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THE EFFECT OF MICROCURRENT STIMULATION ON ATP SYNTHESIS IN THE HUMAN MASSETER AS EVIDENCED BY 31^P MAGNETIC RESONANCE SPECTROSCOPY

By

Jeffrey S. Mannheimer

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Submitted in partial fulfillment of the requirements
for the degree of Doctor of Philosophy in Health Sciences
Seton Hall University 2005

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ABSTRACT

The application of weak, low frequency electrical stimulation at the cellular level in animal, plant and E.Coli has been shown to stimulate the synthesis of adenosine triphosphate (ATP). In direct relationship, microamperage electrical nerve stimulation (MENS) has received anecdotal and clinical support as a pain reducing modality that has not been subjected to scientific study. The theoretical therapeutic effect of MENS is based upon the chemiosmotic theory of ATP synthesis by creating a proton gradient at the inner mitochondrial membrane.

Magnetic resonance spectroscopy (MRS) represents the gold standard to quantify the metabolic effects of exercise and electrical stimulation on ATP synthesis as determined by fluctuation of Pi/PCr. An increase in Pi/PCr is indicative of an elevated rate of ATP production.

31P magnetic resonance spectroscopy (MRS) was used to examine the levels of inorganic phosphate (Pi), phosphocreatine (PCr) and intracellular pH (pHi) of the human masseter muscle. A cohort (n = 23) consisting of normal subjects and those with a temporomandibular disorder (TMD) were tested in a single application, randomized design with active and placebo comparison during a one hour exposure. Data in the form of phosphorus spectra were acquired at baseline, during the 20-32 and 48-60 minute time-points, with active stimulation and placebo protocols administered at a sub-sensory level via surface electrodes adjacent to the masseter muscle, thereby employing repeated measures. Pi/PCr

values were calculated at each time-point and clinical measures consisting of visual analogue scale (VAS), active vertical mandibular range of motion (ROM) and pressure pain threshold (PPT) of the masseter were obtained at pre-and post-exposure for the TMD group.

Exposure to MENS revealed a significant ($p = .05$) elevation of Pi/PCr in both normal and TMD subjects at the 48-60 minute time-point, which was not apparent with placebo exposure. Significant increases in ROM and PPT as well as a decrease in VAS, was apparent for the TMD group exposed to active stimulation. Due to the small sample size and limited statistical power, results should be considered with caution, pending replication and verification by further study.

Chapter I

INTRODUCTION

Background of the problem

The temporary relief of pain by transcutaneous electrical nerve stimulation (TENS) in the milliamperage range is well documented (Mannheimer & Lampe, 1984; Johnson, Ashton & Thompson, 1991; Garrison & Foreman, 1994; Urasaki, Wada, Yasukouchi & Yokota, 1998; Chabal, Fishbain, Weaver & Heine, 1998; Walsh, Lowe, McCormack, Willer, Baxter & Allen, 1998; Cosmo, Svensson, Bornmyr & Wikstrom, 2000; Cheing, Tsui, Lo & Hui-Chan, 2003). During the past fifteen years, a new mode of electrical stimulation, known as microcurrent electrical nerve stimulation (MENS), which is administered at microamperage intensity, has been used with professional athletes and patients with various injuries and pain syndromes. A significant degree of support for this treatment is limited to anecdotal reports, case studies and surveys of returned warranty cards highlighting benefit (Picker, 1990; Morganridge & Chipman, 1990; Wallace, 1990; Weider, 1991; Rossen, 1995; Smith, 2001).

Transcutaneous electrical stimulation administered within the micro-amperage range at pulse frequencies between 0.1-100 Hz has been used with mixed and controversial results to facilitate the healing and to reduce the infection of experimentally induced wounds in animals or ulcers in humans with either positive

or negative direct current paradigms (Carley & Lepley, 1962; Assimacopoulous, 1968; Konikoff, 1976; Cheng, Van Hoof, Bockx, Hoogmartens, Mulier, Ducker, Sansen & Loecker, 1982; Alvarez, Mertz, Smerbeck & Eaglstein, 1983; Carley & Wainapel, 1985). However, more recent studies utilizing rigorous scientific protocol, have not reported benefits with similar stimulation paradigms (Leffmann, Arnall, Holgren & Cornwall, 1994; Byl, McKenzie, West, Whitney, Hunt, Hopf, & Scheuenstuhl, 1994).

Long-term administration of microcurrent stimulation via alternating current has also been effective in healing non-union fractures (Bassett, Pawluk & Pilla, 1974; Rubinacci, Black, Brighton & Friedenberg, 1988; Pilla & Markov, 1994; Ryaby, 1998, Otter, McLeod & Rubin, 1998; Cundy & Paterson, 1990; Aaron, Boyan, Ciombor, Schwartz & Simon, 2004). Alternating current (AC), mediated by bursts of pulses is optimal, although increased protein synthesis and healing processes also occur when a fracture site is exposed to a low intensity electromagnetic field for up to 12 hours per day (Blank 1992; Blank & Soo, 1993; Blank, 1995; Blank & Soo, 1996).

Manufacturers of MENS devices have also promoted their use, stating that they can facilitate tissue healing and decrease muscle spasm in both acute and chronic pain. However, these claims have not been supported by scientific, randomized studies performed on humans. Therefore the value of MENS, due to the low stimulation intensity, has been questioned (Gersh, 1990; American College

of Occupational and Environmental Medicine Ergonomics Committee, 1995).

The physiological effects of milliamperage TENS have been shown to range from gating mechanisms at the dorsal horn to supraspinal endorphin liberation, and are dependent upon stimulation parameters that have been delineated into specific modes (Mannheimer & Lampe, 1984; Johnson, Ashton, Bousfield & Thompson, 1989; Walsh, Foster, Baxter & Allen, 1995; Gopalkrishnan & Sluka, 2000; Sluka & Walsh, 2003; Chesterton, Foster, Wright, Baxter & Barlas, 2003). Each mode has a different sensory perception with indications for varied combinations of pulse rate and intensity based upon pathology as well as acute versus chronic pain states (Mannheimer & Lampe, 1984).

In contrast, the enhancement of ATP synthesis has been a prime mechanism used to explain the physiological action of MENS, but support is based primarily on one study that demonstrated significant increases in ATP synthesis via microcurrent stimulation of rat skin, as opposed to human muscle (Cheng, Van Hoff, Bock, Hoogmartens, Mulier, De Ducker, Sansen, & De Loecker, 1982).

The Need for the Study

Prior to the advent of MENS, the physical therapy profession was well versed in the adjunctive use of TENS as a pain control modality. Numerous clinical reports, randomized double-blind studies and scientific experiments performed on animals and humans, provided significant evidence of its effectiveness (Mannheimer &

Lampe, 1984; Garrison & Foreman, 1994; King & Sluka, 2001; de Tommaso, Fiore, Camporeale, Guido, Libro, Losito, et al, 2003; Koke, Schouten, Lamerichs-Geelen, Lipsch, Waltje, Van Kleef, & Patijn, 2004; Olyaei, Talebian, Hadian, Bagheri & Momedjed, 2004).

Based upon an extensive assessment of the scientific literature, the Food and Drug Administration classified TENS as a Class II medical device in 1979 (U.S. Department of Health & Human Services, 1986). MENS was subsequently included in the FDA classification, as its stimulation parameters were well within the established safety standards. Physical therapists have questioned the benefits of MENS because of its low amperage, the lack of controlled scientific studies; and the supportive physiological theory, which is incongruent to that equated with TENS (Gersh, 1990; American College of Occupational and Environmental Medicine Ergonomics Committee, 1995).

The pain relief and tissue healing claims attributed to MENS include acute and chronic musculoskeletal conditions, temporomandibular disorders, sprains/strains, tendonitis and delayed onset muscle soreness, as well as systemic conditions such as fibromyalgia (Wallace, 1990; Rolle, Alon, Nirschl & Sobel, 1994; Ray, 2001, Lictbroun, Racier & Smith, 2001). In order to scientifically evaluate the merit of a treatment, specific criteria is required (Golden, 1980; Harris, 1996; Sackett, Rosenberg, Gray, Haynes & Richardson, 1996; Sackett, 1997).

According to Harris (1990) and Golden (1996) the treatment approach must be designed for a specific type of population; the potential side effects must be presented; peer-reviewed studies of support should be provided that include well-designed randomized-controlled or single subject experimental trials; the proponents of the treatment should be open and willing to discuss its limitations and the underlying theories must have valid anatomical and physiological support.

A report from the world's largest occupational medical society, as well as a discussion from Golden, states that MENS has not been validated by established research procedures, comes close to displaying the characteristics of unorthodox and controversial therapy, and it does not fulfill the criteria established by randomized, double-blind study needed to validate its effectiveness in pain relief (Golden, 1991 and American College of Occupational and Environmental Medicine Ergonomics Committee, 1995). Any therapy is considered to be unorthdox or controversial if it is not based upon existing knowledge or scientific fact; consists of uncontrolled empirical reports; is stated as effective for a wide variety of problems; is promoted by non-peer reviewed publications or testimonials; and claims no harmful effects (Golden, 1980 & 1991).

Unlike the standard surface electrode application of milliamperage stimulation, MENS is administered in varied paradigms with different mechanisms of physical application (small diameter surface probes, metal rollers or standard electrodes) as well as individually or in combination with cranial

stimulation, all of which hinder any form of direct comparison to the common application of TENS (Rolle, Alon, Nirschl, & Sobel, 1994; Lennox, Shafer, Hatcher, Beil, & Funder, 2002).

The main claim of the manufacturers has been that MENS enhances the synthesis of ATP, the principal energy source for a myriad of metabolic events that occur at the cellular level, such as membrane transport and neuromuscular transmission, which promotes a return to normal function and concomitant pain relief. The proponents of MENS predominately cite the chemiosmotic theory, which relates to stimulating the synthesis of ATP by creation of an electro-chemical gradient between the proton concentrations at the two mitochondrial layers (Mitchell, 1967). A proton flow down the electrochemical gradient releases the energy required to power ATP synthesis from ADP and phosphate. A homeostatic environment is needed to maintain the supply of ATP for healing and normal neuromuscular function, in which the rate of ATP production equals that of utilization.

The enhancement of ATP synthesis has been shown to occur at the cellular level in non-human tissue such as E.coli, yeast, plant seedlings, tobacco shoots and rat skin (Blank, 1992; Tsong, 1992; Berg, 1993; Bolognani, Majni, Costato & Milani, 1993; Platzer, Obermeyer & Bentrup, 1997; Muraji, Asai & Tatebe, 1998; Cogalniceaanu, Radu, Fologea, Moisoi & Brezeanu, 1998). Cellular level research on non-human tissue has been performed by direct as well as alternating currents applied by inert electrodes, such as platinum or stainless steel, that are inserted

into cell suspensions, tissues or single cells by micro-electrodes (Berg, 1995). Since there has not been similar cellular research on human tissue, proponents of MENS have taken a quantum leap by equating events that occur from electrical stimulation at the cellular level in animal and plant tissue, to what may or may not occur via transcutaneous stimulation in human tissue.

Purpose of the Research

Utilizing the human masseter as the region of interest, the prime purpose of this study was to obtain evidence that supports or refutes the claim that MENS enhances ATP synthesis in human tissue. Since MENS is currently being utilized to obtain pain relief in humans, a secondary purpose was to determine if subjective pain relief, elevation of the masseteric pressure pain threshold and increased active vertical range of motion (ROM) of the temporomandibular joints occurred if ATP synthesis was enhanced.

The human masseter is one of the prime elevators of the mandible and thus actively participates in talking, chewing and swallowing. Overuse of the masseter in humans from reflex guarding after trauma or in the presence of a temporo-mandibular disorder (TMD), excessive chewing of dense foods, bruxism or clenching during diurnal or nocturnal periods from stress/tension, as well as other physical and emotional factors, can create discomfort and/or dysfunction of the temporomandibular joints (Attanasio, 1997; Magnussin, Egermark & Carlsson, 2000; Lobbezoo, Drangsholt, Peck, Sato, Kopp & Svensson, 2004). Increased

masseteric electromyographic activity has been shown to be strongly correlated to pain (Glaros & Burton, 2004).

Gold Standard Analysis

Sackett has stated that in order to answer the question of efficacy and hence establish support for the use of a treatment, evidence-based medicine necessitates testing via a gold standard (Sackett, 1997). Quantification of phosphorus metabolite fluctuations can be determined by magnetic resonance spectroscopy (MRS). MRS has been utilized to study muscle metabolism in humans since 1981 and provides the means by which anatomic and metabolic data can be correlated to determine the effects of pathological states, exercise or the application of external stimuli such as electricity, which allow for the assessment of qualitative and quantitative information (Gadian, Radda, Ross, Hockaday, Bore, Taylor & Styles, 1981; Henriksen, 1994, Lenkinski, & Schnall, 1996; Van Den Thillart & Van Waarde, 1996). MRS requires the placement of the tissue of interest inside a strong magnet with a means of stabilization to avoid movement, and its methodology culminates in acquired spectra that are considered to represent the gold standard by which phosphorus metabolite fluctuations are determined.

Metabolic research performed with MRS yields scientific evidence that is more profound than that obtained from subjective measurement. The acquired spectra can also be correlated to the strength of muscle contraction and palpable tenderness (Roy, 1993; Clauw, Hewes, Nelson, Katz & Rajan, 1994; Marcel,

Chew, McNeill, Hatcher & Miller, 1995; Russ, Vandenborne, Walter, Elliott & Binder-Macleod, 2002).

MRS has revealed an increase in the ratio of inorganic phosphate to phosphocreatine (Pi/PCr) in normal subjects who underwent repeated eccentric contractions that caused mild muscle injury. A significant increase in Pi/PCr and a decrease of ATP was seen one-hour post exercise with the greatest elevation occurring one day later. Patients exhibiting muscle damage or stress secondary to neuromuscular disease or strenuous exercise also exhibited an increase of Pi/PCr at rest as evidenced by MRS (McCully, Kakihira, Vandenborne, & Kent-Braun, 1991; McCully & Posner, 1992; McCully & Natelson 1999; Russ, Vandenborne, Walter, Elliott, Binder-Macleod, 2002; Yanagisawa, Niitsu, Takahashi, Goto & Itai, 2003).

In direct relationship to this study, MRS has been utilized to assess metabolic changes in masseteric high-energy phosphates from rabbits, monkeys and humans. Such studies have included metabolic changes that occur with active exercise or electrically induced muscle contraction and have included baseline Pi/PCr values acquired at rest (Lam & Hannam, 1992; Cohen, Lenkinski, Zavatsky & Roberts, 1992; Vestergaard-Poulssen, Thomsen, Sinkjaer, Stubgaard, Rosenfalk & Hendriksen, 1992; Chang, Chew, Decrespigny, Alcantara, McNeill & Miller, 1995; Strom, Holm & Moller, 1994; Brodin & Turker, 1994; Marcel, 1995; Marcel, Chew, McNeill, Hatcher, Miller, 1995; Sappey-Marinier, Dheyriat, Lissac, Frutoso, Mallet & Bonmartin, 1998; Russ, Vandenborne, Walter, Elliott, Binder-

Macleod, 2002). However, previous studies that have used electrical stimulation did so at a milliamperage level, thereby producing a perceptible sensation plus muscle contraction to simulate exercise. In direct contrast, this study utilized non-perceptible (sub-sensory) and non-contractile level stimulation in the micro-amperage range, during which muscle contraction was absent. This specific protocol was utilized to delineate the physiological effects of MENS from that of TENS, which must be considered in all research studies of a similar nature. A considerable degree of pilot work and feasibility testing was performed prior to initiation of the study because of the nature and complex methodology of the research protocol.

Scope of Project

Due to the presence of metallic material on all surface electrodes, initial pilot work with various electrode types was required. The results demonstrated that milli and microamperage stimulation could be used in the magnet with minimal interference during simultaneous spectroscopy. A pilot study was completed, which explored the effects of active and placebo MENS on a cohort of normal subjects (Appendix F). The knowledge gained from the pilot work allowed for the elimination of confounding variables as well as the refinement and standardization of the methodology that was used in the study.

Due to the time-consuming nature of each pilot test, as discussed in Appendix F (4 hours of magnet time plus pre- and post-assessment), it was difficult to recruit normal subjects. It was therefore anticipated that the same

situation would occur with the study, but possibly at a larger magnitude, given the plan to include TMD and normal subjects. Furthermore, the initial plan was to perform a cross-over study, which would require each subject to be tested on two separate occasions. The resultant problems with recruitment, time commitment on the part of each subject, and the need to obtain magnet time among other researchers at the research center, necessitated alteration of the study design into a randomized, double-blind, single application study with repeated measures.

Based upon the results of the study and the difficulty in obtaining normal subjects, a retrospective analysis of the statistical power was performed. A small simulation analysis was calculated to verify the accuracy of the power and necessary sample size calculations, so that the effect size present in the simulated data matched the effect size in the actual data, but with a much larger sample. The analysis revealed that the power for the current effect size found in the pilot study as well as the sample size was 0.175. Therefore the number of subjects required for power levels of 0.8 and 0.5, would necessitate 104-220 subjects, which was not feasible and totally uncommon with MRS research on human subjects (Dalton & Keating, 2000).

The time and cost factors that are inherent in all MRS research involving human subjects necessitates a subject population that cannot approach the proper number to ensure statistical power and avoid Type I and Type II errors. It is thus quite common within the MRS literature to review studies in which the number of participating human subjects ranged from as few as six to eleven (Lam & Hannum,

1992; Greenhaff, Nevill, Soderlund, Bodin, Boobis, Williams & Hultman, 1994; Erkintalo, Bendahan, Mattei, Fabreguettes, Vague & Cozzone, 1998; Kanayama, Minowa, Inoue, Yamaguchi, Yoshida & Kawasaki, 2000; Crowther, Carey, Kemper & Conley, 2002; Vanderthommen, Duteil, Wary, Raynaud, Leroy-Willig, Crielaard & Carlier, 2003). Therefore it is imperative that each study present an in-depth description of the methodology so that replication by others can be used to verify and support all human MRS research.

Hypotheses

It is well known that when animal or human muscle is stressed by exercise, causing Pi to increase and PCr to decrease, there is an increase in Pi/PCr (Chance, et al., 1986; Cohen, et al. 1992; Lam & Hannam, 1992; Plesh, Meyerhoff & Weiner, 1995; Sappey-Mariner et al., 1998). Therefore, when electrical stimulation, sufficient to create muscle contraction is applied to the masseter, it is anticipated that an increase in Pi/PCr will also emerge. Pi/PCr elevation has been demonstrated in the human masseter during prolonged exercise periods with and without electrical stimulation with a concomitant decrease in ATP (Lam, et al.1992; Plesh, et al.1995; Sappy-Marinier, et al., 1998; Russ, et al, 2002). Chronic masseter discomfort and palpable tenderness induced by muscle injury, fatigue and/or hypoxia. These conditions are common in nocturnal bruxism/clenching, and therefore this subject population was included in the research cohort.

During exercise or contractile electrical stimulation, which causes energy expenditure, ATP usage will increase, causing PCr to be broken down and

subsequently release more Pi for the synthesis of ATP. As the Pi level rises and PCr falls, resulting in increased Pi/PCr, the amount of adenosine diphosphate (ADP) also increases, stimulating ATP synthesis. ADP is quickly recycled within the mitochondria to again be converted or synthesized into ATP in order to maintain homeostasis (Bianchet, Pedersen & Amzel, 2000).

However, it is not clear what metabolic effects occur from electrical stimuli administered below the contraction and sensory perception threshold, when stimulation parameters equivalent to those used for cellular level ATP research in non-human tissue, are administered transcutaneously to humans. It is known that MENS will not create enough stimulation to simulate exercise or cause muscle contraction and thus a comparison of the baseline to post-stimulation Pi/PCr changes will provide an indication of the effect solely due to MENS

Utilizing Pi/PCr as the dependent variable and MENS as the independent variable, the following hypotheses are presented:

1. Hypothesis I: MENS, administered to the normal and abnormal masseter of human subjects, will enhance ATP synthesis as evidenced by a significant increase of Pi/PCr.
2. Null Hypothesis I: There will be no demonstrated effect upon Pi/PCr by MENS administered to the human masseter.
3. Hypothesis II: The effect of MENS, as evidenced by a significant increase in Pi/PCr, will be accompanied by a decrease in subjective masseteric pain and pressure pain threshold, with an increase in TMJ vertical ROM.

4. Null Hypothesis II: There will be no effect of MENS upon subjective masseteric pain, the pressure pain threshold or TMJ vertical ROM.

Chapter II

RELATED LITERATURE

Microcurrent Electrical Nerve Stimulation

The majority of published anecdotal and case study reports concerning MENS refers to the initial work of Cheng, who demonstrated the effects of four continuous hours of direct current microamperage stimulation on ATP synthesis in rat skin in-vitro, with currents ranging from 1-30,000μa (30ma) at a constant temperature of 37^F (Cheng, et al. 1982). Protein synthesis was measured and ATP concentration plus amino acid transport (AAT) were determined via chemical assay, as opposed to MRS, which was not yet available. Results demonstrated a 300-500% increase in ATP and AAT concentration at an amplitude from 50-1000 microamps (μa), and a decrease at 5000μa. No change occurred with an intensity of 1000μa while at higher levels ATP and protein synthesis decreased.

Cheng and colleagues (1982) determined that 50μa represented the minimum intensity needed to produce a maximal effect upon protein synthesis in rat skin. A microamp is 1000th of a milliamp. As the strength of the stimulation enters the milliamperage range, contractile effects begin to occur in muscle fibers that require energy in the form of ATP. Although not perceptible or visible with standard size TENS electrodes at 1ma, myofibril stimulation may still occur, which will also require ATP expenditure. In contrast, the low intensity level of

microamperage stimulation is either not perceptible or barely perceptible for a short period of time, depending on the amplitude and electrode size.

Cheng (1982) also demonstrated increased protein synthesis, which can be associated with tissue healing. However, similar research dealing with wound or fracture healing has yet to be equated to concomitant pain relief, increases in ROM or enhanced ATP synthesis (American College of Occupational and Environmental Medicine Ergonomics Committee, 1995).

Cheng's study on rat skin did not provide information about the pulse frequency. It has been demonstrated that ionic concentrations across a cell membrane vary with the frequency of the AC signal, which is considered to be optimal at 100 Hz for all intensity levels, with about 1mV required for activation of ATP synthesis (Blank, 1992). Many of the biological effects produced experimentally upon tissue by magnetic or electric fields are measured in milli or microvolts per centimeter. One millivolt is equal to one thousandth of a volt and one microvolt is equal to one millionth of a volt.

ATP synthesis has been demonstrated to occur within an amplitude range of 30-60 μV/cm at the cellular level, with higher exposures producing a decreased effect (Blank, 1993). Blank proposed the existence of optimal cellular stimulation parameters, which represent a window within which protein synthesis occurs (Blank & Soo, 1996). The paradigm requires a frequency of 15-150 Hz and intensity of > 0.1 mV/cm; however, this was determined using constant not alternating current or electromagnetic fields, and applied in solution to non-human

tissue (Berg, 1995). Robertson and Edwards demonstrated that ATP release from rat neurons occurred at low frequency, which was not delineated in their report, and was inhibited with an increased rate of stimulation (1998). A study on squid mitochondria utilized 20Hz to increase the conductance of the mitochondrial membrane, without information relative to amplitude (Jonas, Buchanan & Kaczmarek, 1999).

Alternating magnetic fields at 5, 10, 20 & 40 Hz, applied to plant seedlings at an unknown intensity and grown in a dark environment, demonstrated increased growth in comparison to a control group, with 10Hz producing the highest growth rate (Muraji, Asai, & Tatebe, 1998). AC stimulation of tobacco shoots at 50 Hz with an intensity of 0.1-50μa, over a 30 day period, yielded a 300% increase in the number of shoots, but the total mass, DNA and protein content were not changed in comparison to a control (Cogalniceanu, Radu, Fologea, Moisoi & Brezeanu, 1998). The intensity of the external field required for these events to occur was determined to be optimal at 0.25V/cm.

Weak AC exposure of pollen tubes revealed a maximal stimulating growth effect at $20mV/^{-1}$ with a frequency of 10Hz (Platzer, et al., 1997). AC stimulation of 10Hz at 10^{-3} to 10^{-1} V/cm^{-1} applied to garden cress roots produced a significant increase in growth and endoplasmic reticular volume in the statocytes, which was attributed to the enhancement of root cell plasma membrane H^{+}- ATPase (Stenz, Wohlwend & Weisenseel, 1998). E-coli cells placed in a liquid medium and depleted of cytoplasmic ATP were exposed to an externally applied alternating

electric field. Electrical parameters of 2.5-50 V/cm at a maximum frequency of 100 Hz for 30 minutes stimulated ATP synthesis with an optimal effect at nine volts (Zrimec, Jerman & Lahajnar, 2002).

Oscillating electric fields have been shown to influence the activity of membrane proteins and are considered to be frequency dependent (Tsong & Astumian, 1988). This effect is based upon the hypothesis that a steady state of enzyme turnover is a function of the frequency of the applied periodic membrane potential of small amplitude (Robertson & Astumian, 1991). This effect is similar to the Arnt-Shulz law, commonly mentioned in reports about MENS, which states that "weak stimuli excite, moderately strong and very strong ones arrest it" (Picker, 1995).

The aforementioned cellular research on non-human tissue provides information relative to the pulse frequency window (< 100 Hz) and to the effectiveness of low intensity stimulation in the microamperage range, but the duration (exposure time) of such stimulation remains questionable. Although the effects of cellular stimulation have been demonstrated to occur within a period of minutes, it is unknown if the same applies to transcutaneous stimulation at equivalent parameters, when administered to human tissue.

Muscle Physiology

Critical physiological and pathological factors consisting of caffeine, fiber type, systemic disease, depressive disorders, pH, salinity, temperature, hypoxia and menstruation can alter phosphorus metabolic activity. Furthermore, the level of

phosphorus containing compounds indicates the metabolic state of tissues with energy status fluctuating between normal, pathological, sedentary and exercise induced states (Henriksen, 1994; Van Den Thillart, & Van Waarde, 1996; Lenkinski & Schnall, 1996).

MRS has become a risk-free means of determining the level of specific metabolites that is devoid of exposure to radiation. It can provide a noninvasive quantification of energy status, acid-base balance, ion transport, fluxes through metabolic pathways and cellular development (Van Den Thillart, & Van Waarde, 1996).

The principal energy carrier in living organisms is ATP, which is required for a myriad of cellular metabolic events, including membrane transport and neuromuscular function. Normal neuromuscular physiology, contraction as well as relaxation, thus necessitates an adequate amount of ATP. A homeostatic environment is required for optimal ATP utilization, with a reduction leading to ion imbalances, cellular dysfunction or ultimately cell death (Kristensen, 1994). Cellular survival therefore depends upon ATP synthesis, which requires active transport of various ions needed to maintain ideal membrane electrical potentials.

Within mitochondria, membrane bound ion pumps functioning as ATPases, split ATP enzymes and control the synthesis of ATP. The Na^{+}/K^{+}-ATPase enzyme is an ion-translocating membrane protein that pumps sodium and potassium across cell membranes (Blank, 1992; Tsong, 1992; & Berg, 1993). Homeostatic alterations can affect the membrane electrical potential and are known

to occur from changes in pHi, potassium or calcium loss, hydrogen ion accumulation, depletion of high energy phosphates (ATP and PCr), glycogen deficiency and hypoxia.

It is well known that an increase in ATP utilization during muscular work (talking, chewing, exercise and clenching as related to the masseter) results in a decrease of the concentration of ATP and a concomitant increase in ADP and Pi concentration. An increase in the concentration of ADP is considered to be the main factor that stimulates ATP production, but other new theories discuss the need for parallel activation by calcium ions and protein phosphorylation (Korzeniewski, 1998; Cho, Thatte, Silvia & Golan, 1999). The effects of MENS administered within the stimulation paradigm of this research, did not simulate exercise and thus any change in phosphate metabolites was attributed to the physiological effect of MENS.

Caffeine and Structural Significance of the Masticatory Musculature

The sarcoplasmic reticulum of the masseter has a relatively high sensitivity to caffeine in comparison to the vastus lateralis (Adnet, Reyford, Tavernier, Etchrivi, Krivosic, Krivosic-Horber & Haudecoeur, 1966). Increased masseter tone leading to trismus, may occur and hence skew the results by depleting ATP. Marchand, Li & Charest (1995) eliminated all dietary caffeine for up to 24 hours prior to TENS, because caffeine is a non-selective adenosine-receptor antagonist (Salter & Henry, 1987). Further-more, the analgesic effect of caffeine on vascular

headache may concomitantly decrease orofacial pain (Ward, Whitney, Avery & Dunner 1991).

Muscle fiber classification, as delineated by physiological and histochemical means, differs between the limb and orofacial regions. The functional properties of muscle fibers vary by oxidative and glycolytic capacity, peak force, speed of contraction, resistance to fatigue, calcium sensitivity and adenosine triphosphatase (APTase) activities (Mao, Stein & Osborn, 1992).

The masseter muscle due to its size, location, function and bony insertion, makes it an ideal muscle for research in animal and human studies (Nordstrom & Miles, 1990; Nordstrom, Miles & Turker, 1990). However, its proximity to bone and teeth make it more difficult to obtain acceptable spectra. The muscles that elevate (close) the human mandible have a fiber type composition that is different from that of the mandibular depressor (opening) muscles as well as the human extremity and trunk musculature (Mao, Stein & Osborn, 1992).

The masseter, medial pterygoid and temporalis comprise the mandibular elevator muscles and are composed predominately of Type I fibers, which are characterized by a larger diameter, high fatigue resistance, increased activity of APTase plus oxidative and glycolytic enzymes as well as slow-twitch motor units, which have the lowest threshold of activation within the range of fiber classification (Nordstrom & Miles, 1990; Mao, Stein & Osborn, 1992; Sappey-Mariner, Dheyriat, Lissac, Frutoso, Mallet & Bonmartin; 1998). The lateral pterygoid and anterior digastric muscles that are involved in jaw opening

movements are composed primarily of Type II fibers, which are smaller in diameter, have a low oxidative capacity and exhibit a fast twitch action. In comparison, the proportion of human fast and slow twitch fibers of the vastus lateralis and medialis are relatively equal (Ruff, 1989). It has been demonstrated that the diameter of the jaw opening muscle fibers is smaller than that of the vastus lateralis and medialis of the human quadriceps (Adnet, et al, 1996). Unlike the jaw closing muscles, the jaw opening muscles are not required to perform tasks that necessitate significant force and therefore the utilization of ATP is not as significant.

Caffeine sensitivity was tested on fragments of masseter and vastus lateralis obtained from normal subjects undergoing orthognathic, parotid gland or orthopedic surgery respectively. It was demonstrated that the normal human masseter reacts to a variety of non-specific stimuli by becoming hyperactive and causing trismus in some cases (Adnet, et al, 1996).

Increased masseter tone and trismus also occur in normal subjects without TMD from exposure to halothane and succinylcholine that are used in anesthesia (Mitsumoto, De Boer, Bunge, Andrish & Cruse, 1990; Adnet et al., 1996). Caffeine is also able to diffuse through cellular membranes and cause the release of calcium from the sarcoplasmic reticulum of muscle therefore inducing a contraction (Mitsumoto, et al, 1990; Cho, et al, 1999). When exposed to caffeine the sarcoplasmic reticulum of Type I masseter fiber displays a higher sensitivity than that of Type II.

A reduction of caffeine resulted in cessation of a five year history of trigeminal neuralgia in a 50 year old female drinking no less than three to four cups of coffee daily with a peak daily caffeine intake of 360mg (Glore & Ricker, 1991). When daily caffeine intake was reduced to 2-3mg, by discussion with a registered dietitian about all sources of caffeine in her diet and medication, symptoms decreased and she remained pain free for two years.

The masseter is innervated by the trigeminal nerve, and since the physiological characteristics of masseteric fibers as well as trigeminal nerve sensitivity are quite relevant to this research, caffeine intake was therefore excluded for 12 hours prior to testing in this study.

Systemic disease processes

Specific systemic disease processes can also alter ATP levels and thus represent a confounding variable. Mitochondrial pathologies can develop in the central and peripheral nervous systems, as well as the eye, heart, liver, kidney and skeletal muscle (Wallace, 1999). A 31^{P} MRS study of patients with eosinophilia-myalgia revealed that resting ATP levels of the calf were statistically lower than that of a control group (Clauw, Hewes, Nelson, Katz & Rajan, 1994).

Exercise tolerance is compromised in both hypo and hyper-thyroidism due to inadequate blood flow, impaired fatty acid release and a reduced skeletal muscle oxidative capacity. Hypothyroidism creates abnormal cardiac function and a possible reduction in the ability of the vasculature to dilate. Therefore, a reduced blood supply to the musculature creates an increased need for glycogen, which in

combination results in a faster rate of ATP depletion (Taylor, Rajagopalan & Radda, 1992). Hypothyroidism has also been shown to impair mitochondrial function causing fatigue and decreased exercise tolerance in an MRS study of humans and rats (Argov, Renshaw, Boden, Winokur & Bank, 1988).

Alterations in energy metabolism also create a reduced exercise tolerance in a hyperthyroid state. Even though cardiovascular function is enhanced in hyperthyroidism, it results in hyperfusion of skeletal muscle during exercise and a more rapid depletion of glycogen (Erkintalo, Bendahan, Mattei, Fabreguettes, Vague & Cozzone 1998). Hyperthyroidism creates a higher internal body temperature during submaximal exercise, with a higher metabolic rate possibly contributing to a reduction in endurance (Das & Harris, 1991; Mao, et al, 1993; Mense, 1993; Bennet, 1993; Simms, 1994; Stohler, 1995; McAllister, Delp & Laughlin, 1995; Lindman, Hagberg, Bengtsson, Hendricksson & Thornell, 1995; McCully, Natelson, Iotti, Sisto & Leigh, 1996; Jurell, Zanetos, Orsinelli, Tallo, & Waylonis, 1996). Subjects with thyroid abnormalities and other systemic disease processes such as diabetes were therefore excluded from the study (Rothman, Schulman, & Schulman, 1992).

Severe pain occurs during exercise in the presence of reflex sympathetic dystrophy (RSD) or chronic regional pain syndrome (CRPS) with impaired oxygen extraction (Heerschap, Hollander, Reynen, & Goris, 1993). MRS analysis revealed an increase in Pi as well as pHi of the leg musculature, considered to be caused by

cellular hypoxia. Subjects with cervical or facial CRPS were therefore excluded from this study.

Depressive Disorders

Major depressive disorders of a chronic and unipolar nature, in combination with muscle pain and mitochondrial function can alter mitochondrial function. In a study of 28 patients with depression, muscular pain, severe tinnitus, hyperacusia and/or hearing loss, the association between these characteristics to that of mitochondrial function revealed a significant decrease in ATP production in comparison to controls (Gardner, Johansson, Wibom, Nennesmo, von Dobeln, Hagenfeldt & Hallstrom, 2003). Therefore subjects who met these characteristics were excluded from this study.

pH

Contractile efficiency, ion channel conductivity and protein synthesis necessitate an optimal pH level. Elevation of pH within the physiological range increases cellular metabolic activity (DNA and RNA synthesis), whereas a decrease in pH towards acidity reduces the Ca^{2+} current, hinders contractility, but may not be a major factor in fatigue (Stackhouse, Reisman & Binder-Macleod, 2001).

The relationship between Pi/PCr and pH is influenced by the level of lactic acid. Increased lactate produces a decrease in pH causing a chemical shift of the Pi resonance, thus altering the critical cytoplasmic environment required for optimal enzymatic activity, conductivity and muscular contraction. Normally exercise will

cause a decrease in PCr and increase of Pi, with tetany creating a pH shift towards acidosis. An acidic state creates a decrease in the sensitivity of myofibrillar components, which results in a reduction of muscle tension.

It is known that tissue damage, inflammation, ischemia and intermittent claudication cause chemical excitation of nociceptors resulting in muscular pain (Lewis, 1931; Simone, Marchetti & Ochoa, 1997; Hoheisel, Reinohl, Unger & Mense, 2004). Therefore, the pH of muscle is an essential factor to track in research of this nature and can be calculated by analyzing the phosphorus spectra produced by MRS. As pH declines, the spectral Pi peak moves closer to the PCr peak and analysis of the pre-and post-stimulation distance allows for the measurement of pH within the muscle that is being studied (McCully & Natelson, 1999; Van Den Thillart & Van Waarde, 1996).

It is important to distinguish pH by its intracellular (pHi) or interstitial-extracellular (pHe) location. The pHi of resting muscle is at least 0.2-0.3 pH units below that of pHe and any change in pHe as a result of exercise is proportional to the degree of muscle contraction (Street, Bangsbo & Juel, 2001). Intracellular pH is determined from the chemical shift of Pi by the following equation: $pHi = 6.75 + \log (\delta - 3.27) / (5.69 - \delta)$, in which δ equals the chemical shift of the Pi to PCr peaks in parts per million (ppm), (Chance, Leigh, Smith, Nioka & Clark, 1986).

Muscle Tenderness

Considering that the subject population would be predominately female and of child bearing age, testing was not performed during menstruation due to

its effect upon muscle tenderness. The number of tender points found in females increases in the follicular stage of the menstrual cycle in women with normal periods, but not in those who take oral contraceptives (Hapidou & Rollman, 1998; Isselee, De Latt, De Mot & Lysens, 2002).

Muscle pain and tenderness can differ during the course of the menstrual cycle and women who seek help for the management of orofacial pain do so more frequently in the presence of menstruation (Riley, Robinson, Wise & Price, 1999; Macfarlane, Blinkhorn, Davies, Kincey & Worthingham, 2002). Gender differences, which may be hormonally mediated, indicate that mechanically evoked discomfort is higher in females than males (Dao & LeResche, 2000; Warren & Fried, 2001). Research has revealed that there are alterations in TMD pain during the menstrual cycle and that the threshold to pressure pain sensitivity is at its peak at the time of the lowest level of estrogen (LeResche, Mancl, Sherman, Gandara & Dworkin, 2003). Therefore, subjects were excluded from testing during the time of menstruation as if they used oral contraceptives. All of the aforementioned pathological and physiological factors played a major role in the development of exclusion criteria for this study.

Chronic Fatigue Syndrome

Patients with chronic fatigue syndrome have demonstrated a reduced oxidative capacity in comparison to sedentary subjects as evidenced by MRS (McCully, et al., 1999). Subjects with chronic masseter hyperactivity and tenderness may exhibit the reduced oxidative capacity found in the presence of

chronic fatigue syndrome (McCully, et al., 1996). Therefore, subjects with chronic fatigue syndrome as well as CRPS of the cervical or facial area were excluded from this study.

Skin Resistance

In the pilot study (Appendix F), tissue resistance at the level of the skin in normal subjects may have been too high for MENS to overcome. Skin resistance is high in normal and decreased in pathological tissue, and areas of subjective pain have been demonstrated to correspond well to overlying regions of low skin resistance (Riley & Richter, 1975; Mannheimer & Lampe, 1984). The electrical resistance of skin varies according to the degree of physical and emotional tension, temperature, circulation, sudomotor activity, surface moisture and segmentally related pathology of somatic or visceral origin (Mannheimer and Lampe, 1984). Decreased skin resistance thus occurs in dermatomal regions that are related to an area of pathology, muscle guarding or pain, characterized by what are known as reactive electropermeable points (Saku, Mukaino, Ying & Arakawa, 1993). Increased concentrations of nitric oxide have recently been shown to occur at acupuncture points which have a low skin resistance (Ma, 2003).

In the presence of normal tissue with homeostasis of the aforementioned factors, skin resistance is normal or elevated (McAllister et al, 1995). Skin resistance; however, will progressively decrease with ongoing electrical stimulation, especially during the four-hour testing period that was utilized in the pilot study (Mannheimer & Lampe, 1984). However, Blank, (1992) found that low

intensity electrical fields applied to tissue surfaces penetrates completely into the interior of the cell and causes an increase in Na,K-ATPase activity which may negate skin resistance as a confounding factor in research of this nature.

Magnetic Resonance Spectroscopy

Magnetic resonance imagery (MRI) detects the energy exchange between an external magnetic field and specific nuclei within atoms. The exchange is measured as a radiofrequency (RF) signal that is translated into an anatomic image by assessing different grey values according to the emitted signal strength. In contrast, MRS can quantitatively determine the concentrations of different chemicals within tissues and specifically the resting and exercise values of phosphorus metabolites as well as their effect upon ATP synthesis (Radda, Odoom, Kemp, Taylor, Thompson & Styles, 1995). The difference between MRI and MRS is that, with MRI, the emitted RF is based upon the spatial position of nuclei, while MRS detects the chemical composition of the scanned tissue. MRS displays the information graphically as a spectrum with peaks consistent with the various chemicals detected, and thus provides a functional image related to the underlying intra and extracellular physiology (Hendricksen, 1994; Lenkinski & Schnall, 1996; Van Den Thillart & Van Waarde, 1996).

The physiological basis of MRS is based upon the fact that various nuclei have different resonance frequencies and magnetic moments, which can be perturbed by a magnetic field and analyzed relative to concentration (Henriksen,

1994; Lenkinski, & Schnall, 1996; Van Den Thillart, & Van Waarde, 1996). Phosphorus 31^P exists as 100% of the naturally present phosphorus in only a few biological molecules involved in energy metabolism, and is therefore used to study cellular metabolism in a variety of tissues. Phosphorus is found in all types of muscle and its various forms such as ATP, PCr and Pi can be measured by MRS (Lenkinski and Schnall, 1996). The generally accepted concentration of ATP in normal muscle is 8.2 mM, and the Pi and PCr concentrations are compared to this standard, which is the same for human slow and fast-twitch fiber types (Soderlund & Hultman, 1990 & 1991; Greenhaff, et al, 1994). The Pi concentration is obtained by dividing 8.2 from the raw ATP value times the raw Pi value. The PCr concentration is determined by dividing the raw ATP value times the raw PCr value, which yields the Pi/PCr.

The pulsing of a particular muscle within an MRI unit by an oscillating magnetic field, at a specific radiofrequency, causes the nuclei to first absorb and then emit some of their energy in the form of a radiofrequency signal, which is amplified, displayed spectrographically and subsequently analyzed as to the amount of intracellular phosphate metabolites (Henriksen, 1994). The in-vivo 31^P spectrum generally reveals seven separate resonances, of which five are discernable by specific peaks (inorganic phosphate, phosphocreatine, alpha, beta and gamma atoms of ATP), and commonly used to assess alterations in energy metabolism as illustrated in Figure 1 (Lam, 1992; Van Den Thillart & Van Waarde, 1996; Lenkinski and Schnall, 1996).

Previous MRS studies of the human masseter have provided both resting and exercise Pi/PCr levels. It has been shown that Pi/PCr of the human masseter significantly increases with clenching and bruxing (Lam & Hannum, 1992; Plesh, et al. 1995; Marcel, et al., 1995). Different metabolite levels were found to be dependent on the region of the masseter that was analyzed, as well as the size of the radiofrequency coil. Elevation of Pi/PCr has also been demonstrated in the human masseter during prolonged exercise periods with a concomitant decrease in ATP (Sappy-Mariner, et al., 1998).

Lam and Hannum (1992) tested 6 normal subjects at rest and during clenching upon an interocclusal stop placed between the first molars. A 2 x 3cm surface coil was used and spectra were acquired from the superficial, intermediate and deep regions of the masseter with a 1.5 Tesla magnet. Plesh (1995) utilized a 2 Tesla magnet with a 4cm surface coil placed over the center of the masseter, which therefore provided an average Pi/PCr reading from a larger area. Marcel (1995) tested 6 non-bruxing subjects with a 1.5 Tesla magnet and 2 x 3cm surface coil placed over the belly of the masseter. A larger surface coil averages the reading over all regions of the masseter and yields a lower Pi/PCr. Table 1 presents a comparison of resting and exercise Pi/PCr levels previously obtained from human masseter muscle in the aforementioned research.

Table 1

Masseter Pi/PCr Levels at Rest and Exercise

Study	Pi/PCr Rest	Pi/PCr Exercise
Lam & Hannum	0.70 +/- 0.17 Deep 0.40 +/- 0.13 Sup. 0.42 +/- 0.13 Inter.	1.70 +/- 0.89 1.09+/- 0.47 1.23 +/- 0.67
Plesh, Meyerhoff & Weiner	0.25 +/- 0.03	0.72 +/-0.21
Sappy-Marinier, et al.	0.30 +/- 0.04	0.63 +/-0.13

The authors agree that the resting Pi/PCr of the masseter is higher than that of the limb musculature. This is due to the predominance of Type I fibers, which have an enhanced oxygen supply, as well as collateral facial circulation that is significantly greater than that of the extremities (Eriksson & Thornell, 1983; Mao, Stein & Osborn, 1992). These factors also account for the high resistance to fatigue that is characteristic of the masseter, but this fatigue–resistance has also been postulated to occur from the development of pain, which causes the subject to decrease the degree of contractility (Plesh, Meyerhoff & Weiner, 1995; Marcel, et al., 1995). A decrease in contraction force may therefore serve to reduce the degree to which Pi/PCr increases as a result of exercise.

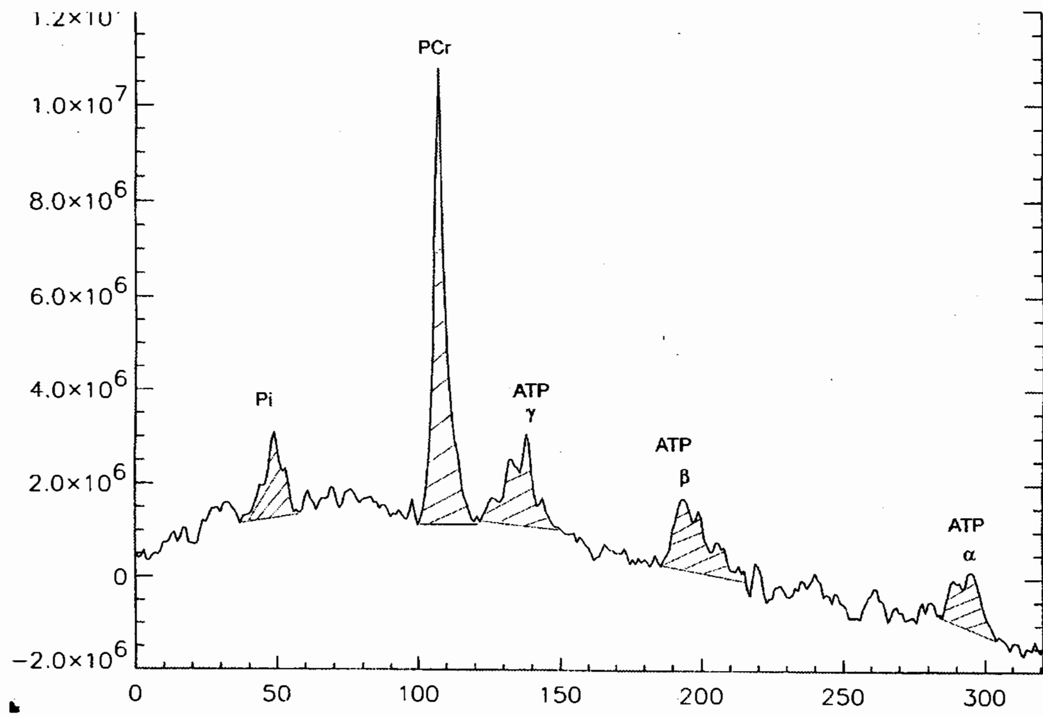


Figure 1. Phosphorus spectra delineating the filled metabolite peaks.

Chapter III

METHODS

Location and Support

The study was performed at the Metabolic Magnetic Resonance Research and Computing Center (MMRRCC) of the University of Pennsylvania. Permission was obtained to perform the study after submission of a research proposal approved by the institutional review board (IRB). The MMRRCC is a regional collaborative research center supported in part by the National Institutes of Health (NIH). The MMRRCC is located in the basement laboratory of the Stellar-Chance Building, which houses a single 2 Tesla magnet, specifically designed for spectroscopy. Support was provided by research associates as well as by faculty of the radiology department relative to the construction of the ancillary components, operation of the magnet and interpretation of acquired spectra.

Electrotherapeutic Generator

Microamperage stimulation was provided by a clinical electrotherapeutic generator known as a My-O-Matic I *, which is a battery-powered, dual-channel direct current stimulator capable of being programmed for stimulation paradigms within the microcurrent range (amplitude 10-600 μa, pulse frequency 0.1-990 Hz). It functions with a 50% duty cycle and a pulse-duration of 2.5 seconds. The 50% duty cycle represents the time period that each pulse is at its highest level, which is dependent upon the waveslope and pulse duration. The pulse duration is fixed at

2.5 seconds and a waveslope control allows for a fast or slow rise time, which was pre-set for each test at the mid-range position ensuring homogeneity for all tests. The stimulating waveform was therefore sinusoidal biphasic, thus providing an alternating current with a zero net direct current effect. The My-O-Matic I also provides for the setting of a net negative or positive direct current, which was not used for this study. Power was supplied by six 1.5v D cell batteries, with standardization performed by voltage measurements prior to each test. Batteries that registered less than 1.5v were replaced before stimulation commenced.

