

## GABAdone® Product Information

### Indication

**GABAdone** is intended for use in sleep disorders involving difficulty in falling asleep, maintaining sleep, falling back to sleep after awakening at night, feeling tired upon awakening, and snoring.

**GABAdone** is a medical food that must be used under the active or ongoing supervision of a physician. Medical foods are developed to address the different or altered physiologic requirements that may exist for individuals who have distinctive nutritional needs arising from metabolic disorders, chronic diseases, injuries, premature birth associated with inflammation and other medical conditions, as well as from pharmaceutical therapies.<sup>1</sup>

Normal patterns of sleep and waking are regulated by neurotransmitters which alter electrical activity in specific areas of the brain. Gamma-aminobutyric acid (GABA) is the major inhibitory neurotransmitter of the central nervous system and the primary neurotransmitter involved in the homeostatic regulation of sleep. Patients with sleep disorders characterized by disrupted patterns of sleep and wakefulness benefit from increased availability of GABA, in addition to glutamate, serotonin, and acetylcholine, to re-establish homeostasis. **GABAdone** is designed to provide a balance of neurotransmitters that have well-defined roles in the modulation of the sleep cycle.

### Ingredients

**GABAdone** is a proprietary blend of neurotransmitters and neurotransmitter precursors (gamma-aminobutyric acid [GABA], L-glutamate, 5-hydroxytryptophan, choline bitartrate); neurotransmitter (GABA) potentiator (valerian); activators of precursor utilization (acetyl-L-carnitine, L-glutamate, cocoa powder); an amino acid uptake stimulator (gingko biloba); activators of amino acid utilization (L-glutamate, cocoa powder); polyphenolic antioxidants (grape seed extract, cocoa powder); anti-inflammatory and immunomodulatory peptides (whey protein hydrolysate); an adenosine antagonist (cocoa powder); and an inhibitor of the attenuation of neurotransmitter production associated with precursor administration (grape-seed extract). The neurotransmitters and neurotransmitter precursors have been carefully selected based on scientific support for their roles in the physiological processes involved in the sleep/wake cycle. These roles are summarized in this monograph in the section *Scientific Support for Use of GABAdone in Management of Sleep Disorders*. The other ingredients in the formulation are involved in neurotransmitter metabolism or are functional components of the *Targeted Cellular Technology*® system.

All of the ingredients included in **GABAdone** 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.

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

## **Targeted Cellular Technology®**

**GABAdone** has been formulated using *Targeted Cellular Technology*, 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 the synthesis and release of neurotransmitters such as acetylcholine, serotonin, nitric oxide, and histamine (1-4).

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 amino acids be added to the formulations. For patients whose precursor requirements were considerably higher than normal, the amounts of exogenous amino acids that were needed were not practical to consume on a daily basis. Moreover, ingestion of large quantities of amino acids increases the potential for adverse effects. Metabolic efficiency is also decreased when large amounts of amino acids are delivered to the cells at one time because intestinal membrane transport receptors would be rapidly saturated resulting in a reduction in fractional amino acid absorption and thus attenuation of the tissue response to the supplemental amounts provided. Improving metabolic efficiency in uptake and utilization of neurotransmitter precursors by target neurons with *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 sleep aids which are not innately involved in the sleep process, and thus may lose their effectiveness in a relatively short period of time, the effectiveness of **GABAdone** is not attenuated.

