

Sentra AM™ Product Information

Indications

Sentra AM is intended for use in the management of chronic and generalized fatigue, fibromyalgia, post-traumatic stress syndrome (PTSD), neurotoxicity-induced fatigue syndrome, and cognitive impairment involving arousal, alertness, and memory. These conditions share an increased requirement for dietary choline, acetyl-L-carnitine, and glutamate to prevent muscle dysfunction, sleep disturbances, cognitive impairment, and disordered hormonal response to these disease states. *Sentra AM* is a medical food that must be used under the active or ongoing supervision of a physician. Medical foods are intended to address the different or altered physiologic requirements that may exist for individuals who have distinctive nutritional requirements arising from metabolic disorders, chronic diseases, injuries, premature birth associated with inflammation, and other medical conditions, as well as from pharmaceutical therapies.¹

Neurotransmitters facilitate the exchange of sensory and metabolic information between the brain, the spinal cord, and peripheral nervous system by propagation of electrical impulses over specific neural pathways. Acetylcholine modulates synaptic transmissions that initiate muscle contraction, regulate circadian rhythms and autonomic nervous system activity, mediate cognitive processes, and modify the stress response. Glutamate interacts with acetylcholine in regulating circadian rhythms and stimulation of arousal and alertness. It is also involved in formation of memory. Acetyl-L-carnitine enhances acetylcholine synthesis and activity and exhibits independent effects on neurotransmission. Patients with disorders that involve imbalances in choline, glutamate, and acetyl-L-carnitine benefit from increased intakes of these nutrients. *Sentra AM* is designed with the complete balance of nutrients needed to meet the increased requirements of patients with muscle dysfunction, sleep disturbances, cognitive impairment, and chronic stress disorders due to imbalances in these neurotransmitters.

Ingredients

Sentra AM is a patented blend of neurotransmitters and neurotransmitter precursors (choline bitartrate and glutamate); activators of precursor utilization (acetyl-L-carnitine, glutamate, and cocoa powder); polyphenolic antioxidants (grape-seed extract, hawthorn berry, cocoa powder); an amino acid uptake stimulator (gingko biloba); an adenosine antagonist (cocoa powder); and an inhibitor of the attenuation of neurotransmitter production associated with precursor administration (grape-seed extract). Each of the nutrients included in the formulation has been specifically selected based on scientific support for its role in modulating cellular processes that

¹ As defined in the guidelines issued by the Center for Food Safety and Nutrition, United States Food and Drug Administration (FDA).

support neuromuscular and neurological activities. These roles are summarized in this monograph in the section *Scientific Support for Use of **Sentra AM** in Disorders Associated with Muscle Dysfunction and Chronic Stress*. The other ingredients in the formulation are functional components of the *Targeted Cellular Technology™* system.

All of the ingredients included in **Sentra AM** are classified as generally recognized as safe (GRAS) by the United States Food and Drug Administration (FDA). To qualify for GRAS status, a substance that is added to a food, including a medical food, has to be supported by data demonstrating that it is safe when consumed in the amounts obtained from these foods as they are typically ingested or prescribed.

Targeted Cellular Technology™

Sentra AM has been formulated using *Targeted Cellular Technology* (TCT), an integrated molecular system that facilitates the uptake and utilization of neurotransmitter precursors by target cells within the nervous system. This 5-component patented system consists of (1) specific neurotransmitter precursors; (2) a stimulus for the neuronal uptake of these precursors by specific neurons; (3) an adenosine antagonist that blocks the inhibitory effect of adenosine on neuronal activity (adenosine brake); (4) a stimulus to trigger the release of the required neurotransmitters from targeted neurons; and (5) a mechanism to prevent attenuation of the precursor response, a well known phenomenon associated with precursor administration.

Use of *Targeted Cellular Technology* improves the metabolic efficiency of neurotransmitter synthesis, thereby reducing the amounts of precursors needed to correct neurotransmitter imbalances. Use of *Targeted Cellular Technology* also ensures that the appropriate amounts of neurotransmitter precursors are delivered to the target neurons with the appropriate timing. As such, *Targeted Cellular Technology* synchronizes the availability of the precursor supply with the fluctuating demand for the corresponding neurotransmitters, which is especially important for processes that are regulated by circadian rhythms and are therefore sensitive to the timing of neurotransmitter synthesis and release such as balanced activity of the autonomic nervous system and diurnal regulation of the hypothalamus-pituitary-adrenal axis (1,2).

Previous attempts to provide an exogenous source of precursor amino acids and other biogenic amines in the quantities required to support neurotransmitter synthesis for individuals with specific needs necessitated that large amounts of these nutrients be added to the formulations. For patients whose precursor requirements are considerably higher than normal, the amounts of exogenous amino acids needed are not practical to consume on a daily basis. In addition, ingestion of large quantities of amino acids is undesirable due to an increased risk of adverse effects. Metabolic efficiency is also decreased when large amounts of amino acids are delivered simultaneously because intestinal membrane transport receptors will be saturated which

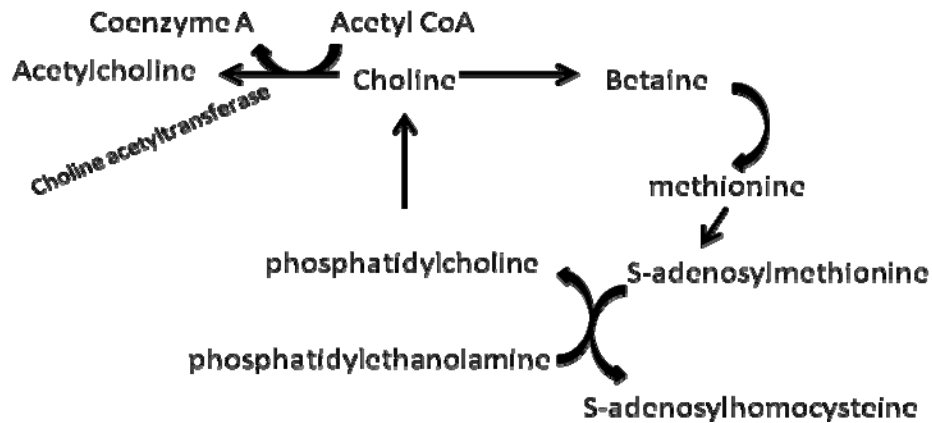
decreases fractional amino acid absorption and attenuates the tissue response to the supplemental amounts provided. Improving metabolic efficiency in uptake and utilization of neurotransmitter precursors by target neurons using *Targeted Cellular Technology* allows ingestion of smaller amounts of amino acids to elicit the same response as larger amounts, making daily dosing more feasible and reducing the potential for tolerance. Unlike pharmaceutical products which are not innate components of the processes that regulate muscular and neurological functions and thus may lose their effectiveness in a relatively short period of time, the effectiveness of *Sentra AM* is not attenuated.

Metabolism

Sentra AM is a source of amino acids, biogenic amines, and other nutrients designed for patients with muscle dysfunction, sleep disturbances, cognitive impairment involving arousal, alertness and memory, and dysregulation of the hormonal response to stress. Patients with these conditions require additional amounts of choline, acetyl-L-carnitine, and glutamate to support neuromuscular function, sleep and wakefulness, cognitive processes, and the stress response. Under normal conditions, these nutrients would be considered nonessential because sufficient amounts can be synthesized from substrates available endogenously through *de novo* biosynthesis, but when needs are altered by increased metabolic demand, the usual rate of synthesis is no longer sufficient and supplemental amounts must be consumed.