A set of twenty-foot long lead wires ran from the electrotherapeutic generator (ETG) through holes in the magnet room underneath the window to the attached masseter electrodes. Current flow through this distance was determined to be the same as programmed on the ETG, by connection of a circuit tester to the ends of the wires inside the magnet room. The ETG remained outside the magnet room and was unaffected by the magnetic field, as depicted in Figure 2. Upon activation, the My-O-Matic I emits a noise consistent with its programmed frequency, even if the output signal from one channel is zero. The unit was activated for each subject, regardless of the experimental paradigm, and therefore represented one method to blind the principle investigator from knowledge concerning active versus placebo stimulation.

Data Recording and Surface Coil

Measurement of the signal from a tissue sample by a magnetic resonance scanner necessitates an antenna to transmit and receive the radiofrequency energy.

The MRS antenna is a surface coil, which can be fabricated in many sizes and shapes dependent upon the region of the body to be studied and the chemical to be analyzed.

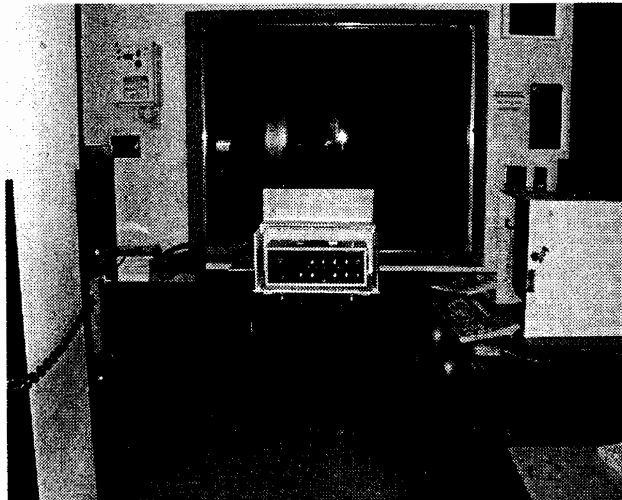


Figure 2. Electrotherapeutic generator situated outside of the magnet room.

* Monad, Inc., Pomona, California

Two double-tuned, linear surface coils with a diameter of 3cm were constructed at the MMRRCC and used for this study. Double-tuned refers to the ability of the coil to measure two distinct radiofrequencies, namely, proton and phosphorus (86MHz and 35MHz respectively). Figure 3 illustrates the surface coils used in this project.

Electrodes

Specialized electrodes were designed by the principle investigator for use with this research. The shape and size of the electrodes as outlined in Figure 4, allowed for placement adjacent to each masseter, with current density relative to the size of the electrode area and the intensity range of 10-60μa calculated at 0.83-5μa. Bilateral electrode placement and stimulation were provided simultaneously to both masseters, as illustrated in Figure 5, even though spectral recording was performed only at the most painful masseter, if both were involved, because the MMRRCC magnet only allows for spectral acquisition at one site. The electrodes were composed of conductive carbon against an adhesive/conductive gel transmission medium with standard cover fabric and female receptacle, as shown in Figure 5.

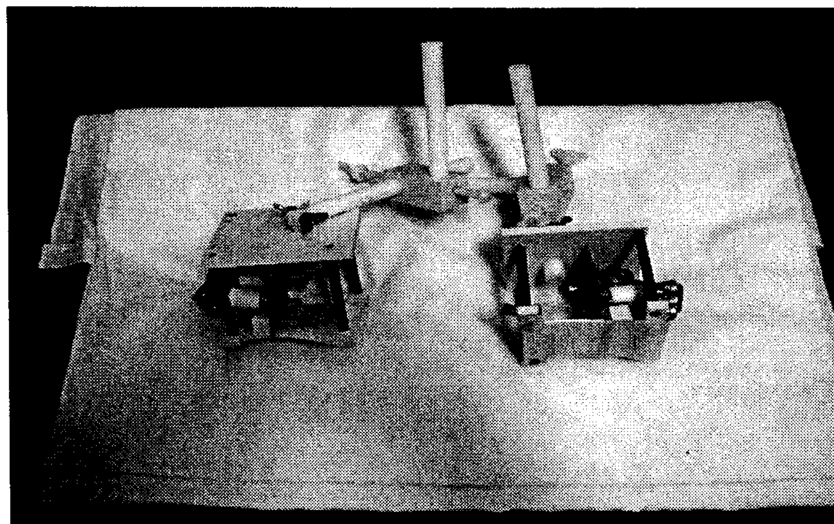
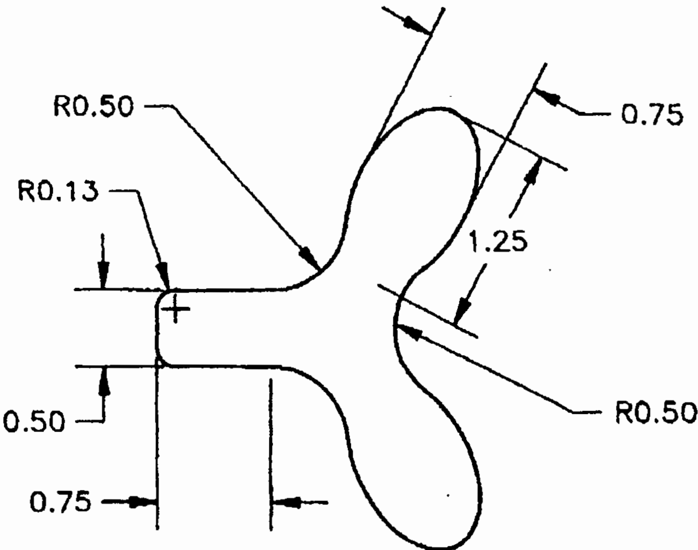


Figure 3. Double tuned RF surface coils mounted in box with positional headgear.

A series of tests with different electrodes were initially performed in the pilot work to determine the presence of interference that would hinder the signal and hence spectral acquisition. The least degree of interference occurred with the rabbit ear electrodeds shown in Figures 4 and 5 that were used in this study.



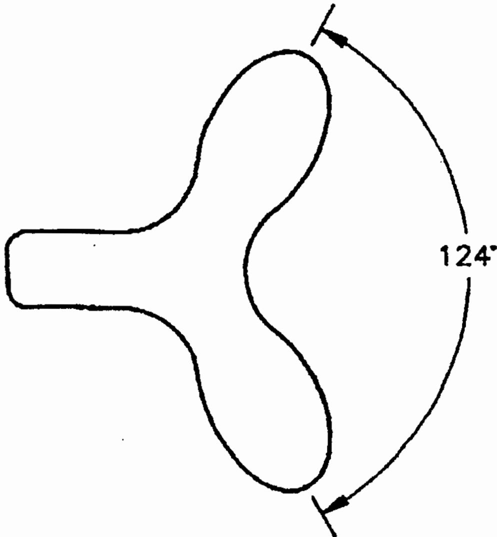


Figure 4. Rabbit ear electrodes.

- Unipatch, Inc. Grand Rapids, Michigan
- Pepin Manufacturing, Lake City, Minnesota

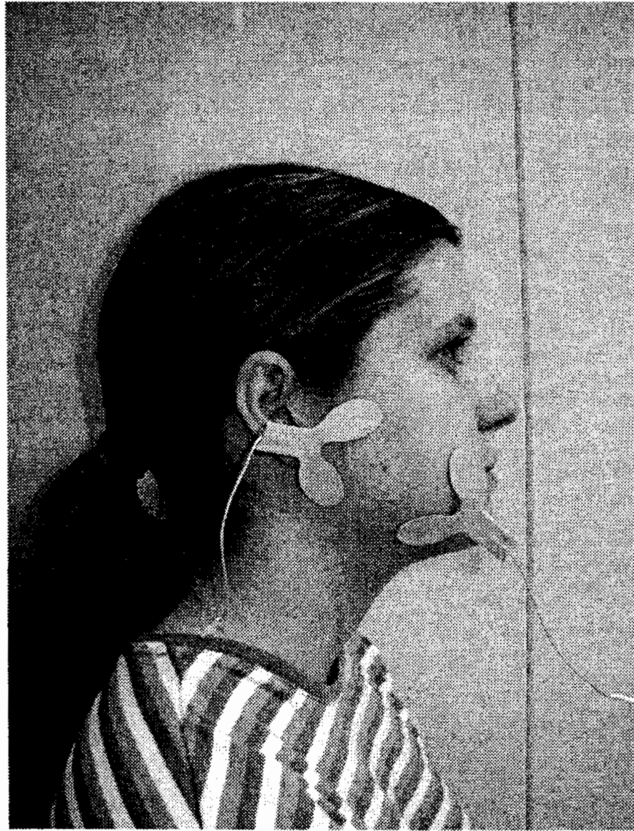


Figure 5. Lateral view of subject with rabbit ear electrodes positioned adjacent to the masseter.

Operational Properties of the Magnet

Shimming

In order to ensure that the magnetic field is as uniform as possible, the proton channel samples water content from the muscle of interest (masseter) via a process known as shimming, which allows for adjustment of the homogeneity of the magnetic field at the region to be studied (Cecil, 1996). The shimming process is accomplished by alteration of gradient magnets, which are located at or surrounding the bore of the magnet, and allow for alteration of the RF signal. The gradients add to or subtract from the static magnetic field to produce greater homogeneity around the region of interest.

Once the proton signal is optimally shimmed to a line-width of 40 Hz or less, pre-stimulation baseline data of phosphorus metabolites can be acquired. If the signal line-width is not reduced close to 40Hz, adequate separation of the phosphorus peaks, especially that of PCr from that of the ATP gamma peak, will be difficult to accomplish, thus making spectral analysis unreliable. The other channel is tuned to phosphorus and allows for observation of phosphorus metabolites. The circular coils have an effective sampling distance of one radius of the coil, which provides for a sampling depth of 1.5cm to prevent signal contamination from oral cavities or tissue adjacent to the masseter. Two equivalent coils were mounted within an adjustable frame overlying each masseter and positioned centrally within all axes of the magnet bore.

The duration of the RF pulse is a few microseconds to many milliseconds, and the bandwidth is large enough to excite all of the nuclei within the required frequency range. The nuclei absorb the RF energy at their resonance frequencies and the resultant energy signal is fed by a transmitter/receiver coil to be analyzed and processed. The pulsed signal is transformed into spectra by Fourier transformation, which is a mathematical procedure performed by the computer software, that separates out the frequency components of a signal from its amplitudes as a function of time, so that it can be analyzed.

Signal Averaging

Signal averaging is used to cancel out any random noise produced by the ETG. Signal repetition times of 2-8 seconds were utilized to create spectral sets in segments of 128 or 256 averaged scans, with data analysis performed off-line, and the spectra zero filled to 4k data points. Signals that originate from molecules in solution exhibit a characteristic line-shape known as the Lorentzian line-shape, and as the molecules become immobilized to a greater degree by the magnetic field, their signals become broader. Exponential line broadening of 5Hz with manual baseline corrections and a Marquardt data analysis was automatically processed with a Lorentzian line-shape assumed for all metabolite peaks (Gadian, Radda, Ross, Hockaday, Bore, Taylor, & Styles, 1995).

The separation of resonance frequencies from that of an arbitrarily close reference frequency is known as the chemical shift, and is expressed in terms of parts per million (ppm) for the different chemical species. The 31^{P} spectrum of

ATP contains three groups of spectral lines that correspond to the alpha, beta and gamma phosphates of ATP, all of which are chemically different and can be seen on the sample spectra of Figure 1 (Gadian, 1995). The value of the peak height, area and chemical shift of the phosphate metabolites are generated with each spectra consisting of 5 peaks as shown in Figure 1.

Most nuclei are at their lowest energy state at a temperature of absolute zero. Although this is not possible for human studies in-vivo, a constant temperature is maintained within the magnet room of 65-70 degrees. Temperature elevation will cause an increase in the line-width due to a more rapid exchange rate of electrons between the different states. Baseline data acquisition followed, after which the ETG was activated by the center engineer and the placebo or microamperage stimulation protocol commenced.

Conductive Phantom

In order to verify and confirm the operational protocol, a conductive agarose phantom was fabricated at the MMRRCC laboratory prior to the start of the study. The agarose was impregnated with phosphorus, which allowed for acquisition of a spectra equivalent to that obtained from a human subject, with or without microamperage stimulation. The phantom was fabricated in a shape that was conducive to the placement of the rabbit ear electrodes at a similar inter-electrode distance to that utilized on each subject.

Rabbit ear electrodes were placed on the agarose phantom as illustrated in Figure 6, which was followed by placement in the magnet as shown in Figure 7.

The same RF coil utilized for spectral acquisition with the human subjects of this study was then placed between the two electrodes, as illustrated in Figure 6. A series of tests in the magnet was performed with the agarose phantom, which verified the presence of a complete electrical circuit, allowed for testing of the enhanced MRS standardization protocol, as outlined in Appendix E, and confirmed the presence of a better signal (spectral acquisition) when the coil did not overlap any part of either electrode.

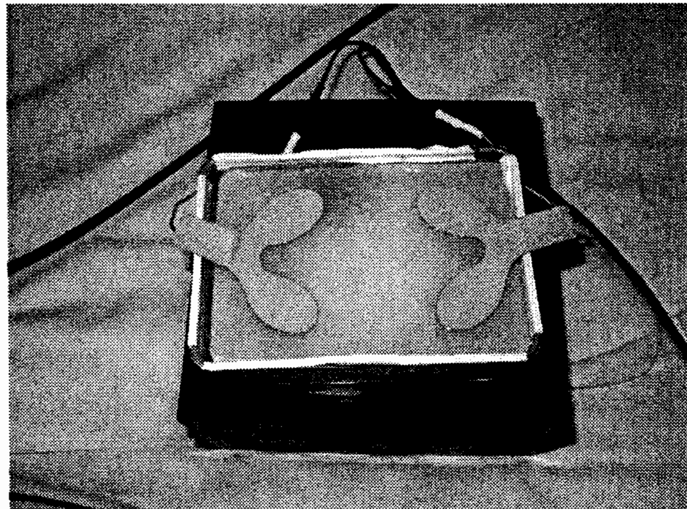


Figure 6. Conductive agarose phantom with rabbit ear electrodes.

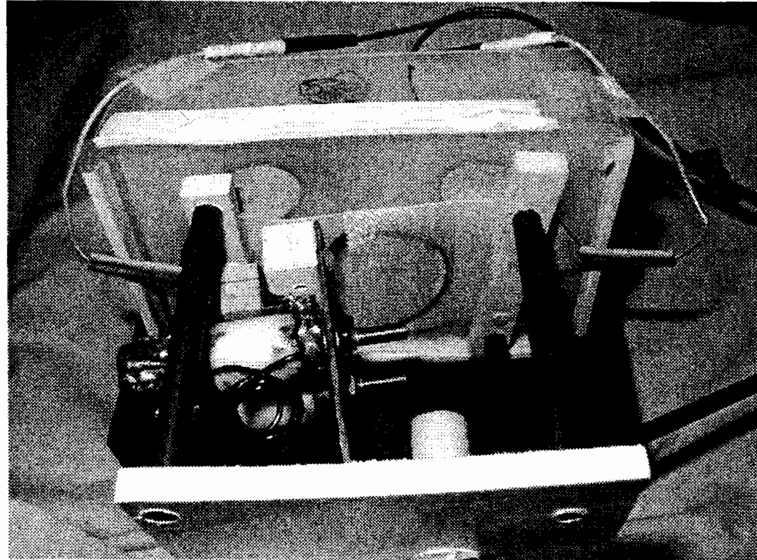


Figure 7. Conductive agarose phantom with RF coil placed between rabbit ear electrodes.

Research Design

This study was designed as a single application, randomized controlled trial, with a cohort of normal and TMD subjects. Testing was performed with both active and placebo stimulation while the subject was positioned within the bore of the magnet. Total testing time varied between 1.5-2 hours, but the actual stimulation or placebo session within the magnet was standardized at 60 minutes.

A pre-test, post-test protocol was designed with data acquisition before stimulation, at the 20-32 and 48-60 minute time-points, thus employing repeated measures. This was a single application study, which negated the effect of any carryover or practice on the part of subjects. Data were recorded across and through the respective time-points with each subject over the entire length of the stimulation session.

Subject Recruitment

The subject population consisted of 25 individual subjects, composed of 20 females and 5 males between the ages of 21-50. All of the TMD subjects were female, while the normal subject groupings were composed of males and females. Subjects were recruited from the patient population of the Oral Medicine Department of the University of Pennsylvania, Delaware Valley Physical Therapy Associates, interning physical therapy students, and the student body of North Georgia State University. Specific recruiting forms, located in Appendix C, were developed to assist the oral medicine staff and provide orientation for the subjects.

As a result of the change in the experimental format, and the dilemma of recruiting subjects, the analyzed data of 23 subjects includes 3 who were tested twice and one three times, of which two were normal and two had a TMD diagnosis. Each of these subjects was exposed to MENS as well as the placebo protocol.

The single subject who was tested on three occasions (active and placebo exposure once and twice respectively) had periodic recurrences of significant TMD inflammation. As a result of the previous explained pathological and physiological factors, specific criteria were developed for subject selection.

Inclusion and Exclusion Criteria

The normal precautions and contraindications relative to the use of TENS as well as human placement within an intense magnetic field are listed in the criteria presented in Tables 2 and 3 for the TMD subjects. Difficulty in obtaining TMD subjects revealed that the initial inclusion criteria, as presented in Appendix C were too stringent and therefore were altered. Additional criteria relative to MRS, as stated in the subject consent forms, can be found in Appendix C, D and F.

Table 2

Exclusion Criteria for TMD and Normal Subjects

Exclusion Criteria
Presence of pacemaker, defibrillator, neurostimulator, metal implants or history of work on or around a metal grinder or construction site
History of systemic disease that may alter normal neuromuscular function (diabetes, hypo or hyperthyroidism, hypertension, etc.)
History of headache/facial pain separate from that of TMD
Mouth breather
Prior utilization of an electrical stimulator for pain control
Claustrophobia, dizziness, vertigo, nystagmus, panic attacks or obesity hindering positioning
Pregnancy, menstruation or lactation at the time of testing
Testing must be performed at least five days prior to or after menstruation
Utilization of prescription or over the counter anti-inflammatory, muscle relaxant or pain medication at the time of testing
Utilization of oral contraceptives

Table 3

Inclusion Criteria for TMD Subjects

Inclusion Criteria
Sub-acute or chronic TMD history > 30 days
Arthrogenous and/or myogenous TMD
Limitation of vertical ROM < 35 mm
Masseteric tenderness and discomfort of at least one masseter
Must be willing to sign the consent form
Males must be clean-shaven and females free of facial cosmetics
Avoidance of any medication at least four hours prior to testing
Avoidance of caffeinated beverages at least 12 hours prior to testing
Avoidance of smoking, vitamin/mineral supplementation, gum chewing and eating at least four hours prior to testing.
No precautions/contraindications to TENS (epilepsy, pregnancy, history of transient ischemic attacks, cerebral vascular accident, cardiac disease or the presence of a cardiac pacemaker

There were 3 TMD subjects who met the inclusion criteria in the clinical setting, but at the time of their actual testing had active vertical ROM beyond 35mm; however they continued to experience considerable discomfort. They were therefore included in the study cohort due to the difficulty of obtaining subjects who met the TMD criteria.

The chosen inclusion and exclusion criteria are similar to a related study on the reduction of TMD pain by high-frequency vibration (Roy, Hollins & Maixner, 2003). That study population was composed of 17 subjects with chronic orofacial pain of at least 6 months, all of whom had a myalgic component, with or without TMJ sounds. The mean vertical ROM was 34.8mm and subjects had to refrain from the use of all centrally acting medications for at least ten days and other medication, including over-the-counter drugs, for two days prior to the testing session. Subjects were excluded if they were taking oral contraceptives or had a major medical condition (Roy, et al, 2003).

Subject Orientation

Upon arriving at the MMRRCC, all metallic objects were first removed and each subject viewed the magnet room, headgear, and testing position that they needed to assume while in the magnet. All metallic objects were removed, the subjects were told to use the restroom prior to the start of the testing period and were again reminded that they could stop the test at any time and for any reason. Each subject was then briefly re-evaluated as to the presence of normal or

abnormal TMJ function by the principle investigator, and then instructed to blindly select one penny from a box, that were labeled as active (A) or placebo (P). The chosen penny was then given to the center engineer, who designated random assignment to either the active or placebo group.

Clinical Assessments

At the baseline time-point, just prior to placement within the magnet and immediately upon exiting, each subject was asked to estimate their degree of discomfort on a visual analogue scale (VAS), after which active vertical range of motion (ROM) and algometric pressure pain threshold of the masseter of interest were assessed by the principle investigator.

Visual Analogue Scale

Subjects were instructed to slide the center portion of a pain rating scale from left to right, to the point which represented the degree of discomfort that they were experiencing before and after each testing session, as illustrated in Figure 8. On the left side of the scale, the words "No pain sensation" appear, and on the right side the words "Most intense pain sensation imaginable" appear. The reverse side of the scale, which was not visible to the subject, delineated the position of the slide via a ten point ordinal scale (Collins, Moore & McQuay, 1997). The primary efficacy variable for the VAS scores was the mean change from the pre to post-stimulation time-points.

Active ROM

Active vertical mandibular ROM to the point of discomfort was recorded by the principal investigator, via manual measurement with a stainless steel millimeter ruler, as illustrated in Figure 8. The rulers were steam autoclaved for each subject and measurements were made from one central maxillary incisor to the corresponding mandibular central incisor prior to and immediately after each stimulation session.

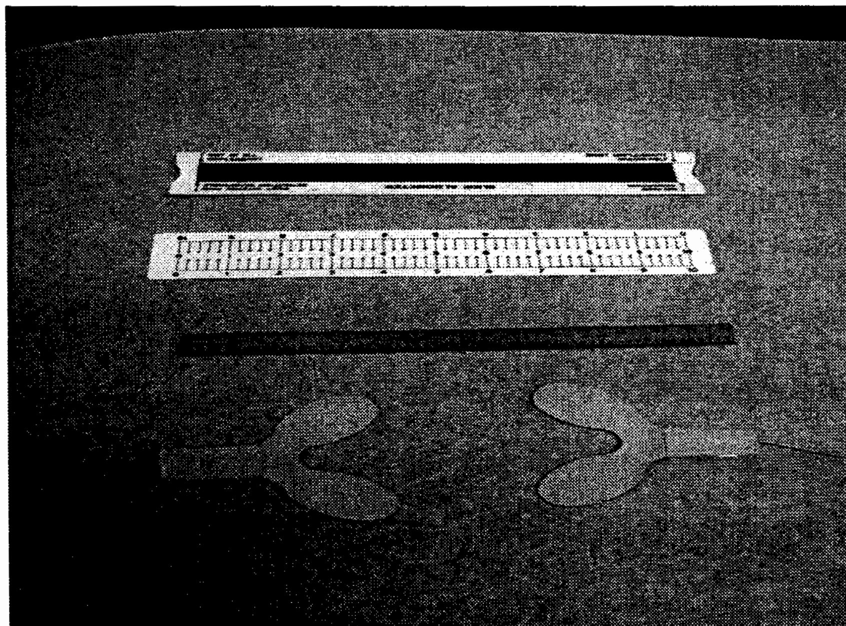


Figure 8. VAS scale, stainless steel millimeter ruler and rabbit ear electrodes

Pressure Algometer

A pressure algometer, illustrated in Figure 9, was used to quantify and document the pressure pain threshold (PPT) of the masseter at baseline (prior to placement within the magnet) and upon removal from the magnet. The pressure

threshold algometer was obtained after the study commenced, which negated the acquisition of algometric data for some of the subjects. The PPT was the amount of pressure in pounds that each subject immediately perceived as painful. Previous research by List, Helkimo and Falk (1989) revealed a PPT masseter range of 1.4 to 3.5kg/cm^2. PPT scores less than 3kg/cm^2 obtained with a 1cm^2 pressure tip is considered to be significant (Fischer, 1988). One kilogram is equal to 2.2 and 3 kilograms is equal to 6.6 pounds respectively.

The PPT was performed by use of a hand-held pressure gauge utilizing a 0.5cm^2 contact- tip, that was applied to the most painful point within the belly of the masseter and marked with an ink spot so that the exact point could be replicated before and after each testing session. The mean of three successive algometric assessments was determined pre and post exposure to the testing protocol. A study comparing VAS scores to that of pressure algometry specific to the masseter found that gender did not affect the reproducibility when testing was performed at short time periods of six and 30 minutes (Goddard, Karibe & McNeill, 2004).

Pressure threshold measurements were determined by positioning the subject with the non-painful side of their head against a wall, so they would not recoil when pressure became painful. Subjects were instructed to say "stop" when the pressure became painful within a three second recording period set on the algometer. The algometer automatically recorded the result upon removal from the masseter, which was retained in its memory for later downloading. There is strong

support in the literature for the reliability and usage of pressure algometers in the evaluation of trigger points, myofascial pain and fibromyalgia (Fischer, 1986, 1987 & 1988; List, Helkimo & Falk 1989 & 1993; McMillan & Lawson, 1994; Reid, Gracely & Dubner, 1994; McMillan, 1995; Svensson, Neilsen, Neilsen & Larsen, 1995; Isselee, De Latt, Bogaerts, et al, 1998; Isselee, De Latt, De Mot & Lysens, 2002).

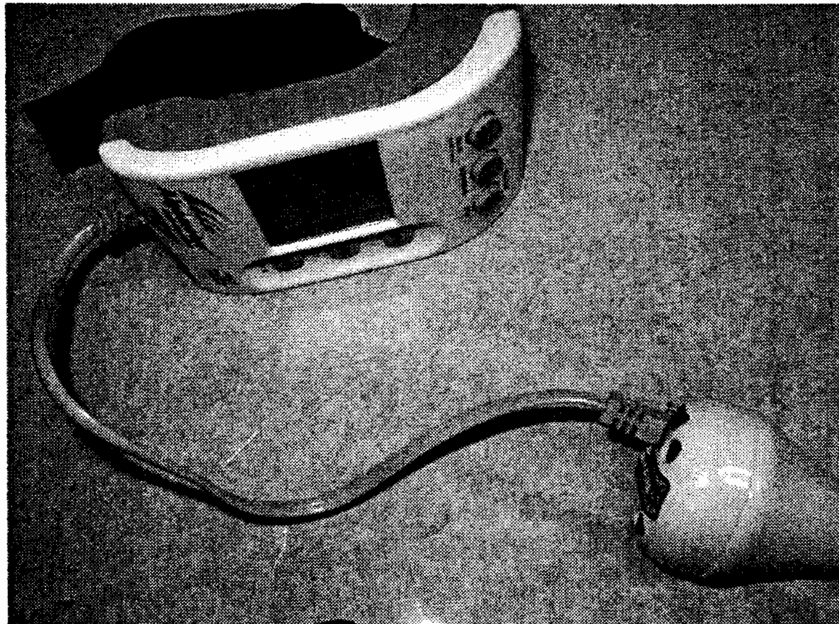


Figure 9. Pressure threshold algometer. The 0.5cm contact-tip is illustrated in Figure 10.

- J Tech Medical. Salt Lake City, Utah 84123

Figure 10. Algometric PPT at belly of masseter with .5cm contact tip.

Electrode Placement

The electrodes were moistened with a wet paper towel, and placed adjacent to the belly of each masseter, delineated by instructing the subject to gently clench on a gauze roll. Electrodes were applied bilaterally and a millimeter ruler measured the distance between each pair to ensure equilibration among each side. Figure 5 illustrates the proper electrode array prior to placement of the RF coils.

Determination of Sub-perception Threshold

After baseline clinical data were obtained, one set of lead wires was connected to the electrodes placed on the masseter to be tested. The lead wires were inserted into the electrotherapeutic generator, which was then activated, and the subject was instructed to let the principle investigator know when they perceived any electrical sensation described as a low rate (3Hz) mild pulsing. This

test of perceptible stimulation intensity commenced at 100μa, and was reduced by the settings on the electrical generator to 80, 60, 40 and 20μa respectively, until the subject did not feel any sensation. The intensity level subsequently utilized for a given subject was set at the next lower increment from the level at which they perceived any electrical sensation and was known as the sub-perception threshold.

The lead wires were detached from the electrical generator and the patient was placed on the MRI plinth with their head positioned optimally on a cervical pillow and within the headgear apparatus. The headgear was constructed of non-magnetic material and allowed for support of the RF coils and their positioning overlying the belly of each masseter. Each coil was stabilized within a specically constructed framed box and attached to the headgear platform by positioning rods that could be moved in any direction, to ensure that they did not overlie any part of either electrode. One or more pillows were placed under the knees of each subject to attain maximal comfort during the time that they remained within the magnet with insrtructions to relax and think of the phrase " tongue–up, teeth apart and lips together ", which promotes mandibular relaxation.

Figure 11 illustrates the headgear apparatus, which stabilized and allowed for positioning of the RF coils. Figure 12 depicts a subject with attached electrodes and the overlying RF coil boxes that are in light contact with the cheek, prohibiting any lateral or rotational cranial movement. Anterior or posterior cranial movement, although possible, was significantly minimized by the snug position of the coil boxes.

Double-blind Format

The ETG was situated outside of the MRI room and hidden within a specially constructed wooden box with holes through which the lead wires were connected to the unit (active group) or just taped to the inside of the box (placebo group) by the center engineer. When viewed from the outside, the lead wires were seen as entering the box through the same holes regardless of their internal connection. After each subject was properly positioned within the magnet, the lead wires were re-attached to the ETG by the center engineer, without the principle investigator or subject in view. The principle investigator and research associate would leave the magnet area and wait in an adjacent section of the MMRRCC, from which the ETG could not be seen. Therefore, neither the subject, research associate nor the principle investigator was aware as to whether active or placebo stimulation was being performed.

At the end of the testing session, the center engineer removed the lead wires from the wooden box outside of the view of the principle investigator and research associate. Both the active stimulation and placebo protocols required activation of the ETG, which produced an audible sound equivalent to the pulse frequency, regardless of whether the lead wires were taped to the inside of the box or inserted into the appropriate receptacle for closed transmission of the microamperage current. Considering that the amplitude of the active stimulation was sub-perception and the placebo protocol did not utilize active stimulation,

none of the subjects were able to perceive any sensation of stimulation, therefore eliminating this factor as a variable.

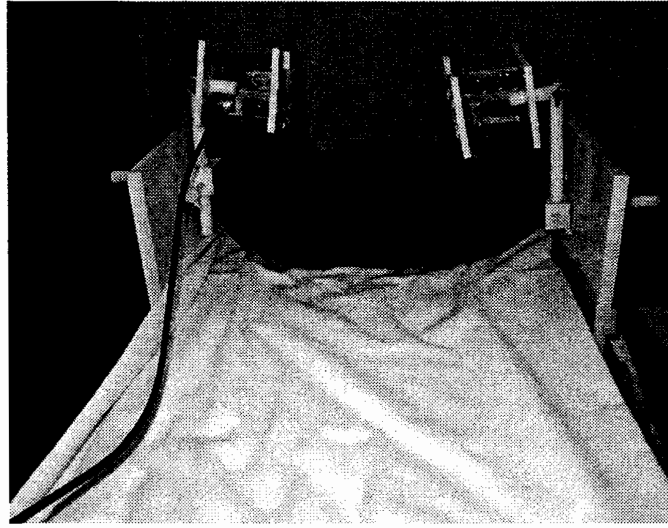


Figure 11. Overhead view of headgear apparatus depicting cervical support pillow, RF coil boxes and positioning rods.

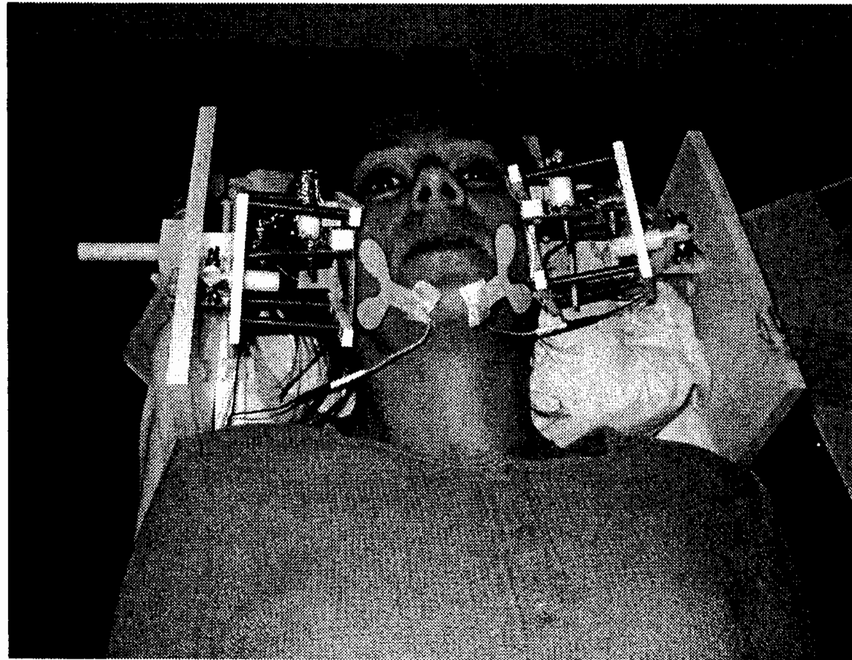


Figure 12. Subject supine with headgear, cervical pillow, electrodes and RF coil boxes placed adjacent to each masseter.

MRS Standardization Process

After the RF boxes that housed each coil were again re- aligned by the principle investigator via manipulation of the positioning rods to ensure that the coils were situated between and not overlying the electrodes, the MRS standardization process commenced. This process consisted of a step by step sequence of events, as outlined in AppendixE, to allow for optimal spectra acquisition. Each RF coil was first oscilloscopically tuned and matched to the resonant frequencies of phosphorus and proton at the 2 Tesla magnet strength, which is also known as the static field strength.

The plinth was then pushed into the magnet bore and proper positioning established in order to ensure that the required area of interest (masseter) was centered and placed 37.5cm below the superior surface of the magnet bore. The magnet room door was then closed and the MRS operational software program was opened and activated with the computer located outside the magnet room, as shown in Figures 13-15.

The process of shimming on the proton signal commenced in order to obtain optimal homogeneity around the masseter of interest. The greater strength of this magnet (2 Tesla) allows for a clearer delineation of the chemical shift of the phosphorus peaks, as shown in Figure 1, thus making spectral analysis better, but not equivalent to that of a 3-4 Tesla magnet.

The shimming and baseline spectra were attained with the electrodes attached adjacent to each masseter and connected to the idle stimulation unit, a process that required about 20-30 minutes. After completing the baseline phosphorus spectral acquisition, the unit was activated by the center engineer in the manner previously presented, which kept the subject, principle investigator and research associate blind to the paradigm of placebo or active stimulation. The testing protocol then proceeded continually for a period of one hour.

Figure 13. Closed wooden box housing the ETG, with lead wires running into the magnet room to the electrodes.

Figure 13 illustrates the wooden box and extruding lead wires, which kept the ETG from view of the principle investigator and research associate. Table 4

highlights the methodology used to maintain a double-blind process throughout the testing period.

Table 4

Double-Blind Format

Double-Blind Format
Subject selects coin from box designating active/placebo group
Pre-stimulation sub-max level test and clinical data obtained
Subject is placed in magnet room and positioned in magnet
Lead wires attached to the ETG by center engineer, without view by the investigator or research associate and the blinding box closed.
Coil tuning procedure is performed
Shimming procedure is performed
ETG is activated by center engineer, while investigator is absent from research area
ETG noise remains consistent for all tests (active or placebo)
Center engineer turns ETG off after 48-60 minute data acquisition and removes lead wires, while investigator is absent from research area

Spectral Acquisition

Stimulation continued uninterrupted for one hour, during which time spectra were acquired at baseline, 20-32 and 48-60 minute time-points, from either masseter (normal subjects) and the most painful masseter (TMD subjects), during the stimulation period. The computer program that controlled the spectrographic process acquired data every four seconds by obtaining 12 sums of the free

induction decay (FID) per RF pulse from 15 FID's. The FID represents the sinusoidal signal generated by spins on the x-y plane that decays exponentially with time so that the amplitude of the signal becomes smaller as the net magnetization returns to equilibrium. Since each FID takes 4 seconds and is summed 12 times, acquisition of the entire spectra for each time-point required 12 minutes.

The MRS standardization process, shimming, baseline data acquisition and the subsequent stimulation period, necessitated that each subject remain within the magnet for approximately 90 minutes. Figure 14 illustrates the shimming console situated outside of the magnet room.

Figure 14. MMRRCC shimming console for signal optimization.

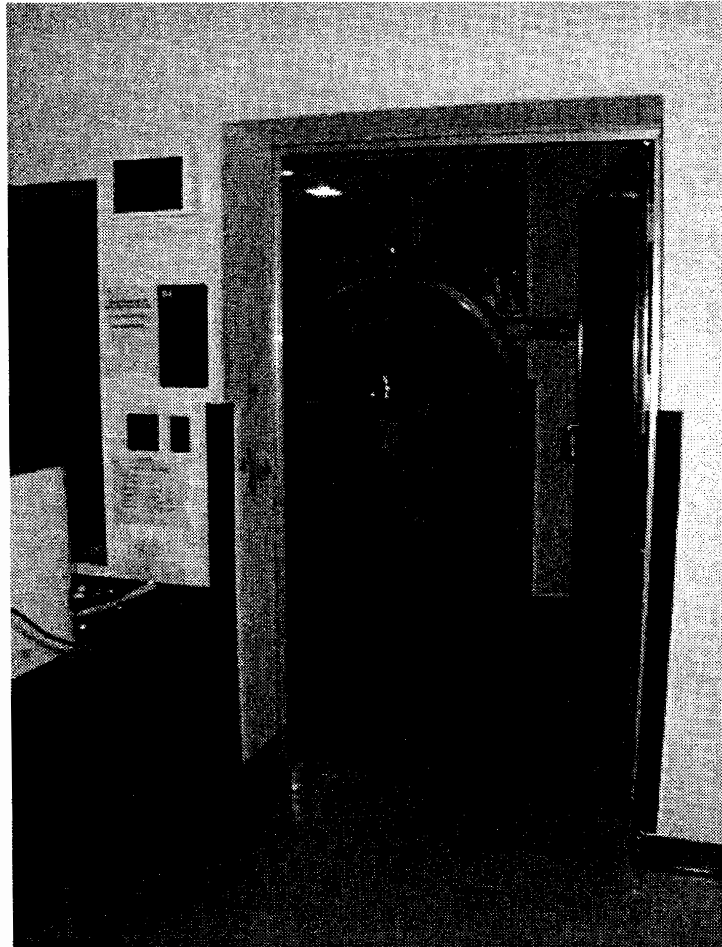


Figure 15. Subject positioned in bore of magnet.

The subject is positioned head first inside the magnet bore with only the feet showing in Figure 15. Upon completion of the testing sequence, each subject was brought out of the magnet, RF coils and electrodes were removed from each cheek, and the subject was allowed to get up. Immediately upon exiting the

magnet room, the principle investigator performed the post stimulation clinical data (VAS, ROM and PPT) acquisition.

Statistical Methodology

The study consisted of a randomized controlled trial that utilized a pre-test-post-test design with repeated measures. This was a single application exposure paradigm, which negated the effect of practice or carryover. The acquired data were obtained from the interaction of the independent variable (MENS) with four dependent variables (Pi, PCr, Pi/PCr, pHi) known as metabolic measures. Pi and PCr values were used to calculate Pi/PCr and perform the statistical tests necessary to test the hypotheses. Alpha was set at .05 for all tests and data was analyzed across and through the three time-points by single subject and group comparisons over the entire length of the stimulation or placebo exposure. A test of significance was completed for each group of subjects, delineated by diagnosis (TMD and normal) as well as by type of exposure (active or placebo) on the key metabolic indicators (Pi/PCr and pHi differences) at and between each time-point. Therefore, three measures of change over time were analyzed in the full data set:

1. Measure at baseline to 20-32
2. Measure at baseline to 48-60
3. Measure at 20-32 to 48-60

Within the cohort of TMD subjects, there were three additional clinical measures taken over a single time period (baseline to 48-60) consisting of the relationship of masseteric PPT, VAS and active ROM. Algometric measurements

are missing for 4 of the TMD/active subjects and 1 TMD/placebo subject who completed testing prior to obtaining the algometer. In addition, due to spectral interference, there were not enough subjects who had acceptable Pi/PCr to compare with algometric pressure pain threshold measurements, therefore negating any statistical analysis for this specific clinical measure.

Data un-blinding of the subjects and subsequent statistical computation were performed upon the completion of all tests. Means and standard deviations of Pi, PCr, Pi/PCr and pHi were calculated for the active and placebo groups at each of three time-points. The Pi/PCr and pHi data were subsequently analyzed using paired and unpaired two-tailed *t*-tests, with alpha set at .05, as previously specified.

When completing many statistical tests within the same "family" of data, there is a compounding effect upon the Type I (alpha) error rate, and that across such a family of tests, the overall rate will be substantially higher than the nominal rate of .05. A family-wise error control method, such as a Bonferroni, can be used when a large number of related statistical tests are being conducted; however, due to the very small samples in this experiment, no such adjustment was made in determining the statistical significance of the test results. However, when applicable, an ANOVA was also completed on the Pi/PCr and pHi differences between each time-point and a summary of the testing methodology follows:

Active Stimulation Comparisons

Normal Subjects:

Baseline Pi/PCr and pHi measures were compared to the 20-32 & 48-60 time-points. H_0: $mean_{baseline} = mean_{time1}$ assessed using a *t*-test of differences (Metabolic $measure_{time1}$-metabolic $measure_{baseline}$. H_0: $mean_{baseline} = mean_{time2}$ assessed using a two-sided *t*-test of differences (Metabolic $measure_{time2}$-metabolic $measure_{baseline}$). The test is Ho : $mean_{difference} = 0$, which is the same as a unpaired t-test of Ho: $mean_1 = mean_2$.

TMD Subjects:

Baseline metabolic measures were compared to the 20-32 & 48-60 time-points. H_0: $mean_{baseline} = mean_{time1}$ assessed using unpaired two-sided *t*-tests of the differences (Metabolic $measure_{time1}$-metabolic $measure_{baseline}$). H_0: $mean_{baseline} = mean_{time2}$ assessed using a *t*-test of differences (Metabolic $measure_{time2}$ – metabolic $measure_{baseline}$).

Group Comparison:

An unpaired two-sided *t*-test of the independence of the change in the mean Pi/PCr and pHi values across the normal and TMD groups was performed, *Ho:* $change_{normal} = change_{TMD}$. This test was conducted under the assumption of equal variance, but the two groups may not have demonstrated constancy of variance (homoscedasticity). (De Angelis, 1990; Portnoy, 2000; Runyon, 1994; Streiner, 1986).

Placebo Comparisons

TMD Subjects:

Baseline Pi/PCr and pHi measures were compared to the 20-32 & 48-60 time-points. H_0: $mean_{baseline} = mean_{time1}$ assessed using unpaired two-sided t-tests of the differences (metabolic $measure_{time1}$-metabolic $measure_{baseline}$). H_0: $mean_{baseline} = mean_{time2}$ assessed using a t-test of differences (Metabolic $measure_{time2}$ – metabolic $measure_{baseline}$).

Normal Subjects:

Baseline Pi/PCr and pHi measures were compared to the 20-32 & 48-60 time-points. H_0: $mean_{baseline} = mean_{time1}$ assessed using unpaired two-sided t-tests of the differences (Metabolic $measure_{time1}$-metabolic $measure_{baseline}$). H_0: $mean_{baseline} = mean_{time2}$ assessed using unpaired two-sided t-tests of the differences (Metabolic $measure_{time2}$ – metabolic $measure_{baseline}$).

Group Comparison:

Two-sided unpaired t-tests of the independence of change in the mean value of Pi/PCr and pHi across the normal and TMD groups was performed, H_0: $change_{normal} = change_{TMD}$. These tests were also conducted under the assumption of equal variance, but again the two groups may not have demonstrated constancy of variance. In addition, ANOVA analyses were performed on two class levels of data (diagnosis and type of exposure) for the combined subject groups (TMD + normal) by the general linear model (GLM).

Pi/PCr and Clinical Data Correlation

Correlation scores can provide an index reflecting a qualitative measure of either a positive (+) or negative (-) relationship between two variables, with a score in either direction close to 1.00, indicative of the strength of the relationship. The correlation of clinical data to Pi/PCr values for the TMD subjects at two corresponding time-points (baseline and at the end of the active or placebo exposure) was anticipated, but there were not enough subjects to adequately analyze, and a comparison of this type must be viewed with great caution as the values will vary substantially in direction as well as magnitude. Furthermore, the small size of the groups (7 active, 4 placebo) can easily create spurious relationships in measures such as correlations.

Data Analysis

Upon finalization of testing, spectral analysis was performed for each of the complete subject exposure tests (active and placebo) that yielded acceptable spectra, to determine the areas under the Pi and PCr spectral peaks, as well as chemical shifts in respect to the PCr resonance, as illustrated in Figure 1. This was followed by calculation of the ratio of Pi to PCr in order to obtain the Pi/PCr for all subjects and each group at the three time-points. The interface description language software program, utilized by the MMRRCC, was used to integrate the areas under each peak from which Pi/PCr was calculated. *

* Interface Description Language. Research Systems Inc. Boulder, Colorado

The data were subsequently organized into tables delineating the Pi, PCr, Pi/PCr and pHi individual and group mean values. Probability values less than .05 for the Pi/PCr and pHi analyses are highlighted in bold within each applicable table. Statistical computations were performed with SAS version 8.2.

Chapter IV

RESULTS

Explanation of Lost Spectra

A total of 41 individual subject tests were attempted, as listed in Tables 7 and 8, but upon subsequent inspection of the raw data and acquired spectra, only 23 tests were deemed sufficient for the determination of the phosphorus peaks, as listed in Table 7. The remaining 18 tests did not yield acceptable spectra and were either aborted due to periodic magnet malfunction or signal to noise ratio (SNR) interference, as illustrated in Table 9. The SNR is the ratio between the amplitude of the received signal and background noise, which can obscure it. However, in nine of these 18 tests the subject completed the full testing period within the magnet, with either the active or placebo protocol without alteration of the type of exposure, which resulted in the acquisition of pre and post clinical data without acceptable spectra. Although incomparable to Pi/PCr, this clinical data was viewed as feasible for analyses without a Pi/PCr comparison.

The spectra acquired after 7/13/01 were unable to be analyzed due to increasing interference problems at the MMRRCC that affected the SNR, which is critical when performing MRS. The microamperage signal is of an extremely low amplitude and when interference was caused by noises that could not be eliminated

it was unable to be clearly delineated from that of other low-intensity noise. This problem is specifically deleterious in the case of phosphorus (^{31}P) spectroscopy, due to its inherent small SNR.

The proton (^{1}H) signal, which is obtained from water, provides a very sharp spectra and has a strong SNR, because it is present in high concentration in all biological tissues. In comparison, the phosphorus (^{31}p) signal is much weaker, as its concentration and gyromagnetic ratio (γ) are much less, hence the signal is quite small and any interference can easily negate obtaining a spectra that can be properly analyzed. The SNR is of greater importance in low power magnets, such as the 2T, which was used in this study.The generating data for all of the statistical calculations and plots that follow are included in the EXCEL file of Appendix B.

Results Specific to Hypothesis I

Tables 5 and 6 contain all of the acquired metabolic and clinical data for each of the 41 subjects that were tested during the entire period of the study. Table 5 is specific for all subjects with both clinical data and acquired spectra sufficient to calculate Pi/PCr values at each time-point. Table 6 lists the remaining subjects in which the test was either aborted or SNR caused spectral interference that did not allow for Pi/PCr analysis, but for whom part or all of the clinical data was able to be acquired.