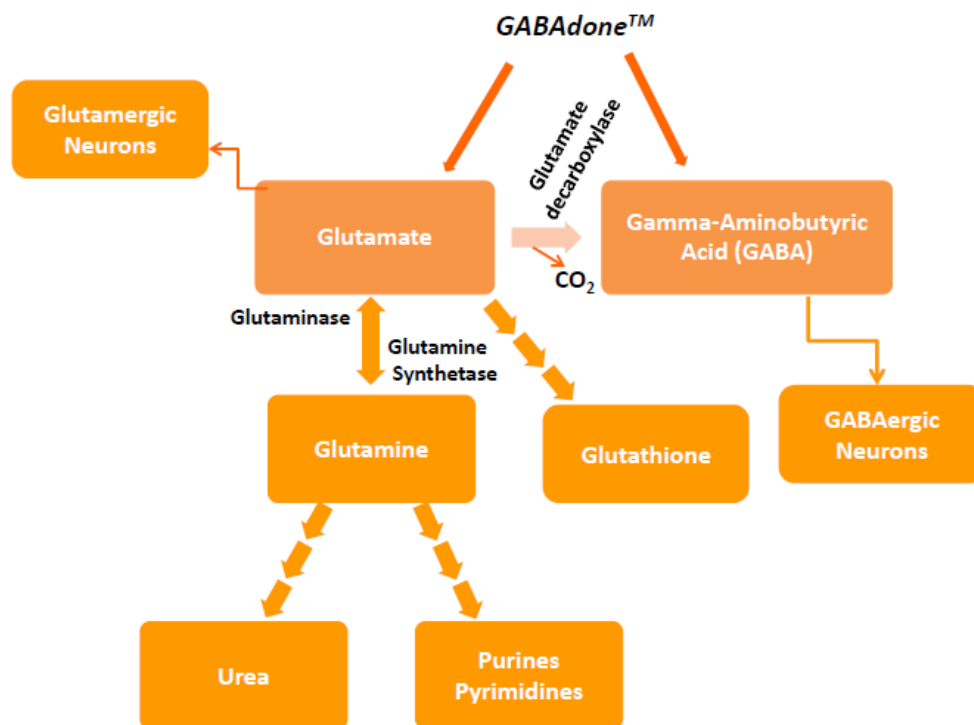
## **Metabolism**

**GABAdone** is a source of amino acids, biogenic amines, and other nutrients formulated for patients with certain types of sleep disorders. These patients require additional amounts of glutamate, tryptophan, and choline to support the synthesis of the neurotransmitters, GABA, serotonin (5-hydroxytryptamine) and acetylcholine, respectively, which are active in the processes that govern sleep and wakefulness. Under normal physiological conditions, these nutrients are considered nonessential because endogenous synthesis is sufficient to satisfy metabolic demand. When needs are altered by conditions that increase

metabolic demand, the usual rate of synthesis is no longer sufficient and these nutrients become conditionally essential, requiring that supplemental amounts be consumed.

**Glutamate.** As a nonessential amino acid, glutamate is not normally dependent on exogenous sources thus metabolic competition for this amino acid develops only under conditions of increased demand. For individuals with some types of sleep disorders, the requirement for glutamate is increased to as maintain activity of glutamatergic neurons well as to provide a precursor for production of additional amounts of GABA. Under normal conditions, glutamate can be supplied by several sources including deamination of glutamine; however, glutamate synthesis competes for glutamine with other pathways that utilize it as a precursor of a number of cellular compounds such as the antioxidant glutathione ( $\gamma$ -glutamylcysteinylglycine), purines, pyrimidines, and urea (Figure 1). These competitive demands for glutamine limit the amount of glutamate, and thus the amount of GABA available to function as neurotransmitters. As a source of both GABA and glutamate, **GABAdone** improves metabolic efficiency by ensuring that there are adequate amounts of both neurotransmitters available while conserving the supply of glutamine for its other uses.

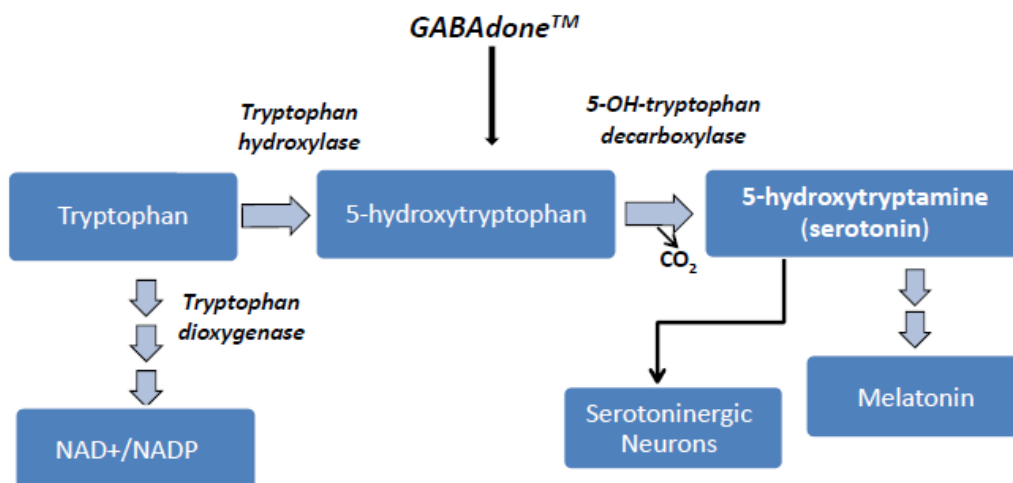
**Figure 1. Competing Pathways of Glutamate Metabolism**