Choline and carnitine are considered nonessential nutrients under normal physiological conditions because they can be supplied in sufficient amounts to meet usual metabolic demand. The requirement for choline is based in part on its use as a precursor in the synthesis of acetylcholine. Acetylcholine is produced from choline in an acetylation reaction catalyzed by choline acetyltransferase with acetyl coenzyme A (CoA) as the acetyl group donor (Figure 1). Under usual metabolic conditions, the primary source of choline for acetylcholine synthesis is from the hydrolysis of the membrane phospholipid phosphatidylcholine (lecithin) which serves as a reservoir of choline to meet short-term demands. When the demand for acetylcholine exceeds the amount of choline that can be supplied by the membrane phospholipid pool, dietary choline becomes an increasingly more important source. *Sentra AM* provides additional amounts of choline to meet the increased needs for acetylcholine when demand is remains high over an extended period. By supplying an exogenous source of choline, *Sentra AM* prevents the depletion of membrane phosphatidylcholine and thus preserves the structural integrity of the cell.

Figure 1. Biosynthesis of Acetylcholine



The need for carnitine is also increased when demands for acetylcholine are high since it is utilized as a precursor of acetyl-L-carnitine which enhances the synthesis and activity of acetylcholine. In a reaction similar to the synthesis of acetylcholine from choline, carnitine is produced by carnitine acetyltransferase in an acetylation reaction involving transfer of an acetyl group from acetyl CoA. Sufficient amounts of acetyl-L-carnitine are normally produced from carnitine, but when the rate of cholinergic activity is elevated over an extended period of time, the demand for acetyl-L-carnitine cannot be met by endogenous synthesis of carnitine.

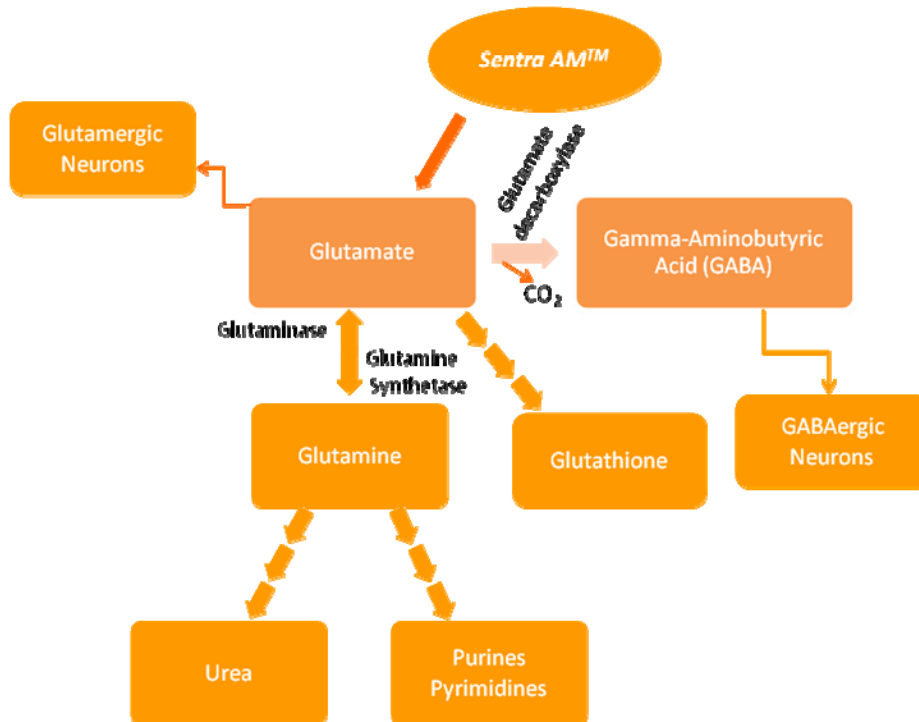
Sentra AM provides additional acetyl-L-carnitine to sustain the increased rate of acetylcholine synthesis and enhance its activity when the rate of cholinergic-mediated activities is increased.

A major role of acetyl-L-carnitine in cellular metabolism is to facilitate transport of acetyl groups into the mitochondria during fatty acid oxidation. The same function would also ensure that a sufficient number of acetyl groups were available to cholinergic neurons at times of elevated activity and could explain how acetyl-L-carnitine enhances the synthesis of acetylcholine. Although the primary role of acetyl-L-carnitine is to facilitate the uptake of acetyl CoA into the mitochondria during fatty acid oxidation, it also supports the synthesis and activity of acetylcholine. This effect may also be involved in its effect on promoting acetylcholine synthesis which may be related to facilitate neuronal uptake of acetyl CoA similar to its effects on mitochondrial uptake of acetyl CoA.

As a nonessential amino acid, availability of glutamate is not normally dependent on dietary sources, but under conditions of increased demand, metabolic competition for this amino acid depletes the supply available. Glutamate functions as a neurotransmitter and is the precursor of gamma-aminobutyric acid (GABA), the major inhibitory neurotransmitter in the central nervous system. Under usual physiological conditions, glutamate is derived from several endogenous sources which include deamination of glutamine; however, glutamine is also utilized as a

precursor for synthesis of other important biological compounds such as glutathione, purines, pyrimidines, and urea Figure 2). These competitive demands for glutamine limit the amount of glutamate available. As a source of glutamate, *Sentra AM* improves metabolic efficiency by ensuring that there is an adequate amount of glutamate available for other uses.

Figure 2. Competing Pathways for Utilization of Glutamate



Dosage

The recommended dose of *Sentra AM* is 2 capsules taken in the morning. An additional dose may also be taken during the day if fatigue continues or returns. As with any medical food, the best dosing protocol should be determined by assessment of individual needs. *Sentra AM* can be taken with other prescription medications. There are no known interactions between *Sentra AM* and any medication.

The amounts of each ingredient consumed at the recommended doses of *Sentra AM* are presented in Table 1.

Table 1. Sentra AM Composition

Ingredient [Select]	mg/kg body weight ¹
choline bitartrate	1.0 – 7.7
L-glutamate	0.4 – 3.1
Acetyl-L-carnitine	0.2 – 4.1
grape seed extract	0.2 – 1.5
cocoa powder	0.2 – 1.5
Hawthorn berry	0.1 – 1.8

¹Dosing range of 2 to 4 capsules daily

Side Effects

As with any amino acid therapy, headache, nausea, or dry mouth may be experienced in some people after beginning treatment with *Sentra AM*. These symptoms are mild and temporary, and readily managed by increasing fluid intake. The development of side effects from *Sentra AM* can be minimized by careful titration of the dosage. All of the ingredients in *Sentra AM* are regularly consumed in amounts normally found in foods or dietary supplements; therefore development of an adverse reaction to *Sentra AM* is not expected.

Abbreviations and Definition of Terms

The definitions for the abbreviations and terms referenced in this monograph are summarized in Table 2.

Table 2. Abbreviations and Definitions of Terms

Term/Abbreviation	Definition
Antioxidant	Protects against oxidative cell damage from exposure to free radicals
Autonomic Nervous System	Component of the nervous system that maintains a steady state within the internal environment
Biogenic amine	Biologically active substances that contain amine groups but do not meet the structural definition of an amino acid
Circadian Rhythm	Consistent pattern of changes in physiological, biochemical, and behavioral processes regularly repeated over a 24-hour period
Cholinergic	Neurons that synthesize and release acetylcholine
Excitatory Neurotransmitters	Transmit neural signals which accelerate the rate and frequency of transmission by depolarizing postsynaptic neuronal membranes
Glutamatergic	Neurons that synthesize and release glutamate

Term/Abbreviation	Definition
HPA axis	Hypothalamus-pituitary-adrenal axis; mediates the physiological and metabolic response to stress
Inhibitory Neurotransmitters	Transmit neural signals which slow the rate and frequency of transmission by hyperpolarization of neuronal membranes
Neurotransmitter	Facilitate the exchange of sensory and metabolic information between the central and peripheral nervous systems by propagation of electrical impulses over specific neural pathways
NMDA receptor	N-methyl-D-aspartate (NMDA) receptors which are present in glutaminergic synapses in the central nervous system
Parasympathetic Activity	Component of the autonomic nervous system which slows body processes to conserve and restore energy reserves
Phosphotidylcholine	A phospholipid component of cell membranes which serves as a reservoir of choline; lecithin
Sarcolemma	A two-part membrane that surrounds the muscle fiber and forms the synaptic cleft at the neuromuscular junction
Suprachiasmatic Nucleus (SCN)	Comprises two pin-sized structures in the hypothalamus which regulates circadian rhythms
Sympathetic Activity	Component of the autonomic nervous system responsible for mobilizing energy stores
Targeted Cellular Technology™	A patent-pending process which facilitates endogenous production, uptake, and utilization of neurotransmitter precursors.