Table 5

Tests with Completed Pi/PCr and Clinical Data

Subject	Type	Parameters	Clinical Data	Baseline Pi/PCr	20-30 Minutes	48-60 Minutes
MKS022500	N	60Hz @ 40µa	N/A	0.215438	0.181186	0.314251
JVH030300	N	60Hz @ 20µa	N/A	0.249714	0.287653	0.225361
GG030700	N	60Hz @10µa	N/A	0.222216	0.251254	0.329478
BC03100	N	40Hz @ 40µa	N/A	0.223767	0.281820	0.349570
SM032100	N	20Hz @ 40µa	N/A	0.150605	0.274311	0.261672
DW032800	N	3Hz @ 40µa	N/A	0.172192	0.259567	0.221588
BP032900	N	3Hz @ 20µa	N/A	0.111394	0.210688	0.231527
WON091500	N	10Hz @ 20µa	N/A	0.287593	0.316012	0.278983
JM040500	TMD	60Hz @ 40µa	A=N/A VAS=2.8 38mm	0.308308	0.191672	0.315992 A=N/A VAS=0.5 42mm
KG041900	TMD	40Hz @ 20µa	A=N/A VAS=6.8 8mm	0.185131	0.392942	0.316942 A=N/A VAS=4.4 16mm
HS050500	TMD	3Hz @ 20µa	A=N/A VAS=2.0 42mm	0.179045	0.312824	0.354415 A=N/A VAS=0.0 45mm
BB052700	TMD	10Hz @ 20µa	A=N/A VAS=3.0 29mm	0.192062	0.262393	0.194709 A=N/A VAS=2.0 29mm
NW110700	TMD	40Hz @ 20µa	A=N/A VAS=5.0 35mm	0.152432	0.283221	0.254107 VAS=1.2 41mm

Table 5 Continued:

<u>Tests with Completed Pi/PCr and Clinical Data</u>

OP020901	TMD	3Hz @ 60μa	A=2.53 VAS=5.4 28mm	0.151427	0.121174	0.232618 A=2.96 VAS=0.0 52mm
KS111000	TMD	3Hz @ 20μa	A=1.0 VAS=5.4 25mm	0.181228	0.182610	0.199435 A=1.56 VAS=1.5 34mm
HS102700	TMD	Placebo	A=N/A VAS=5.6 36mm	0.217637	0.206460	0.216542 A=N/A VAS=5.2 36mm
OP120800	TMD	Placebo	A=2.13 VAS=5.5 24mm	0.278720	0.241237	0.163907 A=1.96 VAS=0.8 30mm
MB020201	TMD	Placebo	A=2.86 VAS=6.2 20mm	0.270713	0.225362	0.250816 A= 1.53 VAS=6.4 20mm
OP032701	TMD	Placebo	A=2.53 VAS=7.0 30mm	0.171605	0.212388	0.184510 A=1.93 VAS=8.0 32mm
BP032800	N	Placebo	N/A	0.221571	0.262074	0.313087
DW032900	N	Placebo	N/A	0.136664	0.132750	0.128368
KI010501	N	Placebo	N/A	0.175952	0.231886	0.265106
TH102000	N	Placebo	N/A	0.201187	0.148722	0.143680

Legend:
N = normal subject
TMD = pathological subject
A = algometer pain pressure threshold
VAS = visual analogue scale
mm = millimeters of vertical ROM
N/A = not applicable

Table 6

Aborted Tests with or without Complete Clinical Data

Subject	Type	Parameters	Problem	Baseline	20-32 Min	48-60 Min
MB022700	TMD	Magnet Malfunction (Active)	Aborted	---------	----------	--------
CS032700	N	Placebo	SNR	--------	---------	---------
DW071301	N	Placebo	SNR	--------	--------	--------
OP071701	TMD	3Hz @ 40μa	SNR	A= 1.53 13mm VAS=9.0	---------	A=2.76 27mm VAS=0.0
JS072100	TMD	10Hz @ 20μa	Magnet Malfunction	A=N/A 24mm VAS=5.2	Aborted	--------
SA080801	TMD	Placebo	SNR	A=2.90 25mm VAS=6.60	---------	A=2.92 29mm VAS=5.0
DG081401	TMD	Placebo	SNR	0.800544 A=0.40 22mm VAS=4.6	---------	----------- A=0.26 22mm VAS=5.2
DG081701	TMD	3Hz @ 20μa	SNR	A=0.33 25mm VAS=3.6	--------	A=0.70 27mm VAS=3.2
OP090401	TMD	3Hz @ 60μa	SNR	A=1.76 25mm VAS=8.0	Trouble Getting Signal	Aborted After 20 Minutes
OP101201	TMD	3Hz @ 20μa	SNR	A=1.83 25mm VAS=6.45	Trouble Getting Signal	Aborted After 20 Minutes
CS020502	N	3Hz @ 60μa	SNR	---------	--------	--------
DB021502	TMD	3Hz @ 60μa	SNR	A=1.36 30mm VAS=8.2	---------	A=2.03 35mm VAS=2.5
OP022602	TMD	3Hz @ 60μa	SNR	A=1.43 20mm VAS=6.5	---------	A=2.63 30mm VAS=0.6
OP030502	TMD	3Hz @ 60μa	SNR	A=1.60 23mm VAS=8.4	---------	A= 3.10 43mm VAS=0.0

Table 7

Pi/PCr Values for Normal Subjects (Active)

Subject	Parameters	DX	Stim	Pi/PCr Base	Pi/PCr 20-32	Pi/PCr 48-60
MKS022500	60Hz @ 40μa	Normal	Active	0.215438	0.181186	0.314251
JVH030300	60Hz @ 20μa	Normal	Active	0.249714	0.287653	0.225361
GG030700	60Hz @ 10μa	Normal	Active	0.222216	0.251254	0.329478
BC03100	40Hz @ 40μa	Normal	Active	0.223767	0.281820	0.349570
SM032100	20Hz @ 40μa	Normal	Active	0.150605	0.274311	0.261672
DW032800	3Hz @ 40μa	Normal	Active	0.172192	0.259567	0.221588
BP032900	3Hz @ 20μa	Normal	Active	0.111394	0.210688	0.231527
WON091500	10Hz @ 20μa	Normal	Active	0.287593	0.316012	0.278983
Mean				0.204115	0.257811	0.276554
SD				0.056530	0.043531	0.049924

The mean ratio of inorganic phosphate to phosphocreatine, in the group of eight normal subjects exposed to the microamperage stimulation protocol, increased from the baseline value at both the 20-32 and 48-60 minute time-points respectively. Descriptive charts highlight the changes from one time-point to the next over the course of the one-hour exposure to MENS.

The Pi/PCr chart of Figure 16 illustrates that in the group of 8 normal subjects exposed to MENS, all except one demonstrated an increase in Pi/PCr from baseline to the 20-32 minute time-point. However, there was a decline in

Pi/PCr for 4 subjects between the 20-32 and 48-60 time-points, but only below the baseline level in 2 of these subjects, as illustrated in Figures 17 and 18. Figure 19 compares the overall change from baseline to the 48-60 time-point and reveals that these same 2 subjects were the only 2 of 8 that did not show an increase above baseline at the end of one-hour of MENS, yet had an elevation at the 20-32 time-point. Therefore exposure to MENS yielded an elevation of Pi/PCr in every one of the 8 normal subjects at one or both time-points, with significant differences between baseline and the 20-32 as well as the 48-60 minute time-points, as shown in Table 16.

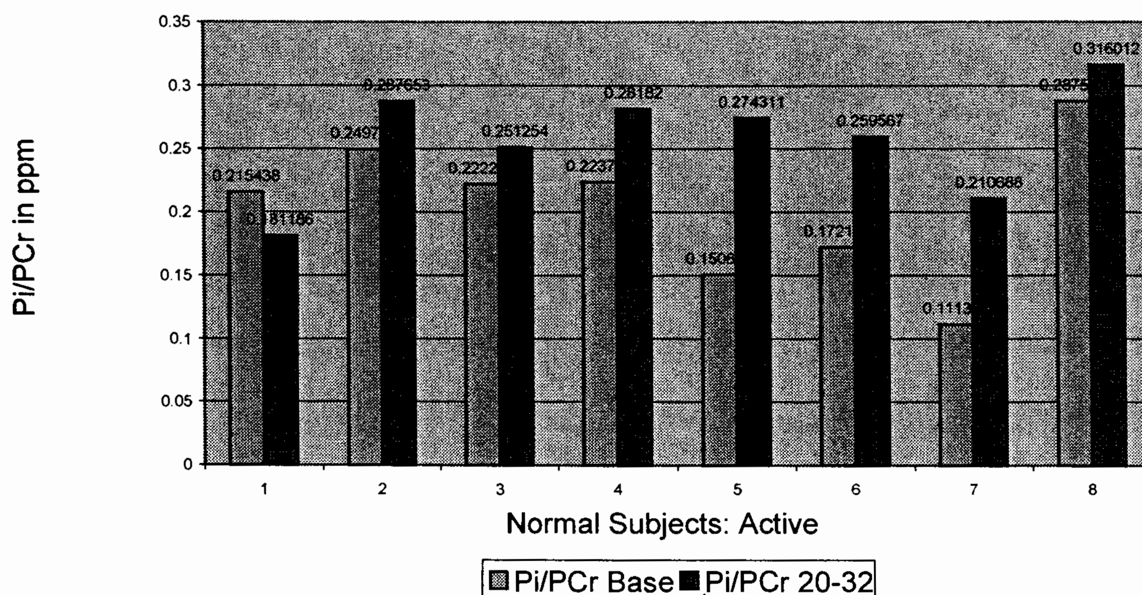


Figure 16. Change in Pi/PCr from baseline to the 20-32 minute time-point for each normal subject exposed to MENS.

All subjects except the first demonstrated increased Pi/PCr values from baseline at the 20-32 minute time-point. The mean change in Pi/PCr values within these time-points was significant as shown in Table 16.

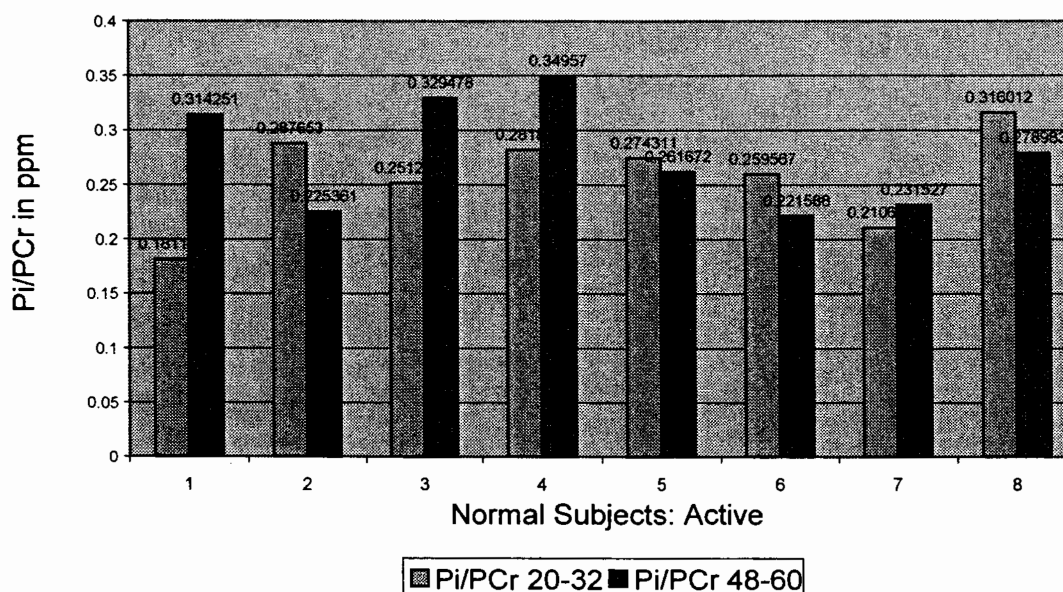


Figure 17. Change in Pi/PCr from the 20-32 to 48-60 minute time-point for each normal subject exposed to MENS.

The chart of Figure 17 reveals that 4 of the normal subjects demonstrated a decrease and 4 an increase in Pi/PCr values between the 20-32 and 48-60 time-points.

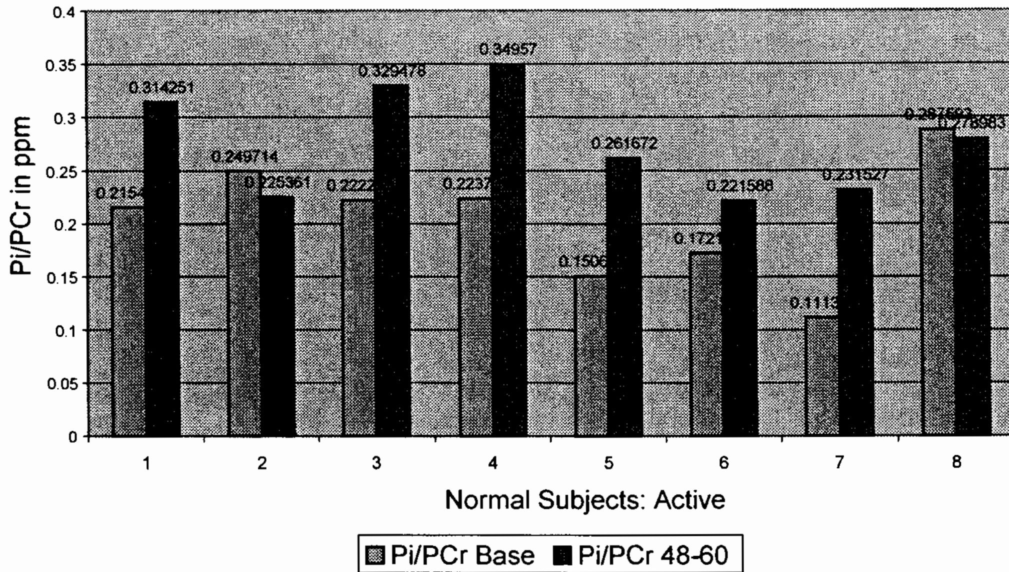


Figure 18. Change in Pi/PCr from baseline to the 48-60 minute time-point for each normal subject exposed to MENS.

The two-sided t-test scores for this group of 8 normal subjects exposed to MENS, revealed a significant elevation in mean Pi/PCr from baseline to the 20-32 as well as the 48-60 time-point with probability scores of 0.01860 and 0.01100, respectively.

Table 8 presents the Pi/PCr data for each of the 7 TMD subjects exposed to microcurrent stimulation and their respective stimulation parameters.

Table 8

Pi/PCr Values for TMD Subjects (Active)

Subject	Parameters	DX	Stim	Pi/PCr Base	Pi/PCr 20-32	Pi/PCr 48-60
JM040500	60Hz @ 40μa	TMD	Active	0.308308	0.191672	0.315992
KG041900	40Hz @ 20μa	TMD	Active	0.185131	0.392942	0.316942
HS050500	3Hz @ 20μa	TMD	Active	0.179045	0.312824	0.354415
BB052700	10Hz @ 20μa	TMD	Active	0.192062	0.262393	0.194709
NW110700	40Hz @ 20μa	TMD	Active	0.152432	0.283221	0.254107
KS11100	3Hz @ 20μa	TMD	Active	0.181228	0.18261	0.199435
OP020901	3Hz @ 60μa	TMD	Active	0.151427	0.121174	0.232618
Mean				0.192805	0.249548	0.266888
SD				0.533320	0.091446	0.062816

The mean ratio of inorganic phosphate to phosphocreatine, in the group of 7 TMD subjects exposed to the microcurrent stimulation protocol, increased from the baseline value at both the 20-32 and 48-60 minute time-points respect- tively. However, there was a decrease from baseline to the 20-32 minute time- point in two subjects, both of whom demonstrated an increase at the 48-60 minute time-point that was above that of their respective baseline value, as highlighted in Figures 19-21.

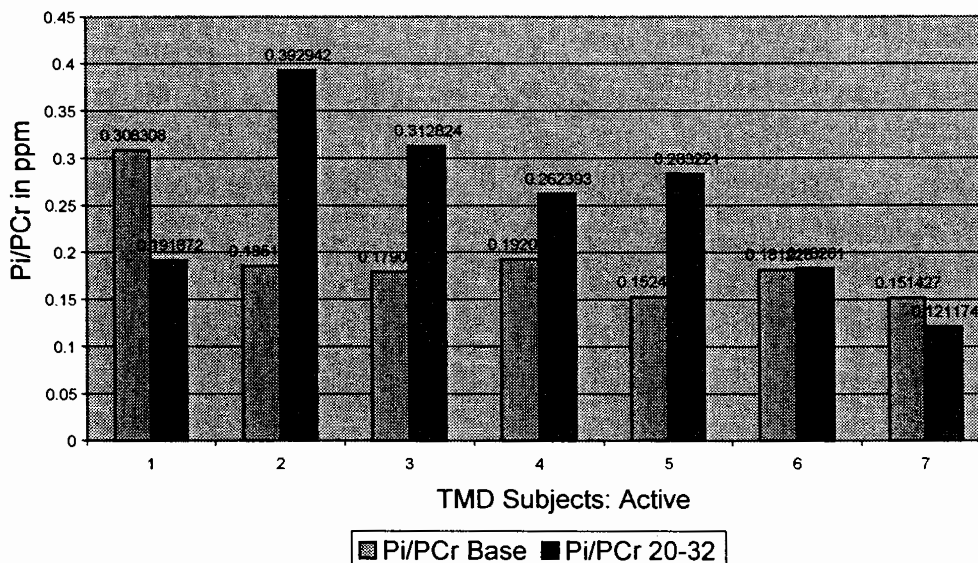


Figure 19. Change in Pi/PCr from baseline to the 20-32 minute time-point for the 7 TMD subjects exposed to MENS.

Subjects 1 and 7 had a decline in Pi/PCr from their baseline values at the 20-32 minute time-point, as shown in Figure 19. However the same subjects demonstrated increased Pi/PCr values at the 48-60 minute time-point, as depicted in Table 9 and Figures 20-21. Therefore all 7 TMD subjects demonstrated increased Pi/PCr values at the one-hour exposure to MENS.

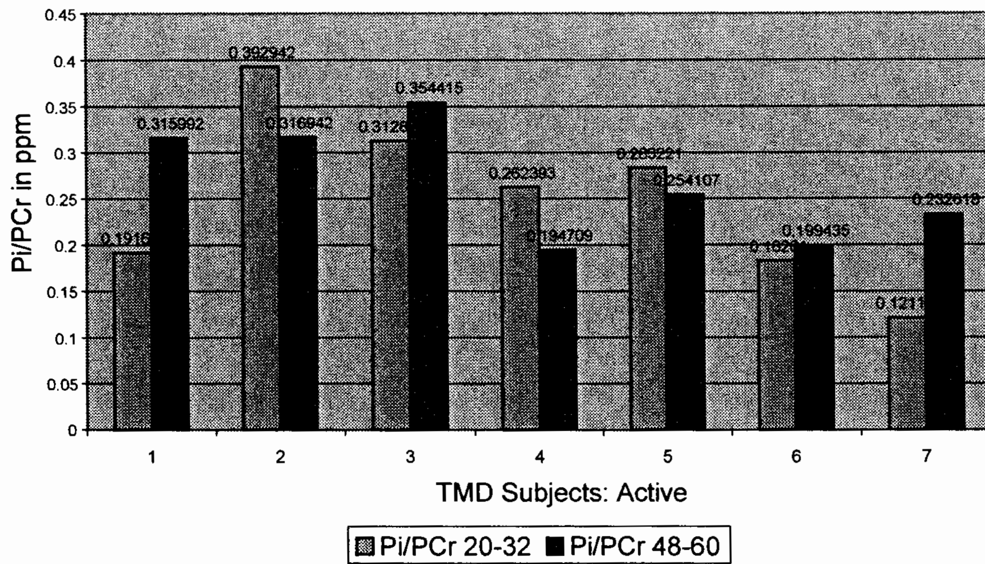


Figure 20. Change in Pi/PCr from the 20-32 to the 48-60 minute time-points for the seven TMD subjects exposed to microcurrent stimulation.

Subjects 1 and 7 demonstrated an elevation of Pi/PCr during this time period, which remained above that of their respective baseline value at the end of the stimulation period. However, subjects 2, 4 and 5 demonstrated a decline in Pi/PCr during the 20-32 to 48-60 minute stimulation period.

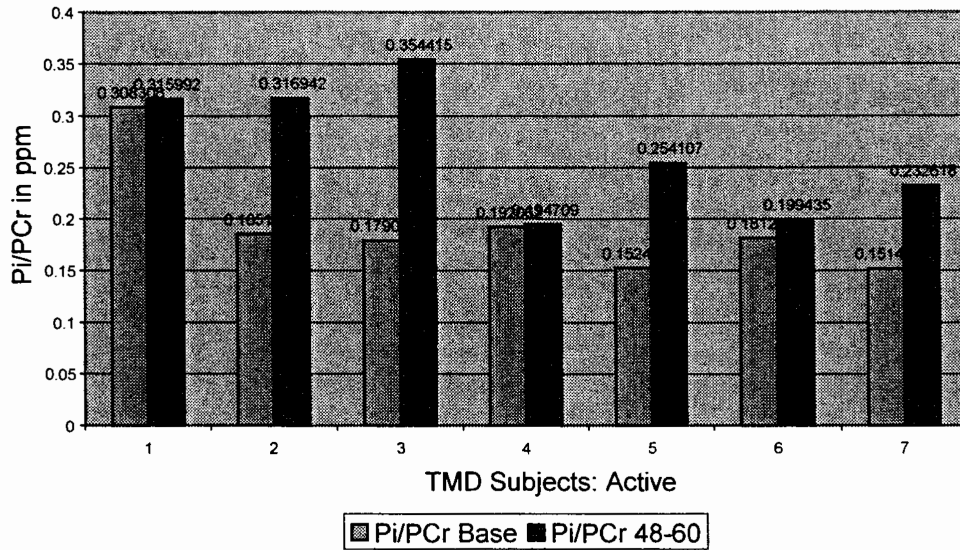


Figure 21. Change in Pi/PCr from baseline to the 48-60 minute time-point for the 7 TMD subjects exposed to microcurrent stimulation.

All 7 TMD subjects demonstrated elevated Pi/PCr from baseline at the end of the one-hour stimulation period, which was significant as shown in Table 8. Table 11 delineates the Pi/PCr data for the 4 TMD subjects exposed to the placebo protocol.

Table 9

Pi/PCr Values for TMD Subjects (Placebo)

Subject	Parameters	DX	Stim	Pi/PCr Base	Pi/PCr 20-32	Pi/PCr 48-60
HS102700	Placebo	TMD	Placebo	0.217637	0.20646	0.216542
OP120800	Placebo	TMD	Placebo	0.278720	0.241237	0.163907
MB020201	Placebo	TMD	Placebo	0.270713	0.225362	0.250816
OP32701	Placebo	TMD	Placebo	0.171605	0.212388	0.184510
Mean				0.234669	0.221362	0.203944
SD				0.050023	0.015423	0.038019

The mean ratio of inorganic phosphate to phosphocreatine, in this group of 4 TMD subjects exposed to the placebo protocol demonstrated a minimal, but progressive decrease from the baseline value at both the 20-32 and 48-60 minute time-points respectively, as illustrated in Figures 22-24.

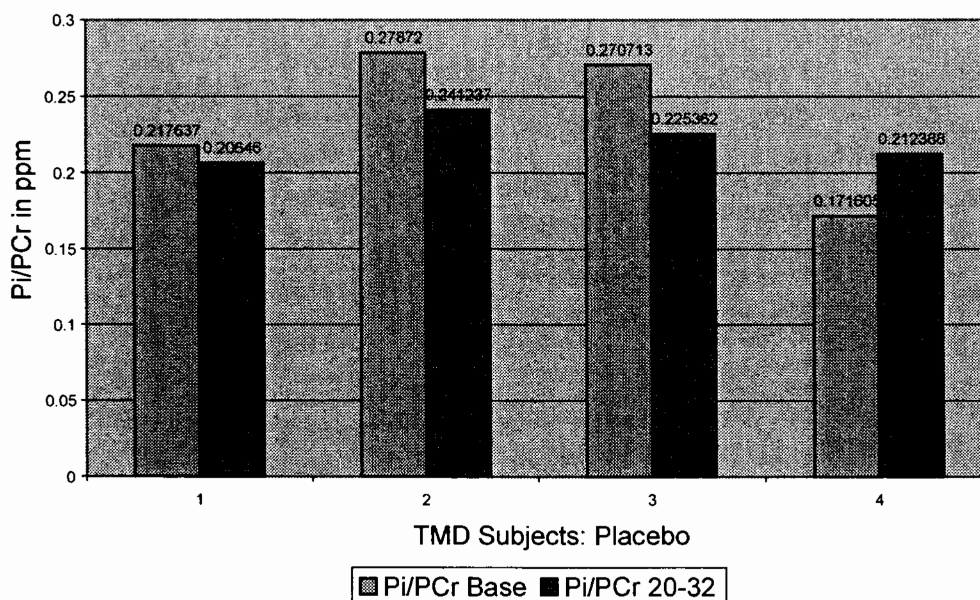


Figure 22. Change in Pi/PCr from baseline to the 20-32 minute time-point for the 4 TMD subjects exposed to the placebo protocol.

Only one of the 4 TMD subjects demonstrated an increase in Pi/PCr at the 20-32 minute time-point from the baseline value with the placebo protocol.

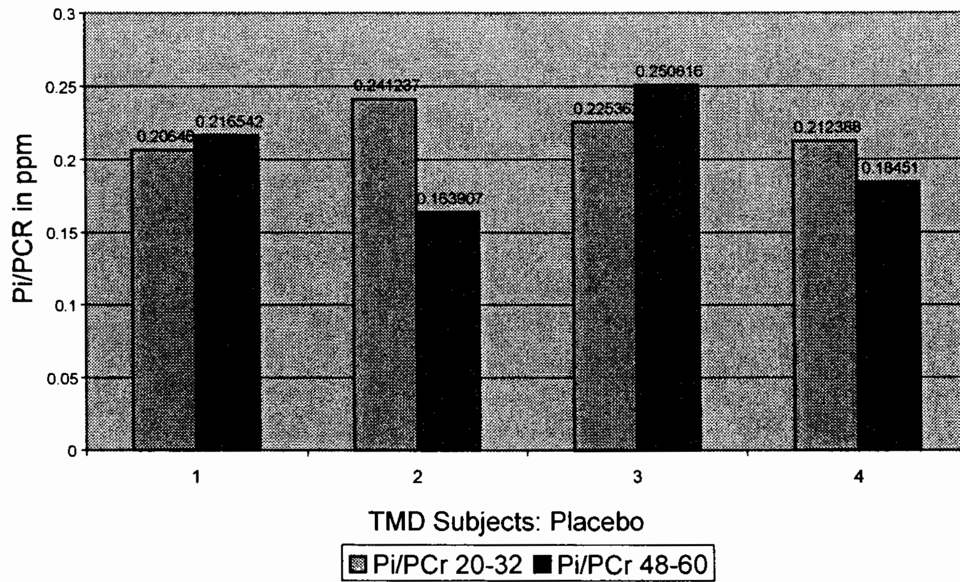


Figure 23. Change in Pi/PCr from the 20-32 to 48-60 minute time-point for the 4 TMD subjects exposed to the placebo protocol.

Two subjects demonstrated elevated Pi/PCr and the other two a decline in Pi/PCr between the 20-32 and 48-60 minute stimulation period.

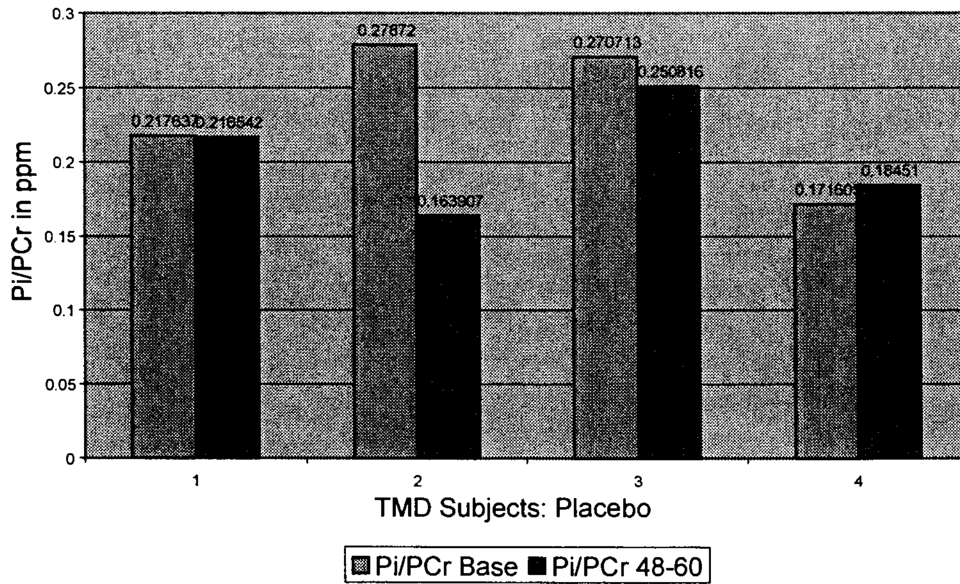


Figure 24. Change in Pi/PCr from baseline to the 48-60 minute time-point for the 4 TMD subjects exposed to the placebo protocol.

Three of the 4 TMD subjects exposed to the placebo protocol demonstrated a decline in Pi/PCr at the end of the one-hour testing period. There was a minimal increase in Pi/PCr at the end of the testing session for the other subject.

Table 10

Pi/PCr Values for Normal Subjects (Placebo)

Subject	Parameters	DX	Stim	Pi/PCr Base	Pi/PCr 20-32	Pi/PCr 48-60
BP032800	Placebo	Normal	Placebo	0.221571	0.262074	0.313087
DW032900	Placebo	Normal	Placebo	0.136664	0.132750	0.128368
TH102000	Placebo	Normal	Placebo	0.201187	0.148722	0.143680
KI010501	Placebo	Normal	Placebo	0.175952	0.231886	0.265106
Mean				0.183849	0.193858	0.212560
SD				0.036571	0.062905	0.090737

The mean ratio of inorganic phosphate to phosphocreatine, in this group of 4 normal subjects exposed to the placebo protocol, increased slightly from the baseline value at both the 20-32 and 48-60 minute time-points respectively. However, there were both increases and decreases in values among the 4 subjects, but none of the group changes was significant. Figures 25-27 illustrate the mean change in Pi/PCr at each respective time-point.

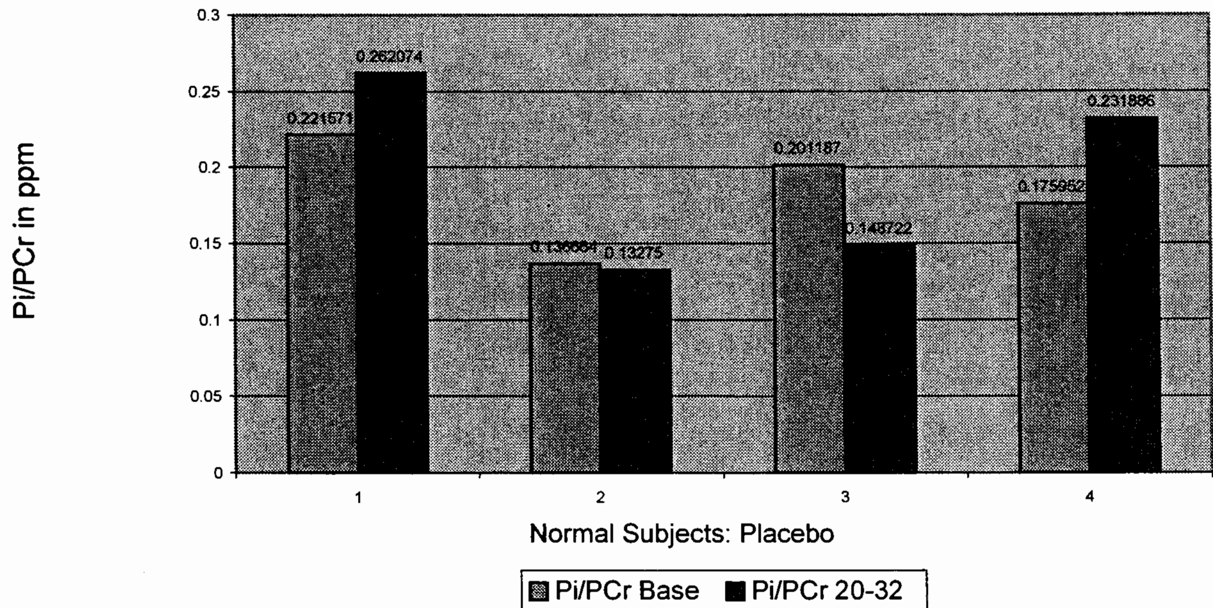


Figure 25. Change in Pi/PCr from baseline to the 20-32 minute time-point for the 4 normal subjects exposed to the placebo protocol.

Normal subjects 1 and 4 demonstrated an increase in Pi/PCr at the 20-32 minute time-point from baseline, while Pi/PCr decreased for subjects 2 and 3.

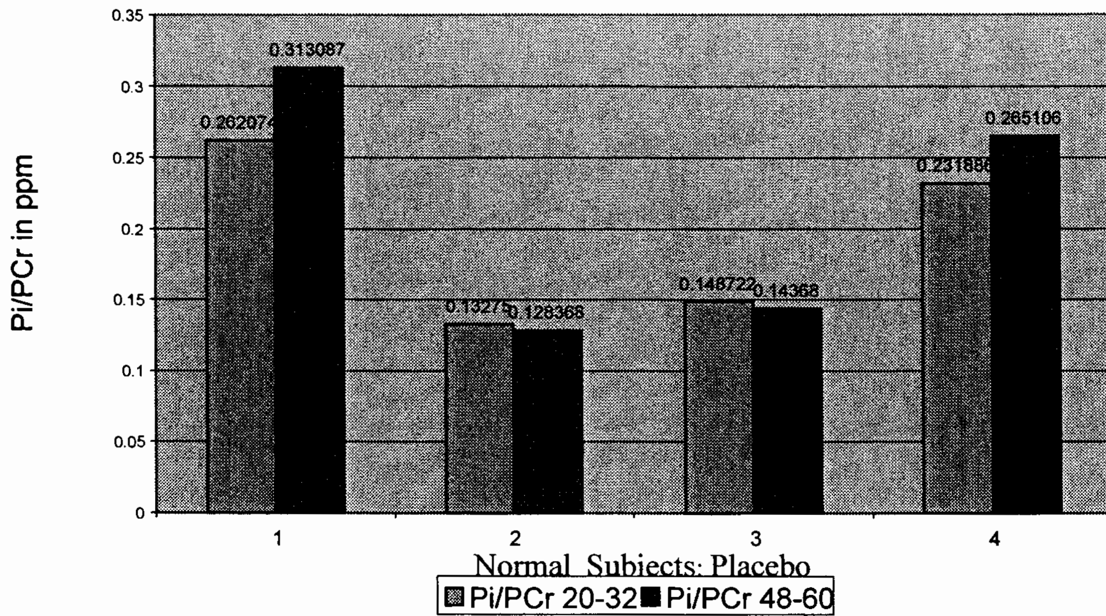


Figure 26. Change in Pi/PCr from the 20-32 to 48-60 minute time-points for the 4 normal subjects exposed to the placebo protocol.

A similar pattern among the 4 subjects was seen during the 20-32 to 48-60 minute time-points, as occured from baseline to the 20-32 minute time-point.

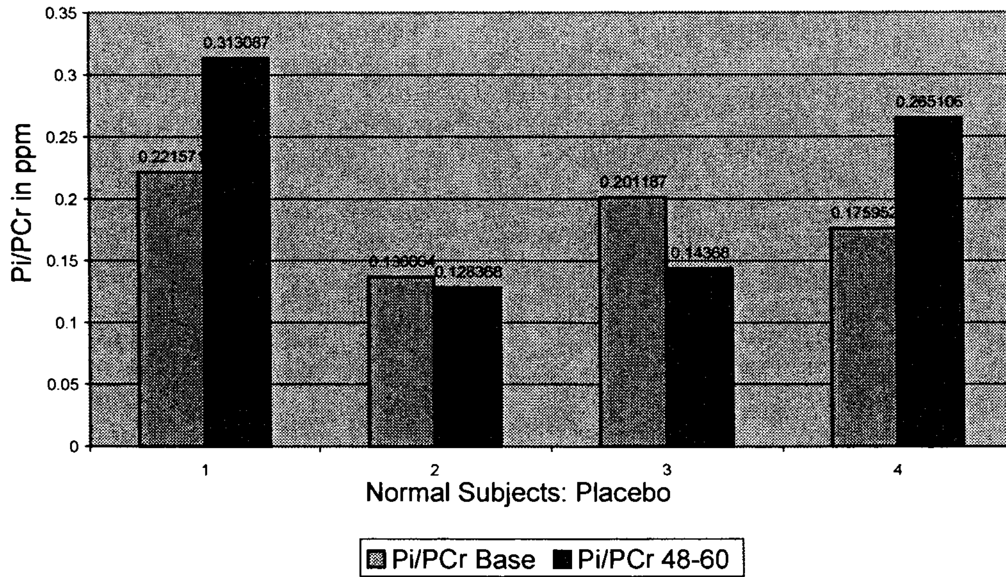


Figure 27. Change in Pi/PCr from baseline to the 48-60 minute time-point for the 4 normal subjects exposed to the placebo protocol.

At the end of the one-hour exposure to the placebo protocol, there was an elevation in 2 and a decline in 2 normal subjects relative to the effect of microcurrent stimulation.

Table 11 delineates the differences in Pi/PCr values between the three time-points, as well as repeated measures, for each of the 23 subjects, according to their diagnostic category and stimulation protocol. ANOVA calculations and two-sided unpaired t-tests were performed to further analyze the data for statistical significance.

Table 11

Pi/PCr Differences Between Repeated Measures

Subject	Parameters	DX	Stim	20/32-Base	48/60-20/32	48/60-Base
MKS022500	60Hz @ 40µa	Normal	Active	-0.03425	0.13307	0.09881
JVH030300	60Hz @ 20µa	Normal	Active	0.03794	-0.06229	-0.02435
GG030700	60Hz @ 10µa	Normal	Active	0.02904	0.07822	0.10726
BC03100	40Hz @ 40µa	Normal	Active	0.05805	0.06775	0.12580
SM032100	20Hz @ 40µa	Normal	Active	0.12371	-0.01264	0.11107
DW032800	3Hz @ 40µa	Normal	Active	0.08737	-0.03798	0.04940
BP032900	3Hz @ 20µa	Normal	Active	0.09929	0.02084	0.12013
WON09150	10Hz @ 20µa	Normal	Active	0.02842	-0.03703	-0.00861
JM040500	60Hz @ 40µa	TMD	Active	-0.11664	0.12432	0.00768
KG041900	40Hz @ 20µa	TMD	Active	0.20781	-0.07600	0.13181
HS050500	3Hz @ 20µa	TMD	Active	0.13378	0.04159	0.17537
BB052700	10Hz @ 20µa	TMD	Active	0.07033	-0.06768	0.00265
NW110700	40Hz @ 20µa	TMD	Active	0.13079	-0.02911	0.10168
KS11100	3Hz @ 20µa	TMD	Active	0.00138	0.01683	0.01821
OP020901	3Hz @ 60µa	TMD	Active	-0.03025	0.11144	0.08119
HS102700	Placebo	TMD	Placebo	-0.01118	0.01008	-0.00109
OP120800	Placebo	TMD	Placebo	-0.03748	-0.07733	-0.11481
MB020201	Placebo	TMD	Placebo	-0.04535	0.02545	-0.01990
OP32701	Placebo	TMD	Placebo	0.04078	-0.02788	0.01291
BP032800	Placebo	Normal	Placebo	0.04050	0.05101	0.09152
DW032900	Placebo	Normal	Placebo	-0.00391	-0.00438	-0.00830
TH102000	Placebo	Normal	Placebo	-0.05247	-0.00504	-0.05751

Mean Pi/PCr values and standard deviations for each group, as shown in Tables 12 and 13, reveals that the 7 TMD subjects exposed to MENS had a mean baseline Pi/PCr of 0.19280, which increased to 0.24855 and 0.26689 at the 20-32 and 48-60 time-points respectively. The paired two–tailed t-test analysis of Table 14 found a significant difference between Pi/PCr values at the 48-60 and baseline time-points, for the group of active TMD subjects, with a probability score of 0.02664. The group of 8 normal subjects also exposed to MENS had a baseline Pi/PCr value of 0.20411, which increased to 0.25781 and 0.27655, at the 20-32 and 48-60 minute time-points respectively. This difference was significant at both the 20-32 and 48-60 minute time-points with probability scores of 0.01860 and 0.01100 respectively. In comparison, the groups of 4 TMD and 4 normal subjects who were exposed to the placebo protocol, did not demonstrate any significant elevation nor difference between successive Pi/PCr values, as shown in Table 14.

Table 12

Mean Pi/PCr Values per Group at each time-point

Group	Stim	N	Mean Base	Mean 20/32	Mean 48/60	Mean 20/32-Base	Mean 48/60-20/32	Mean 48/60-Base
TMD	Placebo	4	0.23467	0.22136	0.20394	-0.01331	-0.01742	-0.03073
Normal	Placebo	4	0.18384	0.19386	0.21256	0.01001	0.01870	0.02872
TMD	Active	7	0.19280	0.24955	0.26689	0.05674	0.01734	0.07408
Normal	Active	8	0.20411	0.25781	0.27655	0.05370	0.01874	0.07424

Table 12 delineates the mean Pi/PCr values for each of the four groups (TMD/placebo, normal/placebo, TMD/active and normal/active), at the three time-points, as well as the differences between each repeated measure.

Table 13

Standard Deviation (SD) Values per Group Pi/PCr at each time-point

Group	STIM	N	SD Base	SD 20/32	SD 48/60	SD 20/32-Base	SD 48/60-20/32	SD 48/60-Base
TMD	Placebo	4	0.05002	0.01542	0.03802	0.03891	0.04580	0.05765
Normal	Placebo	4	0.03657	0.62905	0.09074	0.04877	0.02799	0.07394
TMD	Active	7	0.05333	0.09145	0.06282	0.11189	0.08061	0.06713
Normal	Active	8	0.05653	0.04353	0.04993	0.04981	0.06855	0.05975

Table 13 presents the Pi/PCr standard deviations for each group as well as the differences with repeated measures. The results of two-sided t-tests of the Pi/PCr differences at each time-point for the 4 subject groups as well as the Pi/PCr differences per combined diagnostic group, analyzed by the type of exposure, is delinerated in Tables 14 and 15 respectively.

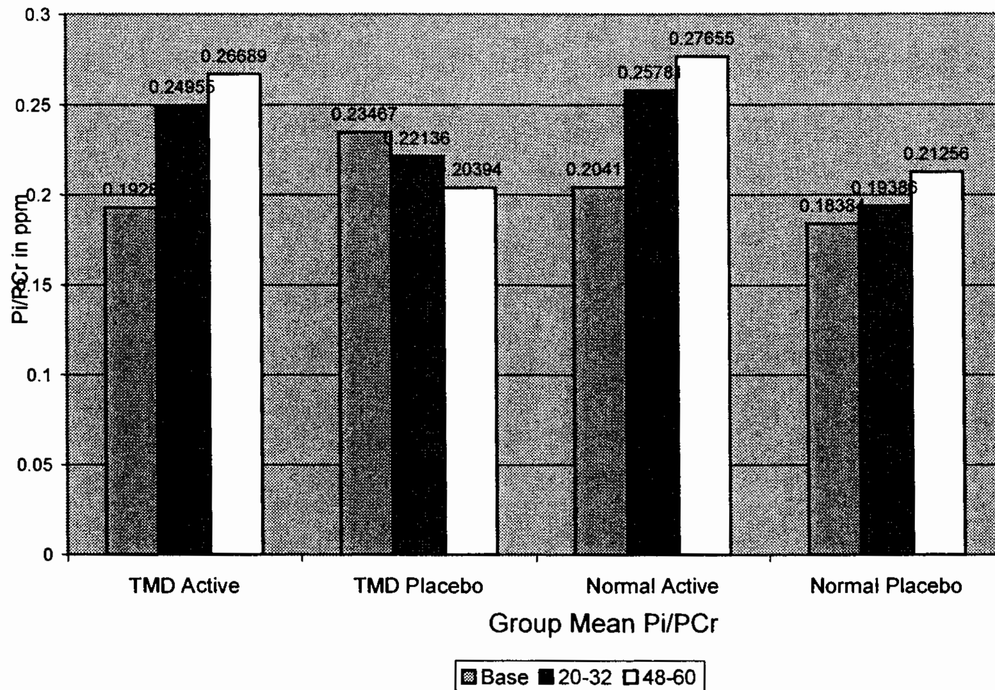


Figure 28. Mean changes in Pi/PCr at each time-point among the four subject groupings, as delineated by the type of exposure.

Figure 28 compares the effect of active and placebo exposure to each subject group as evidenced by the change in Pi/PCr. MENS caused an elevation of mean Pi/PCr for both the TMD and normal subject groups at each time-point, with the larger change occurring between baseline and 20-32 minutes. In contrast, there was a minimal increase in Pi/PCr in the normal and a decrease in Pi/PCr for the TMD subject groups from exposure to the placebo protocol. The difference between the 48-60 and baseline time-points was significant for the TMD group exposed to MENS, as well as the differences between baseline and the 20-32 and 48-60 minute time-points respectively for the normal group exposed to MENS, as shown in Table 14.

Table 14

Two-tailed t-tests (Paired) of the Pi/PCr Differences per Group

Group	Stim	N	T value 20/32-Base	T value 48/60-20/32	T value	DF	P Value 20/32-Base	P Value 48/60-20/32	P Value 48/60-Base
TMD	Placebo	4	-0.68402	-0.76060	-1.06597	3	0.54306	0.50221	0.36461
Normal	Placebo	4	0.41067	1.33604	0.77677	3	0.70888	0.27384	0.49392
TMD	Active	7	1.34175	0.56913	2.91987	6	0.22823	0.58992	**0.02664**
Normal	Active	8	3.04941	0.77333	3.42902	7	**0.01860**	0.46463	**0.01100**

P Values < 0.05 (significant) are presented in bold.

There was a significant difference between the 48-60 and baseline time-points, for the 7 TMD subjects who were exposed to the stimulation protocol. A significant difference in Pi/PCr was also noted between the 20-32 and baseline time-points as well as the 48-60 and baseline time-points, for the 8 normal subjects also exposed to the stimulation protocol, as highlighted in bold. Figure 28 illustrates the mean change in Pi/PCr by type of exposure, at each time-point for the four groups of subjects.

Additional comparisons were performed by combining all of the subjects by diagnosis (TMD and normal) and comparing the changes in Pi/PCr soley by the effects of active or placebo exposure. The results of these comparisons, performed

by two-sided t –tests are presented in Table 15 with subsequent ANOVA results in Tables 16-22.

Table 15

Pi/PCr Mean and SD of all Grouped Active Subjects (Normal & TMD) vs. all Grouped Placebo Subjects (Normal & TMD) by 2-tailed t-test of the Differences

Stim	DX	N	M Base	M 20/32	M 48/60	M 20/32-Base	M 48/60-20/32	M 48/60-Base	
Active	TMD & Normal	15	0.19884	0.25396	0.27204	0.05512	0.01809	0.07321	
Placebo	TMD & Normal	8	0.20926	0.20761	0.20825	-0.00165	0.00064	-0.00100	
Stim	DX		SD Base	SD 20/32	SD 48/60	SD 20/32-Base	SD 48/60-20/32	SD 48/60-Base	
Active	TMD & Normal	15	0.05339	0.06745	0.05443	0.08129	0.07166	0.06097	
Placebo	TMD & Normal	8	0.04882	0.04488	0.06457	0.04270	0.04010	0.06911	
Stim	DX		T 20/32-Base	T 48/60-20/32	T 48/60-Base	DF	P 20/32-Base	P 48/60-20/32	P 48/60-Base
Active	TMD & Normal	15	2.62602	0.97763	4.65044	14	**0.01994**	0.34485	**0.00037**
Placebo	TMD & Normal	8	-0.10904	-0.45300	-0.04109	7	0.91623	0.96514	0.96837

P Values < 0.05 (significant) are presented in bold.

Table 15 presents results of the two-sided t-test analyses of the Pi/PCr differences, when the subjects were grouped according to type of exposure, without separation by diagnostic category. The difference in Pi/PCr values from

baseline to the 20-32 and 48-60 minute time-points were significant at the 0.5 level with probability scores of 0.01994 and 0.00037 respectively, for the combined group of 15 TMD and normal subjects that were exposed to MENS. The combined group of 8 TMD and normal subjects exposed to the placebo protocol did not show a significant change in Pi/PCr.

ANOVA analyses via the general linear model (GLM) procedure, were subsequently performed on two class levels of data (diagnosis and type of exposure) as well as by type of exposure (active or placebo) for all subjects combined, as depicted in Table 16.

Table 16 <u>ANOVA: Delineated by Diagnosis and Type of Exposure</u>

Class	Levels	Values
DX	2	TMD/Normal
Stim	2	Active/Placebo
N	23	Observations

Table 16 delineates the ANOVA analyses that follow in Tables 17-22, which compare the Pi/PCr values (dependent variable) by type of exposure. All subjects (TMD + normals) that were exposed to MENS were compared to all subjects (TMD + normals) that received placebo exposure. ANOVA testing further supported one of the previous findings in that there was a significant elevation of Pi/PCr from baseline to the 48-60 minute time-point, with a

probability score of 0.0238 for the combined group (TMD + normal) of 15 subjects that received MENS as shown in Table 19. Furthermore, as seen in Table 22, the probability score of 0.0170 also represents a significant difference between the Pi/PCr values at the 48-60 and baseline time-points for the combined group that received active stimulation in comparison to the group exposed to the placebo protocol.