**Tryptophan and 5-hydroxytryptophan.** In contrast to glutamate which is nonessential under normal conditions, tryptophan is an essential amino acid that must always be consumed from exogenous sources, as the enzymes required for its synthesis are absent in humans. Because it is an essential amino acid, the amount of tryptophan consumed determines the amount available for utilization by multiple pathways. Tryptophan is a precursor of the neurotransmitter serotonin, as well as of the coenzymes nicotinamide adenine dinucleotide (NAD<sup>+</sup>) and nicotinamide adenine dinucleotide phosphate (NADP) (Figure 2). Since

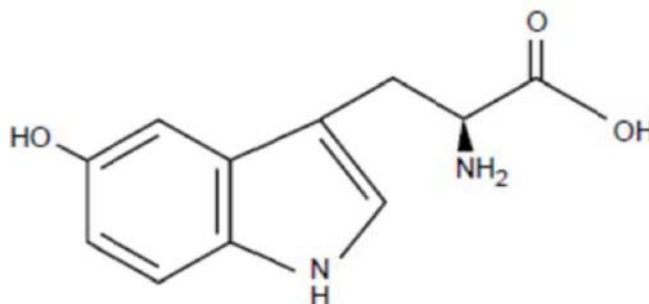
serotonin is further utilized as a precursor of melatonin, an increase in melatonin synthesis will impose a need for additional tryptophan. The competition between these and other metabolic pathways for the supply of tryptophan available restricts the amount of serotonin that can be produced from supplemental amounts of the amino acid.

**Figure 2. Competing Pathways of Tryptophan Metabolism**



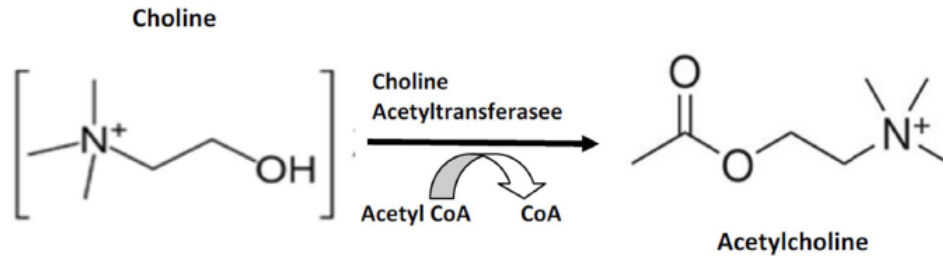
To overcome this limitation, **GABAdone** provides 5-hydroxytryptophan, an intermediate metabolite in the pathway of tryptophan conversion to serotonin, thus bypassing the rate-limiting step dependent on tryptophan availability (5-6). Unlike tryptophan, 5-hydroxytryptophan cannot be shunted into production of niacin or protein (6) (Figure 3). Unlike tryptophan, this intermediate cannot be shunted into production of niacin or protein which eliminates competition by other metabolic pathways for the amount available. Consequently, an increase in 5-hydroxytryptophan lessens the dependence of serotonin levels on the amount of tryptophan consumed. By facilitating production of serotonin without requiring consumption of large amounts of tryptophan, **GABAdone** ensures that adequate amounts of serotonin are produced without compromising synthesis of other important compounds derived from tryptophan, thus improving metabolic efficiency.