Mechanism of Action

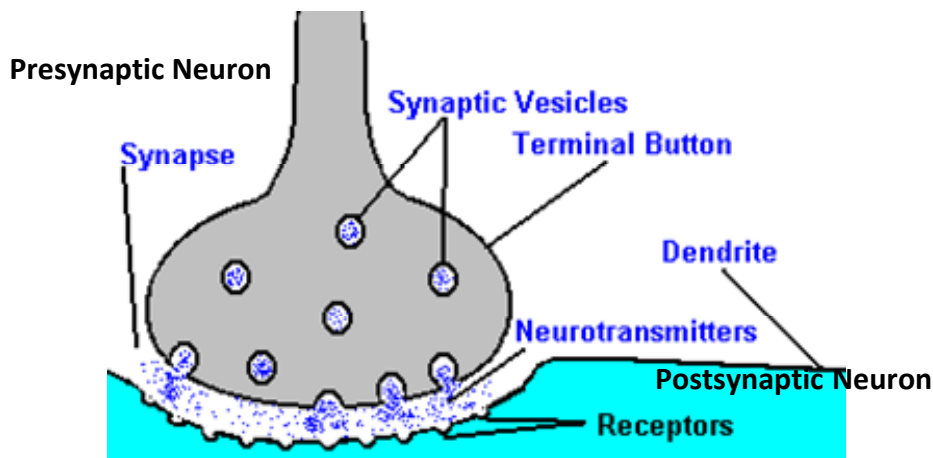
Understanding the mechanism of action of *Sentra AM* in management of disorders characterized by muscle dysfunction, sleep disturbances, cognitive impairment, and dysregulation of the stress response requires a brief overview of the biochemical and physiological events that support muscle function, sleep and wakefulness, cognitive processes, and the stress response, particularly the production of acetylcholine. Dysregulation of the autonomic nervous system, the hypothalamus-pituitary-adrenal (HPA) axis, and circadian rhythms are key elements in the pathophysiology of these conditions all of which are mediated by acetylcholine. Clinical syndromes associated with fatigue are often accompanied by musculoskeletal pain and aggravated by physical or emotional stressors (2-7). Muscle fatigue and pain, sleep disturbances, and impaired memory and concentration are typical of patients with fibromyalgia and chronic fatigue syndrome suggesting a common pathological link between these and other conditions with similar symptomology.

The regulation of neuromuscular activity, autonomic nervous system function, physiological responses to stress, sleep and wakefulness, and certain cognitive functions such as memory, arousal, and alertness are each dependent upon the neurotransmitters acetylcholine and glutamate to transmit signals between the brain and effector organs and over various circuits within the brain (8-15). Neurotransmitters are amino acids or amino acid derivatives that function as mediators of physiological responses to physical, chemical, or electrical stimuli. The interaction

between one or more of these stimuli with specific membrane receptors generates an action potential which is transmitted between presynaptic neurons and postsynaptic neurons by a series of reactions involving neurotransmitters.

Action potentials trigger changes in resting membrane potential at the terminal endings of presynaptic neurons causing the release of neurotransmitters into the synaptic cleft where they rapidly bind to receptors on postsynaptic neurons (Figure 3). Neurotransmitter binding alters the resting membrane potential of postsynaptic neurons triggering a series of action potentials that are sent down the axons of nerve fibers to their terminal endings. This series of electrochemical events is repeated until the signals reach specific centers in the brain where they are processed. Output from the brain is then sent down efferent pathways to innervated effector organs as electrical signals also transmitted by neurotransmitters through a succession of changes in resting membrane potentials to elicit a response. Action potentials that originate within the brain are also relayed by neurotransmitters over internal circuits between different regions or down efferent pathways to effector organs by the same series of electrochemical events.

Figure 3. Neurotransmitter Activity in Presynaptic and Postsynaptic Neurons



The rate of signal transmission between presynaptic and postsynaptic neurons in neural pathways depends on the electrochemical properties of the neurotransmitter and the nature of the postsynaptic membrane receptors. Excitatory neurotransmitters such as glutamate depolarize the membrane which lowers the stimulus threshold for firing and increases the frequency and rate of transmission. Inhibitory neurotransmitters such as GABA have the opposite effect of hyperpolarizing the membrane which raises the stimulus threshold and reduces the frequency and rate of transmission. Acetylcholine can exhibit both excitatory and inhibitory effects on neuronal membranes depending upon the area of the brain where the receptors are located.

The initiation of muscle contraction and synaptic transmission of sensory information on length, tension, tone, and velocity of muscle fibers from the muscle to the brain are the major functional roles of acetylcholine in skeletal muscle (6, 16-17). The events that trigger muscle contraction begin with generation of action potentials at the distal terminal endings of motor neurons which are conducted along the sarcolemma of the muscle fibers. At the neuromuscular junction, these action potentials rapidly depolarize the membrane which opens voltage-gated calcium channels to increase calcium influx. The resulting increase in calcium concentration activates a series of intracellular signaling events which stimulate the migration of acetylcholine-containing vesicles to the membrane surface where they rupture to release the neurotransmitter into the synaptic cleft. Upon binding to receptors on the motor endplate, acetylcholine increases sodium influx which depolarizes the membrane causing the muscle to contract.

Within the brain, cholinergic activity is concentrated in the hypothalamus, hippocampus, and basal forebrain as and in other sites in proximity to areas of glutamatergic activity where a number of critical processes are regulated such as activation of the HPA axis (2, 8, 18-19). In response to psychological, physiological, and environmental stressors as well as to changes in metabolic factors, cholinergic activity increases in the paraventricular nucleus of the hypothalamus to stimulate the release of arginine vasopressin (AVP) and corticotropin releasing hormone (CRH). AVP and CRF bind to receptors on the corticotroph cells in the anterior pituitary and initiate a sequence of biochemical events that begins with the secretion of adrenal corticotrophic hormone (ACTH) into the systemic circulation (2, 5, 9, 19). ACTH is transported to the adrenal cortex where it binds to receptors which trigger the release of glucocorticoids. These hormones act in a number of tissues to stimulate metabolic processes that release glucose into the blood. These events are critical to the control of flight and fight, immune responses, and regulation of neurotransmitter systems.

Activation of the HPA axis is terminated by negative feedback circuits involving glucocorticoid binding to sites in the hypothalamus which suppress CRH release and to sites in the pituitary which inhibit secretion of ACTH in response to CRH (2). These negative feedback loops may be compromised in chronic stress disorders due to long-standing glucocorticoid suppression of CRH release. The adrenal response to ACTH is therefore blunted in stressed subjects who show higher trough levels of cortisol than normal subjects under the same set of conditions. A reduction in adrenal glucocorticoid function is also observed in patients with chronic fatigue syndrome which is consistent with a central nervous system defect in activation of the HPA axis that involves suppression of CRH activity in the hypothalamus. When CRH release is suppressed over the long-term, AVP becomes the more important secretagogue acting on the pituitary. AVP normally acts synergistically with CRH to amplify pituitary release of ACTH, and thus in the absence of CRH, can promote a residual response as long as sufficient cholinergic activity is present.

