

# **Off-Label Drug Booklet for Prokarin™**

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## Introduction

The costs of health care associated with the disease, multiple sclerosis (MS), is a growing concern to the individual patients, their families, the healthcare system, and society. The high cost of the current FDA approved therapies, the increasing prevalence of people being diagnosed with MS and at even earlier ages than in the past, and the many co-morbidities that generally accumulate as the disease progresses all have contributed to the escalating direct and indirect costs of MS.

MS is an incurable neurodegenerative disease that may affect almost any part of the nervous system. Disease of motor pathways leads to weakness, spasticity, and paralysis. Sensory system changes may cause numbness, pain or paresthesias. Autonomic involvement is common with bowel and bladder dysfunction. Visual loss, brainstem dysfunction and cerebellar symptoms are common. Cognitive impairment is being increasingly recognized in MS.

MS affects 200+ persons in 100,000 in the United States. In 1990, it was estimated that 250,000-350,000 persons in the U.S. had a diagnosis of MS. The occurrence of MS cases has steadily increased in the U.S. over the past 30 years from 58 cases per 100,000 persons in 1976 to 175 cases per 100,000 in 1990 (U.S. Congress, Office of Technology Assessment, Neural Grafting: Repairing the Brain and Spinal Cord, Washington, DC: U.S. Government Printing Office, September 1990). According to the National Multiple Sclerosis Society, an average of 200 new cases of MS are diagnosed weekly in the U.S. This data indicates a 26% growth rate in the incidence of MS in the past 30 years with a total of approximately 465,000 people in the U.S. suffering from MS today. This is a smaller number of people than many other disabling illnesses including epilepsy, stroke, diabetes, heart disease, chronic lung disease, and arthritis (Minden et al, 1993). However the impact of this disease on society is disproportionately large because it strikes people during their most financially productive years, 20-50 years of age. Only 29% of MS patients are able to remain in the work force (Minden et al, 1993). Because of the lost earnings and increased healthcare costs, MS is the third leading cause of significant disability in the 20-50 year age range (Cobble et al, 1993). The annual economic cost of multiple sclerosis exceeds \$10 billion a year in the U.S. (National Multiple Sclerosis Society website, [www.nationalmssociety.org/research-factsheet.asp](http://www.nationalmssociety.org/research-factsheet.asp), March 2002).

MS was predominantly diagnosed in women between the ages of 20-50 years. The ratio of female MS sufferers to male MS sufferers was 4:1 in the past, but now the ratio is 2:1. MS is being diagnosed at earlier ages now, perhaps in part due to improved diagnostic criteria. There are 15,000 - 20,000 incidences of MS being diagnosed in adolescents (National Pediatric MS Center, Stonybrook, N.Y., Feb. 22, 2003) and in a few children as young as 3 years of age (reported data from patients and doctors to EDMS, LLC).

Thus, the increasing incidence of MS coupled with the diagnosis of MS being made at an earlier age in life greatly increases the direct and indirect costs of MS. The approved disease-modifying medications Avonex®, Betaseron®, and Copaxone® are high-cost treatments being heavily promoted by their manufacturers and the National Multiple Sclerosis Society. The proponents of these treatments are recommending early intervention with these medications and to continue these treatments throughout the life of an MS sufferer (King, 2000). The current FDA approved MS medications average \$1,000+ per month. The high-cost of these treatments account for the largest percentage of the direct costs (Grudzinski et al, 2000).

Unfortunately, the current FDA approved MS medications Avonex®, Betaseron®, Copaxone®, and Novantrone® are ineffective in alleviating or lessening the symptoms associated with MS. These medications have only shown about 30% effectiveness in decreasing the frequency of exacerbations (Physicians' Desk Reference, 2001). A slow progression in disability associated with MS usually continues despite the continued use of these disease-modifying treatments. The normal course of the disease without drug intervention is similar to the course of the disease with the intervention of these FDA approved MS medications. In 1996, a definition of disease categories was adopted to help describe typical stages of disease progression (National Multiple Sclerosis Society website, [www.nationalmssociety.org/brochures-just%20the.asp](http://www.nationalmssociety.org/brochures-just%20the.asp), March 2002). The four disease categories are:

- **Relapsing-Remitting:** characterized by clearly defined relapses or episodes of acute worsening of neurologic function followed by partial or complete recovery periods. 50% of these cases develop the Secondary-Progressive form of the disease within 10 years of the initial diagnosis
- **Primary-Progressive:** characterized by a nearly continuous worsening of symptoms with no apparent remissions or relapses.
- **Secondary-Progressive:** characterized by an initial period of relapsing-remitting that develops into a steady worsening of the symptoms.
- **Progressive-Relapsing:** characterized by a steadily worsening of symptoms from the onset, but also having apparent flare-ups (relapses).

Note that at least 50% of cases of the Relapsing-Remitting disease category develop Secondary-Progressive type MS within 10 years of the initial diagnosis of MS. Thus, the normal course of the disease without any drug intervention is a steady worsening of symptoms with less apparent exacerbations (flare-ups) and remissions as the length of duration of the disease increases. This is the same course of the disease as claimed with the intervention of the FDA approved disease-modifying treatments Avonex®, Betaseron®, and Copaxone®. These medications are described in their manufacturers' product information and the Physicians' Desk Reference 2001 as "(Avonex®) indicated for the treatment of relapsing forms of multiple sclerosis to slow the accumulation of physical disability and decrease the frequency of clinical exacerbations"; "(Betaseron®) indicated for use in ambulatory patients with relapsing-remitting multiple sclerosis to reduce the frequency of clinical exacerbations"; "(Copaxone®) indicated for reduction of the frequency of relapses in patients with Relapsing-Remitting Multiple Sclerosis". The study by Tolley and Whynes in 1997, addresses this question of proven efficacy of the interferon-beta therapies (Avonex® and Betaseron®) and the lack of cost-effectiveness data. The question is, if the normal course of the disease, without drug intervention with one of the FDA approved medications, is a decrease in apparent relapses (exacerbations) that develops into a steady worsening of symptoms, then how can it be determined that

drug intervention with any of the FDA approved disease-modifying medications had any positive cost effective impact on the outcome of the disease?

The slow steady worsening of symptoms in MS is directly associated with an increase in co-morbidities. The research study conducted by Grudzinski et al (2000) found that the principal determinants of cost were the number of exacerbations, co-morbidities, and the claims for the disease-modifying medications. This study found that the average cost of a disease exacerbation was directly proportionate to the number of co-morbidities present in the patient. The Expanded Disability Status Scale (EDSS) measures the progression of the disease by assessing the worsening or increase in the number of symptoms present in the MS patient. A progression of the disease is calculated in 0.5 increments ranging from 0-10, where 0=symptom free and 10=death. A worsening or increase in symptoms is usually accompanied by an increase in the number of co-morbidities present (Goodin, 1999). Furthermore, the study by Goodin found that fatigue was strongly correlated with disability and accounted for 65% of the disability experienced by the patients. The high-cost FDA approved disease-modifying treatments profoundly contribute to the direct costs related to MS, but yet as discussed previously have only a 30% rate of efficacy in reducing the frequency of exacerbations but do not alleviate or lessen the symptoms. Furthermore, this reduction in frequency may have occurred naturally in the course of the disease without the high-cost of these treatments (Tolley and Whynes, 1997).

The medical visits, hospitalizations, prescription medications other than the disease-modifying treatments, and assistance with Activities of Daily Living (ADL's) through formal and informal care are increased with a worsening or increase in symptoms and complications resulting from these symptoms such as, pneumonia, urinary tract infections (UTI), pressure ulcers wounds, etc. The study by Whetten et al in 1998, reported that in 1994 the annual cost of MS averaged \$34,000 per person, with a total lifetime cost of \$2.2 million per person. The national annual cost of MS in 1994 was \$6.8 billion (Whetten et al, 1998). The National Multiple Sclerosis Society reports that the national annual cost of MS today is in excess of \$10 billion ([www.nationalmssociety.org/research-factsheet.asp](http://www.nationalmssociety.org/research-factsheet.asp)).

Thus, the steadily increasing prevalence of MS, the earlier age of onset of the disease, the high-cost of the FDA approved disease-modifying treatments, the ineffectiveness of these high-cost treatments in alleviating or lessening the MS symptoms, and the increase in the co-morbidities associated with the progression of symptoms in MS greatly impact the cost/benefit ratio. It is vital for the healthcare industry, insurance industry, the patient and his or her family, and society that something is done to improve the cost/benefit ratio. The following is the discussion of the proposed therapy, Prokarin™, which may prove to lessen the direct and indirect costs of MS.

## Prokarin™ Drug Summary Sheet

EDMS, LLC has the licensed patent rights for the method of treatment of U.S Patent 6,277,402. Prokarin™ is the trademark for this method of treatment. Prokarin™ is a proprietary “off-label” compounded prescription medication containing an H2 agonist and a phosphodiesterase inhibitor. The H2 agonist used in Prokarin™ is histamine phosphate and the phosphodiesterase inhibitor is caffeine citrate.

Histamine phosphate, also known as histamine diphosphate, is FDA approved for use as a diagnostic aid for gastric acid function. Histamine phosphate was recognized as an effective “off-label” treatment for neurological disorders, such as, Ménière’s syndrome, multiple sclerosis, and Bell’s Palsy in the United States Dispensatory and Physicians’ Pharmacology 26<sup>th</sup> Edition.

Caffeine citrate is FDA approved as a central nervous system stimulant. Caffeine citrate is a common component in many medications and because of its wide therapeutic index, it is the phosphodiesterase inhibitor of choice used in Prokarin™.

Name	Dose	IND Number	Phase of Testing
Histamine Phosphate	1.65mg / 0.2ml cream b.i.d.	000734	FDA approved prior to 1/1/1982
Caffeine Citrate	100.0mg / 0.2ml cream b.i.d.	020793	FDA approved 9/21/99

**Dosage and Usage:** Prokarin™ is compound containing two active ingredients: 1.65mg of histamine phosphate and 100.0mg of caffeine citrate per 0.2ml of transdermal cream administered via a transdermal patch, applied to the skin for two 8-hour intervals for a total of 16 consecutive hours daily.

More recently Prokarin™ is available in a transdermal disc form called the Prokarin™ Disc. The disc too is a compound containing the two active ingredients: 1.65 mg of histamine phosphate and 1.0 mg of caffeine per disc. One disc is applied to the skin on the torso of the body and covered with an air occlusive adhesive patch. The Prokarin™ Disc is applied in the morning and removed at bedtime daily. The Prokarin™ Disc is stable at room temperature and is easier to apply than the Prokarin™ transdermal cream.

**Potential Adverse Reactions:** Histamine phosphate with average or large doses may cause flushing, headache, local or generalized allergic manifestations, dizziness, hypotension, and abdominal cramps. Small doses may cause bronchial asthma in patients with bronchial disease (Merck Index 7<sup>th</sup> Edition, 1960). Facts and Comparisons, 1988, also lists average or large doses may cause dyspnea, visual disturbances, urticaria, marked hypertension, palpitation, tachycardia, nervousness, diarrhea, vomiting, metallic taste, collapse with convulsions, anginal pain, cyanosis of the face, and peptic ulcer. Histamine phosphate is readily absorbed and metabolized following parenteral administration, thus the possibility of frequent doses causing toxicity by accumulation of histamine phosphate in the tissues and blood is very unlikely. Histamine phosphate is contraindicated in patients with a history of severe allergic reactions, bronchial asthma,

and severe hypertension. Possible adverse reactions of caffeine are insomnia, restlessness, nervousness, headache, excitement, agitation, muscle tremor, twitching, tachycardia, palpitations, extrasystoles, nausea, vomiting, diarrhea, stomach pain, and diuresis.

### **Literature Review of Scientific Rationale for the Proposed Use of Prokarin™ in MS**

Histamine phosphate acts as a histamine<sub>2</sub> agonist (H<sub>2</sub>) when compounded and maintained in a specific pH, protected from hydrolysis and oxidation. H<sub>2</sub> is a potent neurotransmitter and neuromodulator in the central nervous system (Nowak, 1994). H<sub>2</sub> receptor sites are located in the central nervous system (CNS), the hepatic oxidase system, peripheral lymphocytes, and the parietal cells in the intestinal lining (Baer & Williams, 1992).

Research shows that MS patients have an impaired histamine metabolism that results in the inadequate production of the H<sub>2</sub> agonist (Tuomisto et al, 1983). This results in deficient H<sub>2</sub> receptor stimulation throughout the CNS, the hepatic system, the immune system, the gastric/digestive system, and the endocrine system. Deficient H<sub>2</sub> receptor stimulation in the CNS results in atrophy of the pineal gland. Atrophy and calcification of the pineal gland has been found in MS patients studied during an exacerbation or chronic progression of the disease (Sandyk & Awerbuch, 1991). The pineal gland produces melatonin and cyclic AMP. Melatonin is essential in fatty acid metabolism. The pineal gland is the only region of the brain capable of metabolizing polyunsaturated fatty acids via lipoxygenation, which does not produce toxic lipid peroxides. All other regions of the brain are only capable of metabolizing polyunsaturated fats by lipid peroxidation, which is toxic to the myelin and nerve cell membranes (Kim et al, 1999; Sawazaki et al, 1994). Furthermore, the metabolism of histamine in the brain is inhibited by lipid peroxidation up to 60% (Rafalowska & Walajtys-Rode, 1991). Research shows that MS patients have a high level of polyunsaturated fatty acids in the CNS and depleted levels of antioxidants (Syburra & Passi, PubMed). The reactive oxidative stress from the lipid peroxidation depletes the antioxidants and contributes to the myelin destruction. The myelin and nerve cell membranes are very vulnerable to the cytotoxic effects of lipid peroxidation (Smith et al, 1999; Mazierre et al, 1999; Berry et al, 1991; de Kok et al, 1994; Fang et al, 1996; Fernstrom 1999). This increased oxidative stress and depletion of antioxidants may account for the high incidence of hypercholesteremia in MS patients (Sandyk & Awerbuch, 1994). The body will increase the production of cholesterol with stress and inadequate levels of antioxidants, because cholesterol can act as an antioxidant for the body.

Melatonin is also involved in the circadian rhythm. Melatonin regulates the activity of serotonin neurons in the brainstem. Inhibition of melatonin results in the cease firing of the serotonergic neurons during REM (rapid eye movement) sleep which results in sleep atonia associated with REM sleep. MS patients experience cataplexy, which is physiologically and pharmacologically similar to sleep atonia during REM sleep (Sandyk, 1995). Sleep disturbance is a common symptom in MS patients and research shows that MS patients often fail to go into the REM stage of sleep. Low levels of melatonin result in an inadequate swing from a high level to a low level of melatonin, which is necessary for the initiation of REM sleep (Sandyk, 1995).

Melatonin is also necessary for the absorption of zinc from the intestinal tract. A research study by Palm & Hallmans (1982) found that MS patients had lower serum zinc levels compared to age and sex matched controls. Low levels of zinc debilitate the CuZn superoxide dimutase enzyme and this results in the increase in production of lipid peroxides (Johnson, 2000). Furthermore, the demyelinated pathological areas in the CNS of MS patients showed a decreased zinc level (Yasui et al, October 1991). A study by Smith et al (1989, July) showed that there is altered copper and zinc homeostasis in MS patients. The RBC copper concentration was significantly lower in MS patients after receiving steroid therapy. This copper deficiency may correlate with the high levels of cortisol noted with the hyperactivity of the HPA axis in MS patients that increases with disease progression (Then Bergh et al, September 1999; Michelson et al, September 1994).