**Figure 3. Structure of 5-Hydroxytryptophan**



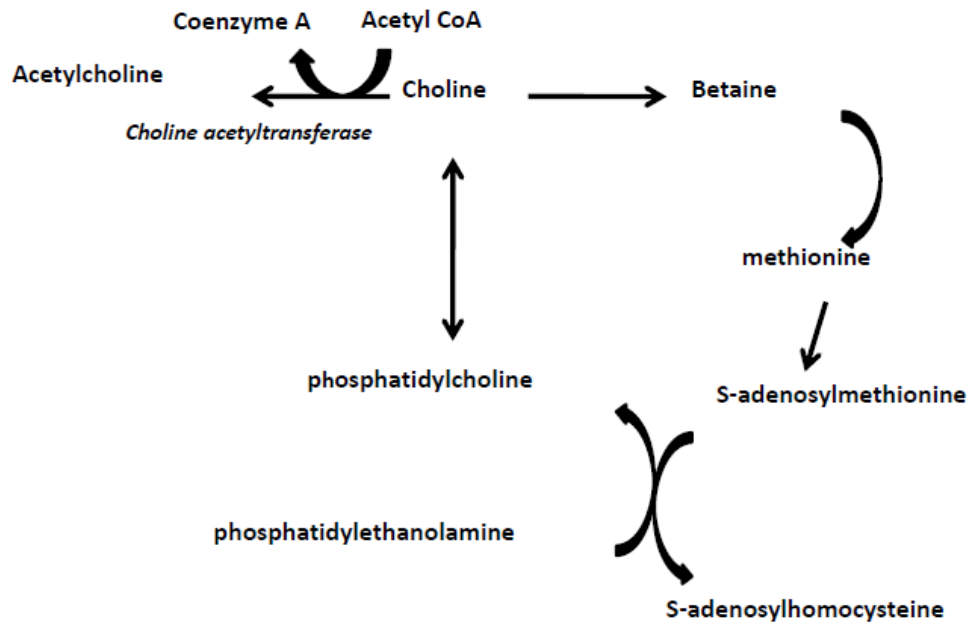
**Choline.** Both choline and carnitine are also considered nonessential nutrients under normal physiological conditions. When the demand for choline is increased to supply additional precursor for synthesis of acetylcholine, supplemental amounts of choline are needed. Acetylcholine is produced from choline in an acetylation reaction catalyzed by choline acetyltransferase with acetyl coenzyme A (CoA) as the acetyl group donor (Figure 4).

**Figure 4. Biosynthesis of Acetylcholine**



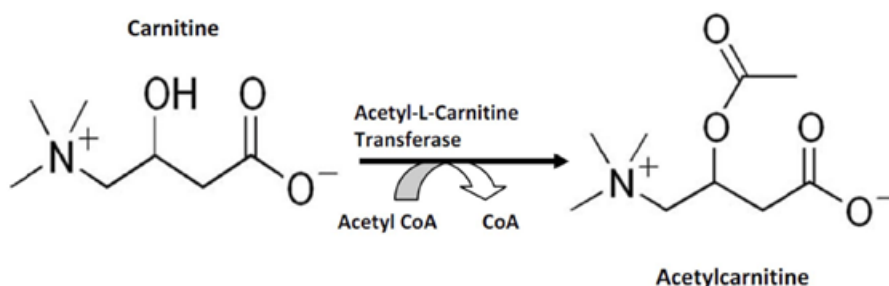
The primary source of choline normally utilized in the synthesis of acetylcholine is phosphatidylcholine (lecithin), a membrane phospholipid which serves as a reservoir of choline for short-term needs (Figure 5). When the demand for acetylcholine exceeds the amount of choline that can be supplied by the hydrolysis of phosphatidylcholine from the membrane pool, dietary choline becomes an increasingly more important source. **GABAdone** provides additional amounts of choline to meet the increased needs for acetylcholine when demand is elevated over an extended time period. By supplying an exogenous source of choline, **GABAdone** prevents the depletion of membrane phosphatidylcholine and thus preserves the structural integrity of the cell.

**Figure 5. Sources of Acetylcholine**



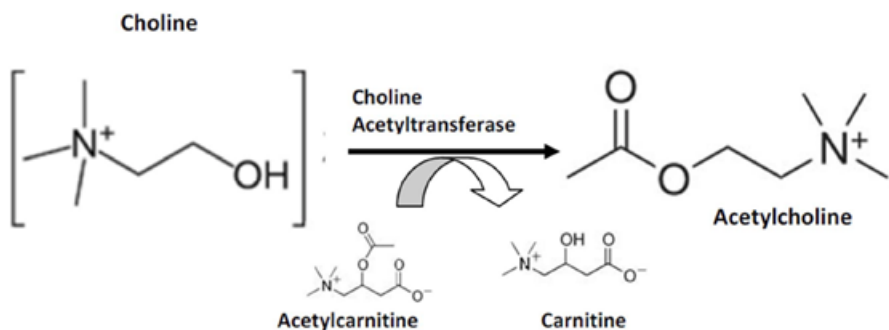
**Carnitine.** The efficiency of the metabolic response to an increased demand for acetylcholine is enhanced by acetyl-L-carnitine (Figure 6). Acetyl-L-carnitine promotes the synthesis of acetylcholine and influences neurotransmitter activity by effects on neurotrophic factors and neurohormones, synaptic morphology, and synaptic transmission of multiple neurotransmitters (7-8). Sufficient amounts of acetyl-L-carnitine can normally be produced from acetylation of carnitine, an amino acid derived from lysine and methionine; however, as essential amino acids, lysine and methionine are utilized by multiple competing pathways and cannot sufficiently accommodate a sustained increase in demand for carnitine. **GABAdone** provides acetyl-L-carnitine to ensure that an adequate supply of acetylcholine is available to support increased cholinergic activity without compromising amounts needed for its other roles in neurotransmission.