The role of acetylcholine and glutamate in modulation of circadian rhythms is an important component of the regulation of autonomic nervous system activity as well as the activation of the HPA axis under nonstressed conditions (20). Both of these systems exhibit diurnal patterns of activity the absence of acute or chronic stress which are closely coordinated with the sleep/wake cycle indicating that circadian rhythms are involved in their regulation (19, 21). At sleep onset, sympathetic activity decreases, parasympathetic activity increases, and cortisol concentration reaches its lowest level. Prior to awakening, sympathetic activity increases, parasympathetic activity decreases, and cortisol concentration increases with peak levels reached shortly after awakening (20, 22). Elevated cortisol levels are accompanied by heightened mental arousal and alertness (22). In addition to interfering with diurnal fluctuations in cortisol levels, impaired cholinergic ganglionic synaptic transmission is an important cause of autonomic failure (23).

The diurnal patterns of the autonomic nervous system and HPA axis implicate the involvement of the suprachiasmatic nucleus (SCN) in modulating the activities of these systems. The SCN receives input from signals generated by changes in light exposure transmitted by acetylcholine, glutamate, and GABA (10, 24). Cholinergic activity is sensitive to changes in light increasing with increased light and decreasing with decreased light. In response to light-induced cholinergic input, the SCN sends out signals that stimulate the release of CRH in the hypothalamus. The effects of the SCN on CRH are consistent with the high cortisol levels observed upon awakening and the low levels observed in the evening and during the night (20). Increases in cortisol promote wakefulness, mental arousal and alertness, all of which sensitize the HPA axis to negative feedback control thereby inhibiting further release of cortisol (2-3, 5, 20, 25). Imbalances in acetylcholine and glutamate disrupt the circadian rhythms which influence cortisol levels and autonomic nervous system activity that regulate normal patterns of sleep and waking (10, 24, 26-27).

Scientific Support for Use of *Sentra AM* in Disorders of Associated with Muscle Dysfunction, Sleep Disturbances, Cognitive Impairment, and Dysregulation of the Stress Response

The effectiveness of *Sentra AM* in the production of the neurotransmitters acetylcholine and glutamate in the management of chronic and generalized fatigue, fibromyalgia, PTSD, neurotoxicity-induced fatigue syndrome, and cognitive impairment involving memory, alertness, mood, and arousal is supported by an extensive body of experimental and clinical data. These data have identified specific roles for each neurotransmitter related ingredient in muscle function, sleep and wakefulness, cognitive processes, and the response to stress. Disorders characterized by muscle pain, fatigue, sleep disturbances, loss of memory, and poor concentration have a common underlying pathology involving deficits in cholinergic activity (2, 7, 19, 21, 28-29). Acetylcholine is the primary neurotransmitter involved in synaptic transmission in the sympathetic nervous system and in the neuromuscular junction, and the only

neurotransmitter with this role in the parasympathetic nervous system (1, 16, 30). Thus, acetylcholine is the central neurotransmitter in the autonomic nervous system and functions as both an excitatory and inhibitory neurotransmitter in the sympathetic nervous system, and as an excitatory neurotransmitter in the parasympathetic nervous system, except in cardiac smooth muscle where it exhibits inhibitory effects that slow heart rate. In regions of the brain where arousal and memory are modulated, acetylcholine functions as an excitatory neurotransmitter (31-32).

Muscle fatigue and weakness is the result of failure of the muscle to generate and sustain force which reflects cholinergic deficits in the motor neurons of the brainstem and the neuromuscular junction (8). Muscle function is also compromised by peripheral nerve damage which decreases motor neuron sensitivity to acetylcholine resulting in neurodegeneration (9). Clinical signs of acute neurotoxicity include fatigue, generalized weakness, inability to concentrate, memory deficits, and confusion attributed to cholinergic neuron damage in the hippocampus (33). Acetyl-L-carnitine protects against neurodegeneration in the hippocampus by facilitating the binding of nerve growth factor and by providing a substrate pool of acetyl CoA for energy production to support tissue repair (34-35).

Muscle pain in PTSD is expressed in a pattern similar to that in fibromyalgia (tender points) suggesting an abnormality common to both conditions involving regulation of the autonomic nervous system and the stress response (3, 7, 25). Muscle pain in fibromyalgia and chronic fatigue syndrome is associated with blunted cortisol release and inappropriate levels of cortisol when exposed to stressors under experimental conditions (2-3, 15). Skeletal muscle nociceptor (pain receptor) dysfunction contributes to heightened pain sensitivity which disrupts the balance in neuroendocrine control of the stress response and contributes to the CRH abnormalities observed in these diseases (3, 4). In non-stressed conditions, patients with these disorders do not show normal diurnal fluctuations in plasma cortisol levels but instead have elevated levels which are sustained throughout the day (2). Administration of exogenous corticosteroids enhances choline uptake by central cholinergic neurons indicating that acetylcholine is involved in the feedback regulation of corticosteroid levels and that this feedback mechanism is less responsive to elevated cortisol levels in chronic disorders associated with muscle pain and fatigue. (5,36). Acetyl-L-carnitine enhances acetylcholine-modulated regulation of cortisol levels by facilitating binding of glucocorticoids in the hippocampus which suppresses release of CRH (34).

Normal diurnal patterns of autonomic nervous system activity are also absent in fibromyalgia, chronic fatigue syndrome, and PTSD which together with absence of these patterns in cortisol levels, indicate a disturbance in circadian rhythms linked to cholinergic activity in patients with these conditions (2, 21). Sleep disturbances are also common among these patients which further implicates altered circadian rhythms as an important pathological feature (25, 28). The effects of acetylcholine on regulation of the diurnal fluctuations in plasma cortisol levels in nonstressed

conditions are consistent with its effects on the sleep cycle and suggest that they may also play a role in modulating the circadian patterns of autonomic activity. After exposure to experimentally-induced psychological stress, sleep patterns were disturbed least in subjects who had the highest density of cholinergic neurons in the hypothalamus where circadian rhythms originate in the suprachiasmatic nucleus (SCN) (15).

Fibromyalgia, chronic fatigue syndrome, and PTSD are increasingly recognized as diseases of disordered autonomic nervous system function in which both sympathetic and parasympathetic activity are suppressed, with a relatively greater suppression of parasympathetic function and thus more pronounced symptoms of sympathetic deficits (3-4, 12). Exposure to different stressors activates efferent sympathetic pathways concurrently with somatomotor efferent pathways within several regions of the brain including the paraventricular nuclei of the hypothalamus where cholinergic activity is concentrated (6). Disorganization of this somatomotor-sympathetic circuitry may be involved in maladaptive physiological and emotional responses to stress.

Many of the activities central to the stress response are mediated by glutamate neurotransmission in the prefrontal cortex where some components of cognitive function such as working memory and vigilance are also regulated indicating that glutamatergic activity may be a common mechanism by which stress affects cognition (19). Patients with fibromyalgia who experience sleep disturbances have significantly higher blood glutamine levels than controls suggesting that altered glutamate metabolism may be an additional factor in this disorder (9, 37). Glutamate interacts with acetylcholine to stimulate wakefulness through synaptic transmission of light-induced signals to the SCN (9-11). Desynchronization of the wake-promoting effects of glutamate and acetylcholine with the sleep-promoting effects of GABA and serotonin disrupts the normal circadian rhythms which modulate the sleep-wake cycle, activation of the HPA axis, and the balance in activity of the autonomic nervous system activity during periods of sleep and wakefulness (10-11, 24, 27, 38-39).

