ergogenic aid. In addition, these results have allowed us to identify appropriate dosing parameters for this Experimental model. To the best of our knowledge a dose-response effect, with respect to muscular endurance, has not been previously identified in the literature

Muscular strength. Interestingly, despite participants being able to sustain the constant position task 26% longer, they experienced similar losses in strength, indicating a similar degree of fatigue, with each treatment (sham, 240 Joule and 480

Joule) following the submaximal isometric contraction. The conservation of muscular strength explained the participant's ability to sustain the constant position task longer. This result also indicates that NIR light therapy was effective at attenuating the onset of muscle fatigue, as a loss in muscular strength is commonly used as the primary outcome variable to quantify fatigue.[15,16,81] These results further demonstrate the ability of NIR light therapy to prevent muscle fatigue when applied before a fatiguing task. Our findings are in agreement with previous studies that have used light therapy to reduce the onset of fatigue.[14,20,22]

Neuromuscular effects. Analysis of electromyography following NIR light therapy application did not result in any statistically significant differences between any of the Experimental treatment sessions. However, there was a trend observed with the amplitude of the EMG signal to increase when active doses (240 and 480 Joules) of NIR light therapy were applied when compared with sham. Similar increases in the amplitude of the EMG signal were observed by Kelencz et al.[85] However, this is the only other study that has analyzed EMG activity in response to phototherapy. Further analysis is warranted for any further conclusions to be made on the ability of NIR light therapy to modulate the neuromuscular system. In addition to the amplitude of the EMG signal, when analyzing the organization of the power spectrum of the EMG signal, there was a trend for the power coming from the 5-13 Hz frequency band to increase with both active doses. Power from 5-13 Hz is indicative of the motor unit discharge rate in the muscle being studied. Although these trends were not significant, they do suggest that NIR light therapy is modulating the neuromuscular system in response to a

fatiguing task. Further analysis, with a larger sample size, is necessary to make any additional mechanistic conclusions.

Preventing and/or postponing the onset of skeletal muscle fatigue through NIR light therapy is a relatively new and novel area of research. As such, the differences observed between the sham and 240 Joule dose of NIR light-therapy demonstrate the ability of this therapeutic intervention to have positive ergogenic effects on muscle function in young adults. In addition, these findings further support the importance of dosimetry when utilizing NIR light therapy for biomodulation, and eventual enhancement of muscle function. This pilot study allowed us to identify 240 Joules as the most effective dose of NIR light therapy to enhance muscular endurance time. Therefore, a dose of 240 Joules was chosen for our second Experiment. This Experiment, and all previous work by other authors have assessed the ergogenic effects of NIR light therapy in young adults. However, there is a void in the literature that needs to address these ergogenic effects of NIR light therapy and how they translate to the phenotypic changes associated with advancing age.

Experiment 2

Empirical evidence from our lab has implicated phototherapy as an effective treatment modality to enhance the time it takes to fatigue a muscle. However, these findings have never been translated into older adults. Giving the progressive age increase of our population, the study of how we can improve the skeletal muscle function in older adults has become an important focus of research.[101] Therefore, the primary goal of this Experiment was to assess if NIR light therapy improves time to task failure and motor output similarly in young and older adults. The secondary goal of this Experiment was to identify the neuromuscular and cardiovascular mechanisms

associated with the improvements observed. The Experimental procedures utilized allowed us to identify the modulatory effects of NIR light therapy and its ability to act as an ergogenic aid in aged muscle. This is the first study, to the best of our knowledge, that has directly assessed the ability of NIR light therapy to modulate muscle function in older adults. The results from this Experiment provide the following novel findings: (a) NIR light therapy increased time to task failure in older adults but not young; (b) NIR also enhanced strength in older adults; (c) fine manual dexterity was improved in older adults following treatment with NIR light therapy; and (d) these improvements in the muscle function of older adults were associated with modulation of the motor neuron pool and cardiovascular system.