**Figure 6. Biosynthesis of Acetyl-L-Carnitine**



The need for carnitine is increased for synthesis of acetylcarnitine to meet the demand for additional acetyl groups to support the increased production of acetylcholine when cholinergic activity is high (Figure 7). Acetyl-L-carnitine is synthesized from carnitine in a reaction similar to acetylcholine synthesis from choline which involves the transfer of an acetyl group from acetyl CoA in an acetylation reaction catalyzed by carnitine acetyltransferase. Sufficient amounts of acetyl-L-carnitine can normally be produced from carnitine, but when the rate of cholinergic activity is elevated over extended periods, the demand for acetyl-L-carnitine cannot be met by endogenous synthesis alone. **GABAdone** provides additional acetyl-L-carnitine to sustain an increased rate of acetylcholine synthesis and enhance its activity when the rates of cholinergic-mediated activities are increased.

**Figure 7. Role of Acetyl-L-Carnitine in the Biosynthesis of Acetylcholine**



In addition to its role as an acetyl group donor in the synthesis of acetylcholine, acetyl-L-carnitine also facilitates uptake of acetyl groups by cholinergic neurons. This role involves a membrane transport

mechanism similar to that utilized for acetyl group transport in the pathway of fatty acid oxidation. In this pathway, acetylcarnitine serves as a membrane transport carrier of acetyl CoA groups which are released in the cytoplasm as endproducts of  $\beta$ -oxidation to undergo further oxidation by the tricarboxylic acid cycle in the mitochondria.

### **Dosage**

The recommended dose of **GABAdone** is 1 or 2 capsules taken at bedtime. An additional dose of 1 - 2 capsules may be taken during the night if the patient awakes and finds it difficult to resume sleep. As with any medical food, the best dosing protocol should be determined by assessment of individual needs. There are no known interactions between **GABAdone** and any medication.

Patients who are taking pharmaceutical agents to initiate and maintain sleep may continue to take these medications with **GABAdone** prior to retiring. If the combination of the drug and **GABAdone** is effective in promoting restorative sleep, then the drug dosage may be further tapered to lower levels under medical supervision. The experience of restorative sleep can be clinically confirmed by the absence of morning grogginess, daytime fatigue, or memory loss upon awakening.

The amounts of each ingredient consumed at the recommended doses of **GABAdone** to initiate sleep and promote restfulness are presented in Table 1.

**Table 1. GABAdone Composition**

<b>Ingredient</b>	<b>mg/kg body weight<sup>1</sup></b>
<b><math>\delta</math>-aminobutyric acid (GABA)</b>	1.8 – 4.6
<b>Choline bitartrate</b>	1.1 - 2.8
<b>L-glutamate</b>	0.7 – 1.8
<b>5-hydroxytryptophan (griffonia seed, 95% w/w)</b>	0.1 – 0.4
<b>Whey protein</b>	0.7 – 1.6
<b>Acetyl-L-carnitine</b>	0.3 – 0.8
<b>Valerian extract</b>	0.4 – 1.0
<b>Ginkgo biloba</b>	0.3 – 0.8
<b>Grape seed extract</b>	0.1 – 0.4
<b>Cocoa powder</b>	0.8 - 2.0

<sup>1</sup>Dosing range of 1 to 2 capsules daily

### **Side Effects and Contraindications**

As with any amino acid therapy, headache, upset stomach, nausea, or dry mouth may be experienced by some people after beginning treatment with **GABAdone**. These symptoms are mild and temporary, and

readily managed by increasing fluid intake. The development of side effects from **GABAdone** can be minimized by careful titration of the dosage. The ingredients in **GABAdone** are regularly consumed in amounts normally found in foods or dietary supplements; therefore development of an adverse reaction to **GABAdone** is not expected to occur.