Unpaired two-tailed t-tests again reveal a significant difference in mean Pi/PCr values at the 48-60 minute time-point for the combined grouping of TMD and normal subjects who received MENS in comparison to the combined group exposed to the placebo protocol, as shown in Table 31. The same statistical analysis reported in Table 34, again shows that the mean difference in Pi/PCr values between the 48-60 and baseline time-points was significantly greater for the combined active stimulation group compared to the combined placebo group.

Table 17

Pi/PCr Value at Baseline for Combined Active and Placebo Groups

Sources of Variation	DF	SS	MS	F	P value	Sig
DX	1	0.00062	0.00062	0.22	0.6441	ns
Stim	1	0.00053	0.00053	0.19	0.6684	ns
Error	20	0.55983	0.00280			

Sig=ns (not significant), if P value > 0. 05, Sig=s (significant), if P value < 0.05.

There was no observed difference in the baseline Pi/PCr values for all subjects that received the active stimulation protocol, in comparison to those that received the placebo exposure.

Table 18

Pi/PCr Values at the 20-32 time-point for the Combined Active and Placebo Groups

Sources of Variation	DF	SS	MS	F	P value	Sig
DX	1	0.00012	0.00012	0.03	0.8730	ns
STIM	1	0.01126	0.01126	2.90	0.1041	ns
Error	20	0.07769	0.00388			

Sig=ns (not significant), if P value > 0. 05, Sig=s (significant), if P value < 0.05.

At the 20-32 time-point a significant difference in Pi/PCr was not observed between the combined active and placebo subjects.

Table 19

Pi/PCr Value at the 48-60 Time-point for Combined Active and Placebo Groups

Sources of Variation	DF	SS	MS	F	P value	Sig
DX	1	0.00050	0.00050	0.14	0.7109	ns
Stim	1	0.02100	0.02100	5.99	**0.0238**	s
Error	20	0.07016	0.00351			

Sig=ns (not significant), if P value > 0. 05, Sig=s (significant), if P value < 0.05.

This analysis reveals that there was a significant increase in Pi/PCr, at the 48-60 minute time-point, for all subjects that received active stimulation in comparison to those that received placebo exposure with the specific values illustrated in Figure 29.

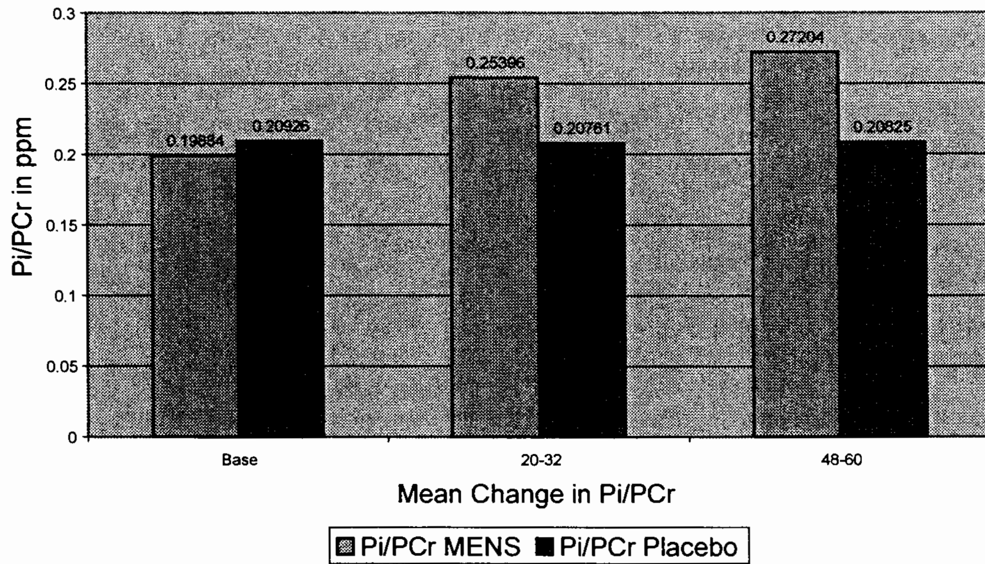


Figure 29. Comparison of the Pi/PCr value for the combined TMD and normal subjects (15 active and 8 placebo) at each time-point.

Mean Pi/PCr remained relatively unchanged for the group of combined TMD and normal subjects that received the placebo protocol as shown in Figure 29. However in the combined group of subjects exposed to active microcurrent stimulation, there was a significant elevation of Pi/PCr from baseline to the 20-32 and the 48-60 minute time-points with probability scores of 0.01994 and 0.00037 respectively in Table 15.

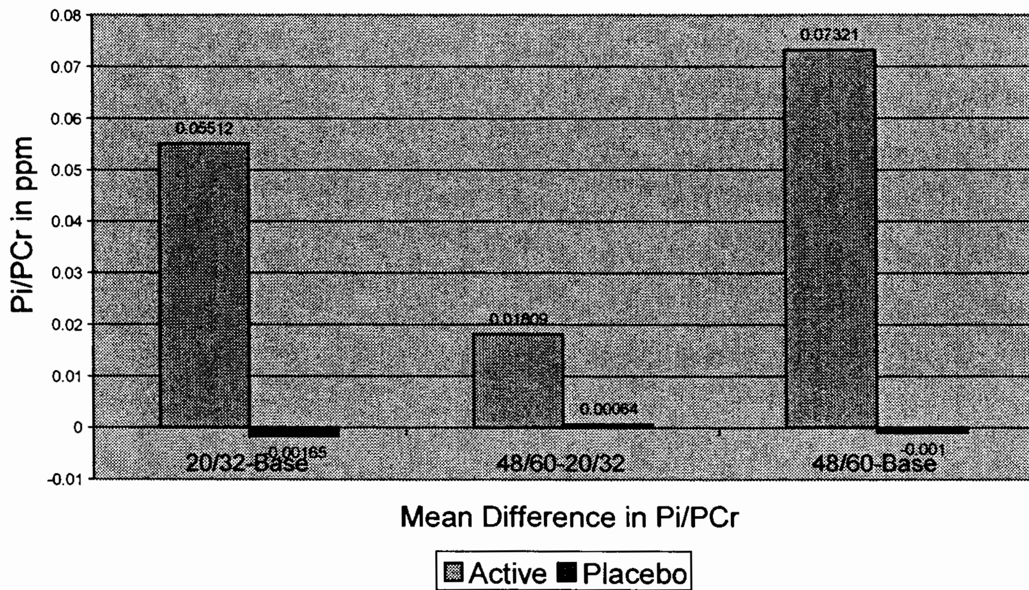


Figure 30. Comparative differences in Pi/PCr at each time-point for the combined groups of TMD and normal subjects delineated by type of exposure.

Figure 30 illustrates the significant difference in Pi/PCr at the 20-32 minus baseline and the 48-60 minus baseline time-points for the combined group of TMD and normal subjects that received exposure to MENS. There was no appreciable difference noted for the combined group that received the placebo protocol. Tables 15, 19 and 22 delineate the specific probability scores.

Table 20

Difference Between Pi/PCr Values at the 20-32 and Baseline time-points for Combined Active and Placebo Groups

Sources of Variation	DF	SS	MS	F	P value	Sig
DX	1	0.00022	0.00022	0.04	0.8410	ns
Stim	1	0.01667	0.01667	3.17	0.0900	ns
Error	20	0.10506	0.00525			

Sig=ns (not significant), if P value > 0. 05, Sig=s (significant), if P value < 0.05.

The difference in Pi/PCr at the 20-32 and baseline time-points for the combined active and placebo groups was not significant either by diagnosis or the type of experimental exposure. However, as previously noted, there was a significant difference in Pi/PCr for the separate groups of active TMD subjects between baseline and the 48-60 time-point, as well as the active normal subjects between baseline and the 20-32 and 48-60 time-points.

Table 21

Difference Between Pi/PCr Values at the 48-60 and 20-32 time-points for the Combined Groups (Active + Placebo)

Sources of Variation	DF	SS	MS	F	P value	Sig
DX	1	0.00105	0.00105	0.26	0.6191	ns
Stim	1	0.00151	0.00151	0.37	0.5516	ns
Error	20	0.08210	0.00410			

Sig=ns (not significant), if P value > 0. 05, Sig=s (significant), if P value < 0.05.

The difference in Pi/PCr at the 48-60 and 20-32 time points for the combined active and placebo groups was not significant by diagnosis or the type of experimental exposure.

Table 22

Difference Between Pi/PCr Values at the 48-60 and Baseline time-points for the Combined Groups (Active + Placebo)

Sources of Variation	DF	SS	MS	F	P value	Sig
DX	I	0.00222	0.00222	0.53	0.4740	ns
Stim	1	0.02829	0.02829	6.77	**0.0170**	s
Error	20	0.08360	0.00416			

Sig=ns (not significant), if P value > 0. 05, Sig=s (significant), if P value < 0.05.

The difference in Pi/PCr for the combined groups, at the 48-60 and baseline time-points was significant for the group that received the active stimulation protocol (p = 0.0170) in comparison to the group that received placebo exposure.

Tables 23-28 present the two-tailed t-test analyses between the combined normal and TMD subject groups without delineation by the type of exposure. The results show that there was no significant difference between mean Pi/PCr values at any time-point or difference between time-points, unless the groups were delineated by active and placebo exposure, as shown in Tables 29-34.

Table 23

Unpaired two-tailed t-tests: Baseline Pi/PCr Values Between the TMD and Normal Subjects by Group

DX	N	Mean	Grouping
TMD	11	0.208	A
Normal	12	0.197	A
Error df	20		
Error MS	0.0028		
Critical Value t	2.0860		
LSD (least significant difference)	0.0461		

Pi/PCr values between the TMD and normal subject groups were not significantly different at baseline.

Table 24

Pi/PCr Values at the 20-32 time-point Between the TMD and Normal Subjects by Group

DX	N	Mean	Grouping
TMD	11	0.239	A
Normal	12	0.236	A
Error df	20		
Error MS	0.0039		
Critical value t	2.0860		
LSD (least significant difference)	0.0543		

Pi/PCr values between the TMD and normal subject groups were not significantly different at the 20-32 time-point.

Table 25

Pi/PCr Values at the 48-60 time-point Between the TMD and Normal Groups

DX	N	Mean	Grouping
TMD	11	0.255	A
Normal	12	0.244	A
Error df	20		
Error MS	0.0035		
Critical value t	2.0860		
LSD (least significant difference)	0.0516		

Pi/PCr values between the TMD and normal subject groups were not significantly different at the 48-60 minute time-point.

Table 26

Pi/PCr Difference Between the 20-32 and Baseline time-points of the TMD and Normal Groups

DX	N	Mean	Grouping
TMD	11	0.0391	A
Normal	12	0.0313	A
Error df	20		
Error MS	0.0525		
Critical value t	2.0860		
LSD (least significant difference)	0.0631		

The differences in Pi/PCr values at the 20-32 and baseline time-points for the TMD and normal groups were not significant.

Table 27

Pi/PCr Difference Between the 48-60 and 20-32 time-points

for the TMD and Normal Groups

DX	N	Mean	Grouping
TMD	11	0.0187	A
Normal	12	0.0047	A
Error df	20		
Error MS	0.0041		
Critical value t	2.0860		
LSD (least significant difference)	0.0558		

The differences in Pi/PCr values at the 48-60 minus the 20-32 minute time-point for the TMD and the normal groups, were not significant.

Table 28

Pi/PCr Difference at the 48-60 and Baseline time-Points for the TMD and Normal Groups

DX	N	Mean	Grouping
TMD	11	0.0579	A
Normal	12	0.0360	A
Error df	20		
Error MS	0.0041		
Critical value t	2.0860		
LSD (least significant difference)	0.0562		

The differences in Pi/PCr values at 48-60 and the baseline time-points, for the TMD and normal groups, were not significant.

Tables 29-34 analyzed the mean Pi/PCr differences at and between each time-point for the combined groups of TMD and normal subjects delineated by the type of experimental exposure. Therefore 15 subjects received microcurrent stimulation and 8 received the placebo protocol. Significant differences in mean Pi/PCr between the two groups occurred at the 48-60 minute time-point (Table 31) and the 48-60 minute minus baseline time-points (Table 34).

Table 29

Baseline Pi/PCr Values by Stimulation (Active vs Placebo Groups)

DX	N	Mean	Grouping
Active	15	0.2093	A
Placebo	8	0.1988	A
Error df	20		
Error MS	0.0028		
Critical value t	2.0860		
LSD (least significant difference)	0.0483		

A difference in the Pi/PCr values at baseline, between the combined active and placebo groups, was not evident when analyzed by the type of exposure.

Table 30

Combined Group Pi/PCr Values at the 20-32 time-point by Stimulation

DX	N	Mean	Grouping
Active	15	0.2540	A
Placebo	8	0.2076	A
Error df	20		
Error MS	0.0039		
Critical value t	2.0860		
LSD (least significant difference)	0.0569		

The difference in Pi/PCr for the combined groups was not significant at the 20-32 minute time-point, when analyzed by the type of exposure.

Table 31

Combined Group Pi/PCr Values at the 48-60 time-point by Stimulation

DX	N	Mean	Grouping
Active	15	**0.2720**	**A**
Placebo	8	**0.2083**	**B**
Error df	20		
Error MS	0.0039		
Critical value t	2.0860		
LSD (least significant difference)	0.0569		

There was a significant effect upon mean Pi/PCr at the 48-60 minute time-point, for the combined TMD and normal subjects who received active stimulation. The combined group exposed to MENS had a significantly greater increase in mean Pi/PCr at the 48-60 time-point as compared to the placebo group.

Table 32

Combined Group Pi/PCr Difference at the 20-32 and Baseline time-points

DX	N	Mean	Grouping
Active	15	0.0551	A
Placebo	8	-0.0017	A
Error df	20		
Error MS	0.0053		
Critical value t	2.0860		
LSD (least significant difference)	0.0662		

Although there was a decrease in mean Pi/PCr between the baseline and 20-32 minute time-point for the combined group of 8 subjects who received the placebo protocol, the comparative difference to that of the combined group of 15 subjects exposed to active stimulation was not significant.

Table 33

Combined Group Pi/PCr Difference at the 48-60 and 20-32 time-points

DX	N	Mean	Grouping
Active	15	0.0181	A
Placebo	8	0.0006	A
Error df	20		
Error MS	0.0041		
Critical value t	2.0860		
LSD (least significant difference)	0.0585		

There was no significant difference in mean Pi/PCr between the combined groups receiving active or placebo exposure from the 20-32 to 48-60 minute time-points.

Table 34

Combined Group Pi/PCr Difference at the 48-60 minus Baseline time-points

DX	N	Mean	Grouping
Active	15	**0.0732**	**A**
Placebo	8	**-0.0010**	**B**
Error df	20		
Error MS	0.0042		
Critical value t	2.0860		
LSD (least significant difference)	0.0589		

There was a significant difference in mean Pi/PCr between the active and placebo groups of 15 and 8 subjects respectively at the 48-60 minute time-point, relative to baseline, for the combined groups (TMD and normal) that received stimulation, in comparison to the combined group exposed to the placebo protocol.

ANOVA comparisons between the group of 7 TMD subjects who received MENS and the 4 exposed to the placebo protocol are presented in Tables 35-41. The only analysis that revealed a significant ($p = 0.0284$) difference in mean Pi/PCr occurred between the 48-60 and baseline time-points among the TMD subjects that received stimulation in comparison to those exposed to the placebo protocol, as shown in Table 41.

Table 35

ANOVA via the General Linear Model (GLM) Procedure

Class	Levels	Values
STIM	2	Active / Placebo
N	11	Observations

Table 36

Pi/PCr Baseline Values for the TMD groups (active vs. placebo)

Sources of Variation	df	SS	MS	F	P value	Sig
Stim	1	0.00446	0.00446	1.63	0.2331	ns
Error	9	0.02457	0.00273			

Sig=ns, if P value > 0.05, Sig=s, if P value < 0.05.

Mean Pi/PCr between the active and placebo TMD groups was not significantly different at baseline.

Table 37

Pi/PCr Values at the 20-32 time-point for the TMD Groups

Sources of Variation	df	SS	MS	F	P value	Sig
Stim	1	0.00202	0.00202	0.36	0.5646	ns
Error	9	0.05088	0.00565			

Sig=ns, if P value > 0.05, Sig=s, if P value < 0.05.

Mean Pi/PCr at the 20-32 minute time-point was not significantly different between the active and placebo TMD groups.

Table 38

Pi/PCr Values at the 48-60 time-point for the TMD Groups

Sources of Variation	DF	SS	MS	F	P value	Sig
Stim	1	0.01009	0.01009	3.24	0.1054	ns
Error	9	0.02801	0.003112			

Sig=ns, if P value > 0.05, Sig=s, if P value < 0.05.

Mean Pi/PCr at the 48-60 minute time-point was not significantly different between the active and placebo TMD groups.

Table 39

Difference in Pi/PCr Values at the 20-32 and Baseline time-points for the TMD Groups

Sources of Variation	DF	SS	MS	F	P value	Sig
Stim	1	0.01249	0.01249	1.41	0.2653	ns
Error	9	0.07966	0.00885			

Sig=ns, if P value > 0.05, Sig=s, if P value < 0.05.

The difference in mean Pi/PCr between the 20-32 and baseline time-points among the TMD groups exposed to the active and placebo protocols was not significantly different.

Table 40

Difference in Pi/PCr Values at the 48-60 and 20-32 time-points for the TMD Groups

Sources of Variation	DF	SS	MS	F	P value	Sig
Stim	1	0.00308	0.00308	0.61	0.4544	ns
Error	9	0.04528	0.005031			

Sig=ns, if P value > 0.05, Sig=s, if P value < 0.05.

The difference in mean Pi/PCr for the active and placebo TMD groups was not significant between the 48-60 and 20-32 minute time-points.

Table 41

Difference of Pi/PCr Values at 48-60 and Baseline time-points

Sources of Variation	DF	SS	MS	F	P value	Sig
Stim	1	0.02796	0.02796	6.80	**0.0284**	s
Error	9	0.03701	0.00411			

Sig=ns, if P value > 0.05, Sig=s, if P value < 0.05.

There was a significant difference in mean Pi/PCr between the 48-60 and baseline time-points among the 7 TMD subjects who received microcurrent stimulation and the 4 exposed to the placebo protocol.

The next set of analyses consists of two-tailed t-tests comparing the 7 TMD subjects that received active stimulation to the 4 TMD subjects that received the placebo protocol. The mean Pi/PCr values of two class levels of data, by diagnosis (TMD or normal) and type of exposure (active or placebo), for these 11 subjects, were analyzed as illustrated in Tables 42-47. Groups that have the same letter do not represent significantly different Pi/PCr values at the respective time-point. It should be noted that cell sizes were not equal. The results provide further support for the previous findings in that the only significant difference in mean Pi/PCr between the active and placebo TMD groups occurred at the 48-60 minute minus baseline time-points as shown in Table 47.

Table 42

Baseline Pi/PCr Values Between TMD Groups by Type of Exposure

DX	N	Mean	Grouping
TMD Placebo	4	0.2347	A
TMD Active	7	0.1928	A
Error df	9		
Error MS	0.0027		
Critical value t	2.2622		
LSD (least significant difference)	0.0741		

There was no difference in mean Pi/PCr between the two TMD groups at baseline.

Table 43

Pi/PCr Values between TMD Groups at the 20-32 time-point

DX	N	Mean	Grouping
TMD Placebo	4	0.2214	A
TMD Active	7	0.2496	A
Error df	9		
Error MS	0.0057		
Critical value t	2.2622		
LSD (least significant difference)	0.1066		

A significant difference was not observed between the two TMD groups at the 20-32 time-point.

Table 44

Pi/PCr Values Between TMD Groups at the 48-60 time-point

DX	N	Mean	Grouping
TMD Placebo	4	0.2039	A
TMD Active	7	0.2669	A
Error df	9		
Erro MS	0.0031		
Critical value t	2.2622		
LSD (least significant difference)	0.0791		

The difference in mean Pi/PCr among the two groups of TMD subjects at the 48-60 minute time-point was not significant.

Table 45

Pi/PCr Differences at the 20-32-Baseline time-points Between the TMD Groups

DX	N	Mean	Grouping
TMD Placebo	4	-0.0133	A
TMD Active	7	0.0567	A
Error df	9		
Error MS	0.0089		
Critical value t	2.2622		
LSD (least significant difference)	0.1334		

The difference in mean Pi/PCr between the 20-32 minute and baseline time-points was not significant among the two TMD groups.

Table 46

Pi/PCr Differences at the 48-60 - 20-32 time-points Between the TMD Groups

DX	N	Mean	Grouping
TMD Placebo	4	-0.0174	A
TMD Active	7	0.0173	A
Error df	9		
Error MS	0.0050		
Critical value t	2.2622		
LSD (least significant difference)	0.1006		

The difference in mean Pi/PCr between the 48-60 and 20-32 minute time-points for the two TMD groups was not significant.

Table 47

Pi/PCr Values at the 48-60-Baseline time-points for the TMD Groups

DX	N	Mean	Grouping
TMD Placebo	4	**- 0.0307**	**B**
TMD Active	7	**0.0741**	**A**
Error df	9		
Error MS	0.0041		
Critical value t	2.2622		
LSD (least significant difference)	0.0909		

The difference in mean Pi/PCr in this comparison was significant. Among the TMD subjects, the group that received active microcurrent stimulation had a significantly higher Pi/PCr at the 48-60 minute time-point relative to baseline, compared to the TMD subjects with placebo exposure. It is important to note that there was a decrease in mean Pi/PCr for the TMD subjects in the absence of microcurrent stimulation.

Tables 48- 54 represent ANOVA summaries of mean Pi/PCr differences for two class levels of data (diagnosis = normal) and the type of exposure (active vs. placebo), between the 12 normal subjects, (active = 8 and placebo = 4). The results failed to show significant differences at any of the time-points. This was further supported by the Two-tailed t-test analyses in Tables 61-66, to control for the Type 1 comparison-wise error rate, but not the experiment-wise error rate.

Table 48

ANOVA via the General Linear Model (GLM) Procedure

Class	Levels	Values
Stim	2	Active / Placebo
N	12	Observations

Table 49

Pi/PCr Baseline Values for the Normal Group (active vs. placebo)

Sources of Variation	DF	SS	MS	F	P value	Sig
Stim	1	0.00120	0.00120	0.42	0.5338	ns
Error	10	0.02638	0.002638			

Sig=ns (not significant), if P value > 0. 05, Sig=s (significant), if P value < 0.05.

There was no significant difference in mean Pi/PCr at baseline, between the two groups (active & placebo) of normal subjects.

Table 50

Pi/PCr levels at the 20-32 time-point, for the Normal Subject Group (active vs. placebo)

Sources of Variation	DF	SS	MS	F	P value	Sig
Stim	1	0.00120	0.00120	4.34	0.0639	ns
Error	10	0.02516	0.00251			

Sig=ns (not significant), if P value > 0. 05, Sig=s (significant), if P value < 0.05.

There was no effect of microcurrent stimulation upon mean Pi/PCr at the 20-32 time-point, between the two groups of normal subjects.

Table 51

Pi/PCr Values at the 48-60 time-point, for the Normal Subject Group (active vs. placebo)

Sources of Variation	df	SS	MS	F	P value	Sig
Stim	1	0.00120	0.00120	2.59	0.1386	ns
Error	10	0.04215	0.00421			

Sig=ns (not significant), if P value > 0. 05, Sig=s (significant), if P value < 0.05.

Mean Pi/PCr at the 48-60 minute time-point for the two groups of normal subjects was not significantly different.

Table 52

Difference in Pi/PCr Values at the 20-32 and Baseline time-points Between the two Groups of Normal Subjects

Sources of Variation	DF	SS	MS	F	P value	Sig
Stim	1	0.00509	0.00509	2.08	0.1801	ns
Error	10	0.02455	0.00245			

There was no effect of stimulation upon mean Pi/PCr at the 20-32 time-point relative to baseline. Pi/PCr values for the two groups of normal subjects were not significantly different.

Table 53

Difference in Pi/PCr Values at the 48-60-20-32 time-points for the Two Groups of Normal Subjects

Sources of Variation	df	SS	MS	F	P value	sig
Stim	1	4.29338	4.29338	0.00	0.9991	ns
Error	10	0.03524	0.00352			

Sig=ns (not significant), if P value > 0. 05, Sig=s (significant), if P value < 0.05.

There was no effect of microcurrent stimulation upon mean Pi/PCr, at the 48-60 minus the 20-32 time-points, between the two groups of normal subjects.

Table 54

Difference in Pi/PCr Values at the 48-60 minus Baseline time-points Between the Two Groups of Normal Subjects

Sources of Variation	df	SS	MS	F	P value	sig
Stim	1	0.00510	0.00510	1.23	0.2931	ns
Error	10	0.41392	0.00414			

Sig=ns (not significant), if P value > 0. 05, Sig=s (significant), if P value < 0.05.

There was no effect of microcurrent stimulation upon mean Pi/PCr at the 48-60 time-point relative to baseline, between the two groups of normal subjects.

Tables 55-60 provide the results of two-tailed t-tests of mean Pi/PCr values, comparing the 12 normal subjects delineated by the type of exposure (8 active vs. 4 placebo) at each time-point. It is important to state that groups with the same letter designation do not represent significantly different Pi/PCr values at the respective time-points and cell sizes were not equal. Neither microcurrent stimulation nor placebo exposure resulted in any significant differences in mean Pi/PCr at any time-point between the normal subject groups.

Table 55

Baseline Pi/PCr Values Between Normal Subject Groups (active and placebo)

DX	N	Mean	Grouping
Normal Placebo	4	0.1838	A
Normal Active	8	0.2041	A
Error df	10		
Error MS	0.0027		
Critical value t	2.2218		
LSD (least significant difference)	0.0701		
Harmonic mean of cell sizes	5.3333		

Mean baseline Pi/PCr between the two normal subject groups was not significantly different.

Table 56

Pi/PCr Values at the 20-32 time-point Between the Normal Subject Groups

DX	N	Mean	Grouping
Normal Placebo	4	0.1939	A
Normal Active	8	0.2578	A
Error df	10		
Error MS	0.0025		
Critical value t	2.2281		
LSD (least significant difference)	0.0684		

Mean Pi/PCr values at the 20-32 time-point for the active and placebo groups of normal subjects were not significantly different.

Table 57

Pi/PCr Values at the 48-60 time-point Between the two Groups of Normal Subjects

DX	N	Mean	Grouping
Normal Placebo	4	0.2126	A
Normal Active	8	0.2766	A
Error df	10		
Error MS	0.0042		
Critical value t	2.2281		
LSD (least significant difference)	0.0886		

Mean Pi/PCr at the 48-60 time-point for the active and placebo groups of normal subjects was not significantly different.

Table 58

Pi/PCr Differences Between the 20-32 and Baseline time-points for the Active and Placebo Groups of Normal Subjects

DX	N	Mean	Grouping
TMD Placebo	4	0.0100	A
TMD Active	8	0.0537	A
Error df	10		
Error MS	0.0025		
Critical value t	2.2281		
LSD (least significant difference)	0.0675		

The difference in mean Pi/PCr between the 20-32 and baseline time-points of the two normal groups was not significant.

Table 59

Pi/PCr Differences Between the 48-60 and 20-32 time-points for the Active and Placebo Groups of Normal Subjects

DX	N	Mean	Grouping
TMD Placebo	4	0.0187	A
TMD Active	8	0.0187	A
Error df	10		
Error MS	0.0035		
Critical value t	2.2281		
LSD (least significant difference)	0.0810		

The differences between mean Pi/PCr values at the 48-60 and 20-32 time-points for the two normal groups were not significantly different.

Table 60

Pi/PCr Differences at the 48-60 and Baseline time-points for the Active and Placebo Groups of Normal Subjects

DX	N	Mean	Grouping
TMD Placebo	4	0.0287	A
TMD Active	8	0.0724	A
Error df	10		
Error MS	0.0041		
Critical value t	2.2281		
LSD (least significant difference)	0.0878		

The difference in mean Pi/PCr values between the 48-60 and baseline time-points for the two normal groups was not significant.

Results Specific to Hypothesis II

Clinical Data

Algometric data

Clinical data consisting of active mandibular ROM, VAS and algometric PPT were acquired for each TMD subject before and after exposure to MENS or the placebo protocol. However, since the algometer was not obtained at the start of the study, PPT data was only acquired for 2 of the 7 active and 3 of the 4 placebo exposures respectively, as shown in Tables 61 and 62. Tables 64-65 contain the breakdown of clinical data per group, while Table 63 and 66 presents the results of t-tests between the baseline (pre-test) score and the 48-60 minute time-point (post-test score) for each group.

Table 61

Algometer, VAS, & ROM Data for TMD Subjects (active) with Pi/PCr Data

Subject	Active Parameters	DX	ALG Base	ALG 48-60	VAS Base	VAS 48-60	ROM Base	ROM 48-60
JM040500	60Hz @ 40μa	TMD	N/A	N/A	2.8	0.5	38mm	42mm
KG041900	40Hz @ 20μa	TMD	N/A	N/A	6.8	4.4	8.0mm	16mm
HS050500	3Hz @ 20μa	TMD	N/A	N/A	2.0	0.0	42mm	45mm
BB052700	10Hz @ 20μa	TMD	N/A	N/A	3.0	2.0	29mm	29mm
NW110700	40Hz @ 20μa	TMD	N/A	N/A	5.0	1.2	35mm	41mm
KS11100	3Hz @ 20μa	TMD	1	1.56	5.4	1.5	25mm	34mm
OP020901	3Hz @ 60μa	TMD	2.53	2.96	5.4	0.0	28mm	52mm

Algometric data was only obtained for 2 of the 7 subjects, which revealed that the pressure pain threshold (PPT) was elevated after exposure to active stimulation. However, in order to determine if there was a significant difference between the pre and post-treatment algometric scores, a two-tailed t-test was performed by including the 6 TMD subjects that completed the full exposure to MENS without acquired Pi/PCr data (Table 66), therefore creating a group of 8 with the 2 subjects that had acquired Pi/PCr data from Table 61. Figure 31

illustrates the pre and post algometric scores for the 8 TMD subjects (6 with and 2 without acquired Pi/PCr values) who received the full exposure to MENS.

Table 62

Algometer, VAS & ROM Data for Subjects (placebo) with Pi/PCr Data

Subject	Parameter	DX	Alg Base	Alg 48-60	VAS Base	VAS 48-60	ROM Base	ROM 48-60
HS102700	Placebo	TMD	N/A	N/A	5.6	5.2	36mm	36mm
OP120800	Placebo	TMD	2.13	1.96	5.5	0.8	24mm	30mm
MB020201	Placebo	TMD	2.86	1.53	6.2	6.4	20mm	20mm
OP032701	Placebo	TMD	2.53	1.93	7.0	8.0	30mm	32mm

In this group of only 4 TMD subjects exposed to the placebo protocol, there were no significant differences in clinical data between the pre-test and post-test recordings.

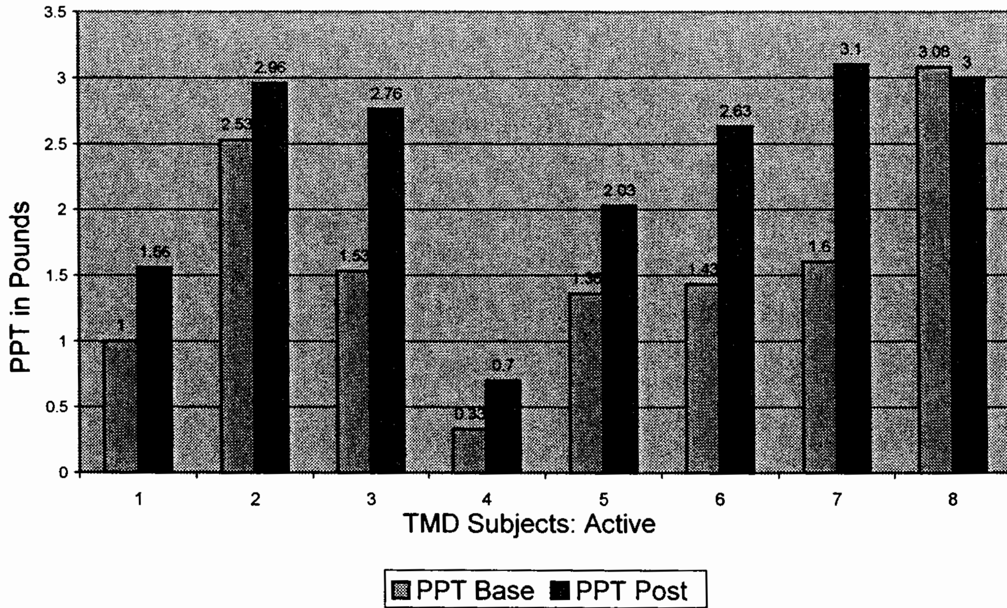


Figure 31. Change in PPT for the 8 TMD subjects exposed to MENS.

A two-tailed t-test of the difference between the baseline PPT and that recorded after exposure to MENS was significant with a probability value of 0.0058, delineated in Table 63. Please note that this comparison consists of the 2 TMD subjects with acquired Pi/PCr and PPT data plus 6 subjects without Pi/PCr, but with PPT data.

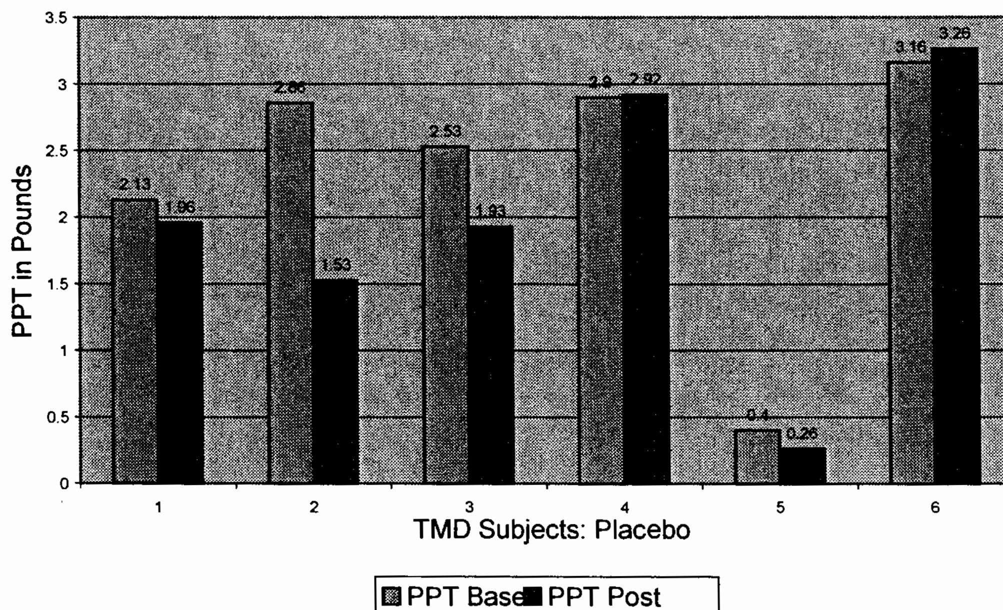


Figure 32. Change in PPT for the 6 TMD subjects exposed to the placebo protocol at baseline and after the placebo protocol.

In this group of 6 TMD subjects (3 with and 3 without acquired Pi/PCr data) exposed to the placebo protocol, the difference in algometric scores was not significant. PPT data is missing for 1 subject who was tested prior to the acquisition of the algometer.

Visual analogue scale

VAS values were recorded for all subjects with acquired Pi/PCr data, as well as those who completed the full exposure without spectra that could be analyzed.

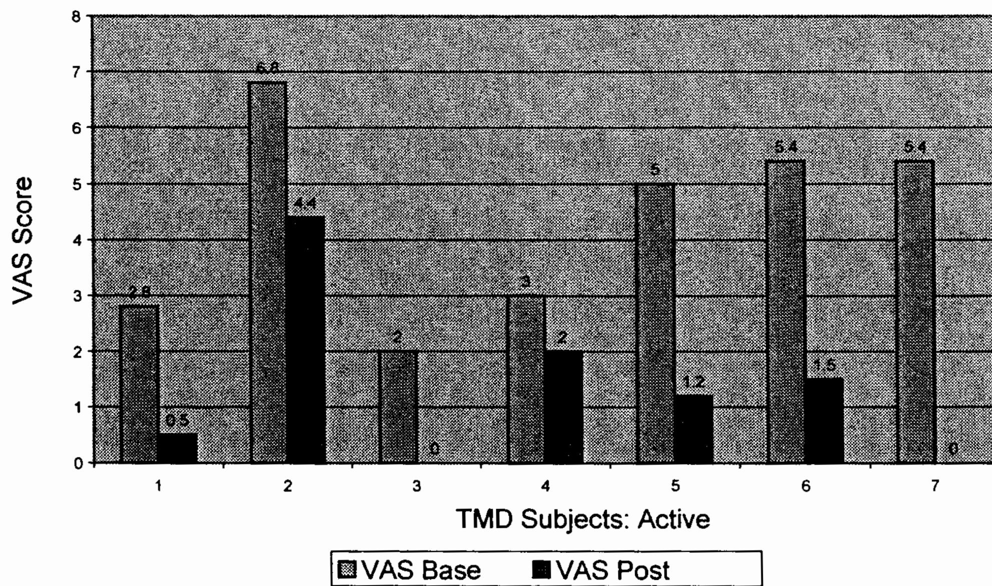


Figure 33. Change in VAS data at baseline and after exposure to MENS for 7 TMD subjects.

There was a decrease in VAS recordings for all 7 TMD subjects exposed to MENS. The differences between VAS values at baseline and after exposure to MENS were significant with a probability score of 0.0018, as shown in Table 63.

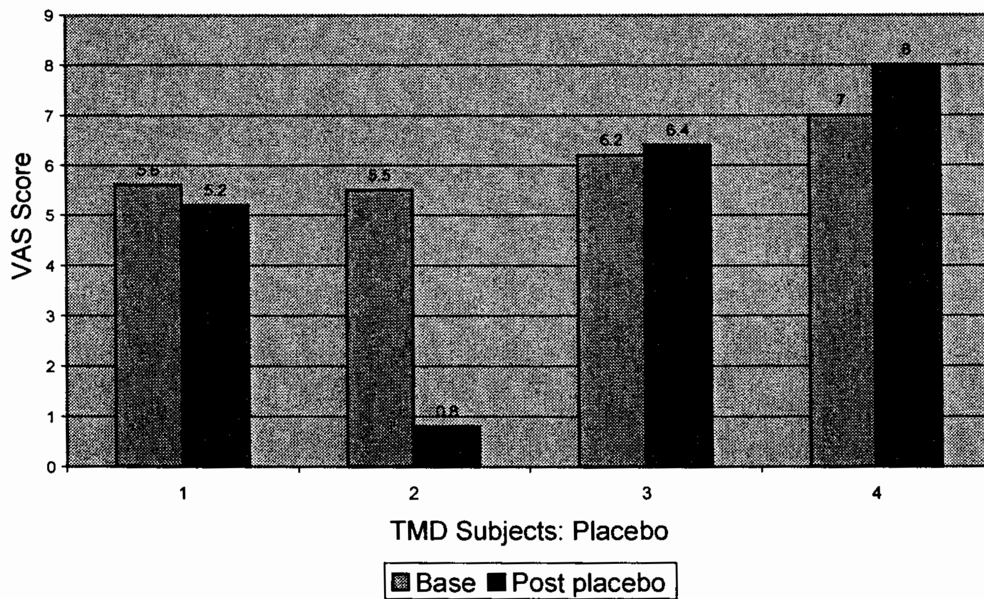


Figure 34. Change in VAS of 4 TMD subjects at baseline and after placebo exposure.

The difference between pre and post VAS values for this group of four subjects exposed to the placebo protocol was not significant.

Range of motion

The data in Table 61, representative of the 7 TMD subjects that received MENS, illustrates an increase in vertical mandibular ROM from baseline to the 48-60 time-point for all subjects except one, in which ROM was unchanged.

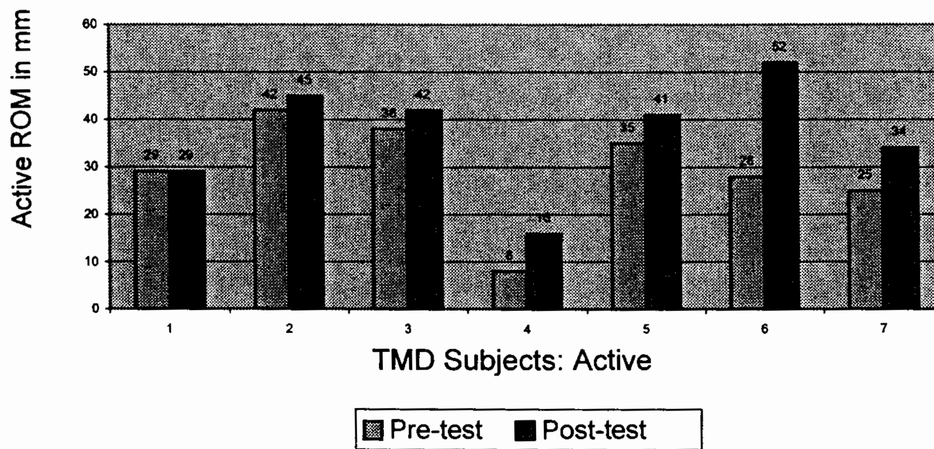


Figure 35. Change in active vertical mandibular ROM of the TMD subjects after exposure to MENS.

The difference between pre-and post-active ROM recordings was significant with a probability score of 0.00398, as shown in Table 63.

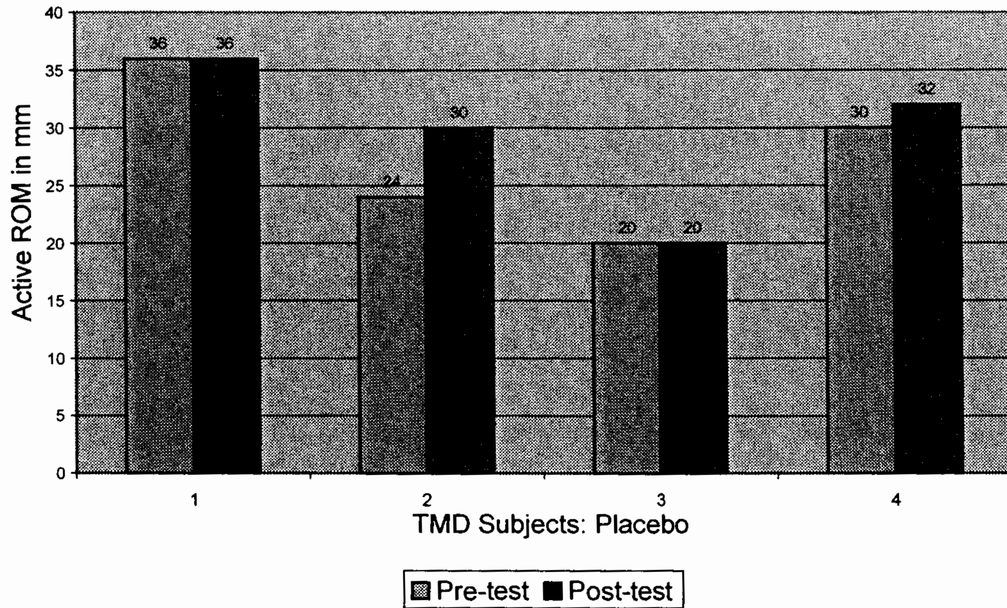


Figure 36. Change in active vertical mandibular ROM for TMD subjects after placebo exposure.

In this group of 4 TMD subjects with acquired Pi/PCr data, the difference between the pre-and post-test ROM after exposure to the placebo protocol was not significant.

Clinical Data for TMD Subjects with Acquired Pi/PCr

Table 63

Two-tailed T-test of the Differences Between pre and post-test Clinical data for TMD Subjects with Acquired Pi/PCr data

Group	Stim	N	Data	Mean Pre	Mean Post	SD Pre	SD Post	df	T Score	P Value
TMD	A	8	PPT	1.6075	2.3425	0.8552	0.8496	7	3.9170	**0.0058**
TMD	P	6	PPT	2.3300	1.9767	1.0099	1.0664	5	1.6134	0.1676
TMD	A	7	VAS	4.343	1.371	1.750	1.535	6	5.3298	**0.0018**
TMD	P	4	VAS	6.075	5.100	0.690	3.088	3	0.7651	0.4999
TMD	A	7	ROM	29.286	37.000	11.131	11.860	6	2.6153	**0.0398**
TMD	P	4	ROM	27.50	29.50	7.00	6.81	3	1.4142	0.2522

P Values < 0.05 (significant) are presented in bold.

Table 63 presents the t-test results for the changes in clinical data for each group. It is important to again note that in order to perform a meaningful analysis, the mean score for PPT in both the active and placebo groups was derived by including the 6 subjects that completed the full exposure to MENS (n = 8) as well as the 3 subjects who received the placebo protocol (n = 6), but in which Pi/PCr values were unable to be acquired. The differences between the pre-test and post-test clinical data for algometric (PPT), VAS and ROM measurements were found

to be significant only for the TMD subjects that received active microcurrent stimulation.

Clinical Data for TMD Subjects without Acquired Pi/PCr

There were 6 additional TMD subjects that received the full hour of MENS and 3 who were exposed to the placebo protocol that did not have Pi/PCr data. Tables 64 and 65 delineate the recorded clinical data for these subjects. Charts of the effect of active and placebo exposure as well as two-tailed t-tests of the differences were completed, without comparison to Pi/PCr values.

Table 64

Algometer, VAS & ROM Data for TMD Subjects (active) without Pi/PCr Data

Subject	Parameters	DX	Stim	Alg Base	Alg 48-60	VAS Base	VAS 48-60	ROM Base	ROM 48-60
OP071701	3Hz @ 40μa	TMD	Active	1.53	2.76	9.0	0.0	13mm	27mm
DG081701	3Hz @ 20μa	TMD	Active	0.33	0.70	3.6	3.2	25mm	27mm
DB021502	3Hz @ 60μa	TMD	Active	1.36	2.03	8.2	2.5	30mm	35mm
OP022602	3Hz @ 60μa	TMD	Active	1.43	2.63	6.5	0.6	20mm	30mm
OP030502	3Hz @ 60μa	TMD	Active	1.60	3.10	8.4	0.0	23mm	43mm
HC081602	3Hz @ 10μa	TMD	Active	3.08	3.00	7.0	7.0	28mm	28mm

In this group of 6 TMD subjects who received MENS, but for whom the Pi/PCr spectra were unable to be analyzed, there was an increase and significant

difference (p = 0.0210) in the PPT at the 48-60 time-point from that at baseline, as shown in Table 66.

There was a concomitant decrease in VAS scores between the pre- and post-test that was significant with a probability value of 0.0269. Active mandibular ROM also increased and the difference between the pre-and post-test recordings was also significant with a probability score of 0.0414, as found in Table 66.

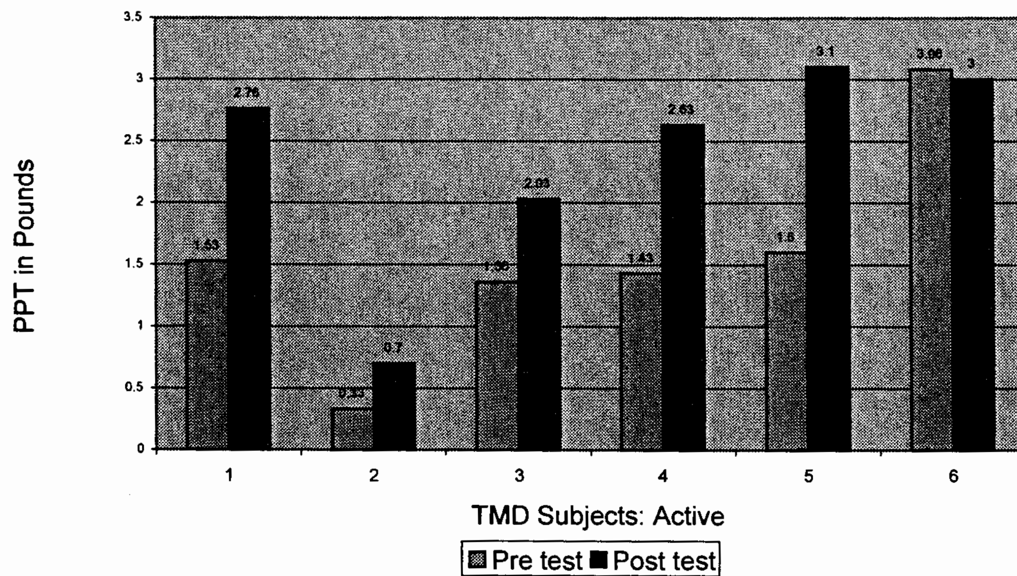


Figure 37. Effect of MENS on pain pressure threshold in the group of 6 TMD subjects, without acquired Pi/PCr data.