Exogenous histamine greatly increases endogenous cyclic AMP production and moderately increases melatonin secretion. The CNS has H<sub>2</sub> receptors that when stimulated increase cyclic AMP production as evidenced by the Nowak and Sek (1994) study that showed histamine to be a powerful stimulator of cyclic AMP production in the chick pineal gland. Cyclic AMP is produced throughout the CNS as well as by the pineal gland. Cyclic AMP stimulates the synthesis of myelin components by oligodendrocytes and Schwann cells (Anderson & Miskimins, 1994; Lyons, Morell, & McCarthy, 1994). The sclerotic lesions of the myelin sheath are found exclusively in the CNS in MS patients and not in the peripheral nervous system (PNS). This phenomenon may be explained by the fact that studies have shown that oligodendrocytes, the myelin producing cells of the CNS, will undergo self-induced degeneration in the absence of cyclic AMP. These degenerating cells will again become viable myelin producing cells if treated with cyclic AMP. These same studies show that the Schwann cells, the myelin producing cells of the PNS, do not undergo self-degeneration in the absence of cyclic AMP, but rather become dormant (Nowak & Sek, 1994). This self-induced degeneration of the oligodendrocytes may explain the presence of macrophages around the myelin lesion sites. (Macrophages are summoned to the site of tissue destruction to clean up the debris.)

Cyclic AMP is involved in the function of all cells not just the myelin producing cells. It is the second messenger for cells, carrying the message from the first messenger receptors located on the surface of the cell membrane to the mitochondria, mRNA, and mDNA (Cecil Textbook of Medicine, 2000). Research shows that a deficiency in cyclic AMP results in a desensitization of the first messenger receptors being, steroid hormone receptors, vitamin D receptors, and peptide hormone receptors (Waki et al, 2001). Research shows that these cell surface receptors are important in modulating and execution of cell death particularly in the nervous system (Deigner et al, 2000). Thus, a deficiency of cyclic AMP may potentially hinder the ability of these cell surface receptors in modulating apoptosis. The desensitization of the surface cell receptors due to a deficiency of cyclic AMP may also explain why increases in the progesterone level such as in pregnancy and exogenous glucocorticoids have shown benefit in lessening symptoms of MS.

The effect of H<sub>2</sub> to stimulate the increase in the production of cyclic AMP is enhanced by the presence of a phosphodiesterase inhibitor (Nowak & Sek, 1994). Methylxanthine agents, such as theophylline, theophylline derivatives, and caffeine inhibit phosphodiesterase, the enzyme that breaks down cyclic AMP. Caffeine is the medication



of choice because it has a longer half-life, less untoward side effects, and a wider therapeutic index (Baer & Williams, 1992).

Cyclic AMP is also produced from ATP with the catalyst adenylate cyclase (Wescott et al, 1979; Wyngaarden et al, 1992). Perhaps an abnormally low level of cyclic AMP in MS patients secondary to the lack of H<sub>2</sub> receptor stimulation results in the energy molecule, ATP, to be catabolized to produce cyclic AMP resulting in the disabling fatigue associated with MS. As mentioned previously, fatigue accounts for 65% of the disability in MS patients.

Histamine is involved in the Na<sup>+</sup> - K<sup>+</sup> pump and action potential for nerve conduction. Histamine can directly stimulate the activity of the Na<sup>+</sup> - K<sup>+</sup> pump that changes the axon membrane ion gradient resulting in nerve impulse conduction. Histamine increases the amplitude of the action potential (Yang et al, 1993). The histamine at the postsynaptic cleft enters the neuronal reuptake system to be retransported into storage vesicles or deaminated (Cecil Textbook of Medicine, 2000). This may explain why it is common that MS patients can perform an activity for a few repetitions and then can't, but then after a brief period of rest, they can perform the activity again.

Histamine via the H<sub>2</sub> receptors modulates many other neurotransmitters such as serotonin and dopamine (Nowak, 1994). H<sub>2</sub> either alone or in combination with serotonin and cyclic AMP maintains the integrity of the blood-brain-barrier (Sharma et al, 1992). Interestingly, recent research has revealed that the integrity of the blood-brain-barrier is impaired in MS patients (Huber et al, 2001).

Histamine is a major heat and stress regulator for the body. H<sub>2</sub> receptors are desensitized with an increase in the core body temperature (Fernandez et al, 1994). Normally this desensitization of the H<sub>2</sub> receptors with heat stress stimulates increased production of the H<sub>2</sub> agonist. The resultant increase in the level of H<sub>2</sub> stimulates the pineal gland to secrete melatonin, which causes the body to sweat and lower the core temperature. The increased H<sub>2</sub> receptor stimulation also dilates the small diameter peripheral arteries, thus allowing a person to perspire (Fernandez et al, 1994). H<sub>2</sub> receptor stimulation increases the brain water content that in turn cools the brain during heat stress and prevents dehydration of the brain (Sharma et al, 1991). Thus, the deficiency of H<sub>2</sub> receptor stimulation in MS explains why heat is a classic stressor shown to worsen symptoms and why it is very common that MS patients have decreased sweating as the disease progresses. Also decreased H<sub>2</sub> receptor stimulation results in constriction of the small diameter peripheral arteries, which may explain the cause of the cold hands and feet in MS patients, the peripheral non-pitting edema, poor skin color, and dry skin. The small diameter arterial constriction may also explain the common occurrence of optic neuritis possibly caused by ischemia-induced inflammation and swelling around the optic nerve.

Histamine via the H<sub>2</sub> receptors also modulates stress. The production of histamine is increased with stress (Ghi et al, 1992). Histamine stimulates the increase of serum corticosterone levels, especially adrenocorticotropin hormone (ACTH) following mild stress (Ghi et al, 1992). The increase in cortisol increases the activity of enzyme; MAO-A 1.5-2.5 fold by progesterone, hydrocortisone, and dexamethasone (Youdim et al, 1989) but this increase is time-dependent as shown in the study by (Edelstein & Breakefield, 1986). MAO-A is involved in the metabolic pathway of histamine in the neurons (Ganong, 1973). Perhaps there is a correlation between these findings and the fact that episodes of relapses in MS patients is often precipitated by stress, such as pregnancy,

infection, emotional stress, or physical injury (Ozuna, 1992). This may also explain why steroid IV treatments have shown some immediate relief in symptoms associated with acute exacerbations of MS, but this beneficial effect doesn't last or necessarily reduce the long term neurological deficits of MS (Ozuna, 1992; Kelley & Smeltzer, 1994). Stress stimulates the endogenous inhibition of MAO-A and inhibition of MAO-A stimulates the activity of the hypothalamus-pituitary-adrenal (HPA) axis, which results in increased cortisol (Clow et al, 2000). The cortisol then increases activity of the MAO-A (Youdim et al, 1989). The increased activity of the MAO-A then decreases the stimulation of the (HPA) axis and balance is achieved. In MS this regulation of the HPA axis is impaired resulting in hyperactivity of the HPA axis (Michelson et al, 1994 September) explaining the high level of cortisol in MS patients. Possibly the inhibition of the MAO-A is too great due to the presence of other factors that inhibit the MAO-A such as lipid peroxidation, low copper levels, high estrogen levels, and stress causing the stimulatory effect of increased cortisol on the MAO-A activity to be inadequate to overcome the inhibitory effect of all the other factors present on the MAO-A activity. The hyperactivity of the HPA axis noted in MS contradicts an inflammatory or autoimmune mediated etiology for MS. This was demonstrated in the LEW/N rat model, where a decreased HPA axis response to inflammatory and immune mediators resulted in the development of experimental allergic encephalomyelitis (the animal model of MS) (Michelson et al, 1994 September).

H2 receptor regulation maintains the balance of the Th1 and Th2 of the immune cytokines, thus it is integral in the regulation of the immune system particularly in the regulation of the T and B cells (Gillson et al, 2000). Beta-adrenergic receptor density on lymphocytes is inversely proportionate to the availability of histamine. Studies show that an increase in histamine results in a decrease in the density of beta-adrenergic receptors on lymphocytes (Galant & Britt, 1984; Mita, Yui, & Shida, 1983). The significance of these findings to MS is that beta-adrenergic receptor density is two to three times greater than normal values in patients with progressive MS or in an exacerbation. The beta-adrenergic receptor density was within normal values in MS patients who were in remission (Karaszewski et al, 1990; Zoukos et al, 1992). Yarosh & Kanevskaya (1992) also established a high level of blood histamine in those MS patients whose disease length was less than five years, and a low level of blood histamine in those whose disease length was greater than five years. Curiously, in the majority of MS cases the onset of the disease is characterized by attacks and remissions during the first five to ten years. Generally after ten to twenty years, some degree of chronic disability is present (Bjork, 1978). Furthermore, a study by Dziuba, Frolov, & Peresadin (1993) indicated that during an exacerbation of MS, patients had marked T-lymphopenia. This contradicts the autoimmune theory that the T-cells are attacking the myelin, which is as yet not a proven hypothesis (National Multiple Sclerosis Society website, 2002).

H2 receptor sites are also located in the hepatic oxidase system and the parietal cells of the gastric mucosa. Histamine at the H2 receptors in the gastric system stimulates the secretion of hydrochloric acid and intrinsic factor. Thus, the stimulation of the H2 receptors is necessary for the absorption of vitamin B12 from the intestinal tract (Baer & Williams, 1992). Numerous studies cited that macrocytosis is common in patients with MS (Crellin, Bottiglieri, & Reynolds, 1990; Reynolds et al, 1992; Goodkin et al, 1994). The binding of histamine to H2 receptors in the intestinal lining also stimulates the secretion of gastrin and pepsin (Baer & Williams, 1992). Thus, H2 is directly involved with the digestion of protein, fats, and carbohydrates. A study by Gupta et al (1977) revealed microscopic fat in 41.6 % of MS patients whose stools had been randomly

screened using Sudan III stain. Also, 40.9% of the MS subjects showed undigested meat fibers in the stools. This study also identified the presence of a measles viral antigen in the nuclei of the epithelial cells in all of the jejunal biopsies performed in 40 MS patients. These findings by Gupta et al (1977) were supported by Yarosh & Kanevskaya (1992) study in which histological abnormalities were identified in all the gastric mucosa biopsies of 32 MS patients.

## **Summary**

Histamine via the H2 receptor stimulation is involved in numerous cellular functions such as:

- The production and maintenance of the myelin.
- Nerve impulse conduction.
- Thermal regulation.
- Stress modulation.
- Cyclic AMP production.
- Immune system regulation.
- Hepatic oxidase system.
- Gastric acid and digestive enzyme production.
- Fatty acid metabolism.
- Small diameter artery vasodilatation.
- Maintenance of the integrity of the blood-brain-barrier

The symptoms associated with MS are manifested as a result of impairment in these cellular functions. These facts and findings are the scientific rationale for the use of Prokarin™ as an “off-label” prescription medication in MS. The results of the double blind study of the effect of Prokarin™ on fatigue in MS patients published in the Multiple Sclerosis Journal Volume 8, Issue 1 supports this scientific rationale.

## **Preliminary Results Studying the Effects of Prokarin™ in MS**

**Feasibility Study Using Prokarin™ in MS:** Daniel Nehls, MD conducted a 90-day feasibility study of 10 MS patients having a baseline score of 5.0-7.5 on the Expanded Disability Status Scale. Eighty percent of the patients were female. The patients were between 38-75 years of age and had 4-35 years of disease length since diagnosis. All patients' diagnosis of MS had been confirmed via a MRI in their past history.

The outcome measures were the MS-Related Systems tool, Fatigue Severity Status Scale, Kurtzke Functional Systems, and the Expanded Disability Status Scale. These outcome measures were tested prior to the initiation of Prokarin™, 45 days after initiation of Prokarin™, and 90 days after initiation of Prokarin™.

Seven of the ten patients (1 male, 6 females) reported improvement in some or all of the following areas: bladder function, balance, coordination, speech, strength in the extremities, ambulation, cognition, and fatigue. These improvements were reported at the 45-day testing point and continued through the 90-day testing date. None of the study patients reported any adverse side effects. Refer to the Feasibility Study Summary Spreadsheet.

Pt. #	MS-RS Tool			FSS Tool			FS Tool			EDSS			Qualitative
	B	45D	90D	B	45D	90D	B	45D	90D	B	45D	90D	
4344	51	55.5	44	57	56	55	11	11	11	5.0	5.0	5.0	No Change
4337	67	42	19	47	31	20	12	12	5	6.0	5.5	5.0	Improved ambulation No longer using cane Increased left side strength
4366	31	7	28	45	36	33	8	8	7	6.0	6.0	6.0	Increased energy Improved ambulation Improved bladder function Reports overall improved
4339	23	33	31	46	54	48	12	12	12	6.0	6.0	6.0	No Change
4336	47	30	27	50	33	Inc	19	16	16	6.0	5.0	5.0	Increased energy Improved ambulation No longer using cane Increased sense of well being
4338	58	50	56	29	25	41	17	17	17	6.5	6.5	6.5	Reports overall improved Improved vision Improved writing function Improved bladder function Improved balance Improved sexual function
4341	34	28	31	33	38	45	6	6	6	6.0	6.0	6.0	Increased energy Improved sleeping at night
4424	63	53	Inc	56	63	Inc	9	9	Inc	7.5	7.5	Inc	Improved bladder function
4340	56	39	41	49	26	28	17	16	16	7.0	7.0	7.0	Decrease in ataxia Improved speech, first time able to sing in 18 years. Increased energy Improved right arm function
4550	46	33	Inc	54	37	Inc	7	7	Inc	6.5	6.0	6.0	Increased energy Improved speech Improved thought process Improved sensory function
	most disabled score	least disabled score		most disabled score	least disabled score		most disabled score	least disabled score		most disabled score	least disabled score		
	110	0		63	9		40	0		10.0	0		

Key: B = Baseline Score  
 45 D = 45 Days on Procarin  
 90 D = 90 Days on Procarin

**A double blind placebo controlled study was published in the Multiple Sclerosis Journal Vol. 8, Issue 1: (See the next 7 pages for the published study.)**

# A double-blind pilot study of the effect of Prokarin™ on fatigue multiple sclerosis

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*In this 12-week study with 29 subjects, the effect of Prokarin™ (n=22), a proprietary blend of histamine and caffeine, was compared to*

*placebo group (n=7) for the following outcomes: 1) fatigue as measured by the Modified Fatigue Impact Scale (MFIS); 2) lower limb function as measured by timed walk test; 3) upper limb function as measured by the pegboard test; 4) cognitive function as measured by the Paced Auditory Serial Additions Test (PASAT); 5) serum caffeine level; 6) change in brain chemistry as measured by quantitative magnetic resonance spectroscopy assay of N-acetyl aspartate (NAA); and 7) safety as measured by routine blood chemistry, TSH and urinalysis. Data were acquired at baseline, 4, 8 and 12 weeks. The Prokarin™ group MFIS mean was significantly different from the mean of the placebo group at 12 weeks (df=24, t=2.08, P=<0.02), with respective means of 37.40, SD=15.18, for the Prokarin™ group and 53.2, SD=11.39 for the controls. For the secondary endpoints (PASAT, 25 foot timed walk, peg test, and magnetic resonance spectroscopy [MRS]), there were no significant differences between the Prokarin™-treated group and the placebo group.*

*However, there were significant improvements within the Prokarin™ group for each of these measures for the pre- versus posttreatment comparison at 12 weeks. Serum caffeine data indicated that caffeine exerted no independent effect on performance. No laboratory abnormalities were seen, and the treatment was well tolerated.*

*Conclusion: There was a modest-size statistical effect of Prokarin™ on fatigue in multiple sclerosis (MS) compared with the placebo group. A larger trial is warranted, based on this pilot study.*

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**Key words:** *clinical trial; fatigue; histamine; multiple sclerosis*

## Introduction

Prokarin™ is a proprietary histamine and caffeine-containing transdermal cream that has been under evaluation for multiple sclerosis (MS) symptom relief over the past 4 years. Uncontrolled studies have found that Prokarin™ appears to have a significant impact on many MS symptoms, including fatigue.<sup>1,2</sup> This paper outlines the results of the first placebo-controlled study of Prokarin™ for symptom relief in MS. Histamine is an important neurotransmitter; its many functions include promotion of mental alertness, temperature regulation, and involvement in brainstem vestibular pathways. We hypothesize that in MS there may be a histamine deficit in the CNS, which may be ameliorated by Prokarin™. A more detailed discussion of this hypothesis has also been presented previously.<sup>1</sup> The use of histamine as a therapeutic agent actually dates back to the 1920s. For example, in 1944, a physician at the Mayo Clinic wrote about his 17 years of clinical use of histamine in a wide variety of settings including MS, Bell's palsy, vasculitis and Meniere's disease.<sup>3,4</sup> Hinton Jonez, MD, also made extensive use of histamine in the late 1940s and early 1950s.<sup>5</sup> Histamine therapy continues to be employed by otolaryngologists for headache and vestibular disturbances.<sup>6</sup>

### Primary outcome measure: fatigue

Fatigue is the most common symptom of MS, being present in 75–95% of cases.<sup>7</sup> It can have an overwhelming impact on the lives of patients with MS and is a major contributor to interpersonal and societal losses suffered from the disease.<sup>8</sup> Because of the tremendous impact fatigue has on the lives of MS patients, their families and society, we chose it as an important symptom to study. The cause of fatigue in MS is unknown, although various mechanisms have been proposed, including intracortical conduction blocks<sup>9</sup> and decreased availability of ATP.<sup>10</sup> The most common definition of fatigue in MS is 'a subjective lack of physical and/or mental energy that is perceived by the individual or caregiver to interfere with usual and desired activities'.<sup>11</sup> This is the type of fatigue addressed in this study.