### **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**

<b>Term/Abbreviation</b>	<b>Definition</b>
<b>Antioxidants</b>	Molecules or enzyme systems that inhibit injury to cells from reactive oxygen or nitrogen species
<b>Autonomic Nervous System</b>	Part of the efferent division of the peripheral nervous system but includes visceral afferent neurons; motor component comprises two-neuron system of preganglionic (myelinated) and postganglionic (unmyelinated) neurons; divided structurally and functionally into parasympathetic and sympathetic nervous systems
<b>Biogenic amine</b>	Biologically active substance that contains an amine group but does not have the characteristic structure of an amino acid, i.e., alpha carbon binding both an amino and carboxyl group
<b>Cholinergic</b>	Neurons that synthesize, package, and release acetylcholine
<b>Circadian Rhythm</b>	A 24-hour cycle of physiological, biochemical, and behavioral processes controlled by the suprachiasmatic nucleus in the hypothalamus
<b>Excitatory Neurotransmitters</b>	Molecules released from presynaptic cells at terminal nerve endings which transmit action potentials to adjacent neurons by depolarization of postsynaptic cell membranes resulting in a decreased stimulus threshold for firing which increases the frequency and rate of transmission of action potentials
<b>GABAergic</b>	Neurons that synthesize, package, and release gamma-aminobutyric acid (GABA)
<b>Glutamatergic</b>	Neurons that synthesize, package, and release glutamate
<b>Inhibitory Neurotransmitters</b>	Molecules released from presynaptic cells at terminal nerve endings which transmit action potentials to adjacent neurons by hyperpolarization of postsynaptic cell membranes resulting in a increased stimulus threshold for firing which decreases the frequency and rate of transmission of action potentials
<b>Melatonin</b>	Hormone synthesized from serotonin which is regulated by output from the SCN generated from processing of changes in light exposure or nonphotic signals
<b>Monoaminergic</b>	Neurons that synthesize, package, and release monoamine neurotransmitters such as norepinephrine and dopamine; serotonergic neurons are monoaminergic
<b>Neurotransmitter</b>	Amino acids, biogenic amines, and other molecules that facilitate communication between the peripheral nervous system, spinal cord, and brain by generating a series of action potentials which are transmitted between neurons

Term/Abbreviation	Definition
<b>NREM Sleep</b>	Non-rapid eye movement period of the sleep cycle; comprises 4 stages differentiated by brain electrical activity
<b>Parasympathetic Nervous System</b>	Component of the autonomic nervous system which functions to conserve and restore energy reserves; synaptic transmission mediated by cholinergic receptors; opposes the activity of the sympathetic nervous system; increases during sleep and subsides with waking
<b>Raphe Nucleus</b>	Mesencephalic nucleus that includes the hypothalamic tract which links ganglion cells to the suprachiasmatic nucleus
<b>REM Sleep</b>	Rapid eye movement period of the sleep cycle; normally follows NREM sleep
<b>Restorative Sleep</b>	Period during the late stages of non-REM sleep (Stage III and IV) when levels of growth hormone and rates of protein synthesis are increased and rejuvenation of cellular processes occur
<b>Reticular Formation</b>	A component of the reticular activating system which regulates vital functions, maintains wakefulness, and supports consciousness; consists of a large network of connected tissue nuclei within the brainstem; includes the cerebral cortex
<b>Serotonergic</b>	Neurons that synthesize, package, and release serotonin (5-hydroxytryptamine)
<b>Sleep Stages</b>	Four distinct periods of non-REM sleep differentiated by changes in brain wave patterns and distinguished by differences in muscular activity, vital signs, and responsiveness to external stimuli.
<b>Suprachiasmatic Nucleus (SCN)</b>	Bilaterally-paired nuclei in the hypothalamus situated above the point where the optic nerves cross; integrates and synchronizes information from peripheral oscillators that respond to temporal changes in environmental and internal cues such as exposure to light, hormone levels, hunger and body temperature, then signals circadian time to the rest of the body
<b>Sympathetic Nervous System</b>	Component of the autonomic nervous system which functions to mobilize energy reserves; synaptic transmission mediated by cholinergic and adrenergic receptors; opposes the activity of the parasympathetic nervous system; responsive to stress; activity decreases during sleep and increases with waking
<b>Targeted Cellular Technology™</b>	A patented process that facilitates endogenous production, uptake, and utilization of neurotransmitter precursors.
<b>Ventrolateral Preoptic (VLPO)</b>	Area of the rostral hypothalamus rich in GABAergic neurons which promote NREM sleep