There was a significant difference in PPT scores (p=0.0210) between the baseline and 48-60 minute time-points for the group of 6 TMD subjects exposed to MENS, but for whom Pi/PCr data was not able to be acquired (Table 66).

Table 65

Algometer, VAS & ROM Data for TMD Subjects (placebo) without Pi/PCr Data

Subject	DX	Stim	Alg Base	Alg 48-60	VAS Base	VAS 48-60	ROM Base	ROM 48-60
SA080801	TMD	Placebo	2.90	2.92	6.6	5.0	25mm	29mm
DG081401	TMD	Placebo	0.40	0.26	4.6	5.2	22mm	22mm
HC080602	TMD	Placebo	3.16	3.26	6.4	5.0	24mm	19mm

Three additional TMD subjects without acquired Pi/PCr data, were exposed to the placebo protocol as delineated in Table 65. The clinical data reveal minimal fluctuations between the pre-and post-test recordings and the differences were not significant, as can be seen in Table 66, which summarizes the two-tailed t-test results among all of the TMD subjects that received the full active or placebo exposure, but for whom acceptable spectra were unable to be acquired.

Similar results were seen among both groups of TMD subjects with and without acquired Pi/PCr data, in that the differences between all three clinical measures were significant, as shown in Tables 63 and 66 respectively.

Table 66

Two-tailed-test of the Differences Between pre-and post-test Clinical data for TMD Subjects Without Acquired Pi/PCr data

Group	Stim	N	Data	Mean Pre	Mean Post	SD Pre	SD Post	df	T score	P Value
TMD	A	6	PPT	1.5550	2.3700	0.8812	0.9004	5	3.3182	**0.0210**
TMD	P	3	PPT	2.1533	2.1467	1.5240	1.6427	2	0.0945	0.9333
TMD	A	6	VAS	7.111	2.217	1.956	2.697	5	3.0999	**0.0269**
TMD	P	3	VAS	5.867	5.067	1.102	0.115	2	1.1390	0.3727
TMD	A	6	ROM	23.17	31.67	6.11	6.31	5	2.7268	**0.0414**
TMD	P	3	ROM	23.67	23.33	1.53	5.13	2	0.1280	0.9098

P Values < 0.05 (significant) are presented in bold.

Additional analyses were performed by combining all of the TMD subjects that received the full active or placebo exposure with or without acquired Pi/PCr spectra, as shown in Table 67. It was again apparent that exposure to MENS produced a significant difference among all three clinical measures as evidenced by the elevation of PPT and ROM values with a decrease in the VAS score between baseline and the 48-60 minute time-points.

Table 67

Two-tailed-test of the Differences Between pre-and post-test Clinical data for all TMD Subjects Combined (with and without Pi/PCr data)

Group	Stim	N	Data	Mean Pre	Mean Post	SD Pre	SD Post	df	T Score	P Value
TMD	A	8	PPT	1.6075	2.3425	0.8552	0.8496	7	3.9170	**0.0058**
TMD	P	6	PPT	2.3300	1.9767	1.0099	1.0664	5	1.6134	0.1676
TMD	A	13	VAS	5.623	1.762	2.280	2.098	12	4.8223	**0.0004**
TMD	P	7	VAS	5.986	5.086	0.809	2.184	6	1.2296	0.2649
TMD	A	13	ROM	26.462	34.538	9.360	9.726	12	3.9300	**0.0020**
TMD	P	7	ROM	25.86	26.86	5.43	6.54	6	0.7354	0.4797

P Values < 0.05 (significant) are presented in bold.

Table 67 highlights the statistical analyses among the combined groups of TMD subjects (with and without acquired Pi/PCr data) who completed the full exposure to the active or placebo paradigm. There were a total of 8 TMD subjects with algometric recordings that were exposed to one hour of MENS and the difference between the pre-and post-test PPT recordings (Table 67 and Figure 31) was significant ($p = 0.0058$). In contrast, there were 6 TMD subjects exposed to the placebo protocol and the difference between their pre-and post-exposure PPT value was not significant (Table 67 and Figure 32).

In total, 13 TMD subjects received the full one-hour exposure to MENS, in which significant pre-and post-test VAS and ROM recordings (Figures 38 and 40) were obtained. In comparison a total of 7 TMD subjects with placebo exposure

did not demonstrate significant changes in VAS or ROM as shown in Table 67 and Figures 39 and 41, respectively. The results of the pre-and post-test clinical analyses were again significant only for the TMD subjects exposed to MENS, as highlighted in Table 67.

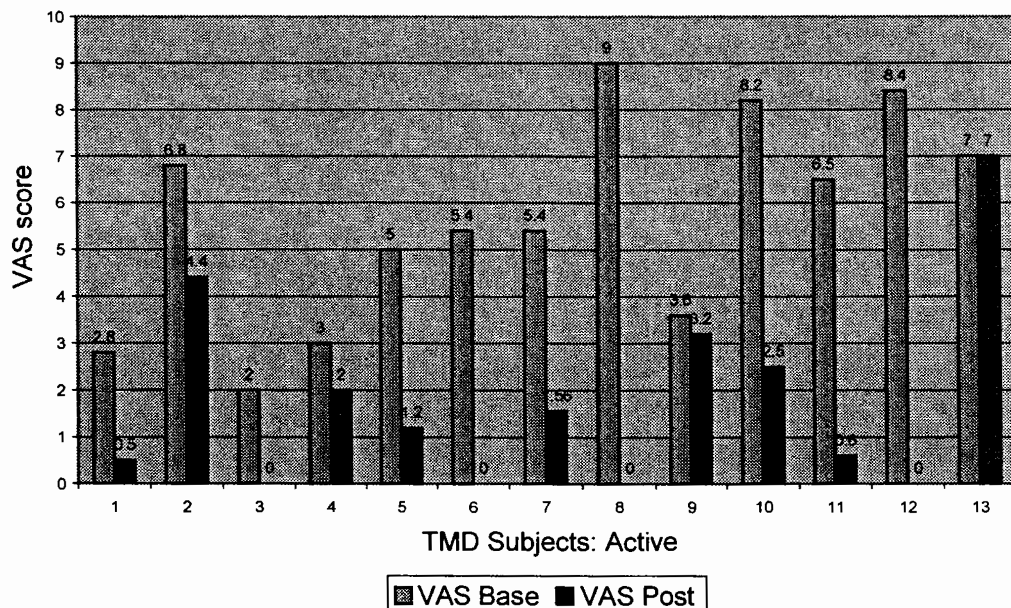


Figure 38. VAS scores for all TMD subjects that completed the full one-hour exposure to MENS, with and without acquired Pi/PCr values.

The difference between the pre-and post-test VAS scores for the combined TMD subjects, 7 with acquired Pi/PCr data and 6 without Pi/PCr data, who completed the full exposure to MENS, was significant with a probability score of 0.0004, as highlighted in Table 67. In contrast, the difference in VAS scores for

the combined group of 7 TMD subjects that received the full one-hour placebo protocol, with and without acquired Pi/PCr data was not significant and the change among each subject is shown in Table 67 and Figure 39.

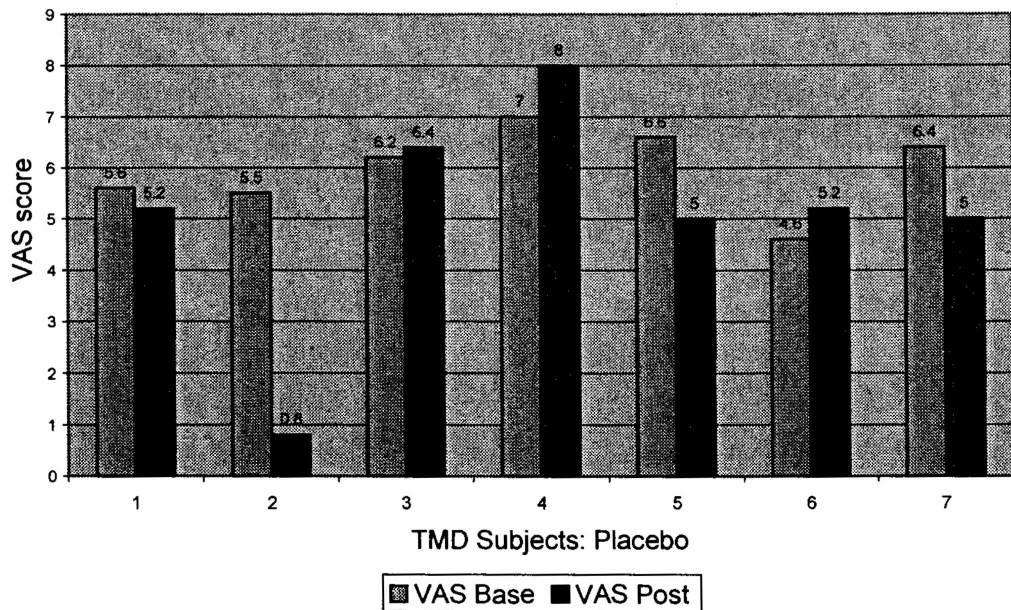


Figure 39. VAS scores for all TMD subjects that completed the full one-hour placebo exposure, with and without acquired Pi/PCr values.

The difference between the pre-and post-VAS scores for the group of 7 TMD subjects with placebo exposure, was not significant, as shown in Table 67 and Figure 39.

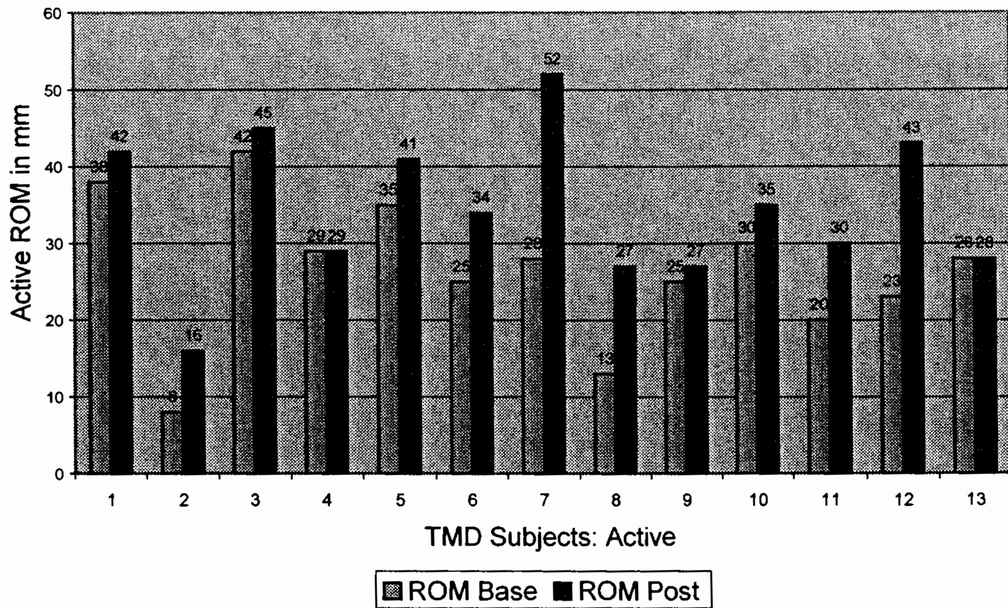


Figure 40. Active ROM values at baseline and after exposure to one-hour of MENS for all TMD subjects, with and without acquired Pi/PCr data.

The change in active ROM after exposure to one-hour of MENS was highly significant in the combined group of 13 TMD subjects, as shown in Table 67 and Figure 40.

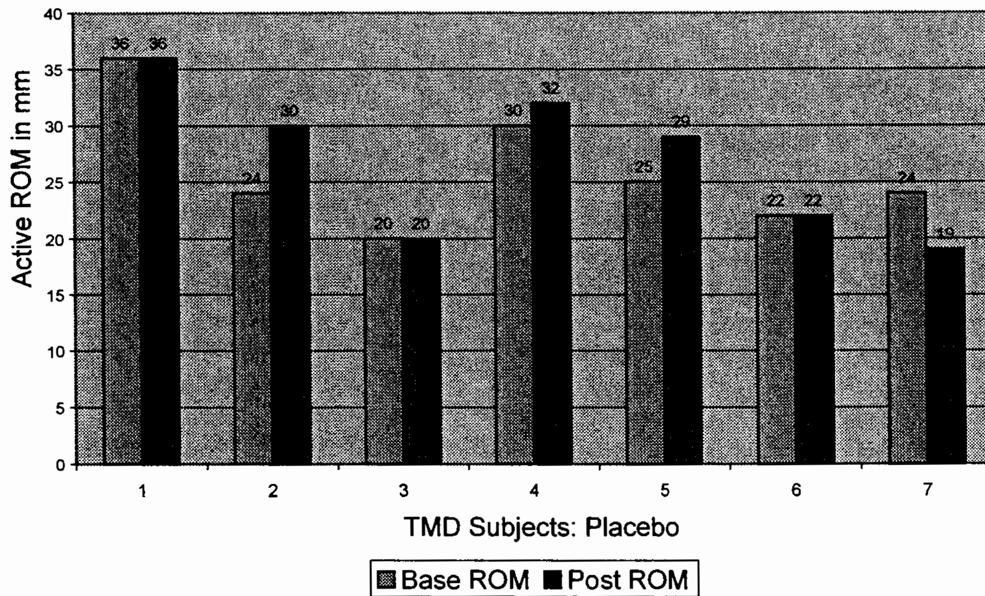


Figure 41. Active ROM values at baseline and after exposure to one-hour of the placebo protocol for all TMD subjects, with and without acquired Pi/PCr data.

Exposure to the placebo protocol did not create a significant increase in active ROM for the combined group of 7 TMD subjects, as shown in Table 67 and Figure 41.

Chapter V

DISCUSSION

Limitations of the Study

Before discussing the results of this study, it is necessary to present the limitations imposed by sample size and statistical power that directly impact the findings and must be stated prior to making any conclusion. .

Statistical Power and Sample Size

Given the results of the pilot study (Appendix F) in which 18 subjects were tested, there remain questions as to the power to detect real effects in the small samples of the study (8 normal-active, 7 TMD-active, 4 normal-placebo and 4 TMD-placebo). As a result, additional analyses were undertaken to determine the statistical properties of the design and the data.

Due to the reduced number of subjects with acceptable spectra to analyze and perform the statistical calculations that would yield enough power to test the hypotheses, it was necessary to determine the effect size. The effect size was calculated as the change in-group means, divided by the pooled standard deviation, which is a common measure used in cases where statistical power may be lacking in the experimental design. As with all statistical measures, it can be affected by sample size, and in very small samples may be unreliable. The effect size for all groups of interest was small and calculated as 0.372. In contrast, the effect size in

the pilot was 0.163, which is even smaller and the larger effect size may be a function of the larger overall sample size (41) in the study.

A small simulation verifying the properties of the test was thus completed as part of this analysis. The probability of a Type II error, indicating that the data analysis cannot document the difference between the groups when the difference is present, is defined as (1-power). The Type II error level for this study is quite high, at 0.825, and indicates that there may be effects of the treatment in the data that simply cannot be detected statistically due to the small sample size.

The effect size for the sample within treatment (active or placebo) across diagnoses (normal or TMD) was also evaluated. For the active group, the effect size was 0.177 (negligible), and the power was 0.07. For the placebo group, the effect size was 1.82 (which is very large), and the power was 0.713. While the power in the placebo group is good, the group is so small (8 subjects) that the results should also be interpreted with great caution. In addition, since the placebo group is the effective control group, the ability to detect changes in this group is equally important.

Null Hypothesis I

The active stimulation protocol of this study utilized electrical parameters that were similar to those in cellular level research with non-human tissue, as cited in the literature review. Elevated Pi/PCr is evidence of increased ATP synthesis and the significant differences in Pi/PCr values that were caused by microcurrent stimulation in this study, although limited by adequate power, leads to rejection of

the null hypothesis which states that there will be no effect of MENS upon Pi/PCr of the human masseter. The foundation and support for this decision is based upon the data and statistical analyses from Tables 14, 15, 19, 22, 31, 34, 41 and 47 as compiled in Table 68.

Table 14 presents the mean Pi/PCr data and probability scores at each respective time-point for the four groups of subjects that completed the active or placebo exposure with acquired phosphorus spectra that was able to be analyzed. The results of the two-tailed t-tests reveals a significant elevation of Pi/PCr between the baseline and 48-60 minute time-points for the group of 7 TMD subjects that received MENS. In addition, the group of 8 normal subjects that received MENS, demonstrated a significant increase in Pi/PCr at both the 20-32 and 48-60 minute time-points. There were no significant increases in either group of normal or TMD subjects with placebo exposure, which were composed of only 4 subjects each.

Table 15 represents further analyses performed by combining both the normal and TMD subjects that received active stimulation (n =15) and those that received placebo exposure (n = 8). This pairing also revealed significant increases in Pi/PCr at the 20-32 and 48-60 minute time-points in comparison to baseline, only for the combined group that received active stimulation. However, there can be a variance between or within the subject groupings, which were determined by ANOVA testing (Tables 16-22), but fluctuations of the dependent variable

(Pi/PCr) can occur from differences in the experimental methodology or the effects of the independent variable (MENS) as well as confounding factors.

The pilot study allowed for the elimination or reduction of the major confounding variables as noted in Appendix F. However, between-group Pi/PCr variances could be due to the manner in which each group was treated, as well as the effects of MENS. The within-group variance reflects upon the Pi/PCr values that may occur from individual subject differences or other unforeseen factors. Calculating the ratio of the between group changes in the dependent variable (Pi/PCr), due to systematic treatment differences (independent variable) or diagnosis, and the within-group changes in the dependent variable due to individual subject differences, provided the means by which variance was assessed.

Subsequent ANOVA testing provided additional support in that a significant increase in Pi/PCr occurred from baseline at both the 20-32 and 48-60 minute time-points for the same group of 15 subjects receiving MENS as shown in Table 19 and 22 respectively. Furthermore, Tables 31 and 34 revealed significant differences between the combined group of 15 subjects exposed to active stimulation, in comparison to the group of 8 subjects exposed to placebo, at the 48-60 and between the baseline and 48-60 minute time-points, respectively.

A significant difference in mean Pi/PCr values was additionally noted in Tables 41 and 47 between the baseline and 48-60 minute time-points among the TMD subjects who received MENS, but not among the group of TMD subjects

exposed to placebo. Two-tailed t-test comparisons within each diagnostic group revealed that the sub-group of TMD subjects exposed to the placebo protocol demonstrated a significant decrease in Pi/PCr at the 48-60 minute time-point (Table 47), that may indicate withholding active stimulation to this group had a negative effect.

Table 68, summarizes the tests and specific time-points at which significant differences between baseline and subsequent Pi/PCr values occurred in the groupings of normal and TMD subjects that were exposed to MENS, as opposed to subjects that received the placebo protocol.

Table 68

Summary of the Significant Differences in Pi/PCr Values Within and Between Subject Groupings

Table	Test	Factor	Group	N	Time-point	P Value
14	Paired 2-tailed t-test	Pi/PCr	TMD active	7	48-60 - base	**0.02664**
14	Paired 2-tailed t-test	Pi/PCr	Normal active	8	48-60 - base	**0.01100**
14	Paired 2-tailed t-test	Pi/PCr	Normal active	8	20/32 - base	**0.01860**
16	2 tailed t-test	Pi/PCr	TMD + normal active	15	20/32 - base	**0.01994**
16	2 tailed t-test	Pi/PCr	TMD + normal active	15	48/60 - base	**0.00037**
19	ANOVA	Pi/PCr	TMD + normal Active vs TMD + normal placebo	15 8	48/60	**0.0238**
22	ANOVA	Pi/PCr	TMD + normal Active vs TMD + normal placebo	15 8	48/60 - base	**0.0170**
31	Unpaired 2-tailed t-test	Pi/PCr	Combined Dx groups Active Placebo	15 8	48/60	**Active ss > placebo**
34	Unpaired 2-tailed t-test	Pi/PCr	Combined Dx groups Active Placebo	15 8	48/60 - base	**Active ss > placebo**
41	ANOVA	Pi/PCr	TMD active TMD placebo	7 4	48/60 - base	**0.0284**
47	2-tailed t-test	Pi/PCr	TMD active TMD placebo	7 4	48/60 - base	**Active ss > placebo**

A more in-depth explanation is required to present support to the Chemiosmotic Theory that has been the focus of this study as well as to analyze the aforementioned results by a physiological and scientific perspective.

Beyond the Chemiosmotic Theory

Cheng used the chemiosmotic theory, to explain the physiological effects of microamperage stimulation (Mitchell, 1966). The chemiosmotic effect produced by electrical stimulation causes electrons to react with water molecules at the cathode, producing hydroxl ions and protons at the anode, plus the creation of a proton gradient across the inner mitochondrial membrane, which then initiates more extensive physiological reactions that ultimately cause Na,K-ATPase to split in order to create ATP synthesis (Tsong, 1992). However, the ATP synthesis process is much more complex, and a summary of the new research that follows, presents interesting information that may provide additional support to the effects of microamperage stimulation at the cellular level, but questions remain as to whether or not the same intracellular process occurs when similar electrical parameters are applied transcutaneously.

Low Frequency Weak AC Stimulation

. There is now general agreement among the researchers in the field of cellular signaling, that alternating electrical fields of weak, low frequency stimuli are the most bioactive, with the ability to gate electrosenitive ion channels and

cause biological effects such as bone growth, cellular transcription, microfilament reorganization, lymphocyte activity, immune system stimulation, neuroendocrine modulation, calcium efflux, ATP synthesis and wound healing (Blank, 1992; Blank, Soo, Lin & Goodman, 1993; Markov & Pilla, 1993; Astumian, Weaver & Adair, 1995; Cho, Thatte, Lee & Golan, 1996; Cho, Thatte, Silvia & Golan, 1999; Pangaopoulos, Karabarbounis & Margaritis, 2002; Gartzke & Lange, 2002).

Electromagnetic (EM) waves of low intensity and low frequency have stimulated biosynthesis in cell and organ cultures, whereas low frequency EM waves produce accelerated bone growth and healing when applied to non-union fractures (Blank and Soo, 1993; Blank 1995; Blank and Soo, 1996; Ryaby, 1998; Otter, et al., 1998). Furthermore, bone growth stimulators apply their electrical fields transcutaneously, and the resultant clinical data support the effect of exogenous electrical and electromagnetic fields upon intracellular mechanisms (Markov, et al. 1993; Markov & Colbert, 2000).

The literature review discussed in chapter two, cited scientific studies that support the stimulation of ATP synthesis at the cellular level in non-human tissue, by the action of alternating current with electrical parameters below 100Hz delivered at a threshold of 1-5 mV/mm with a strength window of less than 3V/m (Blank, Soo, Lin & Goodman, 1993, Polk, 1993). Even very weak 10μV/m AC currents have been shown to affect cell transcription, regardless of their electrical or electromagnetic origin, but a prime hindrance to the alteration of intracellular

physiology is a naturally occurring process known as thermal or shot noise (Polk, 1993, Manwani & Koch, 1999, Steinmetz, Manwani, Koch & Segev, 2000, Francis, Gluckman & Schiff, 2003).

Thermal or Shot Noise

Shot noise is defined as a current source in which the passage of each charge carrier is a statistically independent event (as opposed to a steady flow of many charge carriers) creating a " noisy current " that fluctuates around an average value dependent upon the magnitude of each individual carrier (Austmian, et al., 1995; Francis, et al, 2003). The detection and function of weak stimuli is hindered by thermal noise, which is present in all physical systems and generated by the thermal agitation of electrons in a conductive field, such as a cellular matrix (Manwani & Koch, 1999; Steinmetz, et al, 2000). Neuronal networks are sensitive to sub-millivolt (microcurrent) stimuli and thus in order to cause a significant interaction with an electric field, the effect on the cellular biochemical process has to be equal to or greater than the molecular shot noise driven by macromolecular thermal fluctuations at a threshold of about 100μV/mm (Francis, et al, 2003).

Various mechanisms have been proposed to explain how weak stimuli can overcome thermal noise to produce a change in cellular physiology. It has been proposed that as the duration of an applied electric field increases, it can cause rectification or stochastic resonance that produces stronger inward currents by signal amplification, thus gating ion channels (Westerhoff, Tsong, Chock, Chen, &

Astumian, 1986; Tsong, 1988; Kruglikov & Dertinger, 1994; Cho, et al.,1996; Cho, et al, 1999; Zrimec, 2002; Gartzke, 2002; Lu, 2003).

Counter-ion polarization can also be accomplished with very weak stimuli mediated at low pulse rates, which also serve to overcome thermal noise (Polk, 1993; Gartzke, et al, 2002). Another explanation is that a disturbance in the electrically neutral space between cells causes an ion density fluctuation that is propagated between adjacent cells, producing a neutral charge cloud whose radial width is inversely proportional to the square root of the frequency, and causes cell communication to be more pronounced below 20Hz (Polk, 1993; Gartzke, et al, 2002).

Cellular Microvilli Reorganization and Ion Channels

AC stimulation has been proposed to be the primary stimulus that causes microfilament reorganization (Cho, et al, 1999). These effects may possibly explain how transcutaneously applied electrical or electro-magnetic fields could have an effect on a multicellular area of muscle or bone. Cellular cytoskeletons are characterized by the presence of microvillar structures, that project from and along the entire surface and contain the actual ion channels, including that of the critical Ca^{2+} signal pathway (Lange, 1999; Gartzke, et al., 2002). Transportation proteins exist on the microvilli of differentiated cells that have specific functions, with the cytoplasm on the inside and microvillar surface on the outside, separated by an actin filament bundle, which functions as an electrical wire controlling the diffusion of ions and other solutes (Lin & Cantiello, 1993).

Microvilli of the retina and ear can be activated by weak physical forces such as light or sound respectively. Differential cellular swelling can cause membrane stress, and cellular interaction with weak, low frequency oscillating electrical fields, represent other sources of activation by very weak stimuli (Cho, et al, 1999). A subtle mechanical load that causes very slight bending of the microvilli has been shown to activate the ion conduction pathway of vascular endothelial cells (Cho, 1999).

The cellular sensing nature of microvilli is activated within ion channels by fluxes along electrochemical gradients, thus regulating the influx and efflux of various metabolites including that of calcium, sodium, potassium, magnesium and energy providing substrates such as ATP (Babes & Fendler, 2000; Lange, 2000).

Ion channels therefore function as electrical switches in excitable biological membranes, that open and close with a gate-like manner in response to electrical and chemical stimuli (Blank, 1993; Babes, 2000). The resultant gating current transfers a charge across the membrane between channel surfaces and alters the energetics to favor opening of a constrictive gate in the channel, which is different for sodium and potassium. Sodium channels are fast gating and cause an inward ionic movement, while potassium are slow and produce a delayed outward current (Westerhoff, Tsong, Chock, Chen & Astumian, 1986; Tsong, 1988; Blank, 1992; Blank, 1993; Babes, 2000).

Na $^{+}$/K^{+} -ATPase

Alternating current fields have been shown to alter the ion concentration on the surface of cellular membranes, which control the activity of enzymes such as Na $^{+}$/K^{+}-ATPase (Blank, 1992). Ion concentrations across cell membrane surfaces vary with the frequency of excitable stimuli, which are within the same range as those observed with ATPase. Electric fields produced by AC stimulation therefore induce the redistribution of integral membrane proteins, which are frequency dependent, causing subsequent micro-filament reorganization, which has been shown to occur within 5 minutes from signal transduction cascades initiated by an alternating electrical field of 1Hz at 20v/cm, thereby triggering a calcium influx (Blank, 1987; Cho, et al, 1996; Cho, et al, 1999).

The resultant alteration of cellular membrane concentrations creates a proton gradient, which produces signal transduction that is both extra and intracellular, thereby affecting the composition on the inside as well as the outside of the cell, subsequently activating Na,K-ATPase and creating ATP splitting (Berg, 1993; Blank & Soo, 1993; Blank , Soo & Papstein, 1995; Babes, et al, 2000).

Cellular level research has shown that when the ATPase enzyme is at optimal activity, such as in non-stressed resting muscle or normal tissue, weak, low frequency AC stimulation in the microamperage range causes an increase in ion concentration and lowers the trans-membrane activity thus decreasing the rate of ATP production. In contrast, when the same electrical parameters are applied in a state of decreased ATPase activity, such as in stressed tissue or fatigued muscle,

the net increase in ion concentration increases membrane activity. The increased activity creates a transduction signal that enhances ATP synthesis (Blank, 1992; Tsong, 1992; Blank & Soo, 1993; Tezara, et al, 1999).

However, the results of this study showed that transcutaneous microcurrent stimulation enhanced ATP synthesis in the TMD as well as normal subjects; and therefore may produce a similar effect, although without the necessity of muscle contraction. Considering that the masseter is never at rest and has a constant degree of low tension, required for its participation in the sling-like support of the mandible, separate from its role in chewing, it is always untilizing ATP and therefore always under a minor degree of stress. It is unknown if this mild yet constant degree of activity is sufficient to create an increased need of ATP. Further replication of this research project should therefore be performed upon a muscle other than the masseter.

Mitchondrial Matrix

In the intracellular matrix, further research has uncovered the existence of an outer mitochondrial membrane that is smooth with a degree of elasticity and a voltage-dependent anion channel that is freely permeable to low molecular weight substrates. In direct contrast, the inner membrane is organized in a reticular network containing a gel-like matrix that is 50% protein and composed of invaginations termed cristae, with a permeability barrier to a variety of compounds. In addition there is a inter-membranal space located between the inner and outer membranes (Passerella, Atlante, Valenti & de Barr, 2003). Additional intracellular

physiological steps have therefore been proposed, which provide greater depth to the proton gradient explanation, thus lending more support to the original theory of Mitchell (1966).

It is known that Ca^{2+} triggers the most important intracellular signaling processes, and its release from the sarcoplasmic reticulum enhances the interaction of actin and myosin in muscle cells and secondarily activates various kinases that influence the rate of mitochondrial respiration and ATP levels, of which an increase can persist even after a decline in mitochondrial calcium (Jouaville, Pinton, Bastianutto, Rutter & Rizzuto, 1999; Gartzke, et al., 2002). In this respect the enhancement of ATP synthesis can assist in the ability of a hyperactive muscle to relax as well as contract thus secondarily decreasing discomfort from continual spasm.

Structural reorganization of proteins is known to occur from AC stimulation, creating a change in their electrical properties. Ca^{2+} uptake is driven by the mitochondrial Ca^{2+} uniporter located in the inner membrane, which passes down the electrochemical gradient and highly selective ion channel, thus exerting a control upon the ATP production rate (Jonas, et al., 1999; Lancaster, 2003; Kirchok, Krapivinsky & Clapman, 2004). The resultant potential energy, from this trans-membrane proton-motive force, is used to form ATP from ADP and phosphate, creating an increase in protons that become available for ATP production via oxidative phosphorylation, without altering the pHi (Stock, et al, 1999). Proton efflux occurs during exercise and the subsequent resynthesis of PCr,

results in gradients across the electrogenic mitochondrial membrane that are able to be perturbed by a sinusoidal current as low as 10mV at 20Hz (Kemp, Thompson, Taylor & Radda, 1997; Babes, et al., 2000; Bendahan, Kemp, Roussel, Fur & Cozzone, 2003).

Each side of the inner mitochondrial membrane has a proton translocation channel for ion transport that penetrates to the center of the membrane and involves ATP synthesis. Neither of the two channels traverse along the entire plane of the membrane. They are separated by a short distance, with transport across the gap involving a carrier that reversibly binds the ions (McCabe, Bourgain, & Maguire 2003). The outer mitochondrial layer is permeable to small molecules while the inner layer allows permeability to molecules such as pyruvic acid, ADP and ATP, but not to protons. A resultant electrochemical gradient representing the difference between the proton concentration at the inter-membrane space and matrix occurs, thus providing the power for any metabolic process that has a channel for protons to flow down the gradient back into the matrix (Cooper, 1997).

ATP synthase, contains binding sites for ADP and ATP and is imbedded within the mitochondrial inner membrane thereby capable of creating a channel or pore that allows the passage of protons, which flow down the electrochemical gradient and release the energy needed to power the synthesis of ATP from ADP and phosphate (Blank, 1992; Bolognani et al, 1993; Blank, 1993; Blank & Soo, 1993; Blank, 1995; Blank & Soo, 1996; Horst et al, 1997; Stock, Leslie, & Walker, 1999). The mechanism for transport across the gap requires an electrically driven

rotation of a carrier, and the source of the electric field that drives this carrier is the transmembrane electric potential or proton gradient (McCabe, Bourgain & Maguire, 1993).

The Proton Gradient and Weak AC Stimulation

The the effects of low intensity and low frequency AC on cellular ionic movement creates an efflux of K^+ ions and an influx of Na^+ ions, producing an electrical potential or gradient. One full AC cycle therefore yields a proton gradient across the membrane that causes ATP splitting and the stimulation becomes cumulative due to the continuous reversal of the AC field, which lends support to the view of rectification or stochastic resonance. The proton-motive force required to produce a gradient for ATP synthesis has previously been thought to be composed of a trans-membrane proton concentration (pHi) and the membrane potential. However, support for an electrically induced, as opposed to a pHi gradient, as the prime factor in ATP synthesis, now exists (Kaim & Dimroth, 1998; Dimroth, Kaim & Matthey, 2000).

F_1 and F_0 ATPase

Fluctuation of calcium concentrations, within the physiological range, influence inner membrane phosphorylation events that involve a subunit of F_1 and F_0 ATPase, which is now considered to be the main source of cellular ATP (Azarashvily, Trynela, Baumann, Evtodienko & Saris, 2000). These ATP synthases are two of a family of enzymes that exist in bacterial cytoplasmic membranes, thylakoid membranes of chloroplasts, as well as the inner membrane

of human mitochondria. They catalyze the synthesis of ATP in the last step of oxidative phosphorylation and contain motor subunits that generate the rotational torque required to drive such synthesis, which also necessitates the presence of an adequate concentration of magnesium (Weber & Senior, 2003).

The F_1Fo ATPase is a critical component in cellular energy metabolism and requires a transmembrane proton gradient that it converts into ATP (Capaldi & Aggeler, 2002; Aksimentiev, Balabin, Fillingame & Shulten, 2004). This enzyme is now known to be a composite of a rotor and a stator that function in opposite directions termed protonation and deprotonation (Itoh, Takahashi, Adachi, Noji, Yashuda, Yoshida & Kinosita, 2004; Aksimentiev, et al, 2004). Ion transport across the gap, between the inner membrane and the channels, is produced by an electrical field, which mediates the rotational torque of the Fo rotor of ATP synthase (McCabe, et al, 2003). Dissipation of the electrochemical gradient across the inner membrane stops the action of the F_1Fo ATPase and hence decreases ATP synthesis (Hood, 2001).

Recent experimentation has demonstrated that almost all of the torque sufficient to generate ATP synthesis by the Na^+ F_0 motor is due to the membrane potential and not pHi (Dimroth, Kaim & Matthey, 2000; McCabe, et al, 2003). The F_0 motor converts the transmembrane gradient of Na^+ into a rotary torque (protonation) that releases ATP and in the reverse direction (deprotonation) it functions as a ATP driven Na^+ or H^+ pump (Dimroth, et al, 2000; Fillingame & Dmitriev, 2002). In comparison, the bacterial flagellar motor can change its

direction of rotation forward and back without altering the direction of proton movement through the membrane (Itoh, et al, 2004).

Specific to muscle contraction/relaxation, only one motor head is able to connect to a myosin filament at a time, while the other head remains passive. When the catalytic domain in the head has ADP and Pi bound to its binding site, the action is weak. When the active motor head docks properly to the actin binding site, Pi must be released causing the lever arm to swing counterclockwise, which pushes the actin filament down by about 10nm along its longitudinal axis. The active motor head then releases the bound ADP and another ATP molecule immediately replaces it, again binding to the head of the actin filament, after which the myosin motor dissociates itself from the actin filament and another cycle commences (Cho, et al, 1996; Lang, 1999; Cho, et al, 1999; Dimroth, et al, 2000; Hood, 2001; Fillingame, et al, 2002; Gartzke, et al, 2002; Zrimec, 2002; McCabe, et al, 2003; Weber, et al, 2003; Itoh, et al, 2004; Aksimentiev, et al, 2004). It has been shown that the ATP synthesis rate becomes saturated at a frequency that exceeds 3Hz, which relates to the low end of the frequency window, but the maximal rotary speed of the Fo motor is 130Hz (Itoh, et al, 2004).

The preceeding physiological explanation is now recognized as the mechanism behind ATP synthesis triggered by electrical or electromagnetic microamperage stimulation at the cellular level. Perhaps the same physiological process occurs from the transcutaneous application of MENS, as found in this

study, but replication and further research with a larger subject population utilizing a muscle other than the masseter is required.

Null Hypothesis II

Clinical and Metabolic Correlation

Clinical data consisting of active mandibular ROM, VAS and algometric PPT were acquired for each TMD subject before entering the magnet and after exposure to MENS or placebo. The two groups of TMD subjects were very small (7 active and 4 placebo), and therefore insufficient to perform a viable correlation analysis between Pi/PCr and clinical data. There is a strong possibility of spurious relationships appearing in measures such as correlations in which the sample size is small. The linear relationship between two variables (Pi/PCr and each of the clinical tests PPT, VAS or ROM) is determined by how proportional they are to each other and based upon the size of the sample.

In order to make this assumption, it is critical to have a large sample size, which was not the case with this research. A single outlier or extreme score in a sample of only 4 or 7 subjects can create a profound effect on the value of a correlation (Portnoy & Watkins, 2000). Furthermore, there is a lack of homogeneity between the number of subjects exposed to the active and placebo protocols, which can easily bias the correlation. However, t-test analyses of clinical data pre-and post-exposure to both active and placebo stimulation were performed for all of the TMD subjects.

Recordings and measurements of pressure pain threshold, subjective pain ratings and active vertical ROM of the mandible were obtained before and immediately after each test for all of the TMD subjects. Since the algometer was not available at the start of the study, PPT data was only acquired for 2 of the 7 active and 3 of the 4 placebo subjects. Tables 61 and 62 delineate the clinical data recordings for all TMD subjects with acquired Pi/PCr spectra. Table 63 presents the two-tailed t-test results for each group, which revealed significant increases in active ROM and PPT, plus a significant decrease in VAS levels only in the groups of TMD subjects exposed to MENS.

Please note that there are 8 active TMD subjects (2 with and 6 without Pi/PCr spectra) and 6 placebo TMD subjects (3 with and 3 without Pi/PCr spectra) all of whom had the full exposure to MENS. They were combined in order to calculate differences in PPT, due to the later acquisition of the algometer as compared to those with VAS and ROM data (7 active and 4 placebo), all of whom had acquired Pi/PCr spectra.

The analyses of Tables 64 and 66 reveal similar significant findings for 6 additional TMD subjects who completed the full exposure to active stimulation without acquired Pi/PCr. Table 65 presents the recorded clinical data for the 3 TMD subjects exposed to the placebo protocol, but for whom Pi/PCr analyses were also unable to be determined. The differences in clinical data as determined by the two-tailed t-test analyses of Table 66 were not significant for this group of 3 TMD subjects with placebo exposure.

Table 67 represents the combined group data for all TMD subjects that completed the full one-hour exposure to the active or placebo protocol with or without acquired Pi/PCr spectra. These two-tailed t-test analyses reveal similar findings in that the differences between the pre-and post-test recordings for PPT, VAS and ROM were significantly different only for the combined groups that received MENS. Therefore, the data in Tables 63, 66 and 67, which is summarized in Table 69, provide sufficient support to reject null hypothesis II. In the groups of TMD subjects (with and without acquired Pi/PCr data) that received MENS, t-test analyses showed a significant elevation in PPT and ROM scores, with a decrease in VAS values.

Table 69

Summary of the Significant Differences in Clinical Data by Groups

Table	Test	Data	Group	N	Time-point	P Value
63	2 tailed t-test	PPT	TMD active + Pi/PCr	8	48/60-base	**0.0058**
63	2 tailed t-test	VAS	TMD active + Pi/PCr	7	48/60-base	**0.0018**
63	2 tailed t-test	ROM	TMD active + Pi/PCr	7	48/60-base	**0.0398**
66	2-tailed t-test	PPT	TMD active	6	48/60-base	**0.0210**
66	2-tailed t-test	VAS	TMD active	6	48/60-base	**0.0269**
66	2-tailed t-test	ROM	TMD active	6	48/60-base	**0.0414**
67	2-tailed t-test	VAS	TMD active with & w/o Pi/PCr	13	48/60-base	**0.0004**
67	2-tailed t-test	ROM	TMD active with & w/o Pi/PCr	13	48/60-base	**0.0020**

Optimal Stimulation Windows

The previous review of the literature provided evidence that weak and low frequency AC stimulation enhances ATP synthesis in non-human tissue. One of the major tenents of that research cited the presence of optimal parameters within frequency and intensity windows, typically within 1-100Hz at an intensity ranging from microvolts to millivolts. The duration of the effective stimulation period was commonly within the 5-30 minute range. It must be kept in mind that the optimal windows for these parameters were derived from non-standardized experimentation using single and multiple cellular solutions, as well as plant, bacterial and squid tissue.

Berg provided examples of time windows for both low frequency weak alternating electric and electromagnetic fields on cell metabolism, which ranged from 20-60 minutes and correlates with two of the data collection time-points of this study (Berg, 1995). However, prior to further discussion of the results, it is necessary to present the outcome of additional research performed on non-human and human tissue, with stimulation parameters that were basically equivalent to those utilized in this study.

More recent research has also demonstrated that weak, non-thermal electric fields produce biological effects that are optimized within specific pulse rate and amplitude windows of 10-100 Hz at 0-130$\mu a/cm^2$ (Binhi & Goldman, 2000). In addition, magnetic fields operate optimally at low amplitudes within a frequency

range of 8-60 Hz (Gartzke, et al, 2002). An AC field of 1-10 Hz at 10 V/cm, applied to human hepatoma cells has demonstrated a fourfold increase in Ca^{2+} influx across the plasma membrane from the extracellular medium during a 30 minute exposure (Cho, Hemant, Thaitte, Silvia & Golan, 1999). Continued stimulation beyond 30 minutes did not yield any further increase as it was believed that saturation had occurred.

In order to reach a conclusion regarding the choice of microamperage stimulation parameters for clinical use and further experimental paradigms, the results of this study must be combined with that of the previous research presented in the literature review as well as earlier in this chapter. Figures 42 and 43 illustrate the degree to which Pi/PCr increased among the TMD and normal subjects by different pulse rate and intensity parameters, with the duration of exposure being constant at 60 minutes.

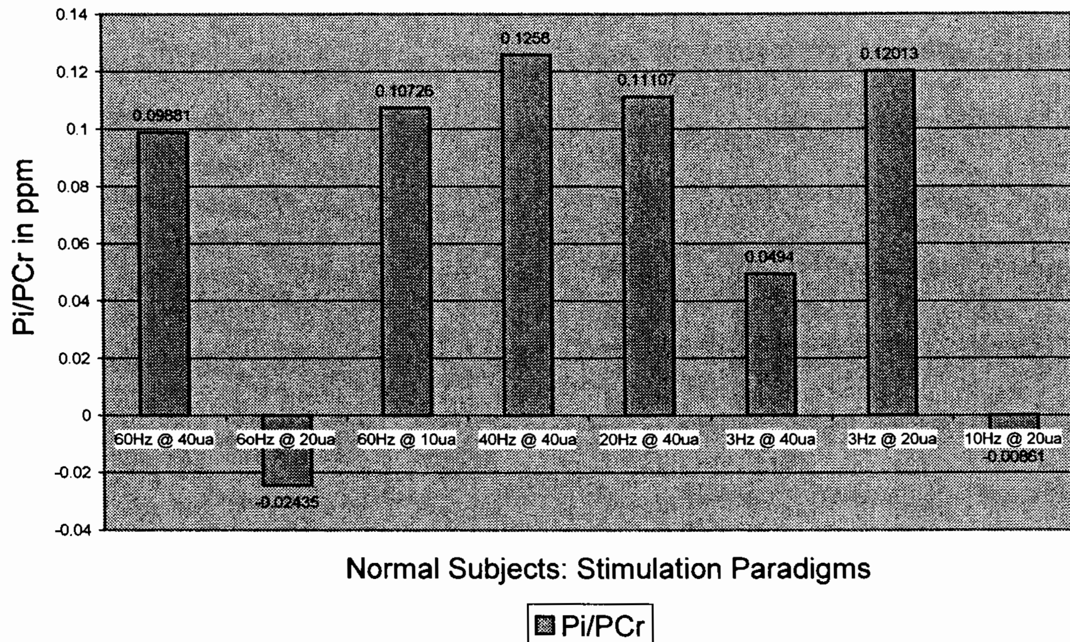


Figure 42. The effect of different stimulation paradigms on mean Pi/PCr for normal subjects after exposure to MENS.

In this group of 8 normal subjects, the largest increases were noted with 40 Hz @ 40μa and 3 Hz @ 20μa, but due to the limited number of subjects without replication of similar paradigms, any statement relative to superiority of one over the other would not be justified.

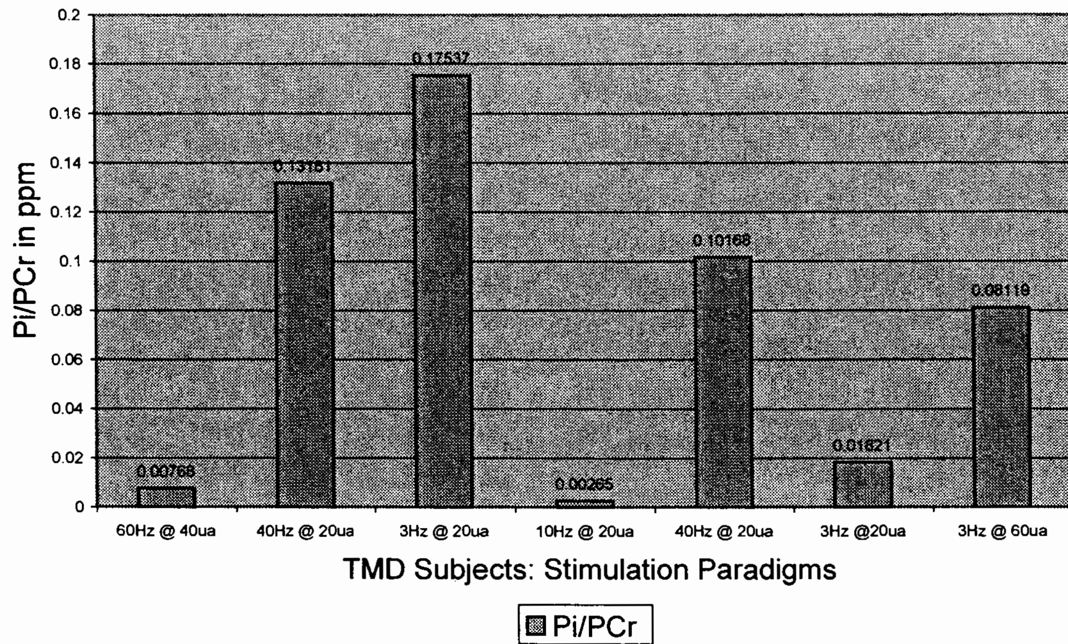


Figure 43. The effect of different stimulation paradigms on mean Pi/PCr for TMD subjects after exposure to MENS.

In this group of 7 TMD subjects, the largest increases were noted with stimulation paradigms of 3 Hz or 40 Hz @ 20μa; but again, due to the limited number of subjects, a pattern is not forthcoming. However, the 3 and 40Hz parameters demonstrated the greatest elevation of Pi/PCr in both the normal and TMD subjects. Ongoing study with a larger subject group is recommended, before any definitive pattern is likely to emerge.

pHi

Appendix A contains all of the tables and figures relative to the analysis of the acquired pHi data as well as the statistical analyses. Although pHi data was not included in the hypotheses, resting pHi levels obtained from the normal and TMD subjects in this study were similar to those obtained from other research. Resting masseter pHi have been found to range between 7.11 +/- 0.06 to 7.17 +/- 0.03 and decline to 6.99 +/- 0.08 to 7.03 +/- 0.06 during clenching, dependent upon the masseteric region analyzed (Lam, et al., 1992). pH levels in another group of normal subjects demonstrated a resting mean of 7.11 +/- 0.06, which decreased to a mean of 7.04 +/- 0.02 with low rate repetitive exercise and 6.99 +/- 0.06 when the contraction was sustained (Plesh, et al., 1995). Sappy-Marinier, et al., (1998), obtained a mean resting pH of 7.02 +/- 0.03, which decreased to 6.93 +/- 0.04 during a biting force exercise. Considering that exercise was not being induced by MENS, the pHi values of this research were similar to those recorded at rest in the above studies with a decline in the TMD subjects from exposure to MENS as well as the normal subjects with placebo exposure.(Table A10 and Figure A7). There was minimal fluctuation in pHi values during the study with the mean ranging from 7.02 -7.06 for all groups.