Various classes of prescription medications are currently used off-label for the treatment of fatigue in MS, including antidepressants, as well as drugs used in the treatment of narcolepsy and attention deficit disorder. Drawbacks to these approaches include side effects and lack of efficacy. Hence, not only is fatigue a common, significant symptom in MS, it is not well treated in our experience.

The primary intent of this study then, was to evaluate the effect of Prokarin™ on fatigue as measured by a standardized questionnaire, the Modified Fatigue Impact Scale (MFIS),<sup>12,13</sup> which measures the impact of fatigue on social, cognitive and physical aspects of daily life. The potential of Prokarin™ to adversely affect metabolic parameters was studied with serial routine laboratory work and urinalysis.

A second phase of this study, in which placebo recipients were crossed over to a 12-week course of Prokarin™, is underway. The object of the second phase is to examine the effect of Prokarin™ on digestive and endocrine parameters, as well as other nutritional biochemical parameters.

*Secondary outcome measures: MS functional composite and cerebral N-acetyl aspartate levels*

Anecdotal experience has indicated that Prokarin™ may also improve motor and cognitive function, hence clinical tests of motor and cognitive function as per the Multiple Sclerosis Functional Composite (MSFC) were chosen as secondary outcome measures. The MSFC is recognized by the National MS Society's Clinical Outcomes Assessment Task Force as a reliable quantitative outcome measure for clinical trials in MS.<sup>12,13</sup>

Proton magnetic resonance spectroscopy (MRS) of the brain has been proposed as a means for monitoring disease activity in MS.<sup>14,15</sup> N-acetyl aspartate (NAA) is one of the cerebral metabolites that can be measured with proton MRS and the reduction in NAA reported in MS subjects by the majority of the authors cited above has been interpreted as a sign of neuronal damage or loss. In this study, NAA levels were measured before and after 12 weeks of Prokarin™ treatment to look for possible effects on underlying neuronal pathology.

## **Methods**

This was a single-center, double-blind, placebo-controlled study of two parallel treatment groups of outpatients with both relapsing–remitting and progressive MS. The study protocol was approved both by the Bastyr University Scientific Review Board, Seattle, Washington, as well as the Western Institutional Review Board, Olympia, Washington. The MRS scanning protocol was approved by the University of Washington Institutional Review Board. Informed consent was obtained. Treatment period was 12 weeks. Subjects were assessed four times: at baseline, 4 weeks, 8 weeks and 12 weeks. Compliance with treatments was monitored by means of patient diaries and periodic telephone follow-ups between assessments. All personnel involved with the study (assessors, subjects and clinical supervisor) were unaware of the treatment allocation in the randomization until after the final assessment. The team performing the clinical assessments was trained and evaluated as per the MSFC handbook. The final statistical analysis was done by a consultant who was unaware of the randomization. The randomization code was broken for patients on the last day of the trial in order to afford them the immediate option of continuing Prokarin™.

Since there proved to be no adverse effects of Prokarin™ relative to placebo, there was no way for participants or examiners to deduce which treatment was being given in any particular case. Also, written comments provided by patients on the last day of the trial, prior to breaking the code, indicated that the concealment of allocation was robust: neither placebo, nor Prokarin™ recipients could predict which treatment was being administered.

### *Patient selection and clinical details*

Potential subjects were recruited via advertisements and flyers. Initial screening was by telephone interview, using a standardized questionnaire. The main inclusion and exclusion criteria are listed in Table 1.

Individuals meeting these criteria underwent an intake assessment consisting of a history, physical exam, routine blood work (CBC, SMAC, TSH) and urinalysis. The same laboratory workup was also done prior to the 12-week assessment. Subjects were then randomized independently to Prokarin™/placebo and to MRS/no MRS. (Subjects randomized to the MRS assessment underwent their first scan prior to the baseline assessment.)

Histamine has been shown to lower serum calcium in animals;<sup>16</sup> hypocalcemia may cause tetany. Previous experience with Prokarin™ indicated that supplementation with calcium is necessary to avoid increased stiffness. Therefore, participants were provided with a 4-month supply of a calcium/magnesium supplement as well as a multivitamin/ multimineral supplement. The multivitamin/mineral supplement was provided to ensure homogeneity, and to rule out potential differences introduced by different nutritional supplementation regimens.

Subjects were advised to continue prescription medications, initiate study dietary supplements and discontinue any other supplements, restrict intake of caffeinated beverages to the equivalent of one cup of regular coffee per day, and consume a diet moderate in saturated fat intake (for those patients on a low-fat diet).

On each study assessment day, blood was drawn for serum caffeine level. On the baseline assessment day, subjects were again instructed on patching technique (they had been given an instructional video to view), and allowed to practice with an inert [nonplacebo] cream until competency at patching was demonstrated. Subjects then applied their first patch and were observed for 30 min before leaving.

**Table 1** Major inclusion and exclusion criteria

*Inclusions*

18 years of age or older

Expanded Disability Status Scale (EDSS) score of 5.0–6.5

Diagnosis of MS confirmed by neurologist exam and the presence of

CNS sclerotic lesions on MRI

Baseline MFIS score greater than 40

Stable clinical course (no relapse in preceding 3 months)

*Exclusions*

Current or previous use of Prokarin™

Current use of antispasmodic agents, corticosteroids, chemotherapeutic

agents, monoamine oxidase inhibitors, histamine (H1 or

H2) blockers

Patients starting antidepressants, interferons, or glatiramer acetate

within the past 3 months

Serious renal, hepatic, endocrine, cardiac or pulmonary disease

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Subjects were provided with a daily diary in which they were to note times of patch application and other details, and were instructed regarding adverse events. They were given a 30-day supply of Prokarin™, a proprietary mixture of 1.65 mg histamine diphosphate and 100 mg of caffeine citrate per 0.2 mL, or an indistinguishable placebo containing only citric acid. The treatments were provided in unit dose syringes containing 0.2 mL of cream. Two consecutive patches, worn for 8 h each, were applied each day, the first patch being applied around 7:00 a.m. if possible. Within 72 h of study inception, all participants were visited at home by an RN, and assessed for vital signs, patching technique, and refrigerator temperature. Subjects were contacted in the week prior to each subsequent assessment day for the purpose of verifying compliance and intent to attend the next assessment.

*Study endpoints*

The primary endpoint was fatigue, as assessed by the MFIS, a standardized 21-item subset of the Fatigue Impact Scale.<sup>12</sup>

All patients had a fatigue score greater than 40 at inception (possible score ranged from 0 to 84) with a higher score indicating a more severe impact of fatigue on daily life. The components of the MSFC (secondary outcome measures) included a timed 25-foot walk, a timed pegboard test (placing and removing nine pegs from a board in under 3 min) and the Paced Auditory Serial Additions Test (PASAT), which involved performing serial additions on a series of 60 numbers presented every 3 s by audiotape. Subjects filled out the MFIS questionnaire and completed the three MSFC assessments at baseline, and at 4, 8 and 12 weeks.

*MRI and MRS*

Magnetic resonance imaging (MRI) and MRS were performed on a 1.5-T Signa scanner using version 5.8 G.E. software. Multislice magnetic resonance images were acquired in the sagittal plane (TR/TE 500/16 milliseconds) and the axial plane (fast spin-echo, TR/TE 2000/100; T1-weighted, TR/TE 500/16, and fluid attenuation inversion recovery (FLAIR), TR/TE/TI 10000/130/2200). Magnetic resonance spectra were acquired using the proton echoplanar spectroscopic imaging (PEPSI) pulse sequence developed by Posse *et al*<sup>17</sup> and were processed as described previously.<sup>15</sup>

*Statistical methods*

The smaller study size was based on a large anticipated effect on fatigue as seen in earlier clinical studies.<sup>1,2</sup> Power of 0.8 is achievable with a small study if the effect is large enough. The statistician remained unaware of both the meaning of the numbers and the desired direction of change, as well as to the randomization itself. The two groups were compared on *t* tests of the means of their MFIS scores going into the study and found to be equivalent ( $t=0.88$ ,  $df=26$ ,  $P<0.48$ ). The statistical analysis was based on the usual assumption of linearity of the data, in which case *F* tests, *r* tests or *t* tests would be appropriate. In the analysis section of the study it was decided to use *t* tests of mean differences, both within groups and between groups.

Drop-outs were initially handled by carrying the scores of the individuals who left the study after 4 weeks forward to 8 and 12 weeks. Note, however, that exclusion of the scores of these individuals in the 8- and 12-week calculations did not make any difference to the findings.

**Results**

A total of 29 patients were recruited from 378 screened. Twenty-two were randomly assigned to receive Prokarin™ and seven to receive placebo. Seven of 22 Prokarin™ recipients and three of seven placebo recipients were randomly assigned to the MRI component of the study. As shown in Table 2, treatment groups were comparable both in demographic characteristics (with the exception of disease classification) and baseline values of clinical parameters, indicating that the randomization was successful.

The main intent of the study was to determine the effect of Prokarin™ on fatigue in MS, without regard to disease classification (relapsing –remitting versus progressive). Since one of the entry criteria was an EDSS score in the range 5.0–6.5 for all subjects regardless of disease type, both disease classification



and time from diagnosis and were less relevant. From Table 2, it is evident that the composition of the groups differed with respect to disease classification. This possible source of bias will be analyzed in the Discussion section. From a statistical standpoint, there was no difference between the groups at baseline for any of the variables of interest.

**Table 2** Baseline characteristics of treatment groups

	<i>Prokarin</i> <sup>TM</sup> (n=22)	<i>Placebo</i> (n=7)
Age	47.7	46.4
Female sex	73%	57%
Coffee consumption ≥ 1 cup/day	77%	86%
Current antidepressant use	50%	43%
Current ABC drug use	54.5%	43%
MFIS score <sup>a</sup>	58.3	61.7
Walk time (s) <sup>b</sup>	12.2	8.6
Peg time (s) <sup>c</sup> (dominant hand)	29.0	28.5
Peg time (s) <sup>d</sup> (nondominant hand)	32.7	27.3
PASAT score <sup>e</sup>	37.8	37.9
Serum caffeine <sup>f</sup> (m g/mL)	1.9	2.5
Relapsing–remitting	9	1
Secondary progressive	11	5
Primary progressive	2	1

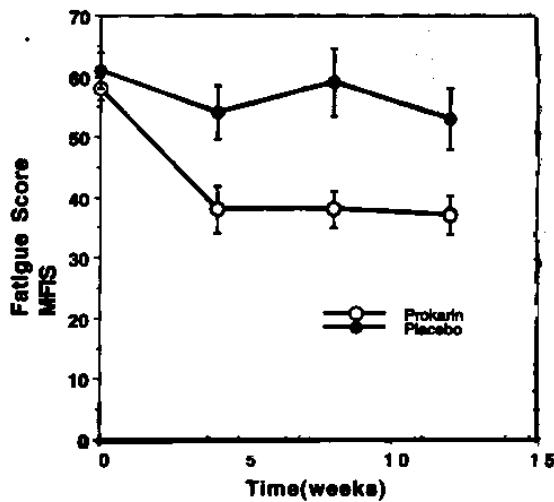
<sup>a</sup> $P < 0.37$  where  $P$  is the probability of rejecting the hypotheses that group means are equal. <sup>b</sup> $P < 0.52$  where  $P$  is the probability of rejecting the hypotheses that group means are equal. <sup>c</sup> $P < 0.91$  where  $P$  is the probability of rejecting the hypotheses that group means are equal. <sup>d</sup> $P < 0.44$  where  $P$  is the probability of rejecting the hypotheses that group means are equal. <sup>e</sup> $P < 1.0$  where  $P$  is the probability of rejecting the hypotheses that group means are equal. <sup>f</sup> $P < 0.46$  where  $P$  is the probability of rejecting the hypotheses that group means are equal.

One patient dropped out of the Prokarin<sup>TM</sup> group at the 8-week mark citing lack of effect and difficulty adhering to the patching schedule. Two subjects dropped out of the placebo group. One subject dropped out just after 4 weeks as she wanted to resume her original regimen of nutritional supplements. The other subject dropped out at 8 weeks citing lack of effect and difficulty adhering to the patching schedule. No adjustments to prescription medications were made during the study, for any of the participants.

#### Primary endpoint

There were 22 MFIS subjects in the Prokarin<sup>TM</sup> group and 7 subjects in the placebo controls. No difference was found between treatment and control groups at baseline, with the Prokarin<sup>TM</sup> group having a mean of 58.38, SD=8.90, and the placebo controls a mean of 61.13, SD=7.49 ( $df=28$ ,  $t=0.77$ ,  $P < 0.22$ ). After the 4-week retest, two of the controls and one Prokarin<sup>TM</sup> recipient dropped out of the study, leaving only 5 control data sets, and 21 Prokarin<sup>TM</sup> data sets for tests on weeks 8 and 12. Carrying the scores of the individuals who dropped out forward did not affect the results.

Prokarin<sup>TM</sup> or placebo group means at each assessment point were compared to their own baseline values (within group comparison). The Prokarin<sup>TM</sup>-treated group improved dramatically at 4 weeks ( $df=20$ ,  $t=5.75$ ,  $P < 0.000008$ ), and sustained that improvement throughout the study (4-week mean=38.49, SD=17.99, 8-week mean=38.30, SD=15.90, 12-week mean=37.40, SD= 15.18). The placebo group means did not differ from baseline group mean at any time during the 12-week period, although when individual percent changes [(final score-initial score)/initial score] × 100% for placebo recipients were calculated at 12 weeks compared to baseline, there was an average 15% improvement. The Prokarin<sup>TM</sup> group mean was significantly different from the mean of the placebo group at 12 weeks ( $df=24$ ,  $t= 2.08$ ,  $P < 0.02$ ), with respective means of 37.40, SD=15.18, for the Prokarin<sup>TM</sup> group and 53.2, SD=11.39 for the controls. Figure 1 presents group mean scores as a function of time for both treatment groups.