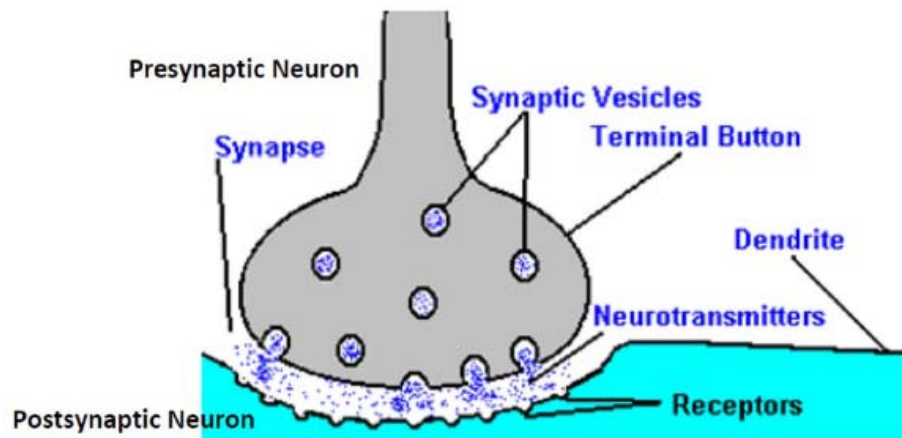
### **Mechanism of Action**

**GABAdone** has been formulated to provide a balance of neurotransmitters that have well-defined roles in the physiology and molecular basis of sleep.

**Mechanisms of neurotransmitter activity.** Neurotransmitters are amino acids, biogenic amines, or amino acid derivatives that function as mediators of physiological responses to physical, chemical, or electrical stimuli (7). Neurotransmitters are released from storage vesicles in presynaptic neurons in response to action potentials at the distal nerve endings where they bind to receptors on postsynaptic

neurons (Figure 8). Neurotransmitter binding alters the resting membrane potential of postsynaptic neurons generating an action potential which is transmitted to the terminal ending of the neuron where the sequence of electrochemical events is repeated until the signal reaches specific processing centers in the brain. The same mechanism of neurotransmitter-mediated electrochemical events is involved in transmission of output from the brain to the target effector organs, and in transmission of signals originating within different regions of brain over the internal circuits between these regions.

**Figure 8. Neurotransmitter Activity in Presynaptic and Postsynaptic Neurons**



The rate of signal transmission between presynaptic and postsynaptic neurons within the central and peripheral nervous systems is dependent upon the chemical nature of the neurotransmitter involved (8). Excitatory neurotransmitters released from presynaptic nerve terminals depolarize the postsynaptic cell membrane which lowers the stimulus threshold for firing and increases the frequency and rate of transmission. Inhibitory neurotransmitters have the opposite effect of hyperpolarizing the postsynaptic membranes which raises the stimulus threshold and decreases the frequency and rate of transmission. Although neurotransmitters can be classified as excitatory or inhibitory based on the primary effects they have on resting membrane potentials, these classifications do not always predict the response of the effector organ to the stimulus. Excitatory neurotransmitters can suppress a response by activation of inhibitory mechanisms and inhibitory neurotransmitters can activate a response by suppression of these mechanisms. Imbalances caused by deficiencies in one or more of the excitatory and inhibitory neurotransmitters, or changes in their binding affinities to postsynaptic receptors, will determine the intensity and duration of the signals transmitted (9-13).

**General roles of neurotransmitters.** The primary neurotransmitters involved in regulation of the sleep/wake cycle are glutamate, GABA, serotonin, and acetylcholine (9, 14). Glutamate is the major excitatory neurotransmitter of the central nervous system and GABA is the primary inhibitory neurotransmitter. Serotonin functions as an excitatory neurotransmitter while acetylcholine exhibits both excitatory and inhibitory effects in the central and peripheral nervous systems depending upon the specific type and location of the cholinergic receptors. Imbalances caused by deficiencies in one or more

of the excitatory and inhibitory neurotransmitters, or changes in their binding affinities to postsynaptic receptors, will determine the intensity and duration of signals generated and thus the response to these signals (10-13, 15-17). Most hypnotic drugs act by increasing the sensitivity of GABA receptors while most stimulants act by increasing the release or inhibiting the reuptake of serotonin or other monoamines (18-22).