Interactions between glutamate and acetylcholine have been identified in mediation of pain, stress, sleep, arousal, vigilance, and memory (8-9, 14, 40-43). Glutamatergic neurons (glutamate secretors) are widely distributed in the brain and concentrated in areas of high cholinergic activity. Under conditions where glutamatergic receptor activity is inhibited, cholinergic transmission is stimulated and its receptors are upregulated in the hypothalamus (9). Both neurotransmitters initiate and maintain arousal in the hypothalamus and both promote vigilance in the basal forebrain through effects on hypocretin- or orexin-secreting neurons which mediate neuroendocrine control of arousal (40-44). Orexins are also involved in the stress response and low concentrations are found in hypothalamic disorders (8, 44). Acetylcholine activates a special type of glutamate receptor, the N-methyl-D-aspartate (NMDA) receptor, which modulates a number of processes including the synaptic changes involved in memory formation and

processing (8). The acetylcholine receptor and the NMDA receptor are coexpressed and anatomically adjacent on cell membranes.

Defects in memory have been attributed to disturbances in cholinergic and glutamatergic activity (19, 45-47). Severe damage to the hippocampus in areas where both types of neurons are found causes profound difficulties in forming new memories (anterograde amnesia), and often affects memories formed prior to the damage (retrograde amnesia) (31, 48-49). Memory is also impaired by diminished activity in the cholinergic centers in the hypothalamus and basal forebrain where wakefulness and the ability to concentrate and stay alert are also regulated (9, 45, 50-52).

Glutamate also has a key role in modulating the processes that establish long term potentiation (LTP), a form of neural plasticity widely believed to be one of the main neural mechanisms by which memory is stored in the hippocampus (8, 53-54). The hippocampus is one of the first areas of the brain to suffer damage in Alzheimer's disease and memory problems and disorientation are among the first symptoms (8). Disorders of acetylcholine metabolism have been associated with Alzheimer's Disease (32, 55-59) and other dementias (48, 60-63). Degeneration of cholinergic innervation in the hippocampus and cerebral cortex is a frequent observation in patients with these conditions. Cholinesterase inhibitors which maintain concentrations of the acetylcholine in cholinergic synapses through inhibiting the breakdown of acetylcholine are frequently used in the pharmacological treatment of Alzheimer's disease (59, 64).

Consumption of supplemental acetyl-L-carnitine has also been shown to improve cognitive function, functional status, and behavior in a study of 23 patients with mild Alzheimer's disease who had been previously nonresponsive to cholinesterase inhibitors. After 3 months of treatment with 2 g/d of acetyl-L-carnitine in combination with a cholinesterase inhibitor, the rate of response increased from 38% to 50% in this group of patients (35). As with cholinesterase inhibitors, acetyl-L-carnitine increases the availability of acetylcholine but in contrast to these drugs, it promotes acetylcholine synthesis instead of inhibiting its hydrolysis. Acetyl-L-carnitine also enhances cholinergic activity by a cholinomimetic effect (34). Although the exact mechanism by which acetyl-L-carnitine enhances acetylcholine synthesis has not been identified, it likely involves the facilitation of acetyl CoA uptake by cholinergic neurons as it functions in mitochondria. By ensuring that sufficient amounts of acetyl groups are available for production of acetylcholine, acetyl-L-carnitine supports an increased rate of synthesis when demand for the neurotransmitter is increased. Acetyl-L-carnitine also blocks the postsynaptic inhibitor potential of cholinergic receptors and directly stimulates synaptic effects. Its independent effects on synaptic transmission involve neurotrophic factors and neurohormones, synaptic morphology, and coordination of activities of multiple neurotransmitters (65).

A summary of the roles of each of the ingredients in *Sentra AM* is presented in Table 3.

Table 3. Roles of the *Sentra AM* Ingredients in Disorders Associated with Muscle Dysfunction, Sleep Disturbances, Cognitive Impairment, and Dysregulation of the Stress Response

Ingredient	Effector Molecule	Function	Role in Neurotransmitter Metabolism
Choline	Acetylcholine	Inhibitory (midbrain reticular formation) and excitatory (basal forebrain) neurotransmitter	Primary neurotransmitter of the autonomic nervous system (1, 30); initiates muscle contraction (16); modulates circadian rhythms (15), activation of the HPA axis (2, 20-21), autonomic nervous system activity (20), and memory, alertness, and arousal (33)
Glutamate	Glutamate GABA	Excitatory neurotransmitter	Interacts with acetylcholine; modulates circadian rhythms and circadian-driven processes, and arousal, alertness, and memory (8-9, 14, 40-43)
Acetyl-L-carnitine	Acetyl-L-carnitine	Precursor uptake stimulator; cholinomimetic agent	Enhances synthesis and activity of acetylcholine (34); promotes binding of nerve growth factor and glucocorticoids in the hippocampus (34-35); blocks post-synaptic inhibitor potential and directly stimulates synaptic transmission (65)
Cocoa Powder	caffeine	Adenosine antagonist	Increases neuronal activity by competitively binding to adenosine receptors which disinhibits the adenosine brake (66-67)
Grape seed extract	Polyphenols	Antioxidant	Preserves receptor membrane integrity and prevents attenuation of responses to neurotransmitter precursors (68-70)
Hawthorn Berry	Flavonoids, oligomeric proanthocyanidins, triterpene acids	Antioxidant	Preserves receptor membrane integrity (71)

Nutritional Requirements in Disorders Associated with Muscle Dysfunction, Sleep Disturbances, Cognitive Impairment, and Dysregulation of the Stress Response

The nutritional requirements of greatest interest to patients with chronic disorders associated with muscle pain, fatigue, sleep disturbances, loss of memory, and poor concentration are nutrients or dietary factors that support muscle function, sleep and wakefulness, cognitive

processes, and the stress response. These include choline, glutamate, and carnitine which contribute to or enhance the synthesis and activity of acetylcholine. A balance in the amounts of these neurotransmitters is important because the interactions between them will determine the intensity of the response to a particular stimulus. *Sentra AM* is formulated to ensure the availability of acetylcholine precursors and glutamate along with the neuromodulator (acetyl-L-carnitine) to support the processes dependent on normal cholinergic and glutamatergic activity.

The concept that nutrient requirements are modified by disease has been recognized for more than 30 years, and is supported by studies which have shown changes in plasma, urinary, and tissue levels of nutrients associated with abnormalities in physiological endpoints reflective of specific pathologies (72). These requirements can be estimated by identifying the level of intake at which alterations in related physiological responses are improved, indicating that the balance between intake and metabolic demand has been favorably modified. For example, improvement in fatigue, muscle pain, sleep patterns, memory, arousal, and alertness following consumption of additional amounts of choline, glutamate, and acetyl-L-carnitine from *Sentra AM* indicates that additional amounts were needed by individuals with muscle dysfunction, sleep disturbances, cognitive impairment, and stress-related disorders. The degree of coordination of activity among these neurotransmitters and the feedback loops involved in homeostasis underscores the importance of modulating the balance and amounts of dietary precursors required for synthesis (73-77).

The presence of a disease with an underlying pathology involving increased requirements for neurotransmitters will increase the requirements for dietary precursors and other dietary factors involved in the metabolism of these neurotransmitters to restore homeostasis (37, 72, 76-81). As blood levels of these dietary precursors rise in response to increased intakes, the concentration-dependent rate of precursor uptake by target neurons is increased, making more substrate available for neurotransmitter production and subsequent release (82). Changes in intakes of dietary precursors of neurotransmitters influence physiological responses through these effects on neurotransmitter production and release (74-75, 79, 83-87).

Neuronal uptake of amino acids and other dietary precursors is a concentration-driven process; therefore, intakes of dietary precursors must be high enough to increase the extracellular to intracellular ratio to a level that will drive a rapid rate of uptake (83-87). The rate of precursor uptake is important because the enzymes involved in neurotransmitter synthesis are confined to specific neurons and thus the amount available to these enzymes is the limiting factor in neurotransmitter production. The balance of neurotransmitters released from these neurons is also important for efficient signal transmission because of the highly interrelated functions of neurotransmitters and the complexity of multiple feedback loops required for homeostasis. These interactions explain why increased requirements for any one neurotransmitter can influence the activities of the others, potentially inducing absolute and relative deficiencies (11, 88).