A crossover, repeated measures study design was used in this Experiment to limit the amount of variability between subjects. This Experiment also used the FDI as the target muscle for NIR light therapy. The advantage of using the FDI is that it is the only muscle that contributes to the abduction of the index finger.[92,93] This allowed us to control for extraneous variables and be sure that the effects observed are a result of irradiating the FDI and not the surrounding musculature. Previous research on joint comparisons has also indicated that older adults are more fatigue resistant than young adults across all joint regions.[102] Therefore, we are confident that the changes we observed with this model are transferrable to other joints. In addition we utilized a percentage of each participant's one repetition maximum to help to standardize the task demands between age, sex, muscle size and ability.[18]

Time to task failure

Muscle fatigue is commonly quantified as a decrease in endurance time or time to task failure.[103] This model allowed us to examine the neural activation and modulation

of muscle function in response to NIR light therapy within the FDI that contributed to improved muscular endurance. There was an increase in time to task failure for older adults when NIR light therapy was applied to the FDI prior to exercise. However, there were no differences in time to task failure for the young adults. The increase in endurance time in older adults in response to NIR light therapy is clinically significant as it lends support for the use of phototherapy in a clinical setting to improve the duration older adults are able to sustain a task. Our results indicate that the improvement in the time older adults were able to perform the task was associated with modulation of the motor neuron pool.In addition, improvements in muscular endurance (TTF) was associated with a blunted pressor response as measured by the rate of change in mean arterial pressure

We focused our power spectrum analysis of the EMG signal from 5-60 Hz because this range is not influenced by the shape of the action potential and reflects the modulation that is occurring in the motor neuron pool.[88,99,100] The increase in time to task failure observed in older adults was explained by an increase in the power of the 5-13 Hz frequency band of the EMG signal. An increase in power from 5-13 Hz during the constant position task following NIR light treatment may reflect an increase in the motor unit discharge rate (MUDR) within the treated FDI. Similarly, increased time to task failure in older adults was explained by an increase in power from 13-30 Hz, commonly known as the beta band, of the EMG signal. An increase in power from 13-30 Hz reflects increased modulation of the motor neuron pool of the FDI muscle and may reflect amplified motor unit synchronization.[98]

Several research projects have observed decreases in motor unit discharge rate with age.[104–106] As such, the results from this Experiment are exciting as they imply that NIR light therapy may help to counteract the decreases in MUDR associated with aged muscle and improve muscle function. Improvements in motor unit synchronization within the FDI has potential to decrease the synaptic noise imposed on the membrane potential allowing older adults to exert a constant force with the limb.[107] The combination of increasing the motor unit discharge rate while simultaneously increasing the synchronicity with which the motor units are discharging is an important finding. These results suggest that NIR light therapy may improve neural signaling within the aged muscle and contribute to improved muscular endurance during sustained isometric contractions.

When individuals sustain a submaximal constant position task the gradual force capacity reductions they experience will eventually lead to task failure. Decreased force capacity is attributable to impairments in the capacity of the neural and muscular processes necessary to sustain the task.[108] Age-related changes in the neuromuscular system potentially increase the aged muscles susceptibility to these impairments. Older adults are commonly perceived as more fatigable, however, resistance to muscle fatigue actually improves with age.[15,102] Improved fatigue-resistance, associated with advancing age, is consistent with several phenotypic changes within a normally aging skeletal muscle. Preferential atrophy of type II fibers, death of α-motor neurons and decreased capillary networks are all associated with the sarcopenic changes observed with age. These changes result in a more oxidative profile of skeletal muscle fibers and an overall loss of strength. As discussed previously, NIR light therapy has the ability to

target many of the physiological processes that are compromised due to these phenotypic changes associated with normal aging. Therefore, it is necessary to study the viability of this therapeutic intervention and its ability to enhance muscular performance in older adults.

Motor output variability. Positional variability remained unchanged for all subjects, young and older, across all Experimental conditions. Similarly, limb acceleration remained unchanged in young adults. However, older adults displayed a increased limb acceleration when they received the NIR light therapy treatment throughout the constant position task. Although the increase in limb acceleration did not reach statistical significance, this difference may have resulted from the enhanced motor unit discharge rates and synchronicity of the motor units. Our results indicate that increased limb acceleration explained the enhanced time to task failure. Therefore, increasing the activity and synchronicity of motor unit activity throughout the task may also indicate additional muscle fibers were recruited and contributed to the ability of older adults to sustain the constant position task. Although these results are supportive of the neuromuscular changes observed, further research is necessary before conclusive statements can be made on the contribution of muscle fiber recruitment to the improved endurance of older adults treated with NIR light therapy.