**Figure 1** The effect of prokarin treatment on fatigue in MS patients. Fatigue was measured using the MFIS. Error bars are standard error of the mean. Prokarin™ group, n=22. Placebo group, n=7 Secondary endpoints

**Table 3** Serum caffeine group means

	Baseline (mcg/mL)	4 weeks (mcg/mL)	8 weeks (mcg/mL)	12 weeks (mcg/mL)
Prokarin™	1.9	2.5	2.2	2.2
Placebo	2.5	2.4	2.8	5.2

There were no significant differences between the Prokarin™ group and the placebo group for any of the secondary endpoints (PASAT, 25-foot walking test, nine-hole peg test, MR spectroscopy). However, there were significant improvements within the Prokarin™ group for each of these measures at 12 weeks compared to baseline.

#### Caffeine

Group means for serum caffeine level are displayed in Table 3. There was no significant difference between group means at baseline, 4 and 8 weeks. At 12 weeks, there was a statistically significant difference between groups ( $P < 0.008$ ) with the level being higher in the placebo group.

#### Other laboratory parameters

No changes were noted in any of the routine lab parameters for the Prokarin™ group compared to placebo.

#### Safety

Prokarin™ was well tolerated. Subjects had been instructed on handling of mild, moderate or severe adverse events (mild adverse events: annoying but not interfering with routine activity or function, e.g., skin rash; moderate adverse events: uncomfortable, intense enough to interfere with routine activity, but carrying no permanent health consequences, e.g., diarrhea with abdominal cramps; severe adverse events: severely uncomfortable, precluding normal activity or function, hazardous to health, and likely requiring hospitalization). No moderate or severe reactions were noted. Reactions among Prokarin™ recipients were limited to headache and skin irritation; both were generally transient. One Prokarin™ and one placebo recipient experienced loose bowel movements and fecal urgency, which resolved after cessation of the calcium/magnesium supplement. Three of the placebo recipients reported significant transient itching that persisted throughout the study.

#### Continuation rate

Ten of the 22 Prokarin™ recipients (45%) elected to continue using Prokarin™. This continuation rate is in keeping with the experience of the principal author. Decision to continue correlated with a large improvement in MFIS score. Seventy-one per cent (10 of 14) of the Prokarin™ recipients with the largest improvements in MFIS score elected to continue the treatment. Conversely, no one whose score improved less than approximately 30% elected to continue the treatment. Interestingly, of the 10 subjects who elected to continue, only 1 was taking a serotonin reuptake inhibitor (SRI). Conversely, 64% (7 of 11) of patients who did not continue Prokarin™ were taking an SRI.

## Discussion

#### Primary endpoint: MFIS

The strong effect on fatigue demonstrated in this study agrees with the large body of anecdotal evidence supporting a role for Prokarin™ in the alleviation of fatigue. The treatment was seen to be well tolerated with

no adverse effects on routine blood parameters. Fatigue is often the first symptom to improve with Prokarin™ use, and the improvement is sometimes obvious within hours of instituting therapy. As discussed in a recent paper,<sup>1</sup> histamine may modify symptoms perceived as fatigue by affecting cerebral blood flow, and hence the onset of the effect might be expected to be rapid. In general, histamine is recognized as an animating, stimulating neurotransmitter.<sup>1</sup> The effect observed here could be consistent with an increased level of histaminergic neuronal activity.

Failure to account for the potential effects of affective disturbances and/or cognitive dysfunction is a potential issue in studies of fatigue in MS. Regardless of exactly what the MFIS questionnaire measures, or whether the MFIS score is partially reflective of depression or cognitive dysfunction, the baseline MFIS scores in the two treatment groups were the same. The rate of antidepressant usage in the two groups was similar, and no patient had started an antidepressant less than 3 months before the start of the trial, or during the trial. It seems irrelevant to question whether the MFIS score purely reflects 'fatigue', or is in part due to unrecognized depression or cognitive dysfunction. The more relevant question is whether individuals have a better quality of life when their MFIS score is lower.

The question of differing group composition according to disease classification is also important. There were proportionately more relapsing–remitting patients in the Prokarin™ group than in the placebo group, and this might have introduced bias in two different ways. Firstly, one could propose that all relapsing–remitting patients were in relapse at baseline, and all subsequently went into a spontaneous, synchronized remission over the next 12 weeks, while the patients with progressive disease classification remained stable. Thus, passage of time alone would explain the apparent improvements in the Prokarin™ recipients. That scenario is unlikely since one of the inclusion criteria was symptom stability, with no relapse in the preceding 3 months prior to the start of the trial. Also, as was pointed out in Table 2, baseline scores for all measures were not statistically different in the two groups. If the differing percentage of relapsing versus progressive patients in the two groups was an important factor for the parameters of interest, this surely would have been reflected by a difference in performance on the various measures at baseline, between the two groups.

A different bias could have been introduced if the following conditions were met: Prokarin™ exerted no effect, all relapsing–remitting patients remained stable, and all progressive patients worsened over 12 weeks. In this case, the groups would once again diverge simply with the passage of time, however the divergence would be the opposite of what was actually found. Placebo patients would worsen, and Prokarin™ recipients would remain unchanged.

We conclude then, that the differing composition of the groups with respect to disease classification was not a significant factor in the differences we observed. The potential stimulant effect of caffeine needs to be considered, especially since the placebo did not contain caffeine. However, serum levels of caffeine were equal in the two groups, at least for the first 8 weeks of the trial, and the percentage of coffee drinkers in each group was high, and similar. Moreover, the caffeine level in the placebo group was higher at 12 weeks than in the Prokarin™ group, yet there was no concomitant increase in the placebo MFIS scores at 12 weeks (or the scores for other measures). Therefore, the effect of Prokarin™ on fatigue does not appear to be correlated to the presence of caffeine in the formulation.

There are 100 mg of caffeine citrate in one dose of Prokarin™, yielding approximately 50 mg of caffeine. The average cup of coffee contains 100 mg of caffeine. Hence, two Prokarin™ patches worn contiguously for 16 h could deliver as much caffeine as a cup of coffee sipped over the same time, assuming 100% absorption. The actual amount delivered is undoubtedly less than this. This too supports the notion that the caffeine in Prokarin™ does not exert a significant independent effect on the central nervous system, especially when the patch is worn by a coffee drinker. Five Prokarin™ recipients did not consume any caffeine during the study. Serum levels of caffeine in all five of these subjects remained below detection limits for the first 8 weeks. At 12 weeks, two of the non coffee-drinking subjects had levels of 1.5 and 2.4 mg/mL, respectively, suggesting that in some patients, there is a gradual accumulation of caffeine, but not before 2 months use of Prokarin™. Significant effects were observed in the first 4 weeks, well before any caffeine could be detected in the blood. The average serum caffeine levels in both groups more likely reflect coffee consumption, rather than Prokarin™ usage.

As noted, the decision to continue Prokarin™ after the study ended was related to the degree of improvement in MFIS score. Those with the largest improvements were more likely to continue using Prokarin™ after the study was over. This indicates that the MFIS score was a clinically significant variable.

#### *Secondary endpoints*

For all of the secondary endpoints, there were no statistically significant differences between the Prokarin™ group and the placebo group. Although there were significant improvements within the Prokarin™ group comparing the baseline to posttreatment time points, the authors acknowledge that these within-group comparisons are not as important as the between-groups comparisons for a controlled evaluation of the effect of Prokarin.

#### *Conclusion*

This study demonstrated a modest statistically significant effect of Prokarin™ on the primary outcome measure, fatigue as measured by the MFIS questionnaire. The average individual per cent improvement in MFIS score was 37%. Improvement in MFIS score correlated well with the decision to continue Prokarin™

after termination of the study, demonstrating that decrease in MFIS score was a clinically relevant parameter. There was no evidence to suggest that the effect of Prokarin™ is exerted by caffeine alone. There was some indication that serotonin reuptake inhibitors might interfere with the action of Prokarin™, but further study is warranted. Side effects were minimal and there were no significant adverse changes in routine blood parameters. Larger studies are certainly indicated, to more fully delineate the role of Prokarin™ in MS, however this study demonstrates that Prokarin™ could reasonably be considered as an alternative for those MS patients who cannot tolerate the side effects of, or have not experienced satisfactory relief with other commonly used medications for fatigue.

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## Comparison of Prokarin™ to Other “Off-Label” Medications Used for Fatigue in MS

Fatigue is considered one of the most debilitating symptoms of MS accounting for 65% of the disability associated with MS (Goodin, 1999). According to the National Multiple Sclerosis Society, “fatigue and cognitive problems, trigger more unemployment than mobility impairments do” (Noyes, Fall 2001). There are currently no FDA approved medications for the treatment of fatigue in MS. It is common medical practice and recognized by the National Multiple Sclerosis Society to prescribe “off-label” medications for symptoms relief such as fatigue in MS (National Multiple Sclerosis Society website, Treatments, Medications Used in MS, 2002).

Furthermore Prokarin™ is a compounded prescription medication that is exempt from FDA approval according to the Modernization Act of the FDA that was signed into law on November 21, 1997. “Section 127 of the Modernization Act added section 503A to the Federal Food, Drug and Cosmetic Act (21 U.S.C. 353a) which exempts compounded drug products from the requirements in sections 501(a)(2)(B) (current good manufacturing practices), 502(f)(1) (adequate directions for use), and 505 (new drug provisions) of the act (21 U.S.C. 351(a)(2)(B), 352 (f)(1), & 355), provided that the compounding is conducted in accordance with, and the drug products meet the requirements in section 503A of the act.” ([www.fda.gov/cder/pharmcomp/12199a.txt](http://www.fda.gov/cder/pharmcomp/12199a.txt)) Section 503A (353a) Pharmacy Compounding is located in Chapter 5 FD&C Act subchapter A Drugs and Devices or it can be accessed at [www.fda.gov/opacom/laws/fdcact/fdcact5a.htm](http://www.fda.gov/opacom/laws/fdcact/fdcact5a.htm).

The double blind study of the effect of Prokarin™ on fatigue in MS was published in the *Multiple Sclerosis Journal* Vol.8, Issue 1 in February 2002. This journal is a recognized peer-reviewed medical journal. The Editor-In-Chief of the journal, Dr. Donald Silberberg, was Chairman of the Department of Neurology at the University of Pennsylvania for 12 years. Dr. Silberberg is the Co-Chairman of the World Federation of Neurology’s Research Group on Organization and Delivery of Neurological Services (<http://ncal.literacy.upenn.edu/sltp/presntr/silberberg.htm>).

Prokarin™ showed a significant statistical effect on fatigue in MS compared with the placebo group,  $p < 0.02$ . Furthermore, the Prokarin™ treated group improved dramatically in fatigue at 4 weeks and sustained that improvement throughout the study,  $p < 0.000008$ . (Note a p value of 0.05 or less is scientifically significant.) Also note that the group of participants receiving Prokarin™ also had significant improvements in cognition, walking, dexterity, and the chemical in the brain called N-acetyl aspartate as measured by magnetic resonance spectroscopy for the pre-versus-post treatment comparison at the end of the 12 week study. Discussions of these pre-versus-post treatment improvements are discussed in detail in the study write up entirety attached as Appendix A.

The “off-label” medications currently used for fatigue in MS are Modafinil (Provigil®), Amantadine (Symmetrel®), and Pemoline (Cylert®) (NMSS website, Treatments, Medications Used in MS, 2002). Amantadine has been used the longest of these three “off-label” medications to treat fatigue in MS. Amantadine is indicated for the treatment of the influenza A virus and Parkinsonism. Suicide attempts, some of which have been fatal, have been reported in patients treated with Amantadine (Physicians’ Desk Reference, 2001). In a study by Krupp et al (1995), Amantadine was shown to have a scientific significant effect in lessening fatigue in MS,  $p = 0.04$ . Note that the study on Prokarin™

showed a significance of  $p < 0.02$  in lessening fatigue in MS, which is a scientifically greater effect than that shown with Amantadine.

Amantadine was shown to have no effect on the cognitive performance in MS (Geisler et al, 1996). Cognitive performance was a secondary endpoint in the double blind study of Prokarin™ in MS. The average gain of cognitive performance tested via the PASAT test among the Prokarin™ group was 35% from baseline to 90 days, and that of the controls was 17%. Chi square testing of these results showed the gain of the Prokarin™ group was significantly greater than that of the controls,  $p < 0.001$ . The average individual percent improvement was 73% in cognitive performance for the Prokarin™ group (Appendix A). Prokarin™ shows to be effective in improving fatigue and cognitive performance, whereas Amantadine was not. Fatigue and cognitive impairment are the leading cause of unemployment in MS patients (Noyes, Fall 2001).

Modafinil (Provigil®) is approved for the treatment of narcolepsy. It is classified as a Schedule IV of the Controlled Substance Act as, “Modafinil is reinforcing, as evidenced by its self-administration in monkeys previously trained to self-administer cocaine” (Physicians’ Desk Reference, 2001). Modafinil showed significant improvement in fatigue in a 9-week crossover study,  $p < 0.001$  (Rammohan et al, 2002). The Modified Fatigue Impact Scale (MFIS) mean scores were 44.7 not taking Modafinil versus 37.7 after taking Modafinil 200mg/day. (The higher the MFIS score the greater the level of fatigue.) The MFIS mean scores at baseline for the Prokarin™ group were 58.38 and 37.40 after 12 weeks of Prokarin™ administration. The Prokarin™ treated group improved dramatically at 4 weeks ( $p < 0.000008$ ) and remained at that level throughout the study. The control group means in the Prokarin™ double blind study did not differ from the baseline group mean at any time during the 12-week period (Appendix A). Thus, in the double blind study of Prokarin™ the MFIS mean scores improved 20.98 points versus only 7 points of improvement in the MFIS mean scores in the Modafinil study.

Pemoline (Cylert®) is indicated for the treatment of Attention Deficit Hyperactivity Disorder. Pemoline has been associated with life threatening hepatic failure. Since Pemoline’s marketing in 1975, there have been 15 cases of acute hepatic failure reported to the FDA as of December 1988 of which 12 resulted in death or liver transplantation (Physicians’ Desk Reference, 2001). Pemoline has not shown to be effective in lessening fatigue in MS (Branas et al, 2000; Krupp et al, 1995;) and it has not shown any benefit in cognitive performance in MS (Geisler et al, 1996).

Thus, Prokarin™ has significant effect in improvement of fatigue in MS and also showed significant improvement in walking, dexterity, and cognitive performance within the Prokarin™ group for pre-versus-post treatment as demonstrated in the double blind study. The “off-label” medications, Modafinil and Amantadine, used to treat fatigue in MS have not been shown to have effectiveness in lessening symptoms other than fatigue in MS. The “off-label” medication, Pemoline, did not show to have any benefit in MS.

**Prokarin™ Potential Beneficial Effect on the Cost/Benefit Ratio in MS**

Anecdotal data from a survey of 421 MS patients using Prokarin™ was performed in 2000-2001. Out of the 421 MS patients surveyed, a total of 285 incidences of decreased use or discontinuation of a medication used for MS symptoms or symptoms of co-morbidities were reported after starting the Prokarin™. Below is a table illustrating the various medications that were decreased in usage or discontinued.