Acetylcholine is produced in the terminal endings of cholinergic neurons and in regions of the brain where choline acetyltransferase is concentrated. Under steady stated conditions, brain enzyme is not completely saturated, thus the rate of acetylcholine production is driven by the availability of choline and acetyl CoA (82, 89). Studies have confirmed that exogenous choline can be utilized as a precursor in acetylcholine synthesis by central cholinergic neurons (90). Dietary choline derived from serum choline that has crossed the blood brain barrier is largely incorporated into the membrane phosphatidylcholine pool; however, the appearance of choline in cerebrospinal fluid confirms the presence of a free choline pool in the brain (36). Most of the free choline is phosphorylated by choline kinase in order to moderate the rate of acetylcholine synthesis in the presence of increased availability of precursor (90). A demand for acetylcholine stimulates hydrolysis of cell membrane phosphatidylcholine to release free choline into the pool (91-92).

Dietary choline is a more important source of precursor for acetylcholine synthesis by the brain than de novo synthesis (93-94). Following treatment with choline chloride, the rate of choline transport across the blood brain barrier is increased by amounts proportional to the serum concentration. The increase in serum choline also stimulates the release of acetylcholine from cholinergic neurons. High levels of serum choline promote the increased expression of high affinity choline transporters on cholinergic neurons which regulate the synaptic availability of choline and facilitate the release of acetylcholine (36). These transporters are inhibited through a feedback mechanism initiated by the increase in synaptic acetylcholine to modulate the amount of choline uptake. Anticholinergic drugs such as chlorpromazine, atropine, and cholinesterase inhibitors decrease acetylcholine release by inhibition of these transporters.

Membrane phosphatidylcholine is the major source of choline for acetylcholine synthesis under steady state conditions and maintains the free choline pool when extracellular choline levels are inadequate to support rapid rates of cholinergic firing (13, 36). Brain phosphatidylcholine levels decrease in parallel with levels of circulating choline indicating that brain choline concentrations are maintained within narrow limits at the expense of larger tissue pools of phosphatidylcholine and other phospholipid precursors (36, 93). If an increased demand for acetylcholine is extended over a prolonged period, dietary choline becomes an increasingly more important source of precursor. Under these conditions, if a supplemental source of choline is not provided, loss of membrane phosphatidylcholine will continue compromising cell membrane function and triggering apoptosis (89, 92, 94-98).

Clinical evidence of a human choline deficiency was first reported in adults receiving total parenteral nutrition (99-100). These patients exhibited hepatic morphologic and aminotransferase abnormalities which were reversed by choline-supplemented TPN. Changes in choline levels in the blood and urine correspond to changes in dietary choline intake and therefore measurements of blood and urine choline levels have been used to evaluate choline status following dietary

deficiency or augmentation. Dietary augmentation can increase serum choline by 52% while a choline-deficient diet can decrease serum choline by 20% (36). Changes in blood and urine markers of organ dysfunction (muscle and liver enzymes) have been observed within 2 weeks of consuming a choline-deficient diet (98, 101). Low blood levels of choline indicate that the requirements for the dietary precursors are not being met at current levels of intake (36, 82, 93, 102). Although serum choline levels are decreased by a choline-free diet, brain choline levels remain relatively stable indicating that the brain is given metabolic priority at the expense of other tissues when available choline is limited (90).

Clinical signs of choline deficiency have also been documented in men with otherwise normal nutritional status who consumed a choline deficient diet for a period of < 2 weeks (100, 102-103). Decreased levels of plasma choline and phosphatidylcholine observed in these men were accompanied by elevated alanine aminotransferase, a biochemical marker of liver damage, and elevated creatine kinase, a biological marker of muscle damage (101,103-107). Muscle and liver damage are the most frequently observed signs of a dietary choline deficiency. Fatty liver results from depletion of the phosphatidylcholine pool which limits membrane fatty acid transport causing fat to accumulate. The fragility of phospholipid-depleted membranes and apoptosis are the primary contributors to muscle damage in a choline deficiency (104). Catabolism of phosphatidylcholine drives cellular uptake of choline indicating that increased hydrolysis of this membrane phospholipid signals an increased demand for choline (36).

Dietary choline deficiency has also been associated with sleep apnea syndromes, disorders of restorative sleep (76, 82, 108), and memory disorders (80, 89, 106). Age-related memory loss is exacerbated by choline deficiency in rats and mice. In a double blind study conducted in normal college students, explicit memory measured by the number of trials in a serial-learning word test was improved after a single dose of 10 g of choline and 25 g phosphatidylcholine (109). Memory enhancing effects were also observed after augmentation with 1000 mg cytidine diphosphocholine (CDP-choline), a precursor of phosphatidylcholine, in a randomized, double-blind, placebo-controlled trial conducted in adults with memory deficits without dementia. The amounts of choline required to maintain cognitive function in humans is unknown and therefore must be individualized for each patient.

There is currently no recommended dietary allowance (RDA) for choline; however, based on a review of the available data, the Food and Nutrition Board of the Institute of Medicine has established 550 mg as the adequate intake level for adults, with an upper tolerable limit of 3000 to 3500 mg (110). Since the richest dietary sources of choline are eggs and high fat meats, many adults, particularly women and those who are on fat-restricted diets, are not consuming the recommended amounts (96). A high degree of individual variation in choline requirements may exist. In one study, 10% of subjects had to consume 850 mg/d of choline to prevent clinical signs of muscle and liver damage.

Several clinical studies have demonstrated statistically significant improvements in musculoskeletal pain, fatigue, and memory in patients with fibromyalgia, chronic fatigue syndrome, mild depression (dysthymia), and multiple sclerosis after consuming 1000-2000 g/d of acetyl-L-carnitine over periods from 8-24 weeks indicating that needs for carnitine are increased in these diseases (111-114). Improvements in functional status have also been reported in elderly adults (>70 years) with debilitating physical and mental fatigue who consumed additional amounts of acetyl-L-carnitine (115). In a double-blind placebo-controlled trial of 102 patients with a clinical diagnosis of fibromyalgia, those who received supplemental acetyl-L-carnitine for a 10-week period had statistically significantly fewer numbers of tender points compared with controls and also had significantly lower total myalgic scores derived from self-assessment of fatigue, tiredness upon awakening, and sleep experience (112). Consumption of supplemental dietary carnitine from acetyl-L-choline for 24 weeks by 30 patients with chronic fatigue syndrome in an open-label study resulted in statistically significant improvements in mental fatigue (p=0.014) and general fatigue (p=0.004) in 59% of patients (113). Two weeks after consumption of supplemental acetyl-L-carnitine was stopped, worsening of fatigue was noted in 52% of patients.

A summary of support for increased requirements of specific nutrients in patients with sleep disorders is found in Table 4.