Cardiovascular regulation. Our results indicate that a blunted pressor response, or decreased rate of increase in MAP during the constant position task was predictive of the increases in endurance time as represented by the increased time to task failure with NIR light therapy. These results may reflect a decrease in metabolite build-up and thus a decrease in metabo-reflex signaling on group III and IV afferents to increase

blood pressure. Blunting of the pressor response in such a way may contribute to the muscles ability to sustain the given task longer. It is well established in the literature that the measurement of heart rate, blood pressure/mean arterial pressure and rate of perceived exertion can be important outcome variables in the detection of changes in the pressor response during fatiguing bouts of exercise.[109] As such, a blunted pressor response during fatiguing exercise has been predictive of increased endurance times in previous research studies.[97]

Although we were able to detect a difference in the rate of change in MAP, it may be advantageous in the future to utilize a more sensitive method of analyzing the discussed metabolic changes that affect the pressor response. Magnetic resonance spectroscopy (MRS) is a valuable tool to monitor changes in intramuscular pH and Pi continuously and non-invasively. The data obtained from MRS is able to reflect metabolite production that can be associated with the chemo and metabo-reflex as a result of continuous exercise. It is reasonable to assume that if NIR light therapy is increasing the total muscular performance and potentially altering motor unit discharge and synchronicity, that there may be changes in the metabolites produced in exercising musculature. Therefore, it would be appropriate to utilize MRS in the future to assess the changes in production of muscle metabolites in response to NIR light therapy.

Motor Output

Net impulse. The secondary goal of this Experiment was to determine if NIR light therapy altered motor output in older adults to a greater extent when compared with young adults. An important outcome from this goal was that the net impulse of the performance in older adults was significantly increased when the FDI of older adults was irradiated with 240 Joules of NIR light therapy. This result indicates that NIR light

therapy is an effective ergogenic aid to enhance the gross musculoskeletal performance of older adults. The net impulse of performance is an important outcome variable for aged muscle. Net Impulse takes into account the changes in force output in addition to endurance time. Previous studies have indicated that older muscle has a greater resistance to fatigue and exhibits increased endurance times.[16,87] However, these studies, as well as data from our laboratory have indicated that older muscle also displays loss in force capacity.[87,95] Therefore, it is necessary to understand both the endurance and the force components of net impulse to properly assess the physiological contributions to the enhancement observed.

Muscle strength. In addition to endurance time, fatigue is also quantified as a decrease in percentage of baseline strength, a fundamental characteristic of skeletal muscle.[81] There were no changes in muscular strength detected in the young participants for this Experiment. However, there were significant increases in strength in the older adults when they received the active dose of NIR light therapy. This increase in strength is important as it contributed to the significant increase in net impulse of the older adult's performance, in combination with their increased endurance time. Therefore, the results from this study show promise not only in improving fatigue resistance with respect to endurance time, but also with respect to enhancing the relative strength at which older adults are able to perform a task. The relative intensity (force) with which the older adults sustained the task contributed to the net impulse of their performance by 57%.Analysis of our EMG power spectrum revealed that a decrease in power from 5-13 Hz was associated with increased strength when older adults received NIR light therapy. Power in this frequency band is commonly thought to

be a reflection of the motor unit discharge rate. It is counter-intuitive to associate a decrease in motor unit discharge rate with increased strength. However, this change may also be a reflection of increased synchronicity with which the motor units are firing and/or additional muscle fibers being recruited. As a consequence of these findings further analysis of the motor unit and muscle fiber activity is warranted.