Medication Cost	Number Of Patients	Average Monthly Cost	Medication	Number Of Patients	Average Monthly Cost
4 AP	2	Price not available	Methotrexate	4	\$20.00
Amantadine	12	\$50.00	Morphine	1	\$240.00
Ambien	1	\$60.00	Naprosyn	2	\$80.00
Amitriptyline	4	\$5.00	Prevacid	2	\$100.00
Antianxiety Drugs	14	\$10.00	Prilosec	1	\$150.00
Anticonvulsants	19	\$200.00	Progesterone	1	\$26.00
Avonex	14	\$872.00	Propranolol	1	\$20.00
Axid	2	\$100.00	Propulsid	1	\$90.00
B-12	2	n/a otc	Provigil	3	\$150.00
Baclofen	82	\$150.00	Prozac	3	\$80.00
Bactrim	1	\$7.00	Quinidine Sulfate	1	\$30.00
Betaseron	6	\$1,000.00	Ranitidine	3	\$90.00
Ca eap	3	price not available	Reglan	1	\$20.00
Celexa	1	\$70.00	Restoril	1	\$5.00
Codeine	2	\$66.00	Roloids	1	n/a otc
Copaxone	5	\$1,000.00	St. Johns Wort	1	n/a otc
Cozaar	1	\$59.00	Steroids	11	\$10.00
Cylert	6	\$100.00	Stool softening	3	n/a otc
Detrol	3	\$80.00	Tagamet	3	\$80.00
DHEA	2	n/a otc	Tamoxifen	1	\$150.00
Dicyclomine	1	\$5.00	Trazodone	1	\$20.00
Ditropan	10	\$20.00	Trental	1	\$50.00
Flexeril	1	\$70.00	Tylenol	4	n/a otc
Laxatives	1	n/a otc	Ultram	2	\$180.00
Levaquin	1	\$126.00	Vicodin	1	\$15.00
Lipitor	1	\$70.00	Vioxx	1	\$160.00
Lithium	1	\$20.00	Wellbutrin	1	\$70.00
Meclozine	1	n/a otc	Zanaflex	26	\$180.00
Methocarbamol	1	\$10.00	Zoloft	3	\$70.00
			<b>Total</b>	<b>285</b>	<b>\$49,983</b>

This data demonstrates the potential beneficial effect of Prokarin™ in decreasing the cost/benefit ratio by decreasing the direct cost associated with the use of many medications for symptoms of MS and co-morbidities.

The study by Grudzinski et al (2000) reported that the total cost to the insurance carrier for the care of MS patients averaged \$9,365 (+/- \$11,047 if any of the current FDA approved disease-modifying medications i.e. Avonex®, Betaseron®, or Copaxone® were being used) per patient for the 2 years study. (The average retail cost of Prokarin™ is approximately 25% of the retail cost of the disease-modifying medications). The principal determinants of cost were the number of exacerbations, co-morbidities, and disease-modifying medication claims. The cost of the exacerbations was directly dependent on the number of co-morbidities present, ranging from \$359 for a patient with no co-morbidities to \$2,839 for a patient with 8 co-morbidities present. The anecdotal data collected from the 421 MS person survey showed a reduction in the use of other medications for co-morbidities and symptoms after the initiation of Prokarin™ use. Baclofen® and Zanaflex®, for the treatment of spasticity, had the largest incidence of decreased use or discontinuation. Antianxiety/anticonvulsants which are often prescribed, “off-label” for the treatment of pain, stiffness, and sleeping disorder in MS patients were the second largest group of medications to be decreased in use or discontinued after the initiation of Prokarin™. Steroids, which are used to lessen the symptoms associated with exacerbations of MS, were also decreased in use or discontinued after Prokarin™ use was started. This is a probable indicator of a decrease in the number of exacerbations of MS that may have coincided with the initiation of Prokarin™. Ditropan® is prescribed for bladder incontinence, which is a common symptom associated with MS. Impaired bladder function is a leading cause of urinary tract infections in MS patients. Urinary tract infections are often the etiology of sepsis in MS that often may result in hospitalization. Thus, the use of Prokarin™ may result in the decreased use or discontinuance of such medications as illustrated and may be indicative of a decrease in present or potential co-morbidities, a decrease in the number of exacerbations, hospitalizations, and doctor visits. The potential for the use of Prokarin™ in decreasing exacerbations, hospitalizations, and doctor visits was confirmed from data collected from a random sample size of 23 MS patients using Prokarin™ for a duration of one year or longer. The chart on page 25 displays this data. The total savings with the use of Prokarin™ equals \$913 per patient per year. This was calculated using the following formula:

Cost of Prokarin™ Patient / year	-	Ave. savings of Rx's decreased or discontinued patient / year	-	Ave. savings of decreased Dr. visits hospitalizations, & exacerbations patient / year	=	Total savings with use of Prokarin™ patient / year
\$250 x 12	-	$\frac{\$49,983 \times 12}{421}$	-	$\frac{\$6,150 + \$36,000 + \$15,078}{23}$	=	\$913 total savings / pt/ yr



<b>Exacerbations – Hospitalizations – Dr. visits/per year with and without Prokarin</b>
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	<i>Average exacerbations per year @ \$359 / exacerbation</i>		<i>Average hospitalization per year @ \$2,000 day / 3 day visit</i>		<i>Average Dr. visits per year @ \$150 per visit</i>	
	Before Prokarin	Using Prokarin	Before Prokarin	Using Prokarin	Before Prokarin	Using Prokarin
	2	0	2	0	10	0
	1	1	0	0	0	0
	2	0	0	0	0	2
	0	0	0	0	0	0
	1	0	0	0	0	0
	1	0	0	0	0	0
	0	0	0	0	1	0
	0	0	0	0	0	0
	1	0	2	0	5	2
	4	0	0	0	6	0
	2	1	0	0	2	0
	12	2	0	0	12	2
	12	3	0	0	0	0
	1	0	0	0	3	0
	2	0	0	0	2	0
	0	0	1	0	0	0
	1	0	0	0	3	0
	1	0	0	0	0	0
	1	0	0	0	0	0
	1	0	0	0	0	0
	2	1	2	1	1	1
	1	0	0	0	3	0
	2	0	0	0	0	0
<b>Total incidence</b>	<b>50</b>	<b>8</b>	<b>7</b>	<b>1</b>	<b>48</b>	<b>7</b>
<b>Total cost per year</b>	<b>\$17,950</b>	<b>\$2,892</b>	<b>\$42,000</b>	<b>\$6,000</b>	<b>\$7,200</b>	<b>\$1,050</b>
<b>Total savings per year using Prokarin</b>	<b>\$15,078</b>		<b>\$36,000</b>		<b>\$6,150</b>	

Prokarin™ showed a significant effect on improving fatigue in MS patients. Fatigue accounts for 65% of the debilitation in MS patients and is a leading cause of unemployment. Thus, fatigue greatly impacts the economic status of the individual MS patient, his or her family, and society. The debilitation resulting from fatigue increases the indirect costs incurred with MS patients. Four of the 23 patients in the chart above reported being able to increase the number of hours worked per week since using Prokarin™. Therefore, Prokarin™ can potentially decrease the indirect costs as well as the direct costs associated with MS.

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Food, Drug and Cosmetic Act website: [www.fda.gov/opacom/laws/fdcaact/fdcaact5a.htm](http://www.fda.gov/opacom/laws/fdcaact/fdcaact5a.htm)

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## Appendix A

**A randomized, placebo-controlled trial of the effect of Prokarin™ on fatigue in multiple sclerosis**

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**Abstract**

In this 12 week study with 29 subjects, the effect of Prokarin™ (n=22), a proprietary blend of histamine and caffeine, was compared to placebo (n=7) for the following outcomes: fatigue as measured by the Modified Fatigue Impact Scale (MFIS), cognitive and motor function as measured by the Multiple Sclerosis Functional Composite (timed walk, pegboard test and paced auditory serial additions test (PASAT)), serum caffeine level, change in brain chemistry as measured by quantitative magnetic resonance spectroscopy assay of N-acetyl aspartate, safety as measured by routine blood chemistry, TSH and urinalysis. Data were acquired at baseline, 4, 8 and 12 weeks. The Prokarin™ treated group mean MFIS scores at 4, 8 and 12 weeks were all highly significantly different from baseline group mean. The control group MFIS means did not differ



from baseline group mean at any time during the 12 week period. Average individual percent improvement at 12 weeks was 37% for Prokarin™. Similar findings were observed for the PASAT scores, with a 73% individual average improvement at 12 weeks. Subgroups of individuals registered increasing, statistically significant improvements from baseline scores in both the timed walk, and pegboard tests. Serum caffeine data indicated that caffeine exerted no independent effect on performance. No laboratory abnormalities were seen, and the treatment was well tolerated. There was a significant change in N-acetyl aspartate in the posterior periventricular white matter region for the treated group.

Conclusion: Prokarin™ is effective for the relief of the symptom of fatigue in Multiple Sclerosis, as measured by the MFIS.

## **Introduction**

Prokarin™ is a proprietary histamine and caffeine-containing transdermal cream which has been under evaluation for Multiple Sclerosis (MS) symptom relief over the past four years. Uncontrolled studies have found that Prokarin™ appears to have a significant impact on many MS symptoms, including fatigue<sup>1,2</sup>. This paper outlines the results of the first placebo-controlled study of Prokarin™ for symptom relief in MS. Histamine is an important neurotransmitter; its many functions include promotion of mental alertness, temperature regulation, and involvement in brainstem vestibular pathways. We hypothesize that there may be a histamine deficit in the CNS, which may be ameliorated by Prokarin™. A more detailed discussion of this hypothesis has also been presented previously<sup>1</sup>.

The use of histamine as a therapeutic agent actually dates back to the 1920's. For example, in 1944, a physician at the Mayo Clinic wrote about his 17 years of clinical use of histamine in a wide variety of settings including MS, Bell's palsy, vasculitis and Meniere's disease<sup>3,4</sup>. Hinton Jonez MD also made extensive use of histamine in the late 1940s and early 1950s<sup>5</sup>. Histamine therapy is still employed by otolaryngologists today for headache and vestibular disturbances<sup>6</sup>.

## **Primary Outcome Measure: Fatigue**

Fatigue is the most common symptom of MS, being present in 75-95% of cases<sup>7</sup>. It can have an overwhelming impact on the lives of patients with MS and is a major contributor to interpersonal and societal losses suffered from the disease<sup>8</sup>. Because of the tremendous impact fatigue has on the lives of MS patients, their families and society, we chose it as an important symptom to study. The cause of fatigue in MS is unknown, although various mechanisms have been proposed, including intracortical conduction block<sup>9</sup> and decreased availability of ATP<sup>10</sup>. The most common definition of fatigue in MS is "a subjective lack of physical and/or mental energy that is perceived by the individual or caregiver to interfere with usual and desired activities"<sup>11</sup>. This is the type of fatigue addressed in this study.

Various classes of prescription medications are currently used off-label for the treatment of fatigue in MS, including antidepressants, as well as drugs used in the treatment of narcolepsy and Attention Deficit Disorder. Drawbacks to these approaches include side effects and lack of efficacy. Hence not only is fatigue a common, significant symptom in MS, it is not well treated in our experience.

The primary intent of this study then, was to evaluate the effect of Prokarin™ on fatigue as measured by a standardized questionnaire, the Modified Fatigue Impact Scale or MFIS<sup>12,13</sup> which measures the impact of fatigue on social, cognitive and physical aspects of daily life. The potential of Prokarin™ to adversely affect metabolic parameters was studied with serial routine labwork and urinalysis.

## **Secondary Outcome Measures: Multiple Sclerosis Functional Composite and Cerebral N-Acetyl Aspartate Levels**

Anecdotal experience has indicated that Prokarin™ may also improve motor and cognitive function, hence the Multiple Sclerosis Functional Composite (MSFC) was chosen as a secondary outcome measure. The MS Functional Composite is recognized by the National MS Society's Clinical Outcomes Assessment Task Force as a reliable quantitative outcome measure for clinical trials in MS<sup>12,13</sup>. The dependent variables to be assessed by the MS Functional Composite are leg function/ambulation, arm/hand function, and cognitive function.

Proton MR spectroscopy (MRS) of the brain has been proposed as a means for monitoring disease activity in multiple sclerosis<sup>14-19</sup>. N-acetyl aspartate (NAA) is one of the cerebral metabolites that can be measured with proton MRS and the reduction in NAA reported in MS subjects by the majority of the authors cited above has been interpreted as a sign of neuronal damage or loss. In this study NAA levels were measured before and after twelve weeks of Prokarin™ treatment to look for possible effects on underlying neuronal pathology.

## **Methods**

This was a single-center, double-blind, placebo-controlled study of two parallel treatment groups of outpatients with both Relapsing-Remitting and Progressive MS. The study protocol was approved both by the Bastyr University Scientific Review Board, Seattle Washington, as well as the Western Institutional Review Board, Olympia, Washington. The MR spectroscopy scanning protocol was approved by the University of Washington Institutional Review Board. Treatment period was 12 weeks. Subjects were assessed four times: at baseline, 4 weeks, 8 weeks, 12 weeks. Compliance with treatments was monitored by means of patient diaries and periodic telephone follow-ups between assessments. All personnel involved with the study (assessors, subjects and clinical supervisor) were blind to the randomization until after the final assessment. The team performing the clinical assessments was trained and evaluated as per the MSFC handbook. The clinical supervisor compiled the data after each assessment, but the final statistical analysis was done by a consultant who was unaware of the randomization. No data lock procedure was employed.

A second phase of this study, in which placebo recipients were crossed over to a 12 week course of Prokarin™ is underway. The object of the second phase is to examine the effect of Prokarin™ on digestive and hormonal function, as well as manifold parameters of interest in nutritional biochemistry.

## **Patient Selection and Clinical Details**

Potential subjects were recruited via advertisements and flyers. Initial screening was by telephone interview, using a standardized questionnaire. The main inclusion and exclusion criteria are listed in Table 1.

***Table 1: Major Inclusion and Exclusion Criteria***

### *Inclusions*

*18 years of age or older*

*Expanded Disability Status Scale (EDSS) score of 5.0-6.5*

*Diagnosis of MS confirmed by neurologist exam and the presence of CNS sclerotic lesions on MRI*

*Baseline MFIS score greater than 40*

### *Exclusions*

*Current or previous use of Prokarin™*

*Current use of anti-spasmodic agents, corticosteroids, chemotherapeutic agents, monoamine oxidase inhibitors, histamine (H1 or H2) blockers*

*Patients starting antidepressants, interferons, or glatiramer acetate within the past three months*

*Serious renal, hepatic, endocrine, cardiac or pulmonary disease*

Individuals meeting these criteria underwent an intake assessment consisting of a history, physical exam, routine blood work (CBC, SMAC, TSH) and urinalysis. The same lab workup was also done prior to the 12 week assessment. Subjects were then randomized independently to Prokarin™/placebo and to MRI/no MRI. Participants were provided with a four month supply of a multivitamin/multimineral and a calcium/magnesium supplement. Subjects randomized to the MRS assessment underwent their first scan prior to the baseline assessment.

Subjects were advised to continue prescription medications, initiate study dietary supplements and discontinue any other supplements, restrict intake of caffeinated beverages to the equivalent of one cup of regular coffee per day, and consume a diet moderate in saturated fat intake (for those patients on a low fat diet). Informed consent was obtained.

On each study assessment day, blood was drawn for serum caffeine level. On the baseline assessment day, subjects were once again instructed on patching technique (they had been given an instructional video to view), and allowed to practice with an inert (non-placebo) cream until competency at patching was demonstrated. Subjects then applied their first patch and were observed for thirty minutes before leaving. Subjects were provided with a daily diary in which they were to note times of patch application and other details, and were instructed regarding adverse events. They were given a thirty day supply of Prokarin™, a proprietary mixture of 1.65 mg histamine diphosphate and 100 mg of caffeine citrate per 0.2 ml, or an indistinguishable placebo containing only citric acid. The treatments were provided in unit dose syringes containing 0.2 ml of cream. Two consecutive patches, worn for eight hours each were applied each day, the first patch being applied around 7:00 a.m. if possible. Within seventy-two hours of study inception, all participants were visited at home by an RN, and assessed for vital signs, patching technique, and refrigerator temperature. Subjects were contacted in the week prior to each subsequent assessment day for the purpose of verifying compliance and intent to attend the next assessment.