ANOVA data for resting pHi at baseline for all four subject groups was not significantly different (Table A17). The ANOVA of Table A18 reveals that at the 20-32 minute time-point there was a significant difference in pHi between the active and placebo TMD groups. The two-tailed t-test of Table A23 revealed a

significant difference at the 20-32 minute time-point between the combined group of 12 normal (7.05) and 11 TMD subjects (7.02) respectively, regardless of the type of exposure.

A decline in pHi was also noted at the 48-60 minute time-point, in which the group of 8 normal subjects exposed to MENS was significantly different (7.02) from that of the normal group of 4 subjects (7.05) that received the placebo protocol as analyzed by ANOVA and two-tailed t-tests respectively (Table A50 and A56). Figure A7 illustrates that there was no change in pHi values for the group of normal subjects exposed to MENS over the three time-points, but a progressive decrease was seen in the normal group from placebo exposure and at the 48-60 minute time-point, with the difference between them significant as shown in Table A56. MENS may have caused a decrease in pHi in the TMD group from 7.06 to 7.02, while placebo exposure showed the opposite effect with an increase from 7.03 to 7.06 at the 48-60 minute time-point (Figure A7).

Because MENS does not induce muscle contraction, any change in pHi may be due to its effect on the proton gradient of the mitochondrial membrane. It is therefore possible that the reduction in pHi that occurred in the TMD group from active stimulation may be due to the effects of MENS, but it is important to keep in mind that a similar reduction in pHi also occurred in the group of normal subjects from placebo exposure.

Current Evidence Relative to pH

An increase in lactic acid production from exercise is no longer considered to be the cause of acidosis and oxidative phosphorylation has been shown to occur without altering the pHi (Stock, Leslie & Walker, 1999). It is known that an experimentally induced acute acid load does not alter pHi, but chronic metabolic acidosis will do so over a period of time (Bailey, England, Long & Mitch, 1996). The current physiological explanation for acidosis due to muscle function is the breakdown of ATP into ADP and Pi, which results in the release of a proton. As the duration or intensity of exercise continues and a greater degree of ATP is consumed, additional protons are released, which is now believed to create the acidosis thereby promoting the onset of fatigue and weakness, as well as the decline in pHi (Norman, Sabina & Jansson, 2001; Robergs, Ghiasvand & Parker, 2004).

The baseline pHi values of this study did not reveal any significant differences between the four groups, nor point to a major degree of acidosis, which may also be a function of muscle fiber type. The accumulation of protons, in lieu of lactate, which occurs more significantly with Type II muscle fibers, gives rise to fatigue, and acidosis occurs when the utilization of ATP is not equivalent to the synthesis rate. The resultant decline in pH in the early recovery phase following intense exercise occurs from the rapid resynthesis of PCr, which also alters pHi by the generation of protons (Taylor, Bore, Styles, Gadian & Radda, 1983; Norman, et al, 2001). A decrease in pHi during exercise and recovery at rest has been

demonstrated in the masseter (Lam & Hannum, 1992; Marcel, Chew, McNeill, Hatcher & Miller, 1995; Plesh, Meyerhoff & Weiner, 1995; Sappy-Mariner, Dheyriat, Lissac, Frutoso, Mallet & Bonmartin, 1998).

The relationship between pHi and inflammatory or ischemic pain has been established, and a recent study demonstrated that unmyelinated group IV mechano-sensitive muscle receptors of the rat gastrocnemius are activated by an increased pHe caused by a proton accumulation (Issberner, Reeh & Steen, 1996; Hoheisel, Reinohl, Unger & Mense, 2004). However, slow-twitch muscles, such as the masseter, are less sensitive to a decrease in pHe towards acidosis than fast-twitch muscles (Baillie & Garlick, 1991; Caso, Garlick, Casella, Sasvary & Garlick, 2004). An experimentally induced drop in pHe (7.43 $\pm$ 0.02 to 7.32 $\pm$ 0.04) has been demonstrated to inhibit protein synthesis by 33% in skeletal muscle as opposed to visceral organs. A decrease in pHe towards acidosis is fiber type specific and slow-twitch muscles such as the masseter, which is composed primarily of Type I fibers are less sensitive. Therefore the decrease in pHi that was exhibited by the TMD group exposed to MENS may not have any influence on ATP synthesis in skeletal muscle. This is supported by the findings of an MRS study that did not reveal any significant differences of in-vivo skeletal muscle pHi at rest and exercise between subjects with essential hypertension and those that were normotensive (Khong, McIntyre, Sagnella, Markandu, Miller, Baker, Griffiths & MacGregor, 2001).

Chapter VI

SUMMARY AND CONCLUSIONS

This study utilized an established method of data acquisition (MRS) that was both quantitative and scientific in order to determine the cellular metabolic effects of microamperage stimulation applied transcutaneously to a human muscle in vivo. The extensive statistical analyses that followed provided support for rejection of the null hypotheses, but with strong concern regarding the strength of the findings in light of the very small sample size and limitation of power.

A summary of the ANOVA and two-tailed t-tests of the differences in Pi/PCr values within and between the different subject groupings, as provided in Table 68, represents the foundation for rejection of null hypothesis I. Significant elevation of Pi/PCr from baseline values were found at the 20-32 (Tables 14 and 16) as well as the 48-60 time-point (Tables 14, 16, 19, 22, 31, 34, 41 and 47) for the groups of TMD and normal subjects exposed to microcurrent stimulation paradigms. Statisitical analyses did not reveal any significant changes in Pi/PCr values for the groups of normal and TMD subjects exposed to the placebo protocol.

Clinical data analysis consisting of the change in pre-and post-test recordings of PPT, ROM and VAS were performed by two-tailed t-tests (Tables 63, 66 and 67) and summarized in Table 69. Significant elevation of PPT and

ROM with a decrease in VAS pain levels were found in each group of normal and TMD subjects exposed to microcurrent stimulation. Exposure to the placebo protocol did not reveal any significant differences in clinical data for the normal or TMD groups, therefore causing rejection of null hypothesis II.

One of the most important aspects of this project concerned development of the scientific methodology which consumed a significant part of the research and necessitated prolonged pilot work as well as the creation and testing of a conductive phantom to verify that the acquired spectra was acceptable for analysis. Despite these efforts, spectral interference resulted in the loss of data that would have increased the subject population and strengthened the power of the statistical analyses.

Although limited by sample size the acquired data and subsequent statistical analyses provided preliminary findings that demonstrated an elevation of Pi/PCr by MENS, thereby supporting the Chemiosmotic Theory of Mitchell (1996) as well as the prime research hypothesis. Various questions and suggestions follow in order to assist others in replicating this research so that additional information can be obtained. Similar research designs with other skeletal muscles allowing for a greater number of subjects and enhanced statistical power are needed to

It is critical to understand that this study only tested a few closely related stimulation paradigms administered at a sub-perception threshold level of intensity, with a fixed stimulation/exposure time on a small number of subjects. However, the range of stimulation paradigms with MENS, as well as other forms

of electrical stimulation, is enormous and necessitates further exploration. Experimentation with larger subject groupings is therefore needed in order to provide greater support to the chemiosmotic theory (Mitchell, 1966), refute the findings of this research or uncover other physiological mechanisms to explain the results.

Additional data analyses may also determine if there is a relationship between the primary and secondary clinical measures or dependent variables. The addition of more frequent measurements (time-points) might provide greater information about patterns in the data that could be obscured by the few time-points that were available. However, it is necessary to state that in order to obtain reliable data per time-point with MRS, an acquisition period of at least 12 minutes is required, which significantly hinders the number of data points for analysis.

Alternative Applications of MENS

Optimal parameters of intensity, frequency and duration, specifc to the application of MENS for pain relief in humans have never been determined through double-blind scientific studies. However, there have been two studies performed with human subjects that did not monitor Pi/PCr and contained numerous confounding variables, but are worthy of discussion.

The use of MENS applied first by surface stimulation with probes and then by common surface adhesive electrodes for the relief of pain due to elbow tendonitis, did not reveal any significant difference over placebo stimulation

(Rolle, et al., 1994). The methodology utilized the same MENS device as this study with application of the active or placebo treatment performed three times per week for two weeks. Subjects were randomly assigned to either an active (n = 15) or placebo group (n = 16), and received 10 minutes of stimulation or placebo via 4mm diameter probes. A wet Q-tip was inserted into the receptacle of each probe and applied to 18 sites for 2.5-5 seconds repeatedly with a total treatment time of 10 minutes.

Electrical parameters consisted of 30Hz at 100μa with polarity reversal every 2.5 seconds. This was followed by 20 minutes of stimulation with two adhesive electrodes applied at a frequency of 0.3Hz at 40μa. In addition, each subject performed a 10-15 minute program of daily active exercise during the course of the study, followed by the application of ice for 15-20 minutes. There was no indication provided as to the nature of any ongoing employment during the course of the study, which may have perpetuated the tendonitis. Although the group that received MENS demonstrated an overall greater increase in grip force and reduction in perceived pain, statistical analysis did not reveal any significant difference between the groups.

Another study utilizing two similar microcurrent devices, involved the application of MENS to 26 patients with chronic radiation induced fibrosis of the cervical spine and/or TMJ's (Lennox, Shafer, Hatcher, Beil, & Funder, 2002). A total of 10 treatments were applied twice each day for five consecutive days with cervical spine ROM data collected at baseline and at the end of the experimental

protocol. Electrical parameters consisted of an initial 20 minute application with the Electro-Myopulse 75F at 0.5-100 Hz (specific settings per subject were not provided) with intensity set at the maximal tolerable level, which was usually 600μa, the highest setting on the device. Stimulation was delivered via a large cylindrical metal roller electrode applied in a moving manner along the involved cervical region, with a fixed electrode was placed on the scapula closest to the affected tissue.

The second phase of the protocol utilized another microcurrent generator, the ElectroAcuscope 80L with stimulation parameters set at 10Hz and 600μa. The fixed electrode of the first application was replaced by two electrodes, upon which the subject placed their hands, while a therapist applied the same metal roller electrode repeatedly over the involved cervical region for a period of 10 minutes. Unlike the study of Rolle, et al., (1994), their experimental protocol did not include any other form of intervention or physical therapy.

The pilot study by Lennox, et al., (2002), did not include a statistical analysis of the data. However, 92%, 85% and 81% of the 26 subjects, demonstrated improvement from baseline in cervical rotation, flexion/extension and sidebending respectively, which was maintained at a re-check three months later. The degree of improvement ranged from 17 - 64% in rotation, 19 - 49% in flexion/extension and 19 – 65% for sidebending. Improvement was most profound in the subjects with the more severe ROM limitations.

Although TMJ ROM was recorded for each subject, only 16 reported it as a problem. There were increases in vertical ROM of the TMJ's in 12 of the 16 subjects averaging 4.6 $\pm$ 2.2mm, three months after the course of treatment. Specific pre-and post-treatment recordings were not provided and it is possible that the reported increase could have occurred independent of the pilot protocol. There were subjective improvements in several other associated factors such as xerostonia (15 of 20 subjects), facial asymmetry (6 of 7 subjects) and pain (9 of 13 subjects), without any degree of measurement provided.

Furthermore, it is possible that MENS may be effective in stimulating ATP synthesis during the healing process. However, two studies that used microcurrent stimulation to enhance wound healing in rats and pigs did not demonstrate any acceleration (Leffmann, et al, 1994 and Byl, et al, 1994). Leffmann, et al., (1994) used 100μa at 0.3Hz for two hours per day over a 14 day period, while Byl, et al., (1994) used the same intensity at 0.1Hz for a one hour period limited to five days, to assess the effectiveness of MENS upon induced wounds.

As previously cited in the research of (Rolle, et al, 1994 and Lennox, et al, 2002), that did not monitor Pi/PCr, MENS is also administered clinically with metal rollers or surface probes that use a Q-tip size electrode for periods of 10-60 seconds per point or region. This application can be performed with or without pressure and/or stretching of the involved tissue, which was not cited, as it presents another variable. Physical therapists that utilize this method really have no

scientific proof that the physiological effects that may occur with probes are equivalent to that attributed to surface electrodes. Surface electrodes are typically active for periods of 10-60 minutes and there is no previous research that has compared the effects of short stimulation via probes to prolonged stimulation by surface electrodes with similar electrical characteristics.

It remains to be demonstrated whether the low frequency and or short period of stimulation that the studies of Rolle, et al., 1994; Leffmann, et al., 1994 and Byl, et al., 1994) were hindering factors, since the optimal frequency window for stimulation of Na,K,ATPase activity was not utilized (Berg, 1995; Blank and Soo, 1996; Cho, et al., 1999; Binhi and Goldman, 2000; Gartzke, et al., 2002).

Recommendations for further Research

Hypoxia and Oxygenation

Ischemia alters many neuromuscular factors, including a decline in pH to the 6.0 level which produces nociceptor activation (Caldwell, 1956; Steen, Reeh, Anton & Handwerker, 1992). Hypoxia from muscle spasm creates a rapid decrease in PCr before any decline of ATP, followed by increased lactate, a subsequent decrease in ATP, alteration of electrical activity and increased Pi/PCr. In a hypoxic state, the only source of ATP is via glycolysis, which can produce decreased oxygenation (Robergs, et al, 2004). Muscle oxidative capacity, which can be altered by environmental, functional or ischemic factors, can be measured by MRS (Van Den Thillart, & Van Waarde, 1996).

Exposure to the active stimulation paradigms in this study revealed evidence of decreased pain increased vertical ROM of the TMJ's and elevation of the pain pressure threshold. Although unable to be directly correlated to Pi/PCr values, MENS may simply enhance masseter oxygenation by causing capillary vasodilation, as opposed to or in addition to ATP synthesis and thus implicate an alternative or dual physiological mechanism that will require confirmation by different research paradigms. Individuals with chronic masseter hyperactivity and tenderness to palpation may exhibit the reduced oxidative capacity found in the presence of chronic fatigue syndrome (McCully, et al., 1996).

Therefore, if MENS stimulates capillary vasodilation, it may offset hypoxia and the subsequent rapid depletion of high-energy phosphates that produce metabolic acidosis caused by muscle spasm. 31^P MRS has demonstrated that oxidative metabolism differs in relation to the level of physical conditioning and long-distance runners exhibit a faster PCr recovery rate in comparison to sprinters, which may be indicative of an enhanced oxidative capacity, thus negating muscle soreness (McCully, et al., 1992; Tartaglia, Chen, Caramanos, Taivassalo, Arnold & Argov, 2000). The assessment of pre-and post-stimulation oxygenation levels is thus worthy of study with MENS and should be considered in future research. Observation of tissue oxygenation may therefore offer an alternative explanation separate from or in addition to that of enhanced ATP synthesis.

Facial Morphology

Masseter muscle metabolism can also be altered by differences in facial types. The study did not consider nor group the subjects according to facial morphology, which may influence the contractile tension of the masseter. A normal appearing face is termed, mesofacial, while one that is disproportionately short or long are known as brachyfacial and dolichofacial respectively Subjects with a deep bite, square jaw appearance and wide nasal aperture commonly have large pharyngeal spaces and a shallow palate. This group of features is known as the brachyfacial type, which is associated with large mandibular elevator muscles that are well developed, aligned vertically over the molars and thus can exert a greater load on the TMJ. The dolichofacial type is the opposite, presenting with an open bite, elongated/narrow facial/jaw appearance, narrow nasal aperture and a deep palate. This group commonly has weaker mandibular elevator muscles that are positioned posterior to the molars and thus exert a weaker loading effect on the TMJ. Furthermore, the dolichofacial complex is commonly associated with mouth breathing, which is usually accompanied by altered tongue position to maintain an open airway (Morrish & Stroud, 1995).

Pi/PCr values, as determined by MRS of the resting masseter, have been found to differ between humans relative to facial pattern. An increase in the maxillary to mandibular divergence (palatal to mandibular angle) has been correlated with a lower resting Pi/PCr. Muscles with a higher Pi/PCr demonstrated a higher level of resting metabolic activity in comparison to those with a lower

Pi/PCr (Al-Farra, Vandenborne, Swift & Ghafari, 2001). Even though masseteric contraction was not part of the protocol for this research, the resting tension of the masseter and medial pterygoid muscles, which constantly maintain a minimal degree of tension to support the mandible, may have been higher in those subjects with a brachyfacial structure. It is therefore possible that differences in craniofacial structure could affect the metabolic activity of the subject, thus representing another variable that will need to be considered in future research of this nature.

Two clinical studies are proposed, utilizing an acute as well as chronic pain paradigm. These research endeavors would follow in an effort to replicate any positive implications of MENS. One design would utilize a miniature microamperge device placed at the TMJ region of subjects who brux or clench during sleep. The device would generate the specific stimulation parameters that demonstrated enhanced ATP synthesis in this study of a Type I muscle. A similar study utilizing a Type II muscle, such as the gastrocnemius, in the presence of nocturnal restless leg syndrome, is also suggested. The effects of active and placebo exposure would be compared among subjects in both of these studies.

Another clinical study would utilize the same miniaturized stimulator on a continuous basis for a 24-48 period immediately following arthroscopic surgical procedures to the same peripheral joint, in order to assess its effect upon Pi/PCr as well as post-surgical inflammation, evidenced by MRS and MRI respectively.

Oxygenation levels in a population of subjects with chronic pain, could also be assessed by infra-red photoelectric tissue spectroscopy, since evidence exists that subjects with chronic muscle pain have slower intramuscular reperfusion after a period of sustained isometric contractions than normals (Delcanho, Kim & Clark, 1996).

Other studies can be designed to determine if different physiological effects occur with alternating current as compared to direct current. Although the stimulation protocol utilized in this research consisted of alternating current, wound-healing protocols have also used various direct current paradigms. It may therefore prove valuable to determine the differences between net positive or negative polarity in the presence of acute soft tissue pathology, which may benefit by immediate application of MENS to enhance healing and simultaneously control pain. If results prove to be positive, an inexpensive, miniature device incorporating both milliamperage and microamperage modes could be designed to allow for 24-48 hours or more of continuous stimulation for healing and pain control. Use of the microamperage mode at night would not interfere with sleep, as there would not be any startle response since the continuous stimulation would be at a non-perceptible level.

Finally it is recommended that additional testing should focus upon other muscles, such as the gastrocnemius, that are larger, easier to test and yield ideal phosphorus spectra, from that which was obtained from the masseter. I would also suggest utilization of an experimental paradigm in which a cohort of normals

undergo baseline Pi/PCr acquisition of a given muscle, and are then subjected to an acute pain producing paradigm, to be followed by immediate re-entry into the magnet to determine if there is a change in Pi/PCr with and without activation of MENS. The same data acquisition methodology developed by this research project would be used to determine the effect of microcurrent stimulation upon Pi/PCr and associated clinical signs therefore supporting or rejecting the findings of this research.

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Appendix A

Pi Data

Tables A1-4, delineate the acquired Pi levels for each subject tablulated by the type of exposure and diagnostic group. Pi values as well as the mean and standard deviation are included.

Table A1

Pi Values for Normal Subjects (Active Stimulation)

Subject	Parameters	DX	Stim	Pi Base	Pi 20-32	Pi 48-60
MKS022500	60Hz @ 40μa	Normal	Active	12.87	9.53	6.99
JVH030300	60Hz @ 20μa	Normal	Active	6.99	6.57	6.90
GG030700	60Hz @ 10μa	Normal	Active	8.15	4.73	12.42
BC031000	40Hz @ 40μa	Normal	Active	6.78	7.39	7.13
SM032100	40Hz @ 20μa	Normal	Active	5.83	9.37	8.70
DW032800	3Hz @ 40μa	Normal	Active	7.20	6.59	7.45
BP032900	3Hz @ 20μa	Normal	Active	3.95	6.90	5.36
WON091500	10Hz @ 20μa	Normal	Active	6.63	7.36	7.58
Mean				7.30	7.31	7.82
SD				2.56	1.56	2.08

In the group of 8 normal subjects who were exposed to the microamperage stimulation protocol, the mean baseline value of inorganic phosphate was

relatively unchanged at the 20-32 time-point, but increased at the 48-60 minute time-point.

Table A2

Pi Values for TMD Subjects (Active Stimulation)

Subject	Parameters	DX	Stim	Pi Base	Pi 20-32	Pi 48-60
JM040500	60Hz @ 40μa	TMD	Active	10.10	5.78	14.26
KG041900	40Hz @ 20μa	TMD	Active	5.96	11.37	9.82
HS050500	3Hz @ 20μa	TMD	Active	4.05	5.05	7.88
BB052700	10Hz @ 20μa	TMD	Active	7.03	11.31	5.95
NW110700	40Hz @ 20μa	TMD	Active	4.05	7.22	7.07
KS111000	3Hz @ 20μa	TMD	Active	5.69	6.99	6.85
OP020901	3Hz @ 60μa	TMD	Active	2.75	3.64	6.11
Mean				5.66	7.34	8.28
SD				2.43	2.99	2.94

In the group of 7 TMD subjects who were exposed to the microamperage stimulation protocol, the mean value of inorganic phosphate increased from the baseline value at both the 20-32 and 48-60 minute time-points.

Table A3

Pi Values for TMD Subjects (Placebo)

Subject	Parameters	DX	Stim	Pi Base	Pi 20-32	Pi 48-60
HS102700	Placebo	TMD	Placebo	10.02	9.41	9.36
OP120800	Placebo	TMD	Placebo	5.90	8.78	6.48
MB020201	Placebo	TMD	Placebo	3.89	7.35	9.00
OP032701	Placebo	TMD	Placebo	6.30	6.39	5.08
Mean				6.53	7.98	7.48
SD				2.56	1.37	2.05

In the group of 4 TMD subjects who were exposed to the placebo protocol, the mean value of inorganic phosphate increased from baseline at the 20-32 and 48-60 minute time-points respectively. Although the value at the 48-60 minute time-point exceeded that of baseline, it declined below the value reached at the 20-32 minute time-point.

Table A4

Pi Values for Normal Subjects (Placebo)

Subject	Parameters	DX	Stim	Pi Base	Pi 20-32	Pi 48-60
BPO32800	Placebo	Normal	Placebo	5.61	7.73	6.77
DW032900	Placebo	Normal	Placebo	4.75	4.63	7.73
TH102000	Placebo	Normal	Placebo	6.10	6.48	7.02
KI010501	Placebo	Normal	Placebo	5.37	6.67	8.07
Mean				5.46	6.38	7.40
SD				0.56	1.29	0.61

In the group of 4 normal subjects exposed to the placebo protocol, the mean value of inorganic phosphate increased at both the 20-32 and 48-60 minute time-points respectively.

Figure A1 illustrates the mean value of Pi at each time-point for the four subject groups respectively. In the group of normal subjects exposed to MENS there is virtually no change in Pi from baseline to the first time-point followed by a small increase at the end of the exposure, while the normal group exposed to the placebo protocol demonstrated an elevation of Pi at each time-point from baseline.

In the group of TMD subjects exposed to active stimulation, there was an increase in Pi at each time-point, which was similar to that of the normal subjects exposed to the placebo protocol, but with different values. The TMD subjects with placebo exposure demonstrate an increase at the 20-32 time-point, followed by a

small decline at the end of the testing period, to a value that remained above that of baseline.

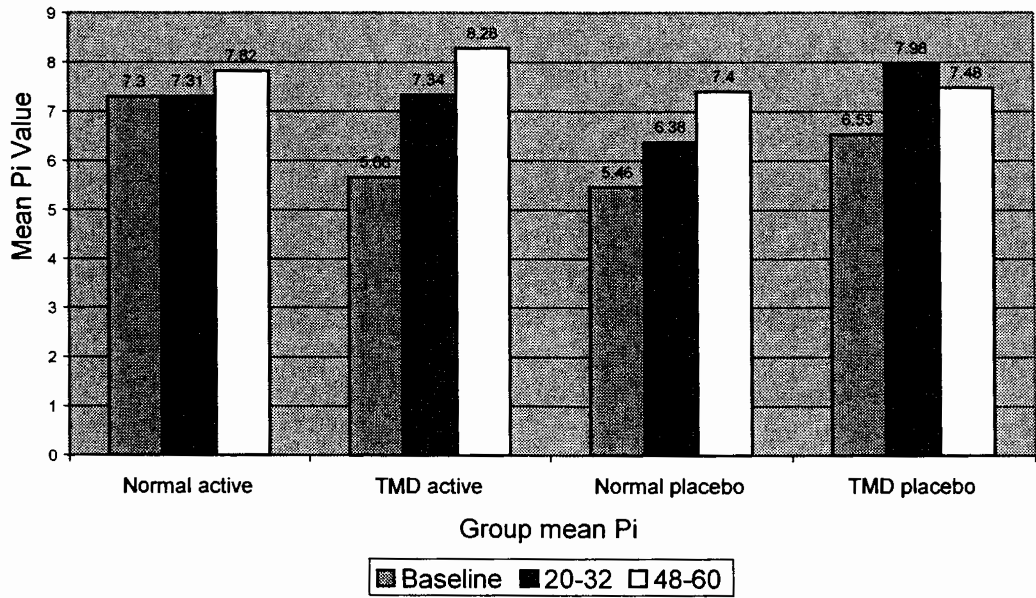

Figure A1. Mean inorganic phosphate values at each time-point among the four groups.

Tables A5-9, contain the PCr data for each subject, delineated by group and stimulation exposure. Mean scores and standard deviations are included, and these data were utilized with the Pi levels from corresponding groups to calculate Pi/PCr at each time-point and subsequently perform the definitive statistical analyses.

PCr Data

Table A5

PCr Values for Normal Subjects (Active)

Subject	Parameters	DX	Stim	PCr Base	PCr 20-32	PCr 48-60
MKS022500	60Hz @ 40μa	Normal	Active	59.96	49.97	20.97
JVH030300	60Hz @ 20μa	Normal	Active	26.69	22.87	29.55
GG030700	60Hz & 10μa	Normal	Active	34.97	18.07	34.98
BC031000	40Hz @ 40μa	Normal	Active	28.89	25.47	19.59
SM032100	20Hz @ 40μa	Normal	Active	36.91	32.39	31.97
DW03280	3Hz @ 40μa	Normal	Active	39.88	24.38	32.65
BP032900	3Hz @ 20μa	Normal	Active	33.81	31.15	22.21
WON091500	10Hz @20μa	Normal	Active	21.97	23.37	25.67
Mean				35.39	28.46	27.20
SD				11.51	9.83	5.86

The mean value of phosphocreatine decreased at both the 20-32 and 48-60 minute time-points respectively in the group of 8 normal subjects exposed to the active stimulation protocol.

Table A6

PCr Values for TMD Subjects (Active)

Subject	Parameters	DX	Stim	PCr Base	PCr 20-32	PCr 48-60
JM040500	60Hz @ 40μa	TMD	Active	31.23	26.32	42.25
KG041900	40Hz @ 20μa	TMD	Active	30.72	27.13	31.06
HS050500	3Hz @ 20μa	TMD	Active	21.58	15.55	20.64
BB052700	10Hz @ 20μa	TMD	Active	34.92	42.03	31.04
NW110700	40Hz @ 20μa	TMD	Active	25.35	24.31	26.53
KS111000	3Hz @ 20μa	TMD	Active	29.96	35.81	32.77
OP020901	3Hz @ 60μa	TMD	Active	17.35	28.64	25.04
Mean				27.30	28.54	29.90
SD				6.16	8.45	6.88

There was a minimal increase in the mean value of phosphocreatine from baseline to the 20-32 and 48-60 minute time-points in the group of 7 TMD subjects exposed to the active stimulation protocol.

Table A7

PCr Values for TMD Subjects (Placebo)

Subject	Parameters	DX	Stim	Base PCr	PCr 20-32	PCr 48-60
HS102700	Placebo	TMD	Placebo	43.89	43.47	41.21
OP120800	Placebo	TMD	Placebo	20.19	34.72	37.67
MB020201	Placebo	TMD	Placebo	13.68	31.08	34.23
OP032701	Placebo	TMD	Placebo	35.02	28.67	26.25
Mean				28.20	34.49	34.84
SD				13.76	6.49	6.40

There was an increase in the mean value of phosphocreatine from base-line to the 20-32 time-point in the group of 4 TMD subjects that received placebo exposure. However, this value was relatively unchanged during the remainder of the test period as evidenced at the 48-60 minute time-point.

Table A8

PCr Values for Normal Subjects (Placebo)

Subject	Parameters	DX	Stim	PCr Base	PCr 20-32	PCr 48-60
BP032800	Placebo	Normal	Placebo	24.14	28.18	20.47
DW032900	Placebo	Normal	Placebo	33.14	33.50	57.15
TH102000	Placebo	Normal	Placebo	28.90	34.97	23.73
KI010501	Placebo	Normal	Placebo	29.12	27.41	29.03
Mean				28.83	31.02	32.60
SD				3.68	3.78	16.75

The mean phosphocreatine value increased minimally from baseline, in the group of 4 normal subjects exposed to the placebo protocol, at both the 20-32 and 48-60 minute time-points respectively.

The comparative PCr chart of Figure A2 illustrates that the group of normal/active subjects demonstrated a sharp decrease at the 20-32 time-point, from baseline, and a minimal decrease at the end of the test. However, the group of TMD/active subjects demonstrated almost the reverse of this pattern. The TMD/placebo group showed an increase in PCr, although virtually unchanged between the 20-32 and 48-60 time-points, while the TMD/active and normal/placebo groups have similar patterns.

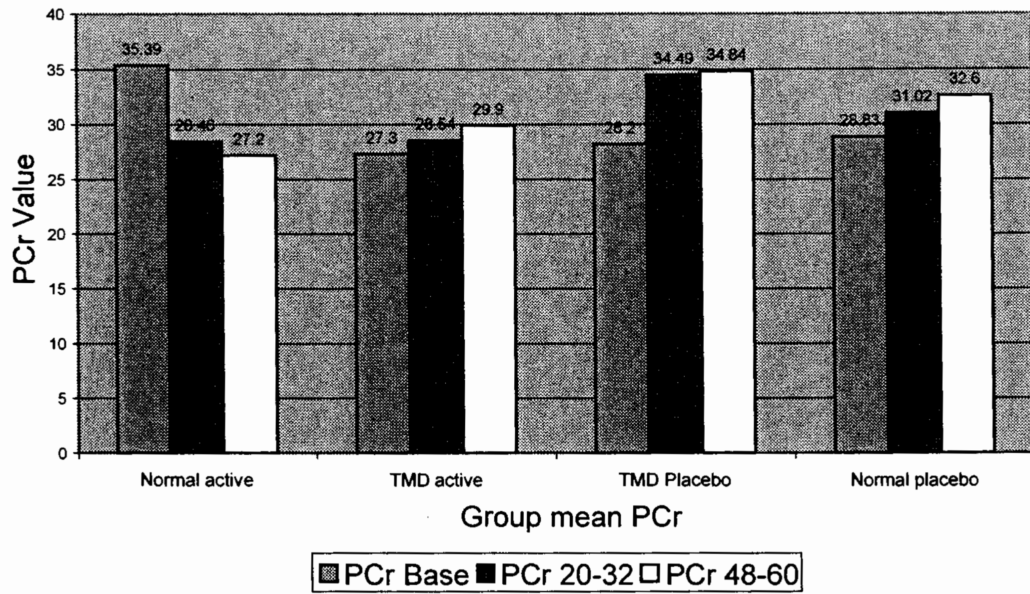

Figure A2. Mean phosphocreatine values at each time-point among the four groups.

pHi Data

Data recording, calculation and subsequent statistical analyses of pHi values, although not relative to the hypotheses, were also calculated at the respective time-points for each subject with acquired spectra.

Table A9

pHi Values for Normal Subjects Exposed to Active Stimulation

Subject	Parameters	DX	Stim	pHi base	pHi 20-32	pHi 48-60	
MKS022500	60Hz @ 40μa	Normal	Active	7.09	7.08	7.05	
JVH030300	60Hz @ 20μa	Normal	Active	7.00	7.05	7.07	
GG030700	60Hz @ 10μa	Normal	Active	7.05	7.09	7.04	
BC031000	40Hz @ 40μa	Normal	Active	6.95	7.02	7.03	
SM032100	20Hz @ 40μa	Normal	Active	6.99	7.03	7.06	
DW032800	3Hz @ 40μa	Normal	Active	6.98	7.04	7.04	
BP032900	3Hz @ 20μa	Normal	Active	7.02	7.01	7.01	
WON091500	10Hz @ 20μa	Normal	Active	7.33	7.11	7.09	
Mean				7.05	7.05	7.05	
SD				0.1206	0.0358	0.0247	

Table A9 delineates pHi changes at each successive time-point, as well as the group mean and standard deviations, for each normal subject that received active stimulation. The mean pHi value remained virtually unchanged throughout the active stimulation protocol, except for a large decrease from baseline to the 20-32 minute time-point in one subject, as can be seen in Figure A3.

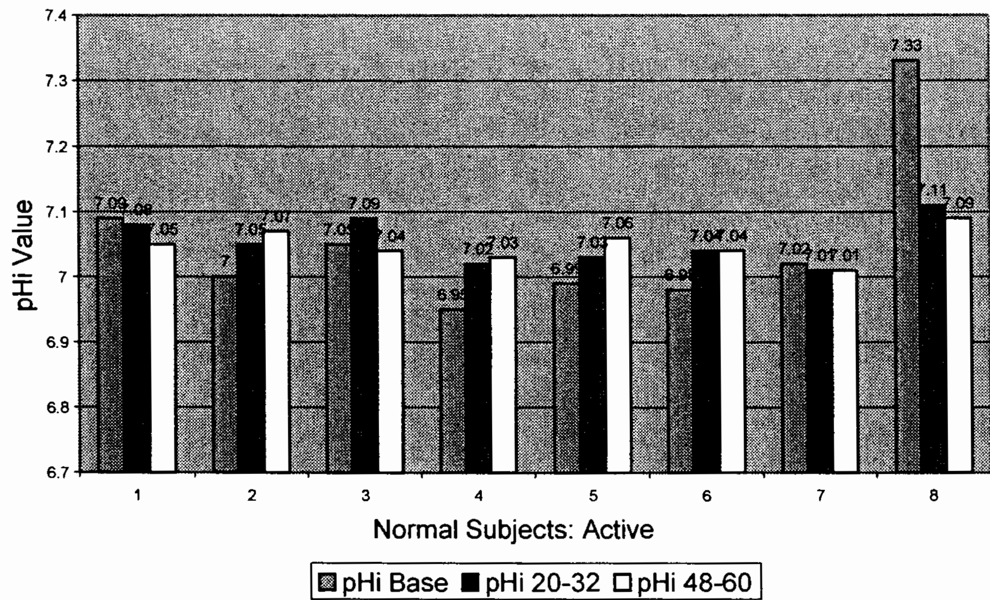

Figure A3. pHi values at the three time-points for the 8 normal subjects exposed to MENS.

Table A10

pHi Values for TMD Subjects Exposed to Active Stimulation

Subject	Parameters	DX	Stim	pHi base	pHi 20-32	pHi 48-60
JM040500	60Hz @ 40μa	TMD	Active	7.02	7.02	7.09
KG041900	40Hz @ 20μa	TMD	Active	7.31	7.04	7.00
HS050500	3Hz @ 20μa	TMD	Active	7.01	7.00	6.96
BB052700	60Hz @ 40μa	TMD	Active	7.04	7.05	7.01
NW110700	40Hz @ 20μa	TMD	Active	7.00	7.02	7.00
KS111000	3Hz @ 20μa	TMD	Active	7.04	7.02	7.10
OP020901	60Hz @ 40μa	TMD	Active	7.01	6.99	7.00
Mean				7.06	7.02	7.02
SD				0.1107	0.0208	0.0519

Table A10 depicts the pHi data at each time-point as well as the group mean and standard deviations for the 7 TMD subjects exposed to active stimulation at successive time-points. Figure A4 shows a large decrease in one subject from baseline to the 20-32 minute time-point, which also occurred in one normal sub ject. Overall, a decrease in pHi from the baseline value occurred at the 20-32 time-point, which remained unchanged at the 48-60 minute time-point. Figure A4 illustrates the change in pHi at each time-point among the 7 TMD subjects.

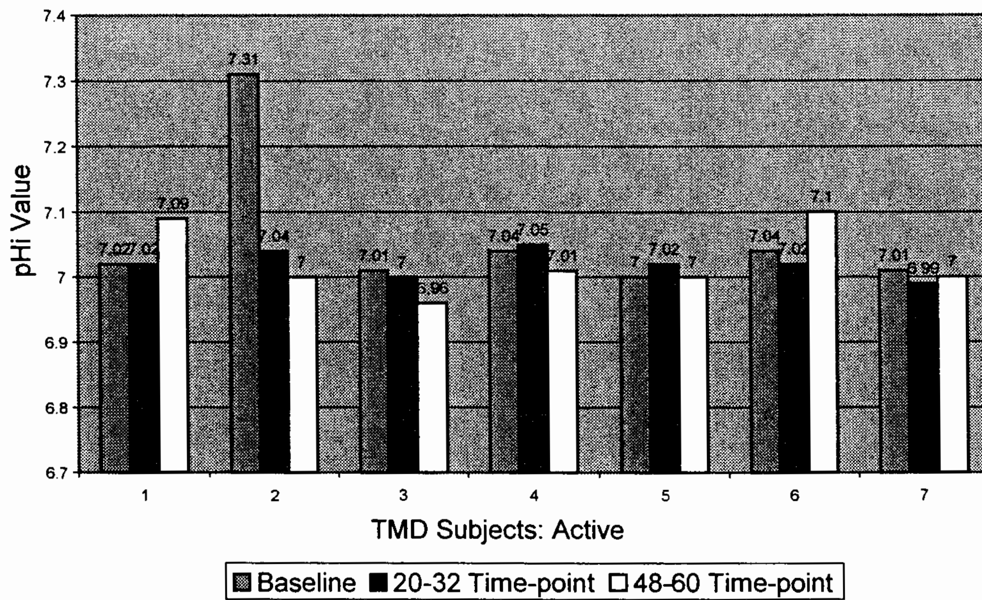

Figure A4. The effect of MENS on pHi values at each time-point for the 7 TMD subjects.

Table A11

Mean and Standard Deviation of pHi Values for TMD Subjects with Placebo Exposure

Subject	Parameters	DX	Stim	pHi Base	pHi 20-32	pHi 48-60
HS102700	Placebo	TMD	Placebo	7.05	7.04	7.05
OP120800	Placebo	TMD	Placebo	6.95	7.04	6.98
MB020201	Placebo	TMD	Placebo	7.07	7.04	7.13
OP032701	Placebo	TMD	Placebo	7.04	6.97	7.06
Mean				7.03	7.02	7.06
SD				0.0532	0.0350	0.0614

Table A11 depicts the pHi value at successive time-points as well as the group mean and standard deviations for the 4 TMD subjects exposed to the placebo protocol. A slight decline in pHi is noted at the 20-32 time-point and an increase from baseline was noted at the 48-60 time-points respectively. Figure A5 illustrates the change for each subject at the respective time-points.

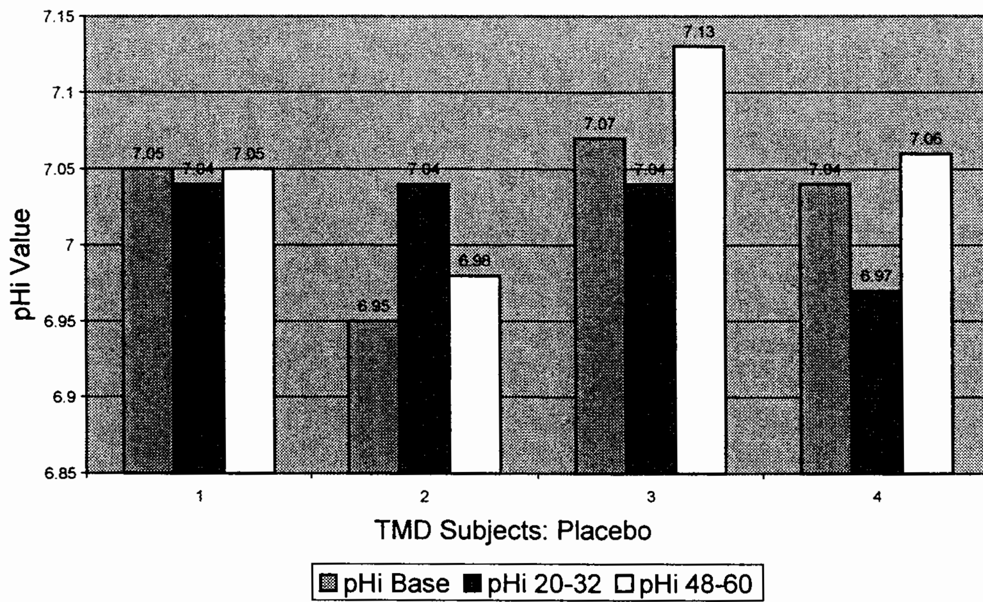

Figure A5. Change in pHi values at each time-point for the 4 TMD subjects exposed to the placebo protocol.

Table A12

Mean and Standard Deviation of pHi Values for Normal Subjects with Placebo Exposure

Subject	Parameters	DX	Stim	pHi Base	pHi 20-32	pHi 48-60	
BP032800	Placebo	Normal	Placebo	7.06	7.04	7.04	
DW032900	Placebo	Normal	Placebo	7.09	7.03	7.01	
TH102000	Placebo	Normal	Placebo	7.04	7.06	7.02	
KI010501	Placebo	Normal	Placebo	6.99	7.04	6.99	
Mean				7.05	7.04	7.02	
SD				0.0420	0.0126	0.0208	

Table A12 illustrates the individual pHi changes as well as the mean and standard deviations for the 4 normal subjects exposed to the placebo protocol. A progressive decline in the mean pHi value is evident as illustrated in Figure A6.

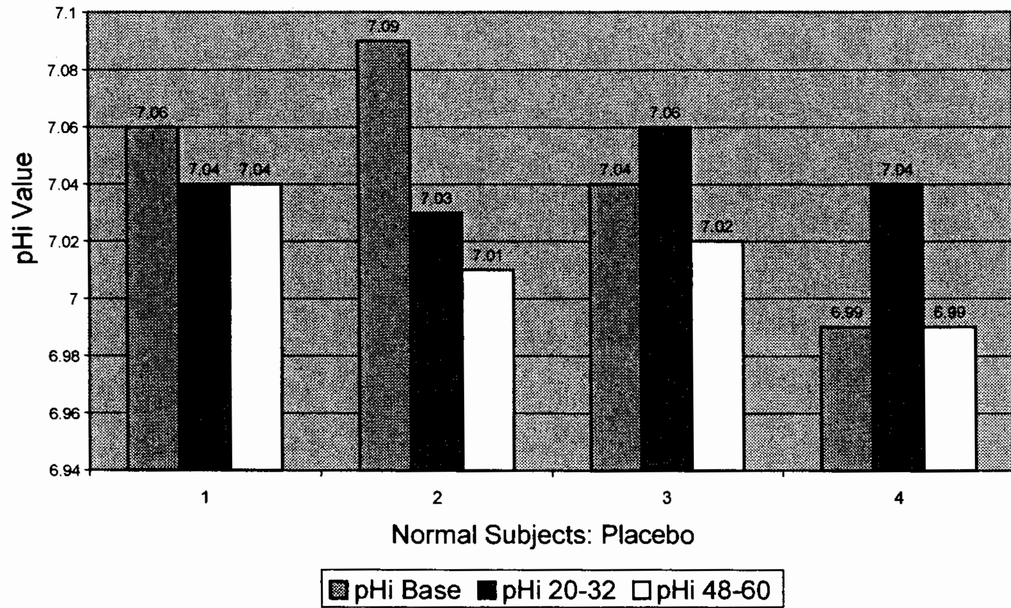

Figure A6. Change in pHi values at each time-point for the 4 normal subjects exposed to the placebo protocol.

Table A13

Differences in pHi Values per Repeated Measures for all Subjects

Subject	Parameters	DX	Stim	20/32-Base	48/60-20/32	48/60-Base
MKS022500	60Hz @ 40μa	Normal	Active	-0.01	-0.03	-0.04
JVH030300	60Hz @ 20μa	Normal	Active	0.05	0.02	0.07
GG030700	60Hz @ 10μa	Normal	Active	0.04	-0.05	-0.01
BC03100	40Hz @ 40μa	Normal	Active	0.07	0.01	0.08
SM032100	20Hz @ 40μa	Normal	Active	0.04	0.03	0.07
DW032800	3Hz @ 40μa	Normal	Active	0.06	0.00	0.06
BP032900	3Hz @ 20μa	Normal	Active	-0.01	0.00	-0.01
WON091500	10Hz @ 20μa	Normal	Active	-0.22	-0.02	-0.24
JM040500	60Hz @ 40μa	TMD	Active	0.00	0.07	0.07
KG041900	40Hz @ 20μa	TMD	Active	-0.27	-0.04	-0.31
HS050500	3Hz @ 20μa	TMD	Active	-0.01	-0.04	-0.05
BB052700	10Hz @ 20μa	TMD	Active	0.01	-0.04	-0.03
NW110700	40Hz @ 20μa	TMD	Active	0.02	-0.02	0.00
KS11100	3Hz @ 20μa	TMD	Active	-0.02	0.08	0.06
OP020901	3Hz @ 60μa	TMD	Active	-0.02	0.01	-0.01
HS102700	Placebo	TMD	Placebo	-0.01	0.01	0.00
OP120800	Placebo	TMD	Placebo	0.09	-0.06	0.03
MB020201	Placebo	TMD	Placebo	-0.03	0.09	0.06
OP032701	Placebo	TMD	Placebo	-0.07	0.09	0.02
BP032800	Placebo	Normal	Placebo	-0.02	0.00	-0.02
DW032900	Placebo	Normal	Placebo	-0.06	-0.02	-0.08
KI 010501	Placebo	Normal	Placebo	0.05	-0.05	0.00

Table A14

Group Mean pHi Values at each time-point

Group	Stim	N	Mean Base	Mean 20/32	Mean 48/60
TMD	Placebo	4	7.03	7.02	7.06
Normal	Placebo	4	7.05	7.04	7.02
TMD	Active	7	7.06	7.02	7.02
Normal	Active	8	7.05	7.05	7.05

Table A14 delineates the composite mean pHi values at the three time-points for each of the four experimental groups.

Table A15

Group Standard Deviations at each time-point

Group	Stim	N	SD Base	SD 20/32	SD 48/60
TMD	Placebo	4	0.0532	0.0350	0.0614
Normal	Placebo	4	0.0420	0.0126	0.0208
TMD	Active	7	0.1107	0.0208	0.0519
Normal	Active	8	0.1206	0.0358	0.0247

The standard deviation values for each group are presented in Table A15. Data from Tables A14-15 represent the pHi values utilized for the statistical analyses that follow. Figure A7 presents a visual comparison of the mean pHi values at each time-point for the four groups of subjects.

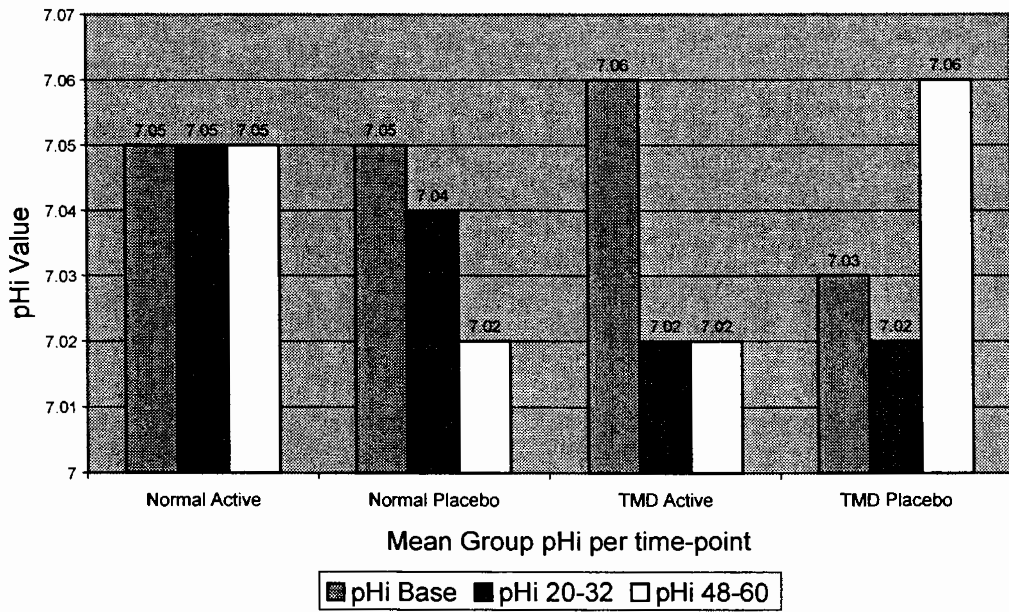

Figure A7. Mean pHi value at each time-point for the four subject groups.

Table A16

ANOVA Analyses of pHi via the GLM Procedure

Class	Levels	Values
DX	2	TMD / Normal
Stim	2	Active / Placebo
N	23	Observations

The pHi data of the 23 subjects were analyzed by diagnostic groupings and type of exposure, as noted in Table A16.