As we know from previous research older adults display enhanced fatigue resistance compared with young adults during constant position tasks.[81,108] However, resistance to fatigue begins to diminish at higher intensities of exercise. The diminished fatigue resistance at higher intensities becomes apparent as older adults attempt to perform tasks at increased relative intensity in their activities of daily living, i.e. rising from a chair and stair climbing. In light of this age-associated reduction in strength, therapeutic interventions, such as NIR light therapy, may target muscle fatigue resistance and affect functional capabilities in the older adults. The ability of NIR light therapy to improve performance to such a large extent, while still maintaining a low-intensity contraction (30% 1RM) is more clinically applicable as high intensity exercise is contra-indicated for many populations, including aging. Utilizing low-intensity contractions to improve muscle function is also more advantageous in a clinical setting as it increases the potential for patient adherence to exercise protocols and participation. In addition, past research has attributed strength losses in older adults directly to the loss of skeletal muscle mass, or sarcopenia. Therefore, it is essential that we are able to identify positive treatment applications to counteract and attenuate these losses in strength and muscle mass to improve functional outcomes.

Purdue pegboard. We utilized the Purdue Pegboard as a functional outcome measure to assess the effects of NIR light therapy and how these effects may translate to clinical hand function. Initially, performance was better for the right hand (dominant hand) than for the left hand (non-dominant hand). These improvements can be associated with a degree of comfort subject's exhibit with their dominant hand. Most importantly, results from this Experiment demonstrated a significant improvement in Purdue Pegboard score when the non-dominant hand (LH) was irradiated with NIR-light therapy. The mean score on the pegboard not only increased, but it also increased to a level greater than standardized norms.[96] In addition, the participant's performed with their left hand to a level greater than their performance with their dominant right-hand.

Our Experiment also observed increased power from 5-13 Hz and 13-30 Hz, when older adults received treatment with NIR light therapy. Enhanced power in these frequency bands may reflect enhancements in MUDR and motor unit synchronization in the FDI. As such, we were also able to associate improvements in Purdue pegboard scores with the increased MUDR (5-13 Hz) and enhanced motor unit synchronicity (13-30 Hz) we observed. Hence, we were able to conclude that improvements in manual dexterity were associated with improved discharge rate and synchronicity of the motor units within the exercising FDI. Previous research has implicated that improvements in motor unit synchronicity can be associated with improved manual dexterity in older adults.[107]

Fine manual dexterity is commonly assessed in the clinical setting for rehabilitative purposes because of its bearing on limb performance and the translation of that performance to functional independence.[96] With the majority of the population aging,

more and more elderly people are at risk of acquiring upper extremity sensorimotor impairments that contribute to deficits in coordination and poor fine manual dexterity. As a result, dexterity, performance and functional independence become especially important with advancing age. Performance with respect to reaching, grasping, and manipulating objects is critical to performing activities of daily living, i.e. buttoning a shirt or writing.[107] Therefore, the ability of NIR light therapy to attenuate the onset of task failure, improve strength and contribute to enhancements in fine manual dexterity provides promise in the attempt to maintain hand function and independence in older adults.

Sinusoidal task. In contrast to the observed modulatory effects of NIR light therapy in older adults during the constant position task we did not see any significant changes in motor control during the sinusoidal task. Previous research has also shown that the differences between young and older adults during dynamic muscle contractions are often abolished.[110–112] The task dependency, with respect to muscle function, is commonly attributed to the age-associated loss in muscle power capacity.[18] A potential explanation may be the age-associated phenotypic shift toward type I fibers and the slowing of contraction and relaxation times in older adults. In addition, it is possible that the speed of task (0.6 Hz) may have been too challenging for the older adults. The velocity-dependent loss of power observed in previous studies may be the result of the phenotypic changes within the muscle that are associated with the normal aging process.[18] Therefore, it is possible that the frequency of contraction used for this Experiment did not allow for adequate assessment of the neuromuscular and behavioral changes that may have resulted from the NIR light therapy intervention. However, these

findings also support our decision to utilize a constant isometric position task to tease out the modulatory effects of NIR light therapy on muscle performance in older adults.