### **Study Endpoints**

The primary endpoint was fatigue, as assessed by the MFIS, a standardized 21-item subset of the Fatigue Impact Scale<sup>12</sup>. All patients had a fatigue score greater than 40 at inception (possible score ranged from 0 to 84) with a higher score indicating a more severe impact of fatigue on daily life.

The components of the MSFC included a timed 25 foot walk, a timed pegboard test (placing and removing 9 pegs from a board in under three minutes) and the Paced Auditory Serial Additions Test or PASAT which involved performing serial additions on a series of 60 numbers presented every three seconds by audiotape. Subjects filled out the MFIS questionnaire and completed the 3 MSFC assessments at baseline, and at 4, 8 and 12 weeks.

### **MR Imaging and MR spectroscopy**

Magnetic resonance imaging (MRI) and MR spectroscopy were performed on a 1.5 tesla Signa scanner using version 5.8 G.E. software. Multi-slice MRIs were acquired in the sagittal plane (TR/TE 500/16 milliseconds) and the axial plane (Fast spin-echo, TR/TE 2000/100; T1-weighted, TR/TE 500/16, and Fluid Attenuation Inversion Recovery (FLAIR), TR/TE/TI 10000/130/2200). MR spectroscopy was acquired using proton echo-planar spectroscopic imaging (PEPSI) pulse sequence developed by Posse et al<sup>20</sup>. The MRIs were used to establish the anatomical coordinate system to define the regions of interest used in the quantitative analysis of the NAA data. PEPSI measurements were performed with the following parameters: TR: 2 seconds; TE: 272 msec; spatial matrix: 32 x 32 voxels; field of view: 24 cm; slice thickness: 20 mm; spectral width: 32 kHz; frame size: 16,384 complex data points (= 32 spatial

points convolved with 512 spectral points); 8 averages; acquisition of partial echoes to permit magnitude reconstruction. A TE of 272 ms was chosen to isolate NAA from surrounding metabolites and to minimize peripheral lipid resonances. A short echo PEPSI water scan was obtained (TR/TE 2000/20 msec) for normalization purposes. The PEPSI data was filtered, Fourier transformed and analyzed as described previously<sup>17</sup>.

### Statistical methods

The sample size of at least 20 treated patients and 5 controls was based on the large effect size (greater than 0.80) for the fatigue score that had been found in earlier clinical studies<sup>1,2</sup>. The statistician received two groups of data with no information as to the positive direction of scoring so that he was blinded as to the meaning of the numbers and the desired direction of change. The two groups were compared on t-tests of the means of their MFIS scores going into the study and found to be equivalent ( $t = 0.88$ ,  $df = 26$ ,  $p < 0.48$ ). The statistical analysis was based on the usual assumption of linearity of the data, in which case, F tests, r tests or t tests would be appropriate. In the analysis section of the study it was decided to use t tests of mean differences, both within groups and between groups.

### Results

A total of 29 patients were recruited from 378 screened. Twenty-two were randomly assigned to receive Prokarin™ and seven to receive placebo. Seven of twenty-two Prokarin™ recipients, and three of seven placebo recipients were randomly assigned to the MRI component of the study. As shown in Table 2, treatment groups were comparable both in demographic characteristics (with the exception of disease classification) and baseline values of clinical parameters, indicating that the randomization was successful.

The main focus of the study was to determine the effect of Prokarin™ on fatigue in MS, without regard to disease classification (Relapsing-Remitting versus Progressive). Since one of the entry criteria was an EDSS score in the range 5.0-6.5 for all subjects regardless of disease type, time from diagnosis and disease classification were less relevant. From Table 2, it is evident that the composition of the groups differed with respect to disease classification. Statistically speaking however, there was no difference between the groups at baseline for the variables of interest.

One patient dropped out of the Prokarin™ group at the eight week mark citing lack of effect and difficulty adhering to the patching schedule. Two subjects dropped out of the placebo group. One subject dropped out just after four weeks, as she wanted to resume her original regimen of nutritional supplements. The other subject dropped out at eight weeks citing lack of effect and difficulty adhering to the patching schedule.

**Table 2: Baseline Characteristics of Treatment Groups**

	<i>Prokarin™ (n=22)</i>	<i>Placebo (n=7)</i>
<i>Age</i>	<i>47.7</i>	<i>46.4</i>
<i>Sex</i>	<i>73%</i>	<i>57%</i>
<i>Coffee consumption</i> <i>≥1 cup/day</i>	<i>77%</i>	<i>86%</i>

<i>Current antidepressant use</i>	50%	43%
<i>Current ABC drug use</i>	54.5%	43%
<i>MFIS score ①</i>	58.3	61.7
<i>Walk Time (sec) ②</i>	12.2	8.6
<i>Peg Time (sec) ③ (dominant hand)</i>	29.0	28.5
<i>Peg Time (sec) ④ (nondominant hand)</i>	32.7	27.3
<i>PASAT score ⑤</i>	37.8	37.9
<i>Serum caffeine ⑥ mcg/ml</i>	1.9	2.5
<i>Relapsing Remitting</i>	9	1
<i>Secondary Progressive</i>	11	5
<i>Primary Progressive</i>	2	1

① $p < 0.37$ : ② $p < 0.52$ : ③ $p < 0.91$ : ④ $p < 0.44$ : ⑤ $p < 1.0$ : ⑥ $p < 0.46$  where  $p$  is the probability of rejecting the hypotheses that group means are equal.

## Results

### Primary Endpoint

All study data was sent to a statistician outside the study who was unfamiliar with MS tests. The data was coded to protect the anonymity of the patients, and the direction of desired change in scores for each data set was not made known to the statistician.

The MFIS data had 22 data sets for the Prokarin™ group and 7 data sets for the placebo controls. After the 4 week retest, two of the controls and one Prokarin™ recipient dropped out of the study, leaving only 5 control data sets, and 21 Prokarin™ data sets for tests on weeks 8 and 12.

No difference was found between treatment and control groups at baseline, with the Prokarin™ group having a mean of 58.38, SD = 8.90, and the placebo controls a mean of 61.13, SD = 7.49 (df = 27,  $t = .77$ ,  $p < 0.22$ ).

Prokarin™ or control group means at each assessment point were compared to their own baseline values (within group comparison). The Prokarin™ treated group improved dramatically at 4 weeks ( $df = 20$ ,  $t = 5.75$ ,  $p < 0.000008$ ), and remained at that level throughout the study (4 week mean = 38.49,  $SD = 17.99$ , 8 week mean = 38.30,  $SD = 15.90$ , 12 week mean = 37.40,  $SD = 15.18$ ). The control group means did not differ from baseline group mean at any time during the 12 week period, although when individual percent changes ((final score-initial score/initial score) x 100%) for placebo recipients were calculated at 12 weeks compared to baseline, the average was a 15% improvement. The Prokarin™ group mean was significantly different from the mean of the control group at 12 weeks ( $df = 24$ ,  $t = 2.08$ ,  $p = < 0.02$ ), with respective means of 37.40,  $SD = 15.18$ , for the Prokarin™ group and 53.2,  $SD = 11.39$  for the controls. Note however, that for small groups with larger variance, such a cross-group comparison is not valid. A comparison of how the two groups fared throughout the study can be seen in Figure 1.

## Secondary Endpoints

### PASAT

An analysis of the PASAT data showed that the treatment and control subjects did not differ on the base line measure (Prokarin™ baseline mean 37.8: control baseline mean 37.9. Perfect score = 60). Further analysis showed that the controls did not differ significantly from baseline at any time over the 12 week period, but the Prokarin™ group improved significantly during every 4 week treatment interval (baseline to 4 weeks,  $t = 2.83$ ,  $p < 0.005$ , 4 weeks to 8 weeks,  $t = 3.96$ ,  $p < 0.0004$ , 8 weeks to 12 weeks,  $t = 2.08$ ,  $p < 0.03$ ,  $df = 20$  in each case). The percent change at 12 weeks was also calculated for each individual. The average individual percent improvement was 73% for Prokarin™. The fact that the PASAT score was inherently non-linear, and was more sensitive for low scoring-subjects was taken into statistical consideration. (A subject whose baseline score is close to the perfect score of 60 will not show a large improvement even if such an improvement has taken place. This subject is already getting most of the answers correct within the 3 second time limit allotted for each calculation. A treatment might enable this subject to give the correct answer in significantly less time, but this improved performance will not be reflected in the score.) To illustrate this idea, the Prokarin™ recipients were sorted into 2 groups according to ranked baseline PASAT score. The average percent improvement for group, as a whole, along with the percent improvement for the lower and higher-scoring halves was plotted in Figure 2, along with the result for placebo. The initially-lower-scoring group (raw scores 2-38) improved by an average of 139% whereas the initially-higher-scoring group (raw scores 40-58) registered an average improvement of only 13.5%.

Due to the small number of controls remaining at the end of 12 weeks ( $n = 5$ ), cross comparison of the two groups with t-score testing of the means was once again inappropriate. Instead the baseline scores of the 5 remaining controls were matched with the closest baseline scores of five members of the treated group (means of 41 and 40.8 respectively). The average gain among the treated group was 35 % from base to 90 days, and that of their matched controls was 17 %. Chi square testing of these results showed the gain of the treatment group was significantly greater than that of the controls ( $\chi^2 = 18$ ,  $p < 0.001$ ). Figure 2 shows the baseline scores as well as the percent change from baseline to 12 weeks for the two treatment groups.

### 25 Foot Timed Walk

There was no statistical difference between the mean of the baseline walk times for the Prokarin™ and placebo groups (Prokarin™: 12.2 +/-13.8 sec; placebo 8.6 +/-3.5 sec;  $t = 0.77$ ,  $df = 26$ ,  $p < 0.52$ ). Both groups improved significantly between baseline and 30 days ( $df = 21$ ,  $t = 2.22$ ,  $p < 0.02$  for the treatment and  $df = 6$ ,  $t = 2.64$ ,  $p < 0.02$  for controls). There was no

further significant change in either group for the duration of the study. At face value then, there was no significant effect of Prokarin™ on walk time compared to placebo.

A recent article by Kaufman<sup>21</sup> discusses the medium-term (one year) variability in Timed 25 Foot Walk score, and puts it at 20% or less. Hence a change in score of more than 20% can be considered significant. When percent change in walk time at 12 weeks was calculated for each Prokarin™ recipient, nine were seen to have gotten faster by more than 20%, whereas only one of the placebo group improved by more than 20%. This invited further analysis of the data.

While the statistician did not have walk time data for individuals without MS, he theorized that the subjects who had the fastest times for the walk, might reasonably have the least disability, and would not register much improvement. The slower individuals in the group, being more disabled would have much more room for improvement. Accordingly the group was divided into thirds based on ranked baseline walk time, and the mean walk times of each group at each assessment point are plotted in Figure 3. Kaufman reported that walk times for minimally affected MS patients ranged between 3 and 5 seconds<sup>21</sup>. The average walk time for the group fastest at baseline was 5.85 seconds over the twelve weeks, close to the performance cited for minimally affected individuals.

The fastest group at baseline increased their speed significantly between baseline and 4 weeks, but then experienced no additional gain. The middle third improved from baseline to 4 weeks ( $t = 3.23$ ,  $p < 0.009$ ,  $df = 6$ ), then again between 4 weeks and 8 weeks ( $t = 3.31$ ,  $p < 0.008$ ,  $df = 6$ ) and also during the final test period, 8 weeks to 12 weeks ( $t = 3.27$ ,  $p < 0.005$ ,  $df = 6$ ). The third of the subjects slowest at baseline improved between baseline and 4 weeks but the change was just short of significance ( $t = 1.84$ ,  $p < 0.06$ ,  $df = 6$ ), and this group did not experience significant improvement during any other phase of the study.

In figure 4 the behavior of the two tertiles fastest at baseline is displayed along with the behavior of the placebo control recipients. The control group means showed no change from baseline at 8 and 12 weeks despite a seeming improvement at 4 weeks. When the percent change for each placebo recipient at 12 weeks compared to baseline was calculated, the average percent change was -12%, a slight improvement.

### **Peg Test**

Data for individuals without MS were available for the Peg Test: mean 18.3 sec with a standard deviation of 3<sup>22</sup>. It was decided to analyze the Prokarin™ scores in two groups: all patient scores that fell within two standard deviations of the normative mean, and those who fell three or more standard deviations away from the normative mean. The reasoning, again, being that a treatment would not be expected to significantly improve patients who were already doing as well as people without MS. It was found that 9 of the 22 treated patients in the study fell within the norm on initial testing, as did 5 of the 7 controls. Figure 5 shows how the treated groups fared during the 12 week study period, compared with the normative mean (a flat line at 18.3 seconds).

The relatively non-disabled Prokarin™ recipients (those whose initial scores fell within 2 standard deviations of the norm) did not change, as they had little room for improvement. The more disabled Prokarin™ recipients (starting 3 or more standard deviations from the norm) experienced a progressive improvement in performance. These changes reached significance between weeks 4 and 8 ( $t = 3.02$ ,  $p = < 0.005$ ,  $df = 12$ ), and experienced another drop between 8 and 12 weeks that almost reached significance ( $t = 1.63$ ,  $p < 0.07$ ,  $df = 12$ ). This gradual change was particularly evident in the case of one individual who could not place any pegs on the board with her more

disabled hand at baseline or 4 weeks. At 8 weeks she managed to place 5 pegs out of 9 in 5 minutes, and at 12 weeks she placed all nine pegs and then removed them within the allotted three minutes. When the placebo group mean at each assessment point was compared to the mean at baseline, no significant change was seen, similar to the Prokarin™ recipients who were fastest at baseline. The average of the individual percent changes for each placebo recipient relative to individual baseline time was -6.8%, a slight improvement.

### Caffeine

Group means for serum caffeine level are displayed in Table 3. There was no significant difference between group means at baseline, four and eight weeks. At twelve weeks, there was a statistically significant difference between groups ( $p < 0.008$ ) with the level being higher in the placebo group.

**Table 3: Serum Caffeine Group Means**

	Baseline (µg/mL)	4 weeks (µg/mL)	8 weeks (µg/mL)	12weeks (µg/mL)
Prokarin™	1.9	2.5	2.2	2.2
Placebo	2.5	2.4	2.8	5.2

### Other Lab Parameters

No changes were noted in any of the routine lab parameters for the Prokarin™ group compared to placebo.

### MR Spectroscopy

There was a significant negative correlation between MFIS scores and NAA values at baseline in brain region 4: a higher baseline MFIS score was associated with a lower baseline NAA value in this region ( $r = -0.68$ ,  $p < 0.02$ ,  $df = 8$ ). This is illustrated in Figure 6. Correlation of MFIS with NAA in the same brain region was not significant, post treatment ( $r = 0.54$ ).

Average NAA change scores with error bars (standard error of the mean) for each brain region are shown in Figure 7. Twelve week NAA scores appear to be lower than baseline (negative change score) for Regions 1,4,6 and 7, for the Prokarin™ recipients. The average twelve week NAA score in Region 4 dropped by approximately 75, and this drop was significant with  $p < 0.001$ . The average twelve week score in Region 7 also appears to have dropped by about 50, but as shown by the larger error bars, this decrease was not significant, as was also the case for Regions 1 and 6. In Region 4, the average twelve week score of the placebo recipients was higher than at baseline (positive change score), as was the case in Regions 1 and 8. The wide separation between average change scores for Prokarin™ and placebo at 12 weeks in Region 4 almost reached significance ( $p < 0.10$ ). Although the error bars on the points are small, the test of significance is more stringent for low n. (The mean scores of the two groups were equal at baseline,  $p < 0.31$ .)