Table 4. Observations Supporting Increased Nutrient Requirements in Disorders Associated with Muscle Dysfunction, Sleep Disturbances, Cognitive Impairment, and Dysregulation of the Stress Response

Nutrient	Biochemical and Physiologic Observations	Clinical Observations Associated with Low Blood and Tissue Levels
Choline	Low blood choline levels; decreased parasympathetic autonomic nervous system activity; increased creatine phosphokinase and alanine transaminase; myocyte and lymphocyte apoptosis (36, 90, 104-107)	Muscle pain, sleep disturbances, and impaired memory (80, 89, 98, 101,106, 108-109); sleep apnea syndromes and disorders of restorative sleep (76, 82, 111)
Glutamate	Elevated blood glutamate (23)	Sleep disturbances (7, 23); anterograde and retrograde amnesia (8, 53-54)
Carnitine	Reduced plasma carnitine levels; cholinergic deficits (34, 65)	Muscle pain, morning grogginess, fatigue associated with mild depression (dysthymia), chronic fatigue syndrome, fibromyalgia, Alzheimer's Disease, and multiple sclerosis (34-35, 111-114)

Clinical Validation of *Sentra AM* in Management of Disorders Associated with Muscle Dysfunction, Sleep Disturbances, Cognitive Impairment, and Dysregulation of the Stress Response

The relationship between intakes of dietary precursors and production of the corresponding neurotransmitters has been validated by observations of improvements in neurotransmitter-mediated clinical outcomes with supplemental intakes of these nutrients (30, 48-49, 73, 80, 86, 116-118). Changes in levels of a neurotransmitter in the blood and/or its metabolites in cerebrospinal fluid following ingestion of a dietary precursor from a medical food reflect uptake and utilization of the nutrient or dietary factor by target cells, thus demonstrating the biological availability of dietary precursors and the clinical utility of the medical food as a source of these precursors (53, 81, 118-122).

The clinical benefits which may be obtained from medical foods can be validated by the observed changes in biological, physiological, and clinical endpoints following ingestion by individuals with specific conditions. For example, a medical food which provides supplemental arginine is clinically validated in individuals with low blood arginine levels when blood levels increase following ingestion (biological availability) accompanied by an increase in nitric oxide production (physiological change) and subsequent improvement in an associated functional parameter (FEV1) (clinical response) following administration. Similarly, if an individual with fibromyalgia or chronic fatigue syndrome shows an increase in choline in blood or urine after ingesting a medical food containing choline (biological availability) which is associated with increased concentrations of acetylcholine or increased cholinergic activity (physiological change) and an increased muscle strength, normal diurnal fluctuations in cortisol or autonomic function (clinical response) or improved memory, the clinical benefit of this medical food as a source of acetylcholine has been validated.

Sentra AM is formulated with specific ratios of choline, glutamate, and acetyl-L-carnitine using *Targeted Cellular Technology* to control the timing of the release of each ingredient. If sufficient amounts of these nutrients are not available, or their availability is not well-synchronized with demand, then deficits in cholinergic activity may develop resulting in muscle pain, fatigue, sleep disturbances, loss of memory, and poor concentration (27, 73, 123).

Biological Availability

Studies conducted in human subjects have confirmed the bioavailability of choline and carnitine from dietary augmentation. In a study of 11 healthy young men, the concentration of choline-containing compounds in the brain measured by proton MR spectroscopy (¹H-MRS) was increased by approximately 6.2% following ingestion of a single dose of 50 mg/kg choline as choline bitartrate (91). This increase in blood levels was estimated to represent a potentially

biologically important increase in phosphatidylcholine of 10-22%. Peak concentrations of free choline, acetylcholine, glycerophosphocholine and phosphatidylcholine were achieved 2 hours after choline ingestion.

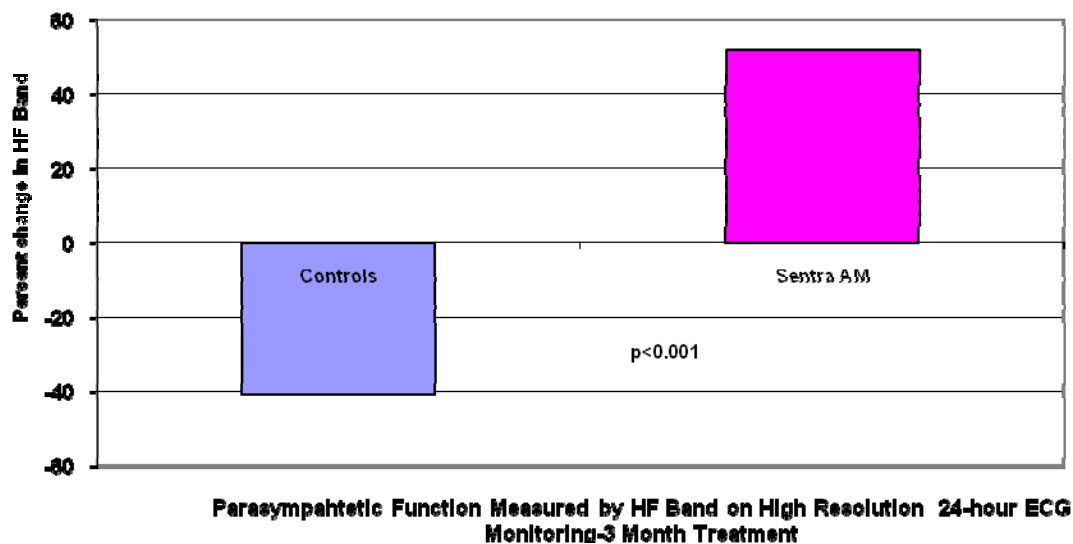
The appearance of choline and acetyl-L-carnitine in cerebrospinal fluid following added choline administration has been reported indicating that dietary sources of these biogenic amines are available for uptake by the central nervous system (31, 36).

Physiological Response

Significant physiologic testing has been performed on patients before and after taking **Sentra AM** to measure parasympathetic function and concentrations of choline and glutamate in centers of the brain. Autonomic nervous system function tests have been used to assess the balance between parasympathetic and sympathetic activities under different conditions. Because autonomic activity cannot be consciously altered, it can be used as an objective indicator of abnormalities in parasympathetic and sympathetic activities and processes which are regulated by these systems. Spectral analysis heart rate variability is a direct measure of parasympathetic activity (124).

High resolution 24-hour ECG recordings were obtained before and after treatment with **Sentra AM** for determination of parasympathetic activity using Heart Rate Variability Analysis. This method has been validated in patients with sleep disorders for assessment of parasympathetic nervous system function. Heart Rate Variability Analysis employs a complex mathematical formula (fast Fourier transform) to analyze rr- intervals (heart rate) for each heartbeat measured over a 24-hour period ECG recording. From this analysis, bands that define total heart rate variability or autonomic function are identified, such as the HF band which represents parasympathetic activity. Without therapy, parasympathetic autonomic nervous system activity is stable with repetitive measurements.

In an open-label study of fibromyalgia patients with abnormal parasympathetic nervous system function at baseline, activity was normalized by treatment with **Sentra AM**. Based on the results of heart rate variability analysis, parasympathetic activity was increased 40% in those patients treated with **Sentra AM** compared with a 20% decrease in controls. This difference was statistically significant ($p < 0.001$).

Figure 4. Effect of *Sentra AM* on Parasympathetic Function

The effects of *Sentra AM* on acetylcholine metabolism were tested by measurement of parasympathetic activity in patients who had a fibromyalgia-like syndrome associated with exposure to an environmental toxin and compared with a control group of patients from the same community who were not exposed to the toxic agent. Quantitative audio vestibular testing was used to confirm the defect in parasympathetic function. Choline and glutamate concentrations in the brains of these patients were measured in a double-blind manner using high resolution positron emission tomography (PET) scanning and quantitative spectral magnetic resonance imaging (MRI). The results of the PET scans identified abnormalities at the origin of the vagus nerve, the hypothalamus, and cerebella-midbrain connections in patients exposed to the toxin where both choline and glutamate concentrations were also reduced (Figure 5 and Figure 6).