Clinical Applications

During rehabilitative exercise programs decrements in skeletal muscle contractile force production may significantly limit functional progression, produce symptoms such as pain and soreness, prolong recovery time and negatively affect clinical outcomes. A therapeutic modality administered just before rehabilitative exercise that has the capability of producing an ergogenic effect on the muscle would be beneficial for patients because it could improve a muscle's capacity to work and also may reduce the risk for exercise-induced muscle damage. Athletes in recovery from musculoskeletal injury or reconstructive surgery rely on therapeutic exercise to reconnect impaired neural networks as well as restore strength levels. As such, decrements in contractile function of skeletal muscle can be a limiting factor to functional progression. Near-infrared light treatments administered before therapeutic exercise improve and sustain muscle performance thereby restoring neuromuscular control and facilitating the return to full function.

The maintenance of skeletal muscle function is essential to the health and independence of older adults. Reduced rates in exercise participation for adults over 65 years, longer life expectancies, coupled with rapid population growth warrants a better understanding of exercise interventions for older adults. To date, performance of high-intensity exercise is the most efficacious method of maintaining skeletal muscle function.[29] However, high-intensity resistance exercise is contraindicated for many older adults. Older adults also exhibit limited self-efficacy in their ability to perform high-intensity resistance exercise.[113] For example, older adults with limited self-efficacy may

give up more readily or decrease their willingness to participate if asked to perform high-intensity exercise protocols. Therefore, it is necessary to identify therapeutic interventions that may facilitate improvements in muscular strength and endurance while utilizing low-intensity loads. The results from this study are supportive of the use of NIR light therapy in older adults to optimize low-intensity training and enhance performance. Photo-irradiation of the FDI with NIR light therapy allowed older adults to perform tasks at greater relative intensities and for an extended amount of time. In addition, the ability for this therapeutic intervention to modulate the motor unit discharge rate and synchronization lends promise to NIR light therapy's ability to offset some of the deleterious effects seen with normal aging. There is great scientific interest in studying the maintenance of muscle mass in older adults, therefore, NIR light therapy shows promise as results from this study displayed positive changes in muscle performance, neuromuscular signaling and clinical hand function.

Conclusion

This study has offered us a greater understanding of the age-associated modulatory effects of NIR light therapy on the neuromuscular system and the related outcomes in muscle performance. The results from this study are important as they may influence clinical decision making and effect the application of therapeutic exercise prescription. Previous research has provided a great deal of evidence on the changes in muscle fatigue associated with aging. However, no other studies on NIR light therapy have examined the magnitude and impact of this potential benefit on musculoskeletal fatigue and/or neuromuscular control. Ultimately this study has provided us with substantive evidence on the ability of NIR light therapy to help offset the deleterious effects of age-associated sarcopenia and loss of muscle strength and function.

One of the most challenging areas of current research is to identify interventions that can promote the quality of life and prevent the age-associated decreases in muscle mass in older adults. Therefore, the results from this Experiment are important in implicating NIR light therapy as an effective ergogenic aid in not only enhancing endurance time in older adults but also force production and fine motor dexterity. Enhancing force production coupled with endurance time is important in older adults because the ability to increase the relative force with which older adults are able to perform a task means we are able to place the muscle under greater stress and create a more beneficial exercise regime. This advantage may translate directly into improvements in functional tasks, such as fine motor tasks, as observed with the Purdue Pegboard. The improvements observed provide clinical promise for NIR light therapy as a non-invasive stimuli for older adults to perform tasks longer, and with increased force, while maintaining low loads. In addition, it is our hope that NIR light therapy would allow older adults to improve their physical capabilities to a greater extent than what they could accomplish in the absence of the stimuli. However, further mechanistic and longitudinal training research is necessary to make conclusions on the precise effects of photo-irradiation. It is important to identify these effects to be able to better harness NIR light therapy's positive effects and successfully apply the modality clinically.

Limitations and Future Research Considerations

The limitations of this study prevent the generalization of these findings to other populations. This study was performed on healthy individuals who were high functioning and had no previous compromise of their hand function. Therefore, the direct application of these findings to the clinical setting should be done with caution. Future

studies are needed to assess the effects of NIR light therapy in a pathological state before these findings can be considered translational to such populations. Also, our Experimental design did not allow us to assess follow up measures such as changes in delayed onset muscle soreness in response to NIR light therapy. Therefore, it would be beneficial if future research examined these effects with a longitudinal study. In addition, our findings implicated NIR light therapy as an effective ergogenic to increase muscular endurance and strength. These effects should be examined more completely with a training study before we know if photo-irradiation prior to exercise may lead to long term enhancements in muscle size, function, and activities of daily living and independence in older adults.