### Safety

Prokarin™ was well tolerated. Subjects had been instructed on handling of mild, moderate or severe adverse events (mild adverse events: annoying but not interfering with routine activity or function, e.g. skin rash; moderate adverse events: uncomfortable, intense enough to interfere with routine activity, but carrying no permanent health consequences, e.g. diarrhea with abdominal cramps; severe adverse events: severely uncomfortable, precluding normal activity or function, hazardous to health, and likely requiring hospitalization). No moderate or severe reactions were noted. Reactions among Prokarin™



recipients were limited to headache and skin irritation; both were generally transient. One Prokarin™ and one placebo recipient experienced loose bowel movements and fecal urgency, which resolved after cessation of the calcium/magnesium supplement. Three of the placebo recipients reported significant itching, which persisted throughout the study.

### **Continuation Rate**

At the time of writing, ten of the twenty-two Prokarin™ recipients (45%) had elected to continue using Prokarin™. This continuation rate is in keeping with the experience of the principal author. Decision to continue correlated with a large improvement in MFIS score. Seventy-one percent (10 of 14) of the Prokarin™ recipients with the largest improvements in MFIS score elected to continue the treatment. Conversely, no one whose score improved less than approximately 30% elected to continue the treatment. Interestingly, of the 10 subjects who elected to continue, only 1 was taking a serotonin reuptake inhibitor (SRI). Conversely, 64% (7 of 11) of patients who did not continue Prokarin™ were taking an SRI.

### **Discussion**

#### **Primary Endpoint: MFIS**

The strong effect on fatigue demonstrated in this study agrees with the large body of anecdotal evidence supporting a role for Prokarin™ in the alleviation of fatigue. The treatment was seen to be well tolerated with no adverse effects on routine blood parameters. Fatigue is often the first symptom to improve with Prokarin™ use, and the improvement is sometimes obvious within hours of instituting therapy. As discussed in a recent paper<sup>1</sup>, histamine may modify symptoms perceived as fatigue by affecting cerebral blood flow, and hence the onset of the effect might be expected to be rapid. In general, histamine is recognized as an animating, stimulating neurotransmitter<sup>1</sup>. The effect observed here could be consistent with an increased level of histaminergic neuronal activity.

Failure to account for the potential effects of affective disturbances and/or cognitive dysfunction has been cited as a potential issue in studies of fatigue<sup>23</sup>. Regardless of exactly what the MFIS questionnaire measures, or whether the MFIS score is partially reflective of depression or cognitive dysfunction, the baseline MFIS scores in the two treatment groups were the same. The rate of antidepressant usage in the two groups was similar, and no patient had started an antidepressant less than 3 months before the start of the trial, or during the trial. It seems irrelevant to question whether the MFIS score purely reflects “fatigue”, or is in part due to unrecognized depression or cognitive dysfunction. The more relevant question is whether individuals have a better quality of life when their MFIS score is lower.

The question of differing group composition according to disease classification is an important one. There were more proportionately more Relapsing-Remitting patients in the Prokarin™ group than in the placebo group. Hence, one could propose that these patients were all in relapse at baseline, and all subsequently went into a spontaneous, synchronized remission, explaining the apparent improvements. This seems unlikely. Also, as was pointed out in Table 2, baseline scores for all measures were not statistically different in the two groups. If the differing percentage of relapsing versus progressive patients in the two groups was an important factor for the parameters of interest, this would have been reflected by a difference in performance on the various measures.

The potential stimulant effect of caffeine needs to be considered, especially since the placebo did not contain caffeine. However, serum levels of caffeine were equal in the two groups, at least for the first 8 weeks of the trial, and the percentage of coffee drinkers in each group was high, and similar. Moreover, the caffeine level in the placebo group was higher at 12 weeks than in the Prokarin™ group, yet there was no concomitant increase in the placebo MFIS scores at 12 weeks (or the scores for other measures).

Therefore, the effect of Prokarin™ on fatigue does not appear to be correlated to the presence of caffeine in the formulation.

There are 100 mg of caffeine citrate in one dose of Prokarin™, yielding approximately 50 mg of caffeine. The average cup of coffee contains 100 mg of caffeine. Hence two Prokarin™ patches worn contiguously for 16 hours could deliver as much caffeine as a cup of coffee sipped over the same time, assuming 100% absorption. The actual amount delivered is undoubtedly less than this. This too supports the notion that the caffeine in Prokarin™ does not exert a significant independent effect on the central nervous system, especially when the patch is worn by a coffee drinker.

Five Prokarin™ recipients did not consume any caffeine during the study. Serum levels of caffeine in all five of these subjects remained below detection limits for the first 8 weeks. At 12 weeks, two of the non-coffee drinking subjects had levels of 1.5 and 2.4 mcg/mL respectively, suggesting that in some patients, there is a gradual accumulation of caffeine, but not before two months use of Prokarin™. Significant effects were observed in the first 4 weeks, well before any caffeine could be detected in the blood. The average serum caffeine levels in both groups more likely reflect coffee consumption, rather than Prokarin™ usage.

As noted, the decision to continue Prokarin™ after the study ended was related to the degree of improvement in MFIS score. Those with the largest improvements were more likely to continue using Prokarin™ after the study was over. This indicates that the MFIS score was a clinically significant variable.

Interestingly, only one of ten patients who elected to continue Prokarin™ was taking a serotonin reuptake inhibitor, as pointed out earlier. This suggests that SRIs may interfere with the action of Prokarin™. A given monoamine transporter exhibits affinity for a variety of monoamine neurotransmitters<sup>24</sup>, hence a serotonin transporter may also transport histamine. Conversely, a serotonin reuptake inhibitor might also interfere with the reuptake of histamine, and thereby interfere with the action of Prokarin™. Excess histamine in the synaptic cleft and surrounding tissue might lead to an eventual downregulation of overall histamine effect, just as the effect of SRIs sometimes declines with time.

## **Secondary Endpoints**

### **PASAT/Walk/Pegs**

The same basic results were seen for the MSFC components. Those individuals least disabled at baseline registered the least improvement. In the Peg test, where data for non-MS controls was available, it was seen that the least responsive group of Prokarin™ recipients were quite close in performance to individuals without MS, hence their scores could not get much better. The same is true for the PASAT and Timed Walk, although non-MS norms were not available.

For the Walk and Pegs, differential results were seen when the groups most disabled at baseline were considered. The walkers slowest at inception did not improve, whereas those with the slowest peg times at inception improved steadily. For those individuals whose lower extremity function was the most impaired at baseline, 12 weeks may not have been long enough to show improvement.

Assumptions of linear response are made in the theory underpinning the calculation of the MSFC score. Due to the obvious nonlinear behavior seen in this study, MSFC scores were not calculated. Sole reliance on the MSFC scores would have obscured important clinical findings. The issue of nonlinearities in these measures (PASAT, walk, pegs) and the effect on the validity of the MSFC score will be discussed in a future publication.

There was some suggestion of effects exerted over different time scales. The MFIS scores improved within the first 4 weeks; no additional improvement was seen after that. Within-group analysis of means (comparison to baseline) of the Prokarin™ PASAT scores indicated significant change (improvement) in mean score as the study progressed. One Prokarin™ recipient posted a gradual, but striking improvement in her Peg Test performance.

Rapid-onset effects may involve an increase in the cerebrospinal fluid level of histamine or other neurotransmitters dependent on histamine such as norepinephrine. Increased cerebral oxygenation due to cerebral vasodilation might also be expected to exert rapid onset effects. Longer timescale improvements might be due to changes in myelination, remyelination, or connectivity of relevant neurons, as well as a gradual improvement in nutritional status through enhanced gastric acidity and pancreatic function <sup>1</sup>.

As pointed out earlier, placebo recipients were seen to improve when the average individual percent change at 12 weeks was calculated. The degree of improvement was modest in each case. Aside from the obvious, classical placebo effect, there are several other possible explanations for an improvement in placebo scores. All subjects received a multivitamin/multimineral supplement as well as calcium and magnesium. There might have been a baseline improvement in all subjects due to this common factor. Although the role of specific nutrients such as Vitamin B12 and Vitamin D in MS has been extensively studied, no studies examining the effect of multivitamin/mineral supplementation on MS symptoms were found in a Medline search. Further research in this area might be warranted.

A training effect also has to be considered, although it is expected to be weak since the subjects were only able to “practice” the PASAT and Peg tests once every 4 weeks. Obviously, in the case of the timed walk, subjects “practiced” every day, so no training effect would be expected.

### **MR Spectroscopy**

N-acetyl aspartate has been previously shown to correlate well with clinical performance in that a higher NAA score correlated with a higher performance or less disability <sup>14,15,17</sup>. In a previous study, Richards has also shown that NAA values correlated negatively with fatigue scores in that higher NAA score correlated with less fatigue in untreated MS patients (unpublished data). In this study also, there was a negative correlation of NAA with MFIS score at baseline but there was no longer a significant correlation after treatment. We interpret this to mean that the brain metabolism/chemistry had not reached steady-state at the time the post-treatment data was collected (12 weeks). In a study by Mader et al, they reported a transient drop in NAA early on after treatment had started with deoxyspergualin <sup>24</sup>. In our study, there was also a significantly low NAA value in a posterior periventricular brain region 4 at 12 weeks compared to baseline in the Prokarin™ group, and as in the deoxyspergualin study, a decline in NAA may have been heralding an eventual increase. It is possible that additional measurements of NAA in a longer study might have shown an increase in NAA, and that the correlation between NAA and fatigue score would have been re-established.

It is interesting to note that the most pronounced NAA changes occurred in brain region 4. Region 4 is located in the posterior periventricular white matter region, which is one of the brain areas most commonly affected by MS, and this has been demonstrated on MRI and MRS <sup>17</sup>. It is possible that this region is more sensitive to a given treatment, as well as being more susceptible to damage.

## Conclusion

This study demonstrated a strong, statistically significant effect of Prokarin™ on the primary outcome measure, fatigue as measured by the MFIS questionnaire. The average individual percent improvement in MFIS score was 37%. Improvement in MFIS score correlated well with the decision to continue Prokarin™ after termination of the study, demonstrating that decrease in MFIS score was a clinically relevant parameter. There was no evidence to suggest that the effect of Prokarin™ is exerted by caffeine alone. There was some indication that serotonin reuptake inhibitors might interfere with the action of Prokarin™, but further study is warranted. Side effects were minimal and there were no significant adverse changes in routine blood parameters.

Prokarin™ use was associated with significant improvements compared to baseline for the cognitive and motor function secondary outcome measures, although only a subgroup was seen to respond in the case of the Walk and Peg tests. A larger, longer trial would be needed to verify these trends. Placebo recipients also posted modest improvements in each measure, and possible explanations for this were discussed. Although larger studies are indicated, to more fully delineate the role of Prokarin™ in MS, this study demonstrates that Prokarin™ could reasonably be considered as an alternative for those MS patients who cannot tolerate the side effects of, or have not experienced satisfactory relief with other commonly-used medications for fatigue.

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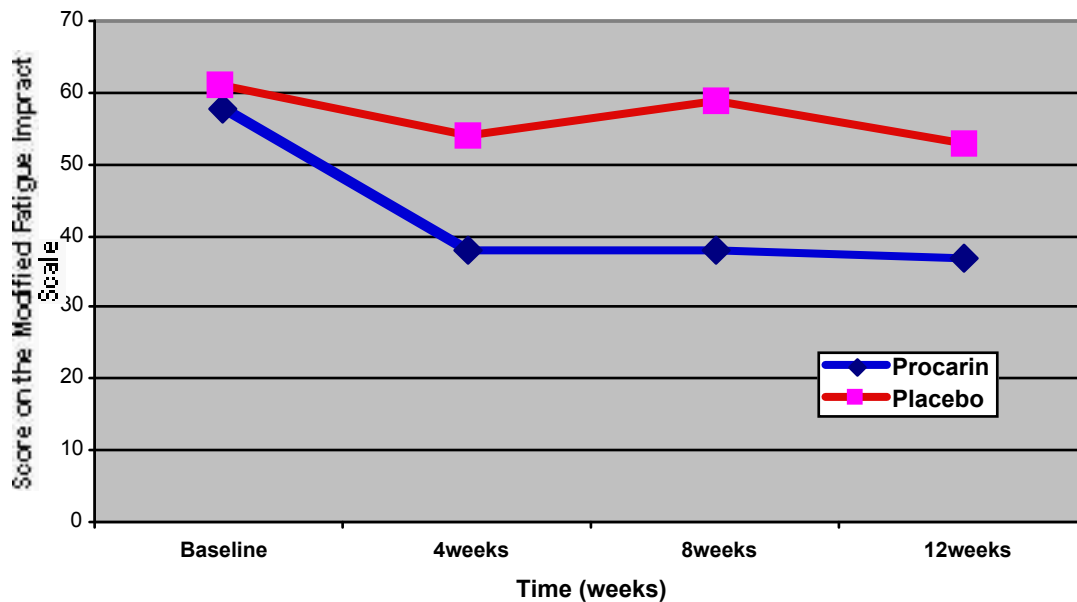
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### **Figure Captions**

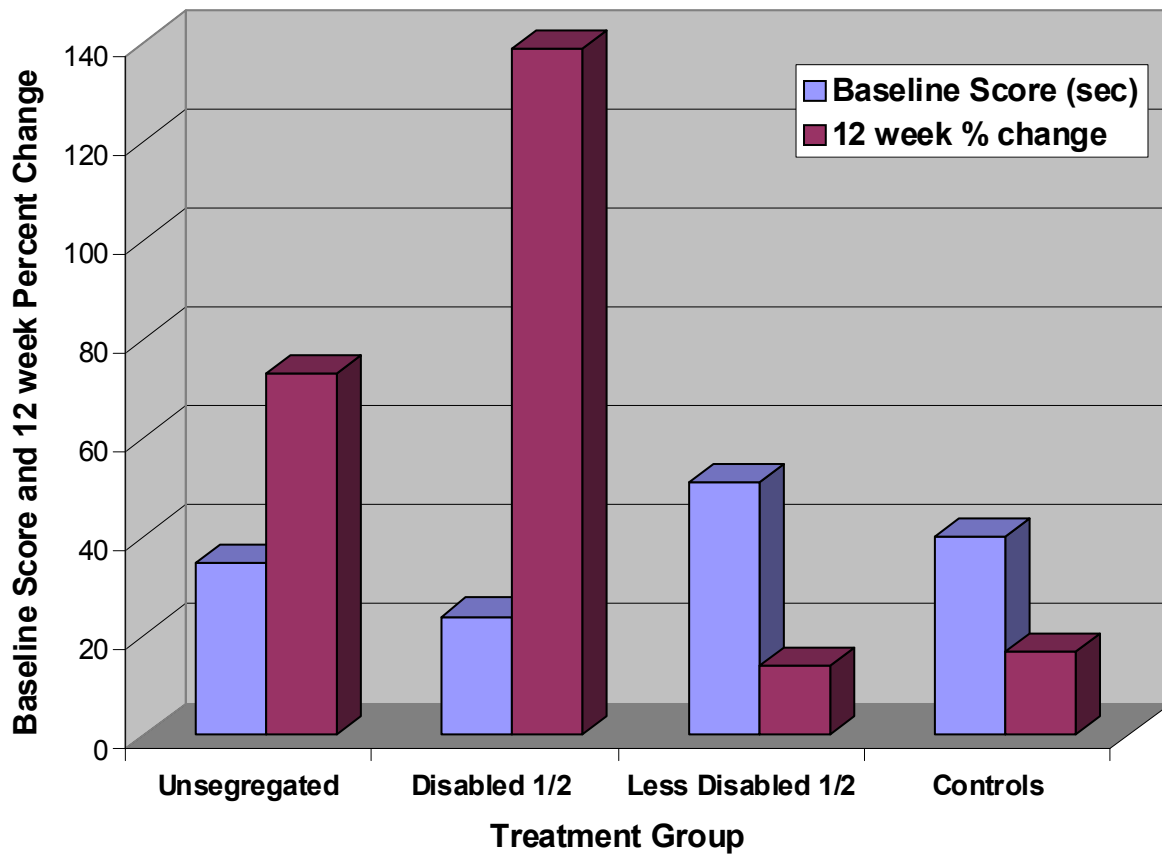
- Figure 1. The Effect of Prokarin™ Treatment on Fatigue in Multiple Sclerosis Patients
- Figure 2. The Effect of Prokarin™ on PASAT Score at 12 Weeks, According to Initial Disability Level
- Figure 3. The Effect of Prokarin™ on Walk Test Scores of MS Patients According to Performance at Baseline.
- Figure 4. The Effect of Prokarin™ Treatment on the Fastest 2 Tertiles of Patients on the Timed Walk Test Compared to Controls
- Figure 5. The Effect of Prokarin™ on MS Peg Test Score According to Performance at Baseline.
- Figure 6. Scatter plot of NAA versus MFIS fatigue score per treatment.
- Figure 7 - Averaged NAA change score versus Brain region for both the treatment and placebo groups. The NAA change score was averaged across 7 subjects for the treatment group and 2 subjects for the placebo group. NAA was normalized to internal water. There was a significant difference between pre and post treatment NAA for the treatment group in brain region 4 (as noted by asterick) which was in the right posterior periventricular white matter region. NAA was measured using pulse sequence proton echo planar spectroscopic imaging (TR/TE 2000/272 msec).