Figure 5. Results of PET Scans of Patients with Fibromyalgia: Neurotoxicity vs. Controls

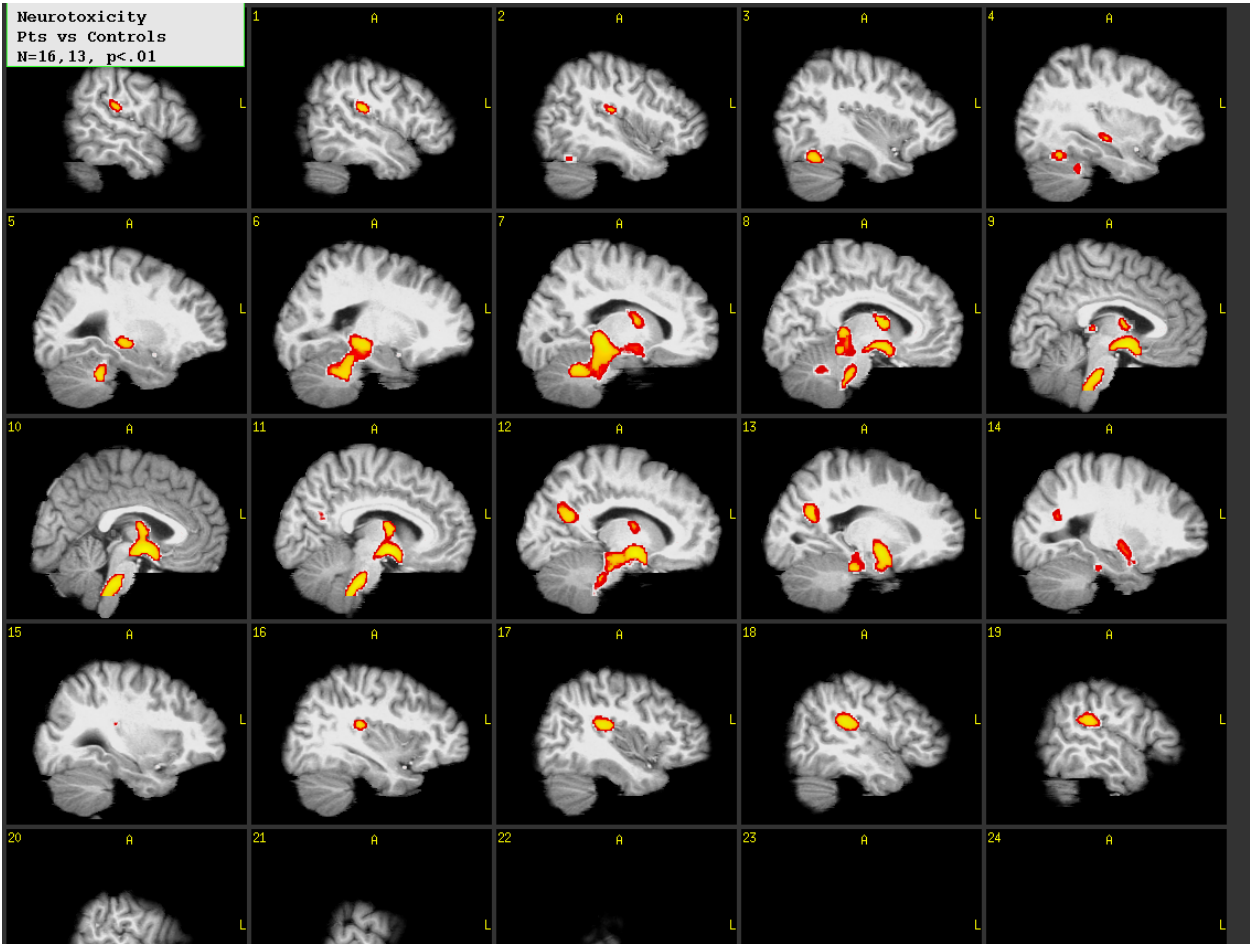
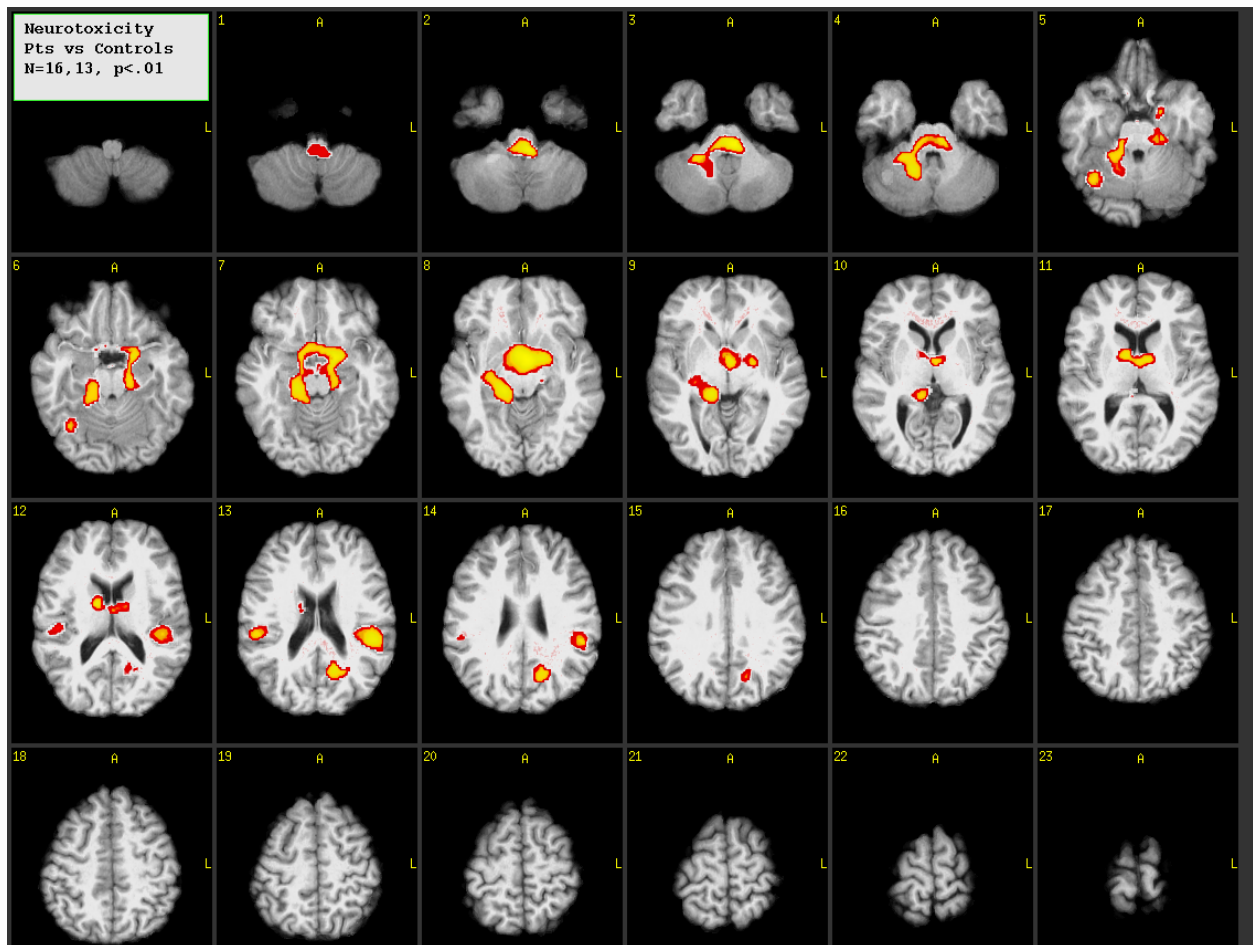


Figure 6. Results of PET Scans of Patients with Fibromyalgia: Neurotoxicity vs. Controls



Clinical Response

Patients with symptoms of chronic fatigue and fibromyalgia show reduced parasympathetic function with reduced concentrations of choline in the brain. In open-label studies, parasympathetic nervous system function was normalized from baseline levels by treatment with *Sentra AM* in patients with reduced acetylcholine function as demonstrated from the results of heart rate variability analysis, PET scans, and spectral MRI data. Symptoms of fatigue, temperature dysregulation, memory dysfunction, cognitive function, and concentration were also improved.

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