In addition, a more thorough understanding of the contributions of blood flow and metabolite production is needed to fully understand the effects of NIR light therapy on musculoskeletal fatigue. Future research should consider using magnetic resonance spectroscopy (MRS) to quantify changes in skeletal muscle energetics non-invasively. The addition of this tool could be useful in future research to monitor the fluctuations in metabolites that may inhibit force production and lead to fatigue. The rate of ATP synthesis, glycolysis and oxidative phosphorylation can also be monitored used MRS, which could add scientific merit to future muscle fatigue research

Furthermore, an adequate maintenance of blood flow is necessary to prevention of muscle fatigue in exercising muscle and conservation of force production. Since older adults are especially reliant on blood flow because of the preference of aged skeletal muscle to rely on oxidative phosphorylation as an energy source rather than anaerobic glycolysis it would be beneficial to quantify changes in tissue oxygenation and blood

flow.[114] Therefore, continuously monitoring changes in blood flow in the exercising muscle following treatment with NIR light therapy would be beneficial to understanding the entirety of the contributing physiological responses that lead to the onset of musculoskeletal fatigue.

Lastly, the results from Experiment 1 positively supported the dose-response effects of NIR light therapy on muscular endurance in young adults as represented by an increased time to task failure. Surprisingly, completion of Experiment 2 contradicted these results. Results from this study and those of others demonstrate that young adults are significantly stronger than older adults as a consequence of increased muscle mass.[87,102,103,110] As such, if time to task failure was enhanced as a result of NIR light therapy influencing blood flow then it is possible that the increased muscle mass observed in young adults may have occluded the vessels supplying blood to the working musculature leading to abolishment of the positive effects of photo-irradiation. In addition, young adults do not typically have compromised motor unit activity. Therefore, their descending drive and neuromuscular strategies are well intact and may not be responsive to external stimuli like NIR light therapy, as was seen in the older adults. Another possibility as to why we observed contradictory results between Experiments 1 and 2 is that the threshold level for stimulation in young adults was not in fact reached. Upon the completion of the second Experiment, and the evidence that contradicts past research, it is clear that there is a necessity to conduct further investigation into an appropriate dose-response for young adults

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BIOGRAPHICAL SKETCH

Kelly Anne Larkin was born in Fergus, Ontario, Canada. The younger of two daughters, she spent the entirety of her childhood in Guelph, Ontario, and graduated from St. James Catholic High School in 2001. She earned her BS in Human Kinetics and a Diploma in Sports Injury Management from the University of Guelph and Sheridan College in June of 2006. In June of 2006 Kelly also attempted and passed the certification examination to become a Certified Canadian Athletic Therapist.

Following graduation Kelly moved to Gainesville Florida to pursue her MS in Health and Human Performance at the University of Florida. During this time Kelly had a graduate assistantship with the Department of Applied Physiology and Kinesiology as a teaching assistant for Human Anatomy and Physiology. She also worked on a dissertation project with Dr. Jeffrey Wight as a research assistant. Kelly completed her MS in August of 2008. At this time she began her PhD with Dr. Paul Borsa in the Sports Medicine Laboratory. Upon commencement of her doctoral degree Kelly was awarded the Charles LaPradd Fellowship and the Grinter Award. As a doctoral student Kelly continued to teach Anatomy, Physiology and Exercise prescription and Fitness assessment within the Department of Applied Physiology and Kinesiology. In addition to teaching she received funding as a research assistant on a water supplement project. During her doctoral studies Kelly also received recognition for her outstanding academics as an international student.

Following completion of her PhD, Kelly continued her career as a post-doctoral fellow at the University of Calgary under the mentorship of Dr. Walter Herzog in the Department of Kinesiology.