**Figure 1: The Effect of Procarin Treatment on Fatigue in Multiple Sclerosis Patients**



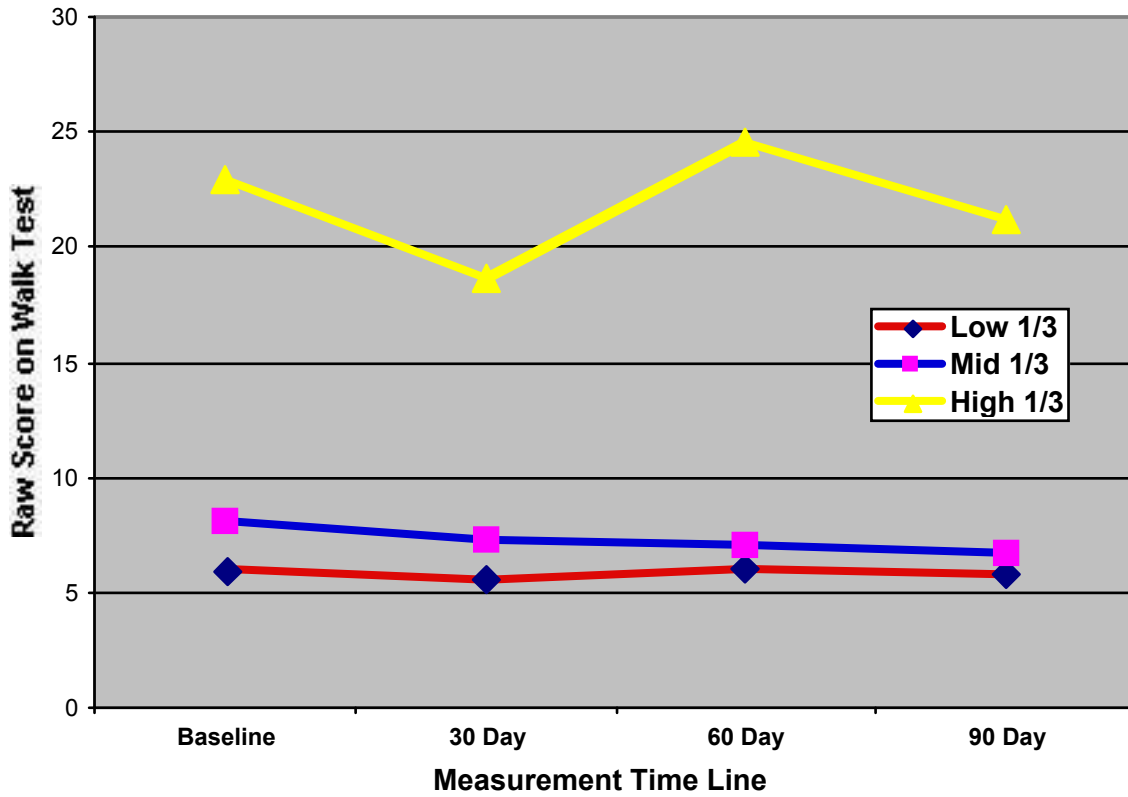
\* Prokarin™ was formerly known as Procarin™. The revised spelling was adopted to avoid potential conflicts with other existing trademarks.

**Fig. 2 The Effect of Procarin on PASAT score at 12 weeks, According to Initial Disability Level**



\* Prokarin™ was formerly known as Procarin™. The revised spelling was adopted to avoid potential conflicts with other existing trademarks.

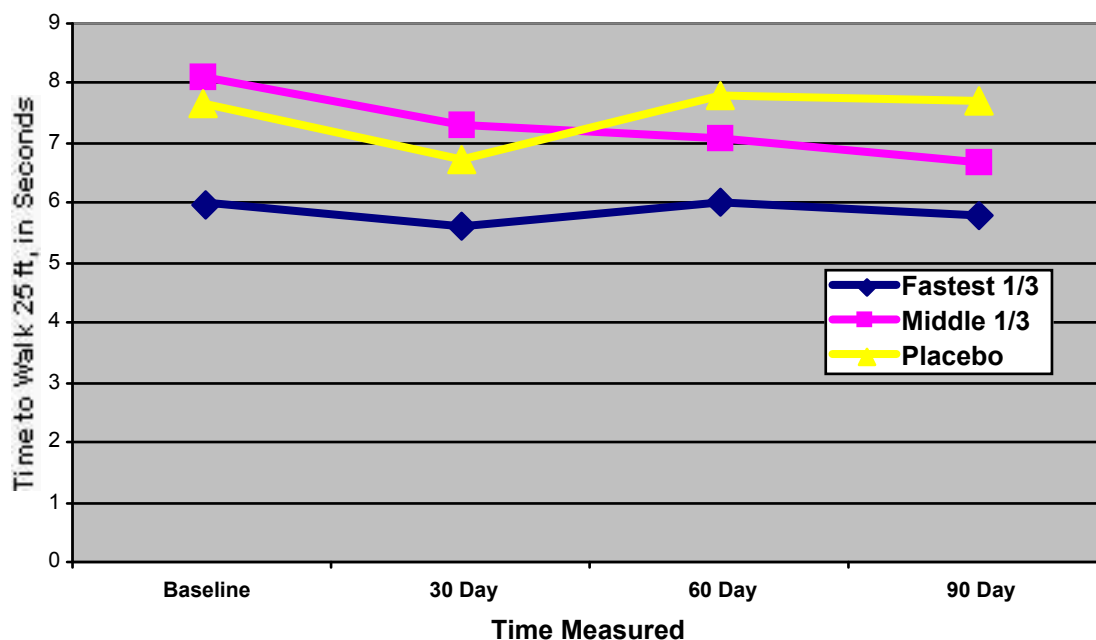
**Fig. 3 The Effect of Procarin Treatment on Walk Test Scores of MS Patients Divided into Thirds According to Performance at Baseline**



\* Prokarin™ was formerly known as Procarin™. The revised spelling was adopted to avoid potential conflicts with other existing trademarks.

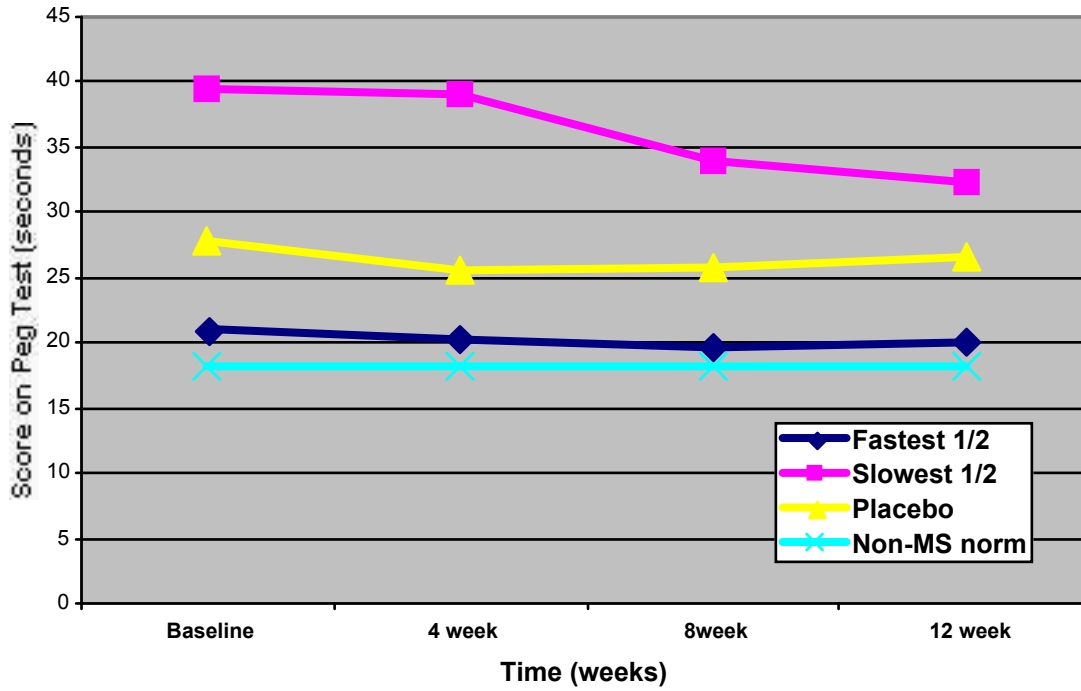


**Fig. 4 The Effect of Procarin Treatment on the Fastest 2 tertiles of Patients on the Timed Walk Test Compared with Contols**



\* Prokarin™ was formerly known as Procarin™. The revised spelling was adopted to avoid potential conflicts with other existing trademarks.

**Fig 5. The Effect of Procarin Treatment on Peg Performance, With group Divided into Slowest and Fastest Halves at Baseline**



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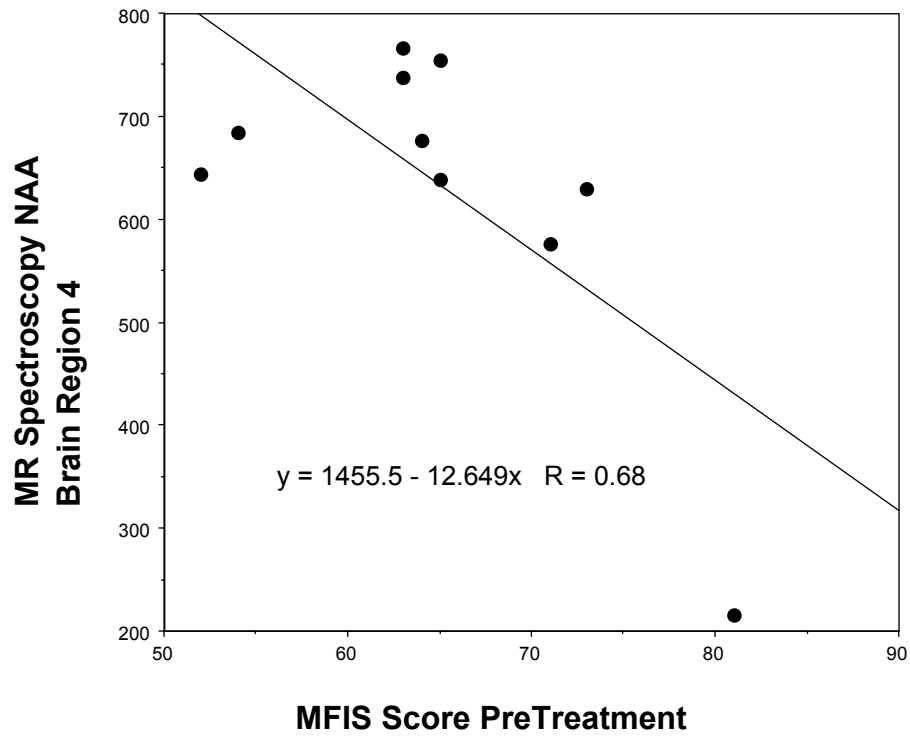
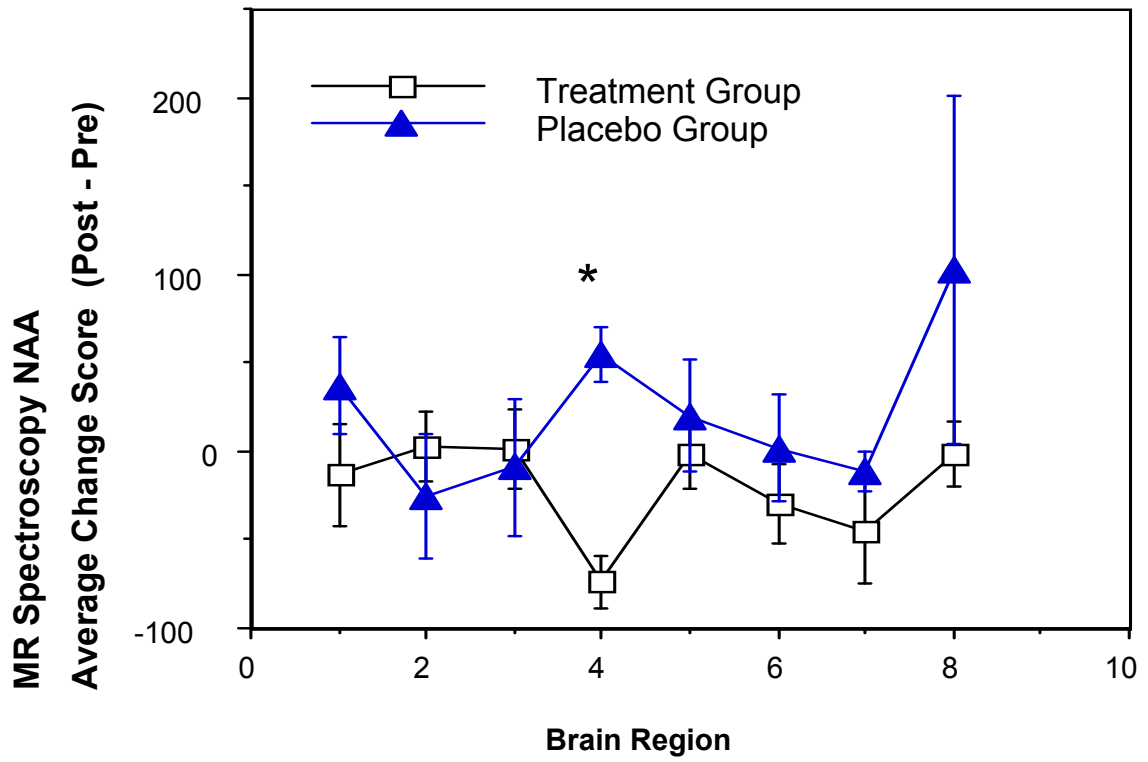


Figure 6- Scatter plot of NAA versus MFIS fatigue score per treatment.

Figure 7- Averaged NAA change score versus Brain region.



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