Table A17

pHi Values at Baseline

Sources of Variation	DF	SS	MS	F	P value	Sig
DX	1	0.000002	0.000002	0.00	0.9899	ns
Stim	1	0.002037	0.002037	0.21	0.6485	ns
Error	20	0.190146	0.009507			

Sig=ns, if P value > 0.05, Sig=s, if P value <= 0.05.

There were no significant differences in baseline pHi values between the four groups of subjects separated by diagnosis and stimulation protocol.

Table A18

pHi Values at the 20-32 time-point

Sources of Variation	df	SS	MS	F	P value	sig
DX	1	0.004806	0.004806	6.01	**0.0235**	**sig**
Stim	1	0.000107	0.000107	0.13	0.7180	ns
Error	20	0.015984	0.000799			

Sig=ns, if P value > 0.05, Sig=s, if P value <= 0.05.

At the 20-32 minute time-point there was a significant difference in mean pHi values among the four groups of subjects separated by diagnosis and exposure protocol. Table A14 delineates the mean pHi values at each time-point by group.

Table A19

pHi Values at the 48-60 time-point

Sources of Variation	DF	SS	MS	F	P value	Sig
DX	1	0.000048	0.000048	0.03	0.8758	ns
Stim	1	0.000013	0.000013	0.01	0.9359	ns
Error	20	0.03868	0.001934			

Sig=ns, if P value > 0.05, Sig=s, if P value <= 0.05.

There were no significant differences in pHi values at the 48-60 minute time-point among the four groups of subjects delineated by diagnosis and stimulation protocol.

Table A20

Difference in pHi values at the 20-32 and Baseline time-points

Sources of Variation	DF	SS	MS	F	P value	Sig
DX	1	0.004982	0.004982	0.67	0.4217	ns
Stim	1	0.001209	0.001209	0.16	0.6904	ns
Error	20	0.14805	0.007402			

Sig=ns, if P value > 0.05, Sig=s, if P value <= 0.05.

There was no significant difference between the baseline and 20-32 minute pHi values among the four groups of subjects.

Table A21

Difference in pHi Values at the 48/60-Baseline time-point

Sources of Variation	df	SS	MS	F	P value	sig
DX	1	0.000067	0.000067	0.01	0.9343	ns
Stim	1	0.001726	0.001726	0.18	0.6774	ns
Error	20	0.19371	0.00969			

Sig=ns, if P value > 0.05, Sig=s, if P value <= 0.05.

There was no significant difference between the 48-60 and baseline pHi values among the four groups.

Tables A22-27 present the results of two-sided t- tests to compare mean pHi values of the combined 12 normal and 11 TMD subjects at each of the three time-points.

Table A22

Comparison of Baseline pHi Values in the Normal and TMD Groups by two-tailed t-tests

DX	N	Mean	Grouping
Normal	12	7.05	A
TMD	11	7.05	A
Error df	20		
Error MS	0.0095		
Critical value t	2.0860		
LSD (least significant difference)	0.0849		

At the baseline time-point, the mean pHi values for the combined normal and TMD subject groups were not significantly different.

Table A23

Comparison of mean pHi values in the Normal and TMD Groups at the 20-32 time-point by two-tailed t-tests

DX	N	Mean	Grouping
Normal	12	**7.05**	**A**
TMD	11	**7.02**	**B**
Error df	20		
Error MS	0.0008		
Critical value t	2.0860		
LSD (least significant difference)	0.0246		

At the 20-32 minute time-point, there was a significant difference in pHi for the TMD group when compared to the normal group. The TMD group had a significantly lower pHi than the normal group.

Table A24

Comparison of Mean pHi values in the Normal and TMD Groups at the 48-60 time-point by two-tailed t-tests

DX	N	Mean	Grouping
Normal	12	7.04	A
TMD	11	7.03	A
Error df	20		
Error MS	0.0019		
Critical value t	2.0860		
LSD (least significant difference)	0.0383		

At the 48-60 minute time-point, the differences in mean pHi values between the combined normal and TMD subject groups respectively were not significant.

Table A25

Comparison of Mean Differences in pHi Values at the 20-32 and Baseline time-points in the Normal and TMD Groups by two-tailed t-tests

DX	N	Mean	Grouping
Normal	12	0.0008	A
TMD	11	-0.0282	A
Error df	20		
Error MS	0.0074		
Critical value t	2.0860		
LSD (least significant difference)	0.0750		

Mean differences in pHi values between the 20-32 and the baseline time-points for the combined normal and TMD subject groups were not significantly different.

Table A26

Comparison of Mean Differences in pHi values at the 48-60 and 20-32 time-points in the Normal and TMD Groups by two-tailed t-tests

DX	N	Mean	Grouping
Normal	12	-0.0125	A
TMD	11	0.0136	A
Error df	20		
Error MS	0.0021		
Critical value t	2.0860		
LSD (least significant difference)	0.04		

Mean differences in pHi values between the 48-60 and the 20-32 time-points, did not reveal a significant difference between the combined normal and TMD subject groups.

Table A27

Comparison of Mean Differences in pHi values at 48-60 and Baseline time-points in the Normal and TMD Groups by two-tailed t-tests

DX	N	Mean	Grouping
Normal	12	-0.0117	A
TMD	11	-0.0146	A
Error df	20		
Error MS	0.0097		
Critical value t	2.0860		
LSD (least significant difference)	0.0857		

Mean differences in pHi values between the 48-60 and the baseline time-points for the combined Normal and TMD subject groups were not significantly different at the 0.5 level.

Tables A28-33, delineating the two-tailed t-test analyses at and between each time-point by the type of exposure, did not reveal any significant differences in pHi values for the TMD subjects. In addition, a significant change was not seen in the ANOVA analyses comparing the TMD subjects receiving active stimulation to those exposed to the placebo protocol, as shown in Tables A34-40.

Table A28

Comparison of Mean pHi Values at Baseline for Active and Placebo Subjects Regardless of Diagnosis

DX	N	Mean	Grouping
Active	15	7.06	A
Placebo	8	7.04	A
Error df	20		
Error MS	0.0096		
Critical value t	2.0860		
LSD (least significant difference)	0.089		

Mean pHi values at baseline did not reveal a significant difference between the combined active and placebo subject groups regardless of diagnostic classification.

Table A29

Comparison of Mean pHi Values at the 20-32 time-point for Active and Placebo Groups Regardless of Diagnosis

DX	N	Mean	Grouping
Active	15	7.04	A
Placebo	8	7.03	A
Error df	20		
Error MS	0.0096		
Critical value t	2.0860		
LSD (least significant difference)	0.089		

Mean pHi values at the 20-32 time-point, did not reveal a significant difference between the combined active and placebo subject groups regardless of diagnostic classification.

Table A30

Comparison of Mean pHi Values at the 48-60 time-point for Active and Placebo Groups Regardless of Diagnosis

DX	N	Mean	Grouping
Active	15	7.04	A
Placebo	8	7.04	A
Error df	20		
Error MS	0.0019		
Critical value t	2.0860		
LSD (least significant difference)	0.0402		

Mean pHi values at the 48-60 minute time-point did not reveal a significant difference between the combined active and placebo groups regardless of diagnostic classification.

Table A31

Comparison of Differences in Mean pHi Values at the 20-32 and Baseline time-points for Active and Placebo Subjects Regardless of Diagnosis

DX	N	Mean	Grouping
Active	15	-0.0180	A
Placebo	8	-0.0038	A
Error df	20		
Error MS	0.0074		
Critical value t	2.0860		
LSD (least significant difference)	0.0786		

Mean differences in pHi values at the 20-32 and baseline time-points, did not reveal a significant difference between the combined active and placebo groups regardless of diagnostic classification.

Table A32

Comparison of Differences in Mean pHi Values at the 48-60 and 20-32 time-points for Active and Placebo Subjects Regardless of Diagnosis

DX	N	Mean	Grouping
Active	15	-0.0013	A
Placebo	8	0.0025	A
Error df	20		
Error MS	0.0021		
Critical value t	2.0860		
LSD (least significant difference)	0.042		

Mean differences in pHi values between the 48-60 and the 20-32 time-points, did not reveal a significant difference between the combined active and placebo groups regardless of diagnostic classification.

Table A33

Comparison of Differences in Mean pHi Values at the 48-60 and the Baseline time-points for Active and Placebo Subjects Regardless of Diagnosis

DX	N	Mean	Grouping
Active	15	-0.0193	A
Placebo	8	-0.0013	A
Error df	20		
Error MS	0.0097		
Critical value t	2.0860		
LSD (least significant difference)	0.0900		

Mean differences in pHi values between the 48-60 and the baseline time-points did not reveal a significant difference for the combined active and placebo groups regardless of diagnostic classification.

The analyses presented in Tables A34-40 did not reveal any significant differences in mean pHi values among the 11 TMD subjects. Comparison was made between the 7 subjects exposed to active stimulation and the 4 that received the placebo protocol.

Table A34

pHi Data for TMD Subjects by Exposure Protocol

Class	Levels	Values
STIM	2	Active / Placebo
N	11	Observations

Tables A34-40 delineate the ANOVA results for pHi values and their differences at various time-points. These were analyzed using the GLM procedure for all TMD subjects, compared by active/placebo exposure.

Table A35

ANOVA: Mean pHi Values at Baseline for TMD Subjects

Sources of Variation	DF	SS	MS	F	P value	Sig
Stim	1	0.002930	0.002930	0.32	0.5844	ns
Error	9	0.081961	0.009106			

Sig=ns if P value > 0.05, Sig=s if P value <= 0.05.

At baseline a significant difference in pHi values was not apparent when comparing the TMD groups delineated by active or placebo exposure.

Table A36

ANOVA: Mean pHi Values at the 20-32 time-point for TMD Subjects

Sources of Variation	DF	SS	MS	F	P value	Sig
Stim	1	0.000016	0.000016	0.02	0.8833	ns
Error	9	0.006275	0.000697			

Sig=ns if P value > 0.05, Sig=s if P value <= 0.05.

At the 20-32 timepoint, a significant difference in pHi values was not apparent when comparing the TMD subjects who received active stimulation to those with placebo exposure.

Table A37

ANOVA for pHi Values at 48-60 time-point for TMD Subjects

Sources of Variation	DF	SS	MS	F	P value	Sig
Stim	1	00.26299	0.0026299	0.86	0.3773	ns
Error	9	0.027443	0.003049			

Sig=ns if P value > 0.05, Sig=s if P value <= 0.05.

At the 48-60 timepoint, a significant difference in mean pHi values was not apparent when comparing the TMD subjects by the type of exposure.

Table A38

ANOVA: Difference in Mean pHi Values at 20-32 and Baseline time-points for TMD Subjects

Sources of Variation	DF	SS	MS	F	P value	Sig
Stim	1	0.003378	0.003378	0.40	0.5433	ns
Error	9	0.076186	0.008465			

Sig=ns if P value > 0.05, Sig=s if P value <= 0.05.

The difference in mean pHi values between the 20-32 and baseline time-points was not significant when comparing the active and placebo TMD groups.

Table A39

ANOVA: Difference in Mean pHi Values at 48-60 and 20-32 time-points for TMD Subjects

Sources of Variation	DF	SS	MS	F	P value	Sig
Stim	1	0.002237	0.002237	0.62	0.4496	ns
Error	9	0.0322218	0.003580			

Sig=ns if P value > 0.05, Sig=s if P value <= 0.05.

The difference in pHi values at the 48-60 and 20-32 time-points was not significant, when comparing the TMD subjects by the type of exposure.

Table A40

ANOVA: Difference in pHi Values at 48-60 and Baseline time-points for TMD Subjects

Sources of Variation	DF	SS	MS	F	P value	Sig
Stim	1	0.011112	0.011112	1.00	0.3424	ns
Error	9	0.099561	0.011062			

Sig=ns if P value > 0.05, Sig=s if P value <= 0.05.

The difference in pHi values at the 48-60 and baseline timepoints was not significant when comparing the TMD subjects by the type of exposure.

Tables A 41-46 present the two-tailed t-test results of the comparison for the TMD active and placebo groups at and between each time-point. Previous t-test analyses comparing the normal active and placebo groups revealed a significant difference in mean pHi values only at the 48-60 timepoint. Table A50 revealed a significant difference ($p = 0.0419$) in pHi values (7.05 active and 7.02 placebo) only at the 48-60 time-point, between the 8 normal subjects who received MENS in comparison to the 4 normal subjects exposed to the placebo protocol.

Table A41

Mean pHi Values at Baseline among TMD Active and TMD Placebo Subjects

DX	N	Mean	Grouping
TMD Active	7	7.06	A
TMD Placebo	4	7.03	A
Error df	9		
Error MS	0.0091		
Critical value t	2.2622		
LSD (least significant difference)	0.1353		

The difference in mean pHi between the TMD active and placebo groups was not significant at baseline.

Table A42

Mean pHi Values at the 20-32 time-point among TMD Active and Placebo Subjects

DX	N	Mean	Grouping
TMD Active	7	7.02	A
TMD Placebo	4	7.02	A
Error df	9		
Error MS	0.0007		
Critical value t	2.2622		
LSD (least significant difference)	0.0374		

The difference in in mean pHi between the TMD active and placebo groups was not significant at the 20-32 time-point.

Table A43

Mean pHi at the 48-60 time-point for TMD

Active and Placebo Subjects

DX	N	Mean	Grouping
TMD Active	7	7.06	A
TMD Placebo	4	7.03	A
Error df	9		
Error MS	0.0031		
Critical value t	2.2622		
LSD (least significant difference)	0.0783		

The difference in mean pHi values between the TMD active and placebo groups was not significant at the 48-60 time-point.

Table A44

Comparison of Differences in Mean pHi Values at the 20-32 and Baseline time-points for TMD Active and Placebo Subjects

DX	N	Mean	Grouping
TMD Active	7	-0.0414	A
TMD Placebo	4	-0.0050	A
Error df	9		
Error MS	0.0085		
Critical value t	2.2622		
LSD (least significant difference)	0.1305		

The difference in mean pHi values between the baseline and 48-60 minute time-points for the TMD active and placebo groups was not significant.

Table A45

Comparison of Differences in pHi Values at the 48-60 and 20-32 time-points for TMD Active and Placebo Subjects

DX	N	Mean	Grouping
TMD Active	7	0.0029	A
TMD Placebo	4	0.0325	A
Error df	9		
Error MS	0.0036		
Critical value t	2.2622		
LSD (least significant difference)	0.0848		

The differences in mean pHi values between the 48-60 and baseline time-points for the TMD active and placebo groups were not significant.

Table A46

Comparison of Differences in Mean pHi Values at Baseline and 20-32 time-points for TMD Active and Placebo Subjects

DX	N	Mean	Grouping
TMD Active	7	-0.0386	A
TMD Placebo	4	0.0275	A
Error df	9		
Error MS	0.0111		
Critical value t	2.2622		
LSD (least significant difference)	0.1491		

The differences in mean pHi values between the baseline and 20-32 time-points for the TMD active and placebo groups were not significant.

The analyses of Tables A41-46 indicates that there were no differences in mean pHi values between the TMD active and placebo groups at any time-point or among two comparative time-points.

Table A47

Mean pHi Values for Normal subjects (Active vs. Placebo groups)

Class	Levels	Values
Stim	2	Active / Placebo
N	12	Observations

ANOVA comparisons of the mean pHi values and their differences among the normal groups of subjects, delineated by the type of exposure, are presented in Tables A47-53.

Table A48

ANOVA: Mean pHi Values at Baseline Between the Normal Active and Placebo Groups

Sources of Variation	DF	SS	MS	F	P value	Sig
Stim	1	0.000104	0.000104	0.01	0.9234	ns
Error	10	0.107188	0.0107188			

Sig=ns, if P value > 0.05, Sig=s, if P value <= 0.05.

There was no significant difference in mean pHi values at baseline between the group of 8 normal subjects exposed to MENS and 4 normal subjects who received placebo exposure.

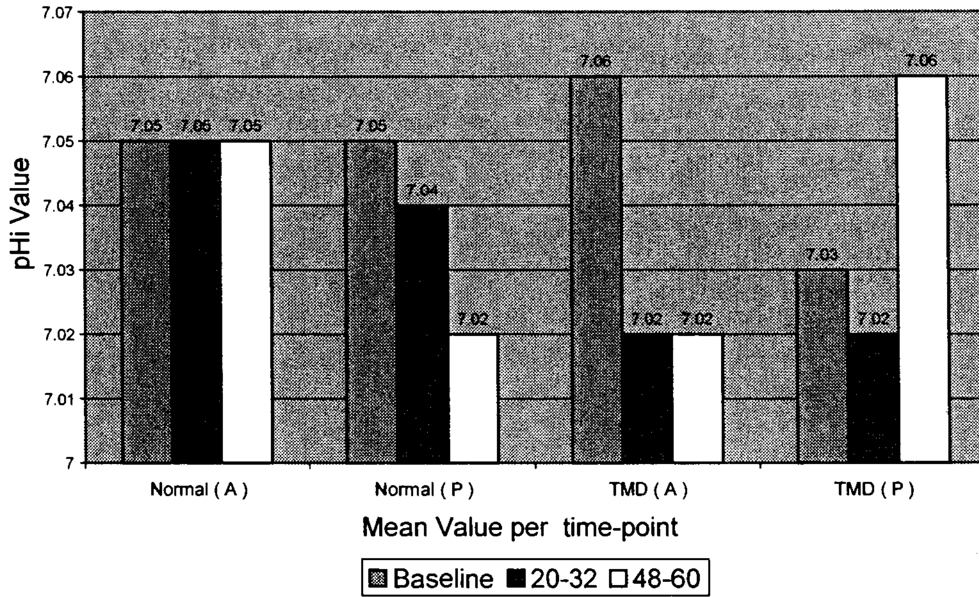

Figure A8. Mean pHi values at each time-point among the four groups.

Table A49

ANOVA: Mean pHi Values at the 20-32 time-point Between the Normal Active and Placebo Groups

Sources of Variation	DF	SS	MS	F	P value	Sig
Stim	1	0.000337	0.000337	0.36	0.5636	ns
Error	10	0.009463	0.009463			

Sig=ns, if P value > 0.05, Sig=s, if P value <= 0.05.

Mean pHi values at the 20-32 minute time-point were not significantly different among the normal active and placebo groups.

Table A50

ANOVA: Mean pHi Values at the 48-60 time-point for Normal Subjects by Type of Exposure

Sources of Variation	DF	SS	MS	F	P value	Sig
Stim	1	0.003038	0.003038	5.44	**0.0419**	**sig**
Error	10	0.005587	0.000558			

Sig=ns, if P value > 0.05, Sig=s, if P value <= 0.05.

There was a significant difference in mean pHi at the 48-60 minute time-point. The group of 8 normal subjects that received MENS and the group of 4 of four exposed to the placebo protocol had a mean pHi of 7.05 and 7.02 respectively.

Table A51

ANOVA; Difference in Mean pHi Bewtween the 20-32 and Baseline time-points for Normal Subjects by Type of Exposure

Sources of Variation	DF	SS	MS	F	P value	Sig
Stim	1	0.000067	0.000067	0.01	0.9240	ns
Error	10	0.069625	0.0069625			

Sig=ns, if P value > 0.05, Sig=s, if P value <= 0.05.

The difference in mean pHi between the baseline and 20-32 minute time-points for the two groups of normal subjects delineated by active and placebo exposure was not significant.

Table A52

ANOVA: Differences in Mean pHi Between the 48-60 and 20-32 time-points for Normal Subjects by Type of Exposure

Sources of Variation	df	SS	MS	F	P value	sig
Stim	1	0.002017	0.002017	0.24	0.6322	ns
Error	10	0.006475	0.000647			

Sig=ns, if P value > 0.05, Sig=s, if P value <= 0.05.

The difference in mean pHi values between the 48-60 and 20-32 minute time-points for the normal active and placebo groups was not significant.

Table A53

ANOVA: Difference in Mean pHi Between the 48-60 and Baseline time-points for Normal Subjects by Type of Exposure

Sources of Variation	DF	SS	MS	F	P value	Sig
Stim	1	0.002017	0.002017	0.24	0.6322	ns
Error	10	0.082750	0.008275			

Sig=ns, if P value > 0.05, Sig=s, if P value <= 0.05.

The difference in mean pHi values between the 48-60 and baseline time-points for the two groups of nomal subjects was not significant.

The previous ANOVA results of Tables A47-53 that compared the two groups of normal subjects by type of exposure revealed that the only significant difference occurred at the 48-60 minute time-point. The mean pHi value of the normal group that received active microcurrent stimulation was 7.02, while that of the placebo group was 7.05 (Table A50). These calculations were further confirmed by two-tailed t-tests, which follow in Tables A54-59.

Table A54

Comparison of Mean pHi Values at Baseline for the Normal Groups Delineated by Type of Exposure

DX	N	Mean	Grouping
Normal active	8	7.051	A
Normal placebo	4	7.045	A
Error df	10		
Error ms	0.0107		
Critical value t	2.2281		
LSD (least significant difference)	0.1413		

There was no significant difference in mean pHi values for the normal groups of 8 active and 4 placebo subjects at baseline.

Table A55

Comparison of pHi Values at the 20-32 time-point Between the Normal Groups of Subjects at 20-32 time-point Delineated by the Type of Exposure

DX	N	Mean	Grouping
Normal Active	8	7.053	A
Normal Placebo	4	7.042	A
Error df	10		
Error MS	0.0009		
Critical value t	2.2281		
LSD (least significant difference)	0.042		

The difference in mean pHi values betwen the two groups of normal subjects (active and placebo) at the 20-32 time-point was not significant.

Table A56

Comparison of Mean pHi Values at the 48-60 time-point among the Normal Subject Groups Delineated by Type of Exposure

DX	N	Mean	Grouping
Normal Active	**8**	**7.048**	**A**
Normal Placebo	4	**7.015**	**B**
Error df	10		
Error MS	0.0006		
Critical value t	2.2281		
LSD (least significant difference)	0.0323		

There was an effect of microcurrent stimulation upon pHi values at the 48-60 minute time-point. The difference in mean pHi for the normal group exposed to the placebo protocol was significantly less (7.02) than that of the normal group exposed to MENS (7.05), thereby supporting the same finding by ANOVA in Table A50.

Table A57

Comparison of Mean pHi Values Between the 20-32 and Baseline time-points for Normal Subjects Delineated by Type of Exposure

DX	N	Mean	Grouping
Normal Active	8	0.00250	A
Normal Placebo	4	-0.00250	A
Error df	10		
Error MS	0.0007		
Critical value t	2.2281		
LSD (least significant difference)	0.1139		

The difference in mean pHi values between the 20-32 and baseline time-points for the two groups of normal subjects (active and placebo) was not significant.

Table A58

Comparison of Differences in Mean pHi Values Between the 48-60 and 20-32 time-points for Normal Subjects Delineated by Type of Exposure

DX	N	Mean	Grouping
Normal Active	8	-0.00500	A
Normal Placebo	4	-0.02750	A
Error df	10		
Error MS	0.0007		
Critical value t	2.2281		
LSD (least significant difference)	0.0347		

The difference in mean pHi values between the 48-60 and 20-32 minute time-points for the two groups of normal subjects (active and placebo) was not significant.

Table A59

Comparison of Differences in Mean pHi Values Between the 48-60 and Baseline time-points for Normal Subjects Delineated by Type of Exposure

DX	N	Mean	Grouping
Normal Active	8	-0.00250	A
Normal Placebo	4	-0.03000	A
Error df	10		
Error MS	0.0083		
Critical value t	2.2281		
LSD (least significant difference)	0.1241		

The difference in mean pHi values between the 48-60 and baseline time-points for the two groups of normal subjects (active and placebo) was not significant.

Appendix B

Excell Data Tabulations

Table B1

Pi Values for all Subjects

Subject	Parameters	DX	Stim	Pi Base	Pi 20-32	Pi 48-60
MKS022500	60Hz @ 40μa	normal	active	12.87	9.53	6.99
JVH030300	60Hz @ 20μa	normal	active	6.99	6.57	6.9
GG030700	60Hz @ 10μa	normal	active	8.15	4.73	12.42
BC031000	40Hz @ 40μa	normal	active	6.78	7.39	7.13
SM032100	40Hz @ 20μa	normal	active	5.83	9.37	8.7
DW032800	3Hz @ 40μa	normal	active	7.2	6.59	7.45
BP032900	3Hz @ 20μa	normal	active	3.95	6.9	5.36
WON091500	10Hz @ 20μa	normal	active	6.63	7.36	7.58
JM040500	60Hz @ 40μa	TMD	active	10.1	5.78	14.26
KG041900	40Hz @ 20μa	TMD	active	5.96	11.37	9.82
HS050500	3Hz @ 20μa	TMD	active	4.05	5.05	7.88
BB052700	10Hz @ 20μa	TMD	active	7.03	11.31	5.95
NW110700	40Hz @ 20μa	TMD	active	4.05	7.22	7.07
KS111000	3Hz @ 20μa	TMD	active	5.69	6.99	6.85
OP020901	3Hz @ 60μa	TMD	active	2.75	3.64	6.11
HS102700	placebo	TMD	placebo	10.02	9.41	9.36
OP120800	placebo	TMD	placebo	5.9	8.78	6.48
MB020201	placebo	TMD	placebo	3.89	7.35	9
OP032701	placebo	TMD	placebo	6.3	6.39	5.08
BPO32800	placebo	normal	placebo	5.61	7.73	6.77
DW032900	placebo	normal	placebo	4.75	4.63	7.73
TH102000	placebo	normal	placebo	6.1	6.48	7.02
KI010501	placebo	normal	placebo	5.37	6.67	8.07

Table B2

PCr Values for all Subjects

Subject	Parameters	DX	Stim	PCr Base	PCr 20-32	PCr 48-60
MKS022500	60Hz @ 40µa	normal	active	59.96	49.97	20.97
JVH030300	60Hz @ 20µa	normal	active	26.69	22.87	29.55
GG030700	60Hz & 10µa	normal	active	34.97	18.07	34.98
BC031000	40Hz @ 40µa	normal	active	28.89	25.47	19.59
SM032100	20Hz @ 40µa	normal	active	36.91	32.39	31.97
DW03280	3Hz @ 40µa	normal	active	39.88	24.38	32.65
BP032900	3Hz @ 20µa	normal	active	33.81	31.15	22.21
WON091500	10Hz @20µa	normal	active	21.97	23.37	25.67
JM040500	60Hz @ 40µa	TMD	active	31.23	26.32	42.25
KG041900	40Hz @ 20µa	TMD	active	30.72	27.13	31.06
HS050500	3Hz @ 20µa	TMD	active	21.58	15.55	20.64
BB052700	10Hz @ 20µa	TMD	active	34.92	42.03	31.04
NW110700	40Hz @ 20µa	TMD	active	25.35	24.31	26.53
KS111000	3Hz @ 20µa	TMD	active	29.96	35.81	32.77
OP020901	3Hz @ 60µa	TMD	active	17.35	28.64	25.04
HS102700	placebo	TMD	placebo	43.89	43.47	41.21
OP120800	placebo	TMD	placebo	20.19	34.72	37.67
MB020201	placebo	TMD	placebo	13.68	31.08	34.23
OP032701	placebo	TMD	placebo	35.02	28.67	26.25
BP032800	placebo	normal	placebo	24.14	28.18	20.47
DW032900	placebo	normal	placebo	33.14	33.5	57.15
TH102000	placebo	normal	placebo	28.9	34.97	23.73
KI010501	placebo	normal	placebo	29.12	27.41	29.03

Table B3

pHi Values for all subjects

Subject	Parameters	DX	Stim	pHi Base	pHi 20-32	pHi 48-60
MKS022500	60Hz @ 40μa	normal	active	7.09	7.08	7.05
JVH030300	60Hz @ 20μa	normal	active	7.00	7.05	7.07
GG030700	60Hz @ 10μa	normal	active	7.05	7.09	7.04
BC031000	40Hz @ 40μa	normal	active	6.95	7.02	7.03
SM032100	20Hz @ 40μa	normal	active	6.99	7.03	7.06
DW032800	3Hz @ 40μa	normal	active	6.98	7.04	7.04
BP032900	3Hz @ 20μa	normal	active	7.02	7.01	7.01
WON091500	10Hz @ 20μa	normal	active	7.33	7.11	7.09
JM040500	60Hz @ 40μa	TMD	active	7.02	7.02	7.09
KG041900	40Hz @ 20μa	TMD	active	7.31	7.04	7.00
HS050500	3Hz @ 20μa	TMD	active	7.01	7.00	6.96
BB052700	60Hz @ 40μa	TMD	active	7.04	7.05	7.01
NW110700	40Hz @ 20μa	TMD	active	7.00	7.02	7.00
KS111000	3Hz @ 20μa	TMD	active	7.04	7.02	7.10
OP020901	60Hz @ 40μa	TMD	active	7.01	6.99	7.00
HS102700	placebo	TMD	placebo	7.05	7.04	7.05
OP120800	placebo	TMD	placebo	6.95	7.04	6.98
MB020201	placebo	TMD	placebo	7.07	7.04	7.13
OP032701	placebo	TMD	placebo	7.04	6.97	7.06
BP032800	placebo	normal	placebo	7.06	7.04	7.04
DW032900	placebo	normal	placebo	7.09	7.03	7.01
TH102000	placebo	normal	placebo	7.04	7.06	7.02
KI010501	placebo	normal	placebo	6.99	7.04	6.99

Table B4

Pi/PCr Values for all Subjects

Subject	Parameters	DX	Stim	Pi/PCr Base	Pi/PCr 20-32	Pi/PCr 48-60
MKS022500	60Hz @ 40μa	normal	active	0.21544	0.181	0.31425
JVH030300	60Hz @ 20μa	normal	active	0.24971	0.288	0.22536
GG030700	60Hz @ 10μa	normal	active	0.22222	0.251	0.32948
BC03100	40Hz @ 40μa	normal	active	0.22377	0.282	0.34957
SM032100	20Hz @ 40μa	normal	active	0.15061	0.274	0.26167
DW032800	3Hz @ 40μa	normal	active	0.17219	0.260	0.22159
BP032900	3Hz @ 20μa	normal	active	0.11139	0.211	0.23153
WON091500	10Hz @ 20μa	normal	active	0.28759	0.316	0.27898
JM040500	60Hz @ 40μa	TMD	active	0.30831	0.192	0.31599
KG041900	40Hz @ 20μa	TMD	active	0.18513	0.393	0.31694
HS050500	3Hz @ 20μa	TMD	active	0.17905	0.313	0.35442
BB052700	10Hz @ 20μa	TMD	active	0.19206	0.262	0.19471
NW110700	40Hz @ 20μa	TMD	active	0.15243	0.283	0.25411
KS11100	3Hz @ 20μa	TMD	active	0.18123	0.183	0.19944
OP020901	3Hz @ 60μa	TMD	active	0.15143	0.121	0.23262
HS102700	placebo	TMD	placebo	0.21764	0.206	0.21654
OP120800	placebo	TMD	placebo	0.27872	0.241	0.16391
MB020201	placebo	TMD	placebo	0.27071	0.225	0.25082
OP32701	placebo	TMD	placebo	0.17161	0.212	0.18451
BP032800	placebo	normal	placebo	0.22157	0.262	0.31309
DW032900	placebo	normal	placebo	0.13666	0.133	0.12837
TH102000	placebo	normal	placebo	0.20119	0.149	0.14368
KI010501	placebo	normal	placebo	0.17595	0.232	0.26511

Table B5

Algometer, VAS and ROM Data for TMD Subjects with Pi/PCr Values

Subject	Parameters	DX	Stim	Alg Pre	Alg Post	VAS Pre	VAS Post	ROM Pre	ROM Post
JM040500	60Hz @ 40μa	TMD	active	N/A	N/A	2.8	0.5	38mm	42mm
KG041900	40Hz @ 20μa	TMD	active	N/A	N/A	6.8	4.4	8mm	16mm
HS050500	3Hz @ 20μa	TMD	active	N/A	N/A	2.0	0.0	42mm	45mm
BB052700	10Hz @ 20μa	TMD	active	N/A	N/A	3.0	2.0	29mm	29mm
NW110700	40Hz @ 20μa	TMD	active	N/A	N/A	5.0	1.2	35mm	41mm
KS11100	3Hz @ 20μa	TMD	active	1.00	1.56	5.4	1.5	25mm	34mm
OP020901	3Hz @ 60μa	TMD	active	2.53	2.96	5.4	0.0	28mm	52mm
HS102700	Placebo	TMD	placebo	N/A	N/A	5.6	5.2	36mm	36mm
OP120800	Placebo	TMD	placebo	2.13	1.96	5.5	0.8	24mm	30mm
MB020201	Placebo	TMD	placebo	2.86	1.53	6.2	6.4	20mm	20mm
OP32701	Placebo	TMD	placebo	2.53	1.93	7.0	8.0	30mm	32mm

Table B6

Algometer, VAS and ROM Data for TMD Subjects without Pi/PCr Values

Subject	Para-meters	DX	Alg Pre	Alg Post	VAS Pre	VAS Post	ROM Pre	ROM Post
OP071701	3Hz @40µa	TMD	1.53	2.76	9.0	0.0	13mm	27mm
DG081701	3Hz @ 20µa	TMD	0.33	0.7	3.6	3.2	25mm	27mm
DB021502	3Hz @ 60µa	TMD	1.36	2.03	8.2	2.5	30mm	35mm
OP022602	3Hz @ 60µa	TMD	1.43	2.63	6.5	0.6	20mm	30mm
OP030502	3Hz @ 60µa	TMD	1.60	3.1	8.4	0.0	23mm	43mm
HC081602	3Hz @ 10µa	TMD	3.08	3.0	7.0	7.0	28mm	28mm
SA080801	Placebo	TMD	2.90	2.92	6.6	5.0	25mm	29mm
DG081401	Placebo	TMD	0.40	0.26	4.6	5.2	22mm	22mm
HC080602	Placebo	TMD	3.16	3.26	6.4	5.0	24mm	19mm

Appendix C

UNIVERSITY OF PENNSYLVANIA IRB SUBMISSION

Date: 12/23/99

Protocol # 700398
(For Committee use)

UNIVERSITY OF PENNSYLVANIA

IRB PROTOCOL "FACE SHEET

Submit the original protocol and 19 copies (22 if Radiation) for full review or one copy of materials for exempted/expedited review to the Associate Director for Regulatory Affairs, Suite 300, 133 S. 36th Street/3246 prior to the initiation of any work involving human subjects or human material. Please limit the title to 2 lines of 50 characters each if possible and answer all items below.

Project Title: The Effect of Microcurrent Stimulation on ATP Synthesis in the Human Masseter as Evidenced by 31P MRS

Funding Agency or Sponsor: NIH Grant #: RR02305
Address: National Institutes of Health, Bethesda, MD
Principal Investigator, Title and Department: John S. Leigh, Ph.D. Professor of Radiology, Department of Radiology.
Campus Mailing Address: 1 Silverstein, 3400 Spruce St.
Telephone: 215-898-2044
Department of Radiology: E-mail Address: jack@mail.mmrrcc.upenn.edu

Other Investigators: Jeffrey S. Mannheimer, M.A., P.T., Martin S. Greenberg, D.D.S, Thomas P. Sollecito, D.M.D., Mel Mupparapu, D.D.S. & Michael Vardaro, B.S.

PLEASE ANSWER THE FOLLOWING QUESTIONS:

1. --- YES X NO Is this application for a fellowship/stipend only?
2. __ YES X NO This project is to be undertaken as part of a previously approved Training, Center, or Program grant. Grant Number, Project Title, and Director:

3. ___YES X NO Does the project involve the administration of personality tests, inventories, or questionnaires? If YES, provide the name of the standard tests or questionnaire or 3 copies of the proposed tests.
4. ___YES X NO Does the project involve the use of human blood, blood products, tissues or body fluids? If YES, contact the Office of Environmental Health and Safety, 898-4453.
4a. __YES X NO Did you attend the Occupational Exposure to Blood borne Pathogen Program?
5. __ YES X NO Does the project involve administration of ionizing radiation to subjects for other than clinical purposes? If YES, you must contact the Radiation Safety Officer, 898-7187.
6. __ YES X NO Does the project involve the testing of investigational drugs or devices? If YES, provide: Name of Drug or Device:
IND# or IDE# ______
Name of Manufacturer:
and 1 copy of an unreturnable Drug Brochure
If this protocol involves the administration of medications to humans for research purposes (not part of general clinical practice), you must obtain an authorization from the Penn Investigational Drug Service (IDS) at 215-349-8817. Authorization number:________ (must obtain from IDS staff).
7. YES X NO Does the proposed study involve the use of electrical apparatus at HUP other than routine patient care equipment? If yes, contact the Director of Clinical Engineering 662-2330 for authorization.
8. YES X NO Will this study involve additional work to the Nursing staff?
9. YES X NO Human Subjects would be involved in the proposed activity as either: None of the following, or including: minors, fetuses, abortuses, pregnant women, prisoners, mentally retarded, mentally disabled, __HIV positive subjects.

SIGNATURES:

______________________________ ______________________________
Signature of Principal Investigator Date

______________________________ ______________________________
Signature of Chairman of Radiology Date

______________________________ ______________________________
Signature of Chairman of Oral Medicine Date

Faculty Sponsor (if required): ________________________
The signature of each department chairperson with faculty involved, is required. A Dean's signature must be obtained if the investigator is also the chairperson

Consent Form

The Institutional Review Board (IRB) of the University of Pennsylvania approved the following informed consent for the participation of subjects in this research. This form is intended to provide you with the necessary information to decide whether or not to participate in this research study.

Title: The efficacy of microamperage electrical nerve stimulation on ATP synthesis in the human masseter muscle, as evidenced by ^{31}P Magnetic Resonance Spectroscopy.

Purpose: The purpose of this study is to determine if very low levels of electricity affect the ability of human muscle to produce energy. This research will investigate if microamperage electricity causes an increase in the levels of energy in human muscle. The muscle to be tested is located on either side of the face and is used for chewing. This study will use magnetic imaging to assess any changes in energy levels. This research will also explore different dosages of electricity. All electrical settings are FDA approved and should produce either no sensation or a very mild, short-lived tingling.

Procedures: This research will require 2 hours of your time and will be done at the Hospital of the University of Pennsylvania (HUP) Metabolic Magnetic Resonance Research and Computing Center in the basement of the Stellar-Chance Building. Tests will be conducted in MRI B1. A special type of MRI will be used to collect data.

You will be required not to eat or chew gum for two hours before reporting for the test or drink any caffeinated beverages for 12 hours before the test. Please report for the testing procedure with clothing that does not have metal on it. This means you should not wear dungarees with rivets or metal buttons. Plastic buttons and Velcro are fine, but should you forget, you may wear clothes available for you at the center. You need to be clean-shaven and wear no make up. You cannot participate in this study if you have any metal implants or a pacemaker. You should bring some reading materials with you to pass the time.

Two pairs of surface electrodes will be placed on your face, one pair on each cheek. You will be asked to lie on a table, which then will be placed in a magnetic field. You will have to lie perfectly still. A baseline reading of the energy in your muscles will be taken. This will take about 20 minutes. After the baseline data has been collected the electrical stimulation will begin. Two more measurements will be taken at the 20-32 and 48-60 minute time-points. Talking should be kept to a minimum. You will be given earplugs to diminish the sound of the MRI machine. It is possible for you to talk to the person running the MRI machine. You will be encouraged to voice any concerns that you might have. If

you have any questions, the technician and research investigators will be available to answer them.

Benefits: There are several possible benefits of this research both to you and others. This research will allow further understanding of the effects of low levels of electricity on human muscle. You will be able to receive an MRI of any part of your body without interpretation by a radiologist for your participation. The MRI film will be given to you at the end of the research session.

Risks: This procedure has minimal risk to you as a participant. Magnetic resonance imaging and spectroscopy is performed without any radiation. The electrical stimulation is at a low level and you should not feel it. The very most that you will feel is a tingling sensation at the area of application. Most people don't feel anything. When taking the measurements you will be in a magnetic field, so all metal objects will be removed to prevent them from being pulled by the magnet. The test will not increase the risk of illness, but it is possible that a person may coincidentally become ill during the testing procedures. If that happens, all personnel have been certified in CPR and first aid. The hospital's emergency systems are also available.

Participation in this research is totally voluntary and you may discontinue the procedure at any time and for any reason. All information gained from this procedure will be held in the strictest of confidence. The results of this study will be made available to the research investigators and the technician. These results will be stored in a filing cabinet at the University of Pennsylvania. Information about adverse side effects will be reported to the manufacturer and the FDA. All information will be kept confidential except where required by law. Publication about the results of this research will occur so that it can be shared with other clinicians but your name will be kept confidential. All of your questions about this research should be answered before your participation begins.

You are encouraged to ask all of your questions. You may direct them to the researchers. The principle investigator Jeff Mannheimer M.A., P.T. may be reached at (215) 968-5981 or (609) 896-9054
I have read and understand the content of this document.

(SUBJECTS SIGNATURE)________________________________ Date______

(Print Name)________________________________

(PRIMARY INVEST. SIGNATURES)______________________________

The solicitation of subjects into this study has been approved by the Committee for the Protection of Human Subjects of the University of Pennsylvania

Purpose

Electrical stimulation is a modality widely used by physical therapists in clinical practice. Transcutaneous electrical nerve stimulation (TENS) units typically utilize currents in the milliamperage range for the purpose of pain reduction. TENS in the microamperage range, henceforth referred to as microamperage electrical nerve stimulation (MENS) has also been used as a means to decrease pain and facilitate tissue healing. Although the use of MENS has increased, evidence of its effectiveness is based primarily on anecdotal information. The mechanism of action is unknown and may be dependent on various stimulation paradigms. Manufacturers of MENS units have claimed that increased adenosine triphosphate (ATP) levels result from microamperage stimulation.

It is generally accepted that increased ATP concentration in damaged tissue is essential to the healing process and normal muscle function. The manufacturers have cited one study in particular to verify their claim about the relationship between microamperage stimulation and ATP. In 1982, Cheng et. al., conducted a landmark investigation that established the link between MENS and ATP synthesis in rat skin. Further research is necessary to supplement the literature and substantiate the relationship between MENS and the generation of ATP in human tissue. The purpose of this study is to examine the effect of MENS on ATP synthesis in the human masseter muscle as evidenced by change in the Pi/PCr ratio.

Background

The application of surface electrodes as a pre-operative assessment, prior to the implantation of a dorsal column stimulator for the control of intractable chronic pain, led to the development of the Transcutaneous Electrical Nerve Stimulator (TENS). The degree of pain relief obtained from this pre-operative evaluation was significant enough to spawn the manufacture of TENS devices in the late 1960's and early 1970's. At that time the use of TENS was primarily confined to the control of chronic pain. Hundreds of scientific publications relative to the mode of action and to the efficacy of TENS began to appear in the literature. Today the number of publications relative to the use of TENS exceeds 1000.

In the early to mid 1970's, Dr. Alan Hymes a thoracic surgeon, began to utilize TENS for the control of post-operative pain and thus pioneered its usage for acute pain. Continued development, technological advancements, approval from

Medicare and educational programs fostered the adjunctive use of TENS for a wide variety of acute and chronic pain situations. The publication of a 600 page medical text in 1984, provided information relative to all aspects of TENS (Mannheimer & Lampe) Clinical Transcutaneous Electrical Nerve Stimulation. FA Davis, Philadelphia 1984. Mr. Mannheimer is the initiator of this project, which is the research component required for the completion of his Ph.D from Seton Hall University.

Initially TENS was delivered via current in the milliamperage range utilizing various stimulation modes (paradigms of pulse rate, pulse duration and amplitude) each of which produces different physiological effects, with scientific published support. However, microamperage current has most recently gained prominence, due to frequent anecdotal and case study support, but without any scientific double-blind studies to explain its mode of action. Other than a study performed on rat skin, which bases the physiological action upon a significant increase in ATP, research at the cellular level, in plant and E. coli, has also demonstrated enhanced ATP synthesis (Cheng, et al., The effects of electrical currents on ATP generation, protein synthesis and membrane transport in rat skin. Clin Orthop & Related Res 171:164-272, 1982), (Blank, Na,K-ATPase function in alternating electric fields. FASEB J. 6:2434-2438, 1992. Blank & Soo, The Na,K-ATPase as a model for electromagnetic effects on cells. Bioelectrochemistry and Bioenergetics, 30:85-92, 1993. Kaim& Dimroth, ATP synthesis by the F_1F_0 ATP synthase of Escherichia coli is obligatorily dependent on the electric potential. FEBS Letters, 434:57-60, 1998.

ATP is a metabolite observable by magnetic resonance spectroscopy techniques. Phosphorus magnetic resonance spectroscopy has been used to investigate many types of tissues *in vitro* for over thirty years. Phosphorus magnetic resonance spectroscopy *in vivo* has been used to study metabolic processes non-invasively in the brain and muscle. ^{31}P MRS techniques provide qualitative and quantitative information regarding chemical content. This project will use this method on the MR scanner for measuring ATP, inorganic phosphate and phosphocreatine content in the masseter muscle prior to and during exposure to MENS.

Studying the effects of MENS at different time points, in the abnormal masseter, with ^{31}P MRS should indicate whether ATP synthesis is enhanced, as claimed by manufacturers of MENS units. The overall goal of this project is to determine if clinically used stimulation paradigms result in a metabolite (proposed ATP) change, which may be equated to pain relief, as determined by VAS and pressure algometer comparison, as well as identification of metabolite variances in normal vs. abnormal subjects.

An initial pilot study with normal volunteers (absence of any masseter pathology or temporomandibular disorder) has been completed (Appendix E). It was determined that TENS at both the milliamperage and microamperage levels, could be used in the magnetic resonance imaging (MRI) unit with simultaneous MRS recording, and spectra were able to be acquired without interference from the electrotherapeutic generator. Testing was initially performed with intensity levels between 8-22mA and data revealed that at a paradigm of 22mA, 85Hz and 75μs for 18 minutes, a decrease in PCr with a concomitant increase in Pi, but no change in ATP concentration occurred (Cecil, Mannheimer, Connick & Lenkinski, 1995). No significant change in metabolite levels were observed with stimulation below 18mA and the assumption was that higher milliamperage levels would continue to reveal similar metabolite alterations, due to contractile energy expenditure and the maintenance of ATP levels.

Rationale

Microcurrent electrical nerve stimulation (MENS) presently lacks the criteria required to determine its scientific merit, yet it is a commonly used physical therapy modality. We are unaware of any controlled, randomized studies with MENS, specific to clinical pain relief in human subjects that have been published in peer-reviewed journals.

Furthermore, no physiological evidence exists to support the use of MENS as a means of clinical pain relief. Proponents of MENS have thus taken a quantum leap by equating physiological events that occur from electrical stimulation at the cellular level in non-human tissue, to that which may or may not occur with similar electrical stimulation parameters via transcutaneous stimulation in man.

The best evidence, which is beyond that obtained by subjective measurement, with which to answer the question of efficacy and hence establish support for the use of a treatment, necessitates testing via a gold standard. The gold standard of ATP synthesis is quantification via MRS, which can be correlated to changes in ROM and palpable tenderness. It is therefore the purpose of this research to develop the protocol for and subsequent performance of, a definitive randomized double-blind trial of MENS with human subjects, and use MRS to quantify its effect upon ATP synthesis via monitoring and analysis of the Pi/PCr ratio.

Magnetic Resonance Spectroscopy can objectively evaluate the efficacy of MENS. Phosphorus magnetic resonance spectroscopy (^{31}P MRS) provides information on metabolism by detecting the presence, chemical environment and relative quantity of chemicals with phosphorus atoms, such as ATP. Other metabolites measured with ^{31}P MRS include inorganic phosphate and

phosphocreatine. These metabolites are known to be utilized in muscle contraction and relaxation.

Duration

Initially we will address the concerns and variables that came to light from the pilot study. The research will commence with creation of RF coils and an agarose phantom for the preliminary testing of normal subjects solely with active stimulation, to evaluate the procedures using the Stellar Chance magnet. Subsequent testing of pathological subjects will be performed to determine the appropriate stimulation paradigms (intensity, duration, frequency), that may produce an increase in ATP synthesis. Stimulation paradigms will be within the parameters suggested by the research performed at the cellular level upon non-human tissue, and will differ only by the duration of the stimulation.

It is anticipated that this research will require a cohort of 30 evaluable subjects that meet strict inclusion criteria relative to the presence of a myogenous temporomandibular disorder. Subjects will be randomized into two groups of 15, one receiving bilateral active MENS at the same stimulation parameters and the other bilateral placebo stimulation.

Subject Recruitment and Selection

Normal subjects as well as those with a temporomandibular disorder (TMD),will be recruited from the principal investigator group, contacts with physical therapy and oral medicine, and from the University population via word of mouth. Subjects must be at least 21 years of age. Potential subjects will be excluded if they have a pacemaker, defibrillator, neurostimulator, metal implants, have worked on or around a metal grinder or construction site. Subjects who have known conditions, which can be exacerbated by stress such as anxiety or panic attacks, heart disease or mental illness will be excluded. Also, these studies will not be performed on pregnant women. Although many of these conditions may not exclude a subject from clinical MRI examination, we have chosen to exclude these conditions from our current study. Other criteria necessary for achieving the goals of this project can be found in the tables that follow. TMD evaluations will be performed by Drs. Greenberg, Sollecito and staff as well as by Jeff Mannheimer, M.A., PT. Subjects who qualify and agree to participate by signing the consent form, will be scheduled for testing.

Table C1

Exclusion Criteria for Normal Subjects

1. Presence of pacemaker, defibrillator, neurostimulator, metal implants or history of work on or around a metal grinder or construction site.
2. History of systemic disease that may alter normal neuromuscular function (diabetes, hypo or hyperthyroidism, fibromyalgia, hypertension, etc.).
3. History of headache/facial pain separate from that of TMD.
4. Mouth breather, sleep disorder
5. Prior utilization of an electrical stimulator for pain control.
6. Claustrophobia, dizziness, vertigo or nystagmus or panic attacks.
7. Currently undergoing physical or psychological therapy.
8. Menstruation or lactation at the time of testing (testing must be performed at least five days prior to or after the menstrual cycle).

Table C2

Inclusion Criteria for Normal Subjects

1. No prior history of a temporomandibular disorder or ongoing orthodonture.
2. No prior history of clenching or bruxism.
3. Normal active range of motion (ROM) of the temporomandibular joints (TMJs), specifically at least 40 mm vertical, 8 mm lateral and 4mm protrusive.
4. No abnormal joint sounds
5. No significant tenderness to palpation of the mandibular elevator muscles.
6. No precautions/contraindications to the use of TENS (see exclusion criteria).
7. Avoid caffeine for at least 12 hours before the testing period.

Table C3

Inclusion Criteria for TMD Subjects

1. Intrinsic or extrinsic dysfunction in the TMJ region as diagnosed through evaluation of the subjects occlusion, active range of motion, mandibular dynamics, and palpatory tenderness of the joint surfaces and muscles of mastication.
2. Males must be clean shaven and females free of cosmetics.
3. Limitation of vertical ROM < 35mm.
4. Tenderness/pain of at least one masseter determined by palpation with a standardized pressure threshold gauge.
5. Avoidance of any medication at least four hours prior to testing.
6. Avoidance of caffeinated beverages at least 12 hours prior to testing.
7. Must be willing to avoid smoking, vitamin/mineral supplements, gum chewing and eating for at least four hours prior to testing.
8. No precautions/contraindications to the use of TENS (epilepsy, pregnancy, history of transient ischemic attacks, cerebral vascular accident or cardiac disease, presence of cardiac pacemaker).

Location

The study will be conducted in the Metabolic Magnetic Resonance Research and Computing Center in the basement of the Stellar-Chance Building at the University of Pennsylvania Medical Center (422 Curie Blvd.), room B-1.

Table C4

Subject Selection Profile

Subject:__________________________ Date:________________
Subject Telephone Numbers: _________ Home ___________Work

Exclusion Criteria

1. Presence of a pacemaker, neurostimulator, metal implants, orthodontics or history of work on or around a metal grinder or construction site.
2. History or presence of systemic disease that may alter neuromuscular function.
3. History of headache or facial pain separate from that of TMD.
4. Mouth breather.
5. Sleep disorder.
6. History of prior use of an electrical stimulator for pain control.
7. Claustrophobia, dizziness, vertigo, nystagmus or panic attacks.
8. Currently undergoing physical or psychological therapy.
9. Pregnancy or lactation.
10. Epilepsy, history of transient ischemic attacks, cerebral vascular accident or cardiac disease

If any of the above criteria exist, the subject must be excluded form the study.

Inclusion Criteria

1. Intrinsic or extrinsic pain/dysfunction of the masticatory region.
2. Vertical TMJ ROM <35mm _________mm.
3. Tenderness of at least one masseter initially determined by palpation and confirmed by algometer.
4. Subjective complaint of masseter/TM pain.
5. Age of 21-50.
6. No medication usage other than NSAIDs or Tylenol.

Please note that the research test must be performed before any therapeutic intervention begins

Diagnosis:____

Dr.____

Table C5

Subject Selection Protocol

If your patient meets the above criteria for participation, please perform the following:

1. Ask the patient if they would like to participate in a university research study related to their pain syndrome, and if they agree, inform them that this single test must be performed before the start of any therapeutic intervention.
2. If they are interested, briefly explain the study and tell them that it will require only 2-2.5 hours of their time.
3. Have them read the consent form plus subject instructions and answer any questions that they may have.
4. If they agree and sign the consent form, please contact Jeff Mannheimer ASAP at 609-896-9054 or 215-968-5981 and he will contact the subject and schedule a testing date.
5. Jeff Mannheimer, will perform a brief final evaluation of each subject on the testing date to confirm that all inclusion criteria continue to be present.

Subjects who were selected and signed the consent form were given the instructions outlined in Table C6, relative to the procedure to follow for testing.

Table C6

Subject Instructions

The Effects of Microcurrent Stimulation on ATP Synthesis in the Human Masseter as evidenced by 31P MRS

The study that you have agreed to participate in is being conducted at the Metabolic Magnetic Resonance Research and Computing Center (MMRRCC) of the University of Pennsylvania.
The MMRRCC is located in the basement (B-1) of the Stellar-Chance Research Building (422 Curie Blvd.) at the University Medical Center.
You will be contacted by Jeffrey Mannheimer, MA, PT, to arrange for a testing date ASAP.

One of the researchers will meet you in the lobby of the Penn Tower Hotel at 8:45am on the morning of________________________________ and escort you to the MMRRCC.

1. Do not chew gum on the morning of your testing.
2. Finish eating breakfast by 7:00am on the morning of your testing.
3. Male subjects must be clean-shaven.
4. Female subjects must be free of cosmetics on the cheeks.
5. If you are or anticipate that you will be menstruating at the time of your test, please let the investigator know when scheduling the test date.
6. Do not take any medication after midnight on the night before testing.
7. Do not drink any caffeinated beverage upon awakening
8. Do not smoke after getting up on the morning of the testing.
9. Do not take any vitamin, mineral or herbal supplements on the morning of your testing.

You will receive lunch immediately following the test and a cup of coffee if desired. In addition, all subjects will receive $50.00 for participating in each phase of this research study. Thank you very much for your cooperation.

Sincerely,

Jeffrey S. Mannheimer, MA, PT

Table C7

Masseter/MRS Data Collection Form

Subject: Date : Arrival Time:

FILE NAME	File #	File #	File #	Comments
FACIAL TYPE	Base	20-32	48-60	
ACQUIRE DATA FROM ___________MASSETER				
# OF FILLINGS SIGNED CONSENT:		XXX	XXX	
MAGNET PROTOCOL: STANDARDIZATION CHECKOUT		XXX	XXX	
BATTERY CHECK		XXX	XXX	
MASSETER ALGOMETRIC ASSESSMENT		XXX		
VAS 0___________________10		XXX		
ROM VERT.____MM		XXX		
ELECTRODE PLACEMENT		XXX	XXX	
HEADGEAR PLACEMENT		XXX	XXX	
SHIM SETTING TIME FREQUENCY (Hz)		XXX	XXX	
DATA ACQUISITION TOTAL STIM TIME:________	TIME START STOP	TIME START STOP	TIME START STOP	
STIM. AMPLITUDE				
STIM. FREQUENCY				

Table C8

Masseter/MRS Post-Processing Data Collection Form

	FILE#	FILE#	FILE#	Comments
File Name:	Base	20-32	48-60	
Subject:				
Acceptable spectra from _____masseter				
pHi				
PCr				
Pi				
Pi/PCr				

Appendix D

University of Pennsylvania

Revised Consent Form

The Effect of Microcurrent Stimulation on ATP Synthesis in the Human Masseter Muscle as Evidenced by ^{31}P MRS

Purpose

You are being asked to participate in a research study, which will be performed using an MRI machine. MRI stands for Magnetic Resonance Imaging. It uses magnets to measure the amount of certain chemicals in your body. We will be using MRI to study phosphorous levels in muscle with a procedure called phosphorus magnetic resonance spectroscopy (MRS). This allows us to measure the amount of various phosphorous containing chemicals in your body. Phosphorus chemicals are used in your body to provide energy.

Treatment with low levels of electricity is one method used by physical therapists instead of drugs for the relief of acute and chronic pain. The strength of the electricity at this low level cannot be perceived. This treatment is believed to increase the levels of energy in muscle. The purpose of this study is to determine if very low levels of electricity affect the ability of human muscle to produce energy. We have selected the muscle used for chewing, the masseter, for our study. We will use the MRI machine to look at phosphorous levels (and in turn energy) in the masseter muscle to determine if low levels of electricity affect the ability of human muscle to produce energy.

Selection of Subjects

You have been asked to participate either as a subject with a temporo-mandibular (TMD) condition or a control subject without a TMD condition. You should not participate if you have a pacemaker, defibrillator, neurostimulator or a metal implant of any kind (foreign metal objects in your body such as bullets, shrapnel, metal slivers, etc.). In addition, you should not participate if you have a condition which can be exacerbated by stress such as an anxiety or panic attack, including fear of enclosed spaces (claustrophobia), uncontrolled high blood pressure or suffer from seizure disorders. If you are a female, you should not participate if you are pregnant, trying to become pregnant or are currently breast feeding. Although many of these conditions may not exclude you from a clinical imaging examination known as a Magnetic Resonance Imaging (MRI)

examination, we have chosen to keep out individuals with these conditions from our current study. In order to participate, you must be 18 years of age or older.

Procedures

This procedure will be performed at the Metabolic Magnetic Resonance Research and Computing Center located in the basement of the Stellar-Chance Building at the University of Pennsylvania. After giving informed consent, you will be checked to see if you have any metal objects on your clothes or in your pockets and will be asked to remove such items. Soft electrode pads will be attached to each cheek. Long cables will connect the electrodes to a stimulation unit outside the magnet room. You will then be placed on the magnet bed and a small coil of wire placed next to the masseter muscle of each cheek to detect the radio signals that the chemicals in the masseter muscle emit in the scanner field. You will be given earplugs to wear to dampen the banging noise caused by the machine being adjusted. The earplugs do not block out all sound since we wish to stay in two-way voice communication at all times. You are then placed inside the scanner until the region under investigation, the masseter, is in the center of the magnet, after which the study will proceed.

The machine operator will be in constant two-way voice communication with you. You will be given periodic updates throughout the examination and encouraged to report any discomfort immediately. You will not be asked to remain in the magnet for more than 90 minutes. It will take 20 minutes for the first measurement without electrical stimulation. This will be immediately followed by a 60 minute period during which time additional information will be acquired. Each cheek will receive very low levels of electrical stimulation that you will most likely not feel. If you do feel the stimulation, it will either be extremely mild and comfortable or short-lived. When the study is complete, you will be brought out of the scanner, the listening tools moved away from your cheeks, electrodes removed, and you will be allowed to get up.

Risks:

All studies performed under this project will not exceed the Food and Drug Administration of the USA (FDA) guidelines for MRI machines. The single greatest risk to normal healthy subjects is the possibility of a metal object flying towards the scanner. In order to assure against such an event, all persons present at the study, subject, investigators, technicians, etc., are thoroughly screened for metal objects, which are removed and placed in a safe location away from the scanner room. In addition, once you are in the scanner, the door to the room will be closed so that no one accidentally walks into the magnet room. The only known adverse reactions to electrical stimulation are skin irritation in the form of

erythema or itching as well as allergic responses to the electrode interface material. These side effects occur in less than 2 percent of usage. In addition, skin reaction is significantly reduced with amplitude ranges at the microamperage level, which will be utilized in this study.

Benefits:

Your participation in this study will not benefit you in any way. However, as a result of your participation there may be benefits to health care and relief of chronic pain of future patients and TMD subjects.

Alternatives:

The alternative to participating in this study is not to participate.

Confidentiality:

All information will be kept confidential except as may be required by law. It will be available only to the investigators involved in this study. NIH may also have access to the study records. Any publications resulting from this study will identify you by study number, not by name. Information will be released to others only after you have been given written permission.

Withdrawal:

You are free to decide whether or not you would like to participate and are free to withdraw at any time. Your decision not to participate will not harm future dealings with the University of Pennsylvania or the University of Pennsylvania Medical Center in any way.

Injury/Complications:

You understand that in the event of any physical injury resulting from the research procedures, medical treatment will be provided without cost to you, but financial compensation is not otherwise available from the University of Pennsylvania.

You or your third party, if any, may be billed for medical expenses associated with this study if they are deemed medically necessary and such expenses would have been incurred independent of the study, or if your third party payer agrees in advance to pay for such expenses.

Questions:

You should make sure that all your questions are answered before participating in this study. The research technician and the investigators are available to answer any of your questions before, during and after the study.

Subject's Rights:

You understand that if you wish further information regarding your rights as a research subject, you may contact the Director in the Office of Regulatory Affairs at the University of Pennsylvania by telephoning 898-2614. If you have a problem, you may contact the hospital research subject 24-hour telephone line 662-6059.

Consent to Participate:

I have read and understand the consent form. Upon signing below, I will receive a copy of the consent form.

I agree to participate in this research study.

____

Signature of Subject Date

____

Name of Subject (Print)

____

Signature of Investigator Date

____

Name of Investigator (Print)

____

Signature of Witness Date

____

Name of Witness (Print)

Appendix E

MRS Operational Protocol

Optimizing Coil Placement, Tuning and Equipment Check-Out

1. Computer Logon: mike
2. Password: go_hoyas
3. Turn on shimming console (allow for a 10 minute warm-up). Maintain shim console in Standby Mode.
4. Make sure that RF amp is on in adjacent room
5. Set power attenuation at 10db (make sure that other buttons are out)
6. Gradients should be off
7. Set up coil (phantom or subject)
8. Connect coil tune lead cable (use the one that has " legkick label " or tape attached)

Oscilloscopic Coil Tuning

Determine sub-max intensity level. If actual subject, active/placebo blind selection chosen, sub-max microamperage level, baseline vertical ROM, VAS and Algometer data obtained prior to positioning in magnet

Phantom/Subject should be appropriately positioned in magnet before coil tuning

9. Oscilloscopic coil check and match: Test phosphorus (34) and proton (86)
   a. Turn on marker
   b. Signal peak is optimal if it touches superior line on scope (use middle dial to narrow peak)
   c. Ten dials to set frequency
   d. Optimize (tune) phosphorus and proton coils. (Tune with middle dial of 3 on the middle console, large left dial and large bottom dial).
   e. Turn scope off & turn first knob back to 1 from 0. Disconnect cable and place in magnet proximal to phantom or subject.
10. Center coil in magnet to obtain optimal signal/noise. Move plinth to the opposite side of the testing muscle, to ensure that the masseter to be tested is centered on the plinth. Plinth should be down to the point where the distance from the subject/masseter is 37.5 inches from the top of the bore.
11. Push subject in to marker.
12. Attach coil cable to magnet (use top TR switch at rear of magnet)

Optimizing Spectra

Shim on proton

(magnitude domain, but measure in real domain)
Domains expressed as magnitude in white, imaginary in blue and real in red
Z (in & out), X (↑↓) and Y (←→)
One peak with phantom and two with human subject

Proton 86235844-gain 32
Phosphorus 34901398/34906400-gain 93

1. In Winterm, type newnmr (Proton). Right click activates menus. Resize ↑ to 25%. Two windows should now be open.
2. Click on adjust frequency in upper left window, which opens a third window.
3. From bottom right window, open processing window and pull down under frequency window.
4. Click on overwrite and show sums. Highlights in yellow when activated.
5. In processing window (bottom left) remove DC.
6. Go to sequence file = continue, choose tune sequence & accept. (opens upper left NMR tuning window).
7. Switch shim console from standby to RUN and use protocol # 28.
8. Choose 1 and click-start sums and time to commence counting. The magnet is now sending RF pulse and receiving information.
   Nothing (minimal) should be on the pulse/spectral screen at this time.

Table E1

MRS/Proton Magnet Settings

Nucleus	Proton
RF Power	100
RF Width	0.15 (0.1) may need to ↑ with masseter
TR time	1
Acquisition Points	1024
SW	50k first ↓ to 3000
BW	50k first ↓ to 3000
Acquisition gain	0
Sums	1
Saves	1

9. Commence Zoom & Shift (left side for vertical and right for horizontal)
10. Click Zoom down then shift up to level followed by horizontal shift for data view.
11. Data inside spectra window is now within 50k (left –25k and right +25k). Click at signal peak = X coordinate. Goal is now to optimize the signal to the lowest frequency (line width) with the highest peak, via shimming. Time domain is a product of the frequency and time.
12. Click on NMR processing in lower left window to rephase.
13. Add Exp.Weight to remove excess noise.
14. Right click on Shim Aid to Show Zero Line in Plot Options.
15. Open bottom left to show Clip Line & right click = green peak height line. Yellow lines represent frequency domain (click on frequency domain) are clip lines = output. Zoom to see yellow lines. If the signal is reaching the clip line, the output must be rectified by decreasing the receiver gain. (34906400)
16. Click on Plot Type (top) in lower right window (data is now in Real plot type) To create a Phase change, use the lower left window (Real) PHO: root mean square of signal = magnitude = positive deflection. <u>Use magnitude to shim on Proton</u>. Goal is to again maximize peak height. ↓ the line width = ↑ real height, (frequency domain), which is the narrowest line width.
17. Optimizing the magnetic field: ZO is main magnetic field. Cycle between Z1 (when peak is highest), X, Y, XY, Z1. (If unable to locate signal, hit re-set scale and start over). If X & Y change, go back to Z1 and re-shim. (Cycle back and forth between Z1-X-Y-Z1-X-Y at least 3X) <u>When optimized ↓ BW to 5k.</u>
18. Click on peak = real X value. Use Proton frequency scale (left middle window) to move signal to center line. To move signal to the right use (+) and to the left use (-). 10 = first > button. Due to the 3Hx noise, the signal should be moved 200 Hz away from the center line.
19. Click frequency domain in bottom left window. If signal is hitting clip line ↓ receiver gain. When signal is centered, decrease SW & BW to 3k or 5k. ↓ BW = ↑ in resolution and ↓ noise. If it is ↓ too much, the spectra will be out of the window.
20. Left click = shim line to show previous signal (may need to re-phase). Set cursor at top of peak and measure width at 50% of peak height (left for X and right for Y values) Difference = shim frequency. (Read line-width from REAL domain).
21. When shim is acceptable, click on Pause and stop, go to PHO, left click on Select Nucleus and right click on PHO.
22. Type PHO in upper right box and hit enter. PHO is less sensitive than Proton, due to a lower concentration. The magnetic moment of the phosphorus nucleus is 3X less sensitive than proton.
23. ↑ Exp. Weight to 5-6 Hz.

24. Stop sequence file
25. Commence Data Acquisition of baseline, 20-32 & 48-60 time-points.

Table E 2

MRS/Phosphorus Magnet Settings

Nucleus	Phosphorus
RF power	100
RF width	0.15 or 0.25
TR time	4
Acquisition points	1024
SW	3000 or 5000
BW	3000 or 5000
Acquisition gain	5
Sums	240
Saves	1

1. Choose masseter rest sequence and change magnet settings as in table 2.
2. Re-name data file. Type in name initials-date-rest (Cannot overwrite, click O.K., re-name, hit O.K. to star).
3. Hit run and accept (baseline/rest acquisition sequence).
4. Start double-blind test sequence (active or placebo). MMRRCC engineer to set lead wires in stimulation box, according to active or placebo acquisition, followed by activation of stimulation unit.
5. After 20 minutes select sequence file. Choose masseter 12S exercise sequence (20-32 minute datapoint). Adjust NOE scale to 1 and AD 90 = 1.
6. Click O.K. (12 minute acquisition time)
7. Repeat data acquisition (re-name new data file) again at the 48-60 minute timepoint.
8. Turn off sequence at 60 minute timepoint.
9. Turn off shim console
10. Turn off computer
11. Quit and exit (hit 3 quit buttons).
12. Move cursor to side of data screen and Log Out.
13. Data is saved under MMR user in BAT files.
14. Disconnect cable from rear of magnet.
15. Pull phantom or subject out.

16. Disconnect lead wires, remove electrodes and assist the subject to sitting position and getting off the plinth.
17. Remove cable from RF coil.
18. Acquire post-test VAS, Algometer and ROM data.
19. Remove bedding from plinth and replace in magnet room.
20. Remove lead wires from magnet.
21. Replace stimulation unit and RF coils.

Appendix F

Pilot Study Informed Consent

This form is intended to provide you with the necessary information to decide whether or not to participate in this research study.

Title: The effect of microamperage electrical nerve stimulation on ATP synthesis in the human masseter muscle, as evidenced by ^{31}P Magnetic Resonance Spectroscopy.

Purpose: The purpose of this study is to determine if very low levels of electricity affect the ability of human muscle to produce energy. This research will investigate if microamperage electricity causes an increase in the levels of energy in human muscle. The muscle to be tested is located on either side of the face and is used for chewing. This study will use magnetic imaging to assess any changes in energy levels. This research will also explore different dosages of electricity. All electrical settings are FDA approved and should produce either no sensation or a very mild, short-lived tingling.

Procedures: This research will require 4 hours of your time and will be done at the Hospital of the University of Pennsylvania (HUP) Metabolic Magnetic Resonance Research and Computing Center in the basement of the Stellar-Chance Building. Tests will be conducted in MRI B1. A special type of MRI will be used to collect data.

You will be required not to eat or chew gum for two hours before reporting for the test or drink any caffeinated beverages for 12 hours before the test. Please report for the testing procedure with clothing that does not have metal on it. This means you should not wear dungarees with rivets or metal buttons. Plastic buttons and Velcro are fine. But, should you forget, you may wear clothes available for you at the center. You need to be clean-shaven and wear no make up. You cannot participate in this study if you have any metal implants or a pacemaker. You should bring some reading materials with you to pass the time.

You will be asked to lie on a table, which then will be placed in a magnetic field. You will have to lie perfectly still. A baseline reading of the energy in your

muscles will be taken. This will take about 20 minutes. The table will then be removed from the MRI unit and your face will be washed with warm water. Then, two pairs of surface electrodes will be placed on your face, one pair on each cheek. After the baseline data has been collected the electrical stimulation will begin. Four more measurements will be taken over the next four hours.

A bathroom break will be given after 1 hour. Talking should be kept to a minimum. You will be given earplugs to diminish the sound of the MRI machine. It is possible for you to talk to the person running the MRI machine. You will be encouraged to voice any concerns that you might have. Each measurement takes about 15-20 minutes. If you have any questions, the technician and research investigators will be available to answer them.

Benefits: There are several possible benefits of this research both to you and others. This research will allow further understanding of the effects of low levels of electricity on human muscle. You will be able to receive an MRI of any part of your body without interpretation by a radiologist for your participation. The MRI film will be given to you at the end of the research session.

Risks: This procedure has minimal risk to you as a participant. Magnetic resonance imaging and spectroscopy is performed without any radiation. The electrical stimulation is at a low level and you should not feel it. The very most that you will feel is a tingling sensation at the area of application. Most people don't feel anything. When taking the measurements you will be in a magnetic field, so all metal objects will be removed to prevent them from being pulled by the magnet. The test will not increase the risk of illness, it is possible that a person may coincidentally become ill during the testing procedures and if that occurs all personnel have been certified in CPR and first aid. The hospital's emergency systems are also available.

Participation in this research is totally voluntary and you may discontinue the procedure at any time and for any reason. All information gained from this procedure will be held in the strictest of confidence. The results of this study will be made available to the research investigators and the technician. These results will be stored in a filing cabinet at the University of Pennsylvania. Information about adverse side effects will be reported to the manufacturer and the FDA. All information will be kept confidential except where required by law. All of your questions about this research should be answered before your participation begins.

You are encouraged to ask all of your questions. You may direct them to the researchers. The principle investigator, Jeff Mannheimer M.A., P.T. may be reached at (215) 244-4603 or (609) 896-9054.

I have read and understand the content of this document

(SUBJECTS SIGNATURE)________________________________ Date______

(PRINT NAME)________________________________

(PRIMARY INVEST. SIGNATURES)________________________________

Date_______ Jeff Mannheimer, MA, PT.

The solicitation of subjects into this study has been approved by the Committee for the Protection of Human Subjects of the University of Pennsylvania

Pilot Study

Subjects

Participants consisted of 18 normal college students between the ages of 18-32, who met the criteria for admission, agreed to participate and signed the consent form. They were then scheduled for the pilot study, at which time the inclusion and exclusion criteria were reviewed and a re-evaluation of the temporomandibular complex performed, to determine if there had been a change in status between the selection and test dates that would necessitate expulsion.

Subjects with various physical and psychological factors that presented confounding variables, as well as those with systemic conditions such as fibromyalgia, chronic fatigue syndrome and thyroid dysfunction, were excluded in order to ensure as homogeneous a group as possible (McAllister, et al. 1995, Radda, et al., 1995). Tables F1-2 list the inclusion and exclusion criteria respectively.

Table F1

Inclusion Criteria for Normal Subjects

Normal TMJ function, consisting of:
a. Active vertical ROM to 40mm b. 8mm lateral and 4mm protrusive c. Absence of joint sounds (clicking, crepitus or subluxation) d. Normal Class I occlusion
Age range 18-50
Agree not to eat or drink any caffeinated beverage or food, nor chew gum two hours prior to the beginning of the test or during the stimulation period
Sign the informed consent
Clean-shaven and not wearing cosmetics

Table F2

Exclusion Criteria for Normal Subjects

Exclusion Criteria
Unwilling to sign the informed consent
Determined to have TMD via the questionnaire or screening evaluation
Currently ongoing orthodontics
Had dental work within a two-week period prior to the test date
Currently taking prescription or over-the –counter anti-inflammatory, muscle relaxant or pain medication
Currently menstruating
Presence of a pacemaker or other metal implants
Precautions or contraindications to the use of TENS: epilepsy, pregnancy,transient ischemic attacks, post cerebral vascular accident
Decreased sensation to temperature, cardiac disease, clinical diagnoses of stress,anxiety or mental illness
Pain upon palpation of the mandibular elevator musculature
The presence of systemic disease that is known to influence energy metabolism

Procedure

Location, Support and Operation of the MRS Unit

Testing was performed in the Devon MRI Center of the Hospital of the University of Pennsylvania. A university post-doctoral fellow, who operated the MRI and collected the spectra, provided support. MRI and MRS measurements were performed with a General Electric Signa scanner at a field-strength of 1.5 Tesla.

Testing

Subjects reported to the Devon MRI Center on the designated test day at least one-half hour prior to their test time and were reminded that they could discontinue participation at any time and for any reason. Researchers checked for and subjects removed all metal jewelry, watches, coins, etc. with instruction given to use the restroom before the start of the testing period.

The skin of each masseter was checked for abrasions or cosmetics, the area swabbed with a moist paper towel and subjects instructed to clench mildly on a gauze roll for one second to determine the specific location of the masseteric belly, after which a pair of electrodes were applied adjacent to each masseter. Subjects were told that they may or may not feel a mild and short-lived tingling sensation when the electrotherapeutic device was activated.

Double-blind Randomized Format

Prior to placement within the magnet subjects were instructed to blindly pick one piece of paper from 18 placed within a brown paper bag, which designated the active or placebo side and the stimulation parameter setting. They were then properly positioned on the MRI plinth, ear-plugs offered to dampen the sound of the MRI unit and twenty- foot long lead wires connected to the electrodes from the electrotherapeutic generator by the operator of the magnet.

The electrotherapeutic generator was situated outside of the MRI room and hidden within a specially constructed wooden box with holes through which the lead wires were connected to the unit (active group) or just taped to the inside of the box (placebo group) by the operator of the magnet. The principle investigator and subjects were therefore blind to knowledge of which masseter was receiving active or placebo stimulation.

Data were coded by subject number and baseline phosphate measures performed, followed by activation of MENS, with the start-time noted on the data collection form. Due to the four-hour stimulation procedure, subjects were removed from the MRI after each period of data recording and allowed to sit outside the magnet room and read. Verbalization was kept to a minimum to avoid masseteric function and subjects were not allowed to eat, chew gum, drink coffee or other caffeinated beverages (water was permitted) suck on mints or smoke at any time.

Each of the 18 subjects were tested using one of the six parameters of stimulation as illustrated in table F3 for a duration of 4 hours, equivalent to that employed by Cheng, et al (1982). Because there are numerous combinations of pulse rate and intensity that could be delivered, paradigms were chosen that were similar to those clinically reported with MENS protocols. They covered a fairly wide range of intensities at either a low (3Hz) or high frequency (85Hz) and the only variable between the right and left masseter was the microcurrent stimulation. MRS data were collected at baseline, 20 minutes, 1 hour, 2.5 hours and 4 hour time-points and kept blinded until all subjects were tested.

Confidentiality

Subject consent forms were kept on file at the Devon MRI Center by the primary investigator. Collected data were stored for each subject by number on the computer hard drive as well as a floppy disc. The consent forms and collected data were to be disposed of 3 years after completion of the research.

TABLE F 3

Parameters of MENS for Pilot Study

Subject	Frequency	Intensity	Waveform	Time
1	85 Hz	300 μa	sinusoidal	4 hours
2	85 Hz	100 μa	sinusoidal	4 hours
3	85 Hz	60 μa	sinusoidal	4 hours
4	85 Hz	300 μa	sinusoidal	4 hours
5	85 Hz	100 μa	sinusoidal	4 hours
6	85 Hz	60 μa	sinusoidal	4 hours
7	85 Hz	300 μa	sinusoidal	4 hours
8	85 Hz	100 μa	sinusoidal	4 hours
9	85 Hz	60 μa	sinusoidal	4 hours
10	3 Hz	300 μa	sinusoidal	4 hours
11	3 Hz	100 μa	sinusoidal	4 hours
12	3 Hz	60 μa	sinusoidal	4 hours
13	3 Hz	300 μa	sinusoidal	4 hours
14	3 Hz	100 μa	sinusoidal	4 hours
15	3 Hz	60 μa	sinusoidal	4 hours
16	3 Hz	300 μa	sinusoidal	4 hours
17	3 Hz	100 μa	sinusoidal	4 hours
18	3 Hz	60 μa	sinusoidal	4 hours

Results

Data Acquisition

The research protocol for the pilot study consisted of a within-subjects design, in which baseline phosphate measurements were taken for each subject prior to the introduction of the experimental variable (MENS) and at successive time-points during the testing period. In addition a between-group design allowed for a comparison of the differences among the active and control groups. The raw

data, in the form of spectra, were analyzed by a standard procedure for determining ATP levels, which required measurement of the amplitude and area under the five different phosphorus containing peaks. The gamma ATP/PCr ratio was used as the representation of ATP concentration, because it indicated the presence of all three phosphorus nuclei, thereby differentiating ATP from other phosphate containing compounds (ADP & AMP).

A ratio comparing gamma ATP to an internal standard, PCr, accounts for any potential shift in the spectral baseline (Gadian, 1995, Lenkinski & Schnall, 1996). The PCr peak normally will decrease with muscular work or energy expenditure, but because the energy produced by MENS is considered not to be great enough to alter PCr concentrations, it thus was used as the internal standard. The generated MRS data were then summed and entered into Microsoft Excel 7.0

Results and Statistical Analysis

No identifiable trend in the raw data for either the stimulated or non-stimulated masseter, indicative of ATP alteration, was observed. Table F4 presents the gammaATP/PCr ratio of the stimulated and non-stimulated masseter for each of the 18 subjects at all five time-points. Ten of the subjects appeared to have an increase in ATP levels and 8 a decrease when the gamma-ATP/PCr ratio was considered. Means and standard deviations for the gamma-ATP/PCr ratio for stimulated and non-stimulated muscles were calculated at each successive time interval and appear in Table F5. A subsequent review of these calculations was not indicative of a significant increase or decrease in ATP levels, and the

magnitude of change did not have any reproducible trend, nor was there any association with a specific time interval or stimulation parameter.

Table F4.

GammaATP/PCr Ratio at each time-point for Stimulated (Stim) and non-stimulated (Con) Masseter

I D	Con - Stim	Baseline	20 minutes	1 hourt	2.5 hours	4 hours
		(gam/pcr)	(gam/pcr)	(gam/pcr)	(gam/pcr)	(gam/pcr)
1 Right	Con	0.322	0.495	0.670	0.450	0.270
1 Left	Stim	0.424	0.498	0.476	0.488	0.158
2 Right	Stim	0.240	0.545	0.574	0.303	0.323
2 Left	Con	0.420	0.286	0.389	0.331	0.476
3 Right	Stim	0.386	0.420	0.383	0.458	0.311
3 Left	Con	0.450	0.284	0.313	0.193	0.224
4 Right	Con	0.265	0.236	0.470	0.409	0.305
4 Left	Stim	0.432	0.262	0.240	0.259	0.273
5 Right	Con	0.237	0.405	0.337	0.327	0.259
5 Left	Stim	0.300	0.528	0.302	0.275	0.330
6 Right	Stim	0.248	0.371	0.279	0.338	0.242
6 Left	Con	0.381	0.363	0.717	0.207	0.413
7 Right	Con	0.307	0.295	0.355	0.148	0.360
7 Left	Stim	0.541	0.333	0.551	0.386	0.343
8 Right	Stim	0.443	0.326	0.434	0.400	0.325
8 Left	Con	0.333	0.428	0.289	0.412	0.298
9 Right	Con	0.331	-	0.327	0.281	0.312
9 Left	Stim	0.241	0.195	0.362	0.393	0.273
10 Right	Stim	0.459	0.482	0.380	0.325	0.327
10 Left	Con	0.326	0.454	0.071	0.437	0.320
11 Right	Stim	0.518	0.378	0.550	0.247	0.443
11 Left	Con	0.322	0.333	0.485	0.466	0.276
12 Right	Stim	0.234	0.380	0.330	0.478	0.297
12 Left	Con	0.321	0.204	0.244	0.202	0.281
13 Right	Stim	0.310	0.324	0.560	0.421	0.396
13 Left	Con	0.281	0.221	0.875	0.454	0.259
14 Right	Con	0.268	0.584	0.349	0.655	0.331

Table F4 Continued

GammaATP/PCr Ratio at each time-point for Stimulated (Stim) and non-stimulated (Con) Masseter

14 Left	Stim	0.372	0.202	0.384	0.310	0.596
15 Right	Con	0.514	0.299	0.303	0.471	0.325
15 Left	Stim	0.279	0.130	0.282	0.366	0.347
16 Right	Stim	0.567	0.119	0.294	0.424	0.453
16 Left	Con	0.360	0.313	0.320	0.671	0.355
17 Right	Con	0.116	0.288	0.217	0.166	0.304
17 Left	Stim	0.432	0.184	0.292	0.239	0.248
18 Right	Stim	0.552	0.396	0.368	0.174	0.304
18 Left	Con	0.279	0.402	0.294	0.280	0.394

(-) = data not obtained

Table F5

Mean and Standard Deviations for Gamma-ATP/PCr ratio

Datapoint	Stimulated muscles		Non-stimulated muscles	
	Mean	Std Dev	Mean	Std Dev
Baseline	.39	.12	.32	.09
20 minutes	.34	.13	.33	.13
1 hour	.39	.11	.39	.19
2.5 hours	.35	.09	.36	.15
4 hours	.33	.10	.32	.16

The paired t-tests of the active and placebo groups comparing baseline data to each subsequent measurement interval were not significant for an increase or decrease in ATP concentrations as shown in Table F6.

Table F 6

Paired t-test Results for Stimulated and non-stimulated Muscle

Time-point	Stimulated Muscle			Non-stimulated Muscle		
	Corr Sig	t-value	2-tail Sig	Corr Sig	t-value	2-tail Sig
Baseline to 20 min	.630	1.15	.267	.674	-.08	.935
Baseline to 1 hour	.622	-.10	.921	.893	-1.34	.197
Baseline to 2.5 hours	.642	-1.02	.323	.313	1.01	.328
Baseline to 4 hours	.433	.17	.864	.429	1.74	.101

An ANOVA with repeated measure of gamma-ATP/PCr, indicated that there was no significant change in ATP concentrations even when frequency and intensity were isolated. All of these tests compared stimulated to non-stimulated muscle on the same subject for changes over the five measurement intervals with results depicted in Table F7.

A simple t-test was also performed at each time interval comparing stimulated and non-stimulated muscle. Curiously, at the baseline time-point, prior to the start of stimulation, there was a significant difference in the gamma-ATP/PCr ratio between the right and left masseter ($P = .035$). This retrospective factor may have been due to the spectral acquisition process or alteration of the RF coil position, when subjects were moved in and out of the magnet during the

testing period. The t-tests of the other four measurement intervals were not significant and a summary appears in Table F8.

An additional double difference analysis of the post-baseline-minus baseline gammaATP/PCr for the stimulated group, minus the post-baseline-minus baseline ratio for the placebo group, was calculated for each subject, as shown in Table F8. This calculation did not reveal a trend toward an increase or decrease in ATP levels. Negative values indicated an increase in ATP on the placebo side with a positive value depicting an increase on the stimulated side. Ten subjects demonstrated ATP elevation at the placebo side at the 20 minute and four-hour data-points, while 8 subjects revealed an increase at the stimulation side for those same time intervals. Eleven placebo and 7 active subjects had an increase at both the 1-hour and 2.5-hour time intervals. Greater variability would be expected on the stimulated side, due to the different stimulation protocols, but the reverse occurred. There were no significant trends noted in the time intervals and the amount of change was not consistent with either a specific time interval or stimulation parameter as shown in Table F8.

Table F7

ANOVA with Repeated Measures Comparing Stimulated and non-stimulated Muscle with Values for Intensity and Frequency Considered Separately

	Comparison of changes in ATP levels between stimulated and non-stimulated muscle	Comparison of changes in ATP levels in stimulated muscle only between 3Hz and 85Hz	Comparison of changes in ATP levels in stimulated muscle only between 60, 100 and 300μa
F - value	.52	.05	.60
Significance of F	.475	.828	.559
Chi-square	10.66	7.08	8.61
Multivariate Sig. of F between control & experimental	.649	.218	.491
Multivariate Sig. of F over time	.197	.822	.173
Average Multivariate Sig. of F	.695	.788	.632

Table F8

Simple t-tests at each Measurement Interval Comparing ATP Levels

Between Stimulated and non stimulated Muscle

	p - value	Sum of squares
Baseline	.035	.036
20 minutes	.409	.001
1 hour	.494	.000
2.5 hour	.349	.002
4 hours	.321	.001

Table F9 reveals the mean and standard deviations of the double difference values for each subsequent time interval.

Table F9

Double Difference (DD) Calculations for each Subject at the six Different Stimulation Parameters.

Stimulation Parameters	ID #	DD (20 min)	DD (1 hour)	DD (2.5 hour)	DD (4 hours)
3 Hz @ 60μa	3	.200	.140	.329	.151
	4	-.141	-.397	-.317	-.199
	9	NA	.125	.202	.051
3 Hz @ 100μa	11	-.151	-.131	-.145	-.029
	13	.074	-.344	-.062	.108
	15	.066	.214	.130	.257
3 Hz @ 300μa	8	-.212	.035	-.122	-.083
	10	-.105	.176	-.245	-.126
	12	.263	.173	.363	.103
85 Hz @ 60μa	1	-.099	-.296	-.064	-.214
	7	-.196	-.038	.004	.251
	14	-.486	-.069	-.449	-.161
85 Hz @100μa	2	.439	.365	.152	.027
	16	-.401	-.233	-.454	-.109
	18	-.279	-.199	-.379	-.363
85 Hz @300μa	5	.060	-.098	-.115	.008
	6	.141	-.305	.264	-.038
	17	-.420	-.241	-.243	-.372

Table F10

Mean and Standard Deviations on each Group of Three Subjects:

Double Difference Calculations

Stimulation Parameters	ID #s	Mean & SD 20 minutes	Mean & SD 1 hour	Mean & SD 2.5 hours	Mean & SD 4 hours
3 Hz at 60 μa	3, 4, 9	.029 (.241)	-.046 (.304)	.071 (.342)	.001 (.180)
3 Hz at 100 μa	11,13, 15	-.004 (.128)	-.087 (.282)	-.116 (.276)	.112 (.143)
3 Hz at 300 μa	8, 10, 12	-.018 (.249)	.128 (.081)	-.001(.321)	-.035 (.122)
85 Hz at 60 μa	1, 7, 14	-.260 (.201)	-.134 (.141)	-.170 (.244)	-.101 (.228)
85 Hz at 100 μa	2, 16, 18	-.080 (.454)	-.022 (.336)	-.227 (.330)	-.148 (.198)
85 Hz at 300 μa	5, 6, 17	-.073 (.303)	-.215 (.106)	-.031 (.264)	-.134 (.207)

Statistical Re-run

The raw data from Table F4, was re-entered into SPSS version 8 and a repeated measures ANOVA, in lieu of multiple t-tests, was performed with time as the repeated variable and the experimental condition (MENS) as the between subjects variable. This accounted for all the effects of interest relative to time-points, intensity and frequency within any of the subjects in either group, and decreased the possibility of performing Type I errors. The results, which are depicted in tables F11-14, again did not reveal any significant effects of the independent variable (MENS) upon the dependent variable (gammaATP/PCr) at any of the time-points nor with any specific stimulation parameters.

Table F11

Minimal and Maximal gammaATP/PCr ratio, Mean and SD for all 36 tests (18 active & 18 placebo)

Timepoint	N	Minimum	Maximum	Mean	S.D
Baseline	36	.116	.567	.35686	.10446
20 minutes	36	.000	.584	.33231	.12907
1 Hour	36	.071	.875	.39072	.15475
2.5 Hours	36	.148	.671	.35676	.12494
4 Hours	36	.158	.596	.32642	.97500

Table F12

Repeated Measures ANOVA of Within-subjects Effects Across all Treatment Conditions

Source	Sum of Squares	Degrees of Freedom	Mean Squares	F	Sig.
Time: Con & Stim					
Sphericity Assumed	0.9311	4	0.236	1.641	.167
Greenhouse-Geisser	0.9311	3.533	0.263	1.641	.175
Huynh- Feldt	0.9311	4.000	0.238	1.641	.167
Time & parameters					
Sphericity Assumed	0.2984	4	0.746	.526	.717
Greenhouse-Geisser	0.2984	3.533	0.844	.526	.695
Huynh-Feldt	0.2984	4.000	0.746	.526	.727

The results were relatively the same with both the Greenhouse-Geisser and Huynh-Feldt correction factor.

Table F13

Between-subjects Effects

Source	SS	Degrees of Freedom	MS	F	Sig.
Intercept	.0223	1	.0223	.1259.215	.000
Condition (Time)	.0881	1	.0880	.495	.486

Table F14

Interaction Between the Control and Stimulated Masseter in each Subject

Group		Base	20 Min	1 Hour	2.5 Hours	4 Hours
Con.	Mean	.32606	.32722	.39028	.36444	.32011
	N	18	18	18	18	18
	SD	.0847	.12866	.19349	.15498	.06187
Stim.	Mean	.38767	.33739	.39117	.34911	.33272
	N	18	18	18	18	18
	SD	.11512	.13281	.10892	.08941	.09582
Total	Mean	.35686	.33231	.39072	.35678	.32642
	N	36	36	36	36	36
	SD	.10466	.12907	.15475	.12494	0.7975

The analysis shown in Table F14, did not reveal a significant difference between the group that received stimulation (Stim) and the one receiving placebo (Con) exposure. The overall effect of MENS, collapsing across time and lumping the subjects of both the stimulation and placebo groups together regardless of the stimulation paradigm, did not yield any significant difference between the groups over the entire testing period. It is however, possible that there were competing effects between the stimulated and non-stimulated masseter of each subject, because the pattern of means is stable across the levels (data-points) of the experimental variable. Due to the fact that this revealed no effect of the independent variable, there was no need to perform a post-hoc test.

Knowledge from Pilot Study

The pilot study was performed with normal volunteers who presented with absence of any masseter pathology or TMD. Prior to commencing the pilot study, experimentation with various electrodes determined that TENS at the milliamperage level could be used in the magnetic resonance imaging (MRI) unit with simultaneous MRS recording. Spectra were acquired and analyzed without any significant interference from the electrotherapeutic generator. Testing was initially performed with intensity levels between 8-22mA and data revealed that a paradigm of 22mA, 85Hz and 75μs for 18 minutes produced a decrease in PCr with a concomitant increase in Pi, but without change in ATP concentration between the pre and post stimulation readings (Cecil, Mannheimer, Connick & Lenkinski, 1995). No significant change in metabolite levels were observed with stimulation below 18mA and the assumption was that higher milliamperage levels would continue to reveal similar metabolite alterations, due to contractile energy expenditure and the maintenance of ATP levels, thus maintaining consistency with previous findings utilizing muscle contraction stimulation. The pilot study utilized intensities of 300, 100 and 60μa at two different pulse rates (3 and 85 Hz) with normal human subjects to determine the effect with stimulation solely within the microamperage range.

The experience gained from the pilot work allowed for the elimination of confounding variables as well as refinement and standardization of the methodology to be employed in the study.

Confounding Variables

Retrospective analysis and continued literature review, revealed several confounding factors, (contralateral stimulation effects, caffeine and masseter fiber structure, possible magnetic effect of the magnet, cranial movement, alteration of subject position, RF coil movement and perception of the stimulation), which may have explained some of the unusual findings or at least cause concern about validity. These variables necessitated the creation of a conductive phantom to further analyze and confirm the acquired spectra prior to the initiation of subsequent testing with normal subjects as well as a cohort with masseteric pain and TMD. The knowledge gained from analyzing the data necessitated major changes in the research methodology that was used for the study.

Contralateral Effects

In the pilot study, subjects served as their own controls as MENS was applied to one masseter with concomitant placebo stimulation contralaterally. Ipsilateral stimulation via TENS has demonstrated contralateral effects that may approximate 50% of the relief obtained ipsilaterally (Lehmann & Strain, 1986). This is however, more likely to occur with acupuncture-like stimulation (low frequency and high intensity) rather than the parameters of MENS. Even though it is unlikely that crossover occurred with the parameters employed in the pilot study, the close proximity of each masseter and the prolonged continuous stimulation, cannot entirely rule out that a contralateral effect was evident. In addition, the metabolite fluctuations noted in the data analysis of the pilot could have been

caused by a contralateral effect. Therefore, the study compared bilateral active stimulation to bilateral placebo stimulation, using a parallel group design to avoid any possible contralateral effects from ipsilateral stimulation of one masseter.

Headgear Apparatus

A unique headgear apparatus and new radiofrequency (RF) coils were designed and constructed for utilization in the study (Figures 11-12). The headgear platform served to eliminate cranial movement and maintain a standardized position of the cranium, electrodes and RF coils, while the subject was in the magnet. Therefore the possibility of coil movement over the stimulating electrodes was negated along with movement of the subject's head. The headgear apparatus was previously discussed in greater detail in the methods section of the study.

It was also important to note that since subjects in the pilot were moved in and out of the magnet, an alteration of head position took place. When the subjects were in a supine position within the magnet, the effect of gravity on the mandible was eliminated. However, at the time that the subject was taken out of the magnet, brought to a sitting position, walks out of the magnet room and subsequently sits in a chair awaiting their return to the magnet, the mandible is in a gravity dependent position. This factor may have created a significant change in masseteric rest activity, since the mandible is supported by a sling-like mechanism composed of the temporalis, masseter and medial pterygoid. The protocol for the study was therefore designed to eliminate any possible change in the position of

the subject during the testing period, and therefore negated any increase in resting masseter activity that may have required increased muscle contraction and hence greater utilization of ATP.

Baseline Standardization

A decision to increase the baseline standardization process, testing protocol and measurement techniques for the study was also prompted by the statistical analysis of the pilot data, which revealed a significant difference in baseline ATP levels for each group (Table F6). Furthermore, the unusual fluctuations noted at the 20-minute and 4-hour time-points (Table F8), which may have been caused by movement of the subjects in and out of the magnet, coil repositioning or a change in the position of the mandible, prompted the need for an enhanced standardization process.

GammaATP/PCr vs. Pi/PCr

The pilot study used gammaATP/PCr as the dependent variable in comparison to Pi/PCr designated in the study. A review of the research citations in the reference list of the study reveals that both ratio's have been utilized in the past. However, the majority of the most recent research consistently tests for changes in Pi/PCr, which was the choice for the study.

Summary

The information and knowledge gained from the pilot study necessitated refinement of the research methodology as well as fabrication of and testing with a

conductive phantom in order to enhance spectral acquisition. A unique headgear apparatus was built to eliminate cranial movement during subject position within the magnet. The data acquisition period was reduced from 4 hours to 1 hour, which allowed for a sustained placement in the magnet and a stable cranial-mandibular position.

The incorporation of subjects with TMD in the study required alteration of the inclusion and exclusion criteria. A significantly enhanced step by step MRS process with greater standardization, strengthened the homogeneity of the testing process, and eliminated the aforementioned variables.