**The sleep cycle.** Sleep is an active process consisting of 2 phases that are differentiated by brain electrical activity on an electroencephalogram (EEG). During non-rapid eye movement (NREM) sleep, brain wave patterns progress through 4 distinct stages beginning with the fast, medium-amplitude alpha waves that characterize the waking state then shifting to theta and delta waves as sleep progresses. The transition from wakefulness to light sleep (Stages I and II) is characterized by the appearance of medium-velocity, high-amplitude theta waves interspersed with alpha waves which eventually shift to large, high amplitude, slow-moving delta waves that signal the onset of deep sleep or slow-wave sleep (Stages III and IV). The transition from NREM to REM (rapid eye movement) sleep is marked by a shift in brain electrical activity to desynchronized, low-voltage, fast waves. The corresponding pattern of eye movements consists of slow movements during light sleep becoming nearly undetectable or completely absent in deep sleep. The appearance of rapid, jerky eye movements is a hallmark of REM sleep.

The progression through each stage of NREM sleep to the end of REM sleep comprises a sleep cycle, which repeats at 90-110 minute intervals. The first period of REM sleep is initiated 70 to 90 minutes after the onset of non-REM sleep. During the first few sleep cycles, the time spent in REM sleep is short relative to the period of deep sleep. As sleep duration increases, the amount of time spent in the REM period is extended while the amount spent in deep sleep is shortened. Just prior to awakening, nearly all of the sleep cycle is spent in Stage II and REM sleep. A healthy adult spends an average of approximately 20% of time asleep in the REM period and 50% in Stage II with the remaining time divided between the other stages of NREM sleep. Restorative sleep occurs during deep sleep (Stages III and IV) when growth hormone levels are elevated and the metabolic activities associated with cellular rejuvenation are increased.

**The suprachiasmatic nucleus (SCN) and circadian patterns.** Normal patterns of sleep and wakefulness reflect the synchronized activity between sleep-active and wake-active neurons integrated with circadian patterns generated by input from the hypothalamus to the suprachiasmatic nucleus (SCN). The SCN consists of bilaterally-paired nuclei comprising more than 20,000 neurons with a high concentration of serotonergic receptors situated in the hypothalamus above the point where the optic nerves cross (5, 8, 10-13, 15-17, 20-22). The SCN is the master clock which integrates and synchronizes input from peripheral oscillators responding to temporal changes in environmental and internal cues such light exposure, hormone levels, hunger and body temperature, and then signals circadian time to the autonomic nervous system and the rest of the body (10-11, 23-25). Diurnal balance in the functions of autonomic-innervated organs are therefore regulated by neurotransmitters which transmit circadian information to the SCN (26-27).

**Neurotransmitter modulation of SCN activity.** The sleep/wake cycle is regulated by the SCN from input originating from interactions between ultraviolet light and photoreceptor cells in the retina, and

from nonphotic stimuli generated by food intake, fluctuations in body temperature, and activity of the sympathetic nervous system (10-11, 25, 28). Acetylcholine, glutamate, and GABA are involved in transmission of signals generated by changes in light exposure while serotonin is the primary neurotransmitter involved in transmission of signals generated by nonphotic stimuli from peripheral oscillators (12, 19, 22, 27, 29-30). Light-induced signals are transmitted directly from the retina to the SCN by glutamate through the retinohypothalamic tract and indirectly through the geniculohypothalamic tract by GABA. Experimental manipulation of the sleep-wake cycle in healthy volunteers has revealed a dependence of sleep latency, sleep efficiency, and REM sleep propensity on circadian phase (31).

As light exposure decreases, acetylcholine, serotonin, and glutamate are withdrawn from the reticular formation while GABA is increased in the cerebral cortex. The specific patterns of brain electrical activity observed during sleep are modulated by variations in the levels of neurotransmitters which regulate the duration of each stage and the timing of the transitions between stages (32). The changes in the levels of these neurotransmitters alter brain electrical activity to wave patterns associated with drowsiness and the initiation of NREM sleep. Many sleep disorders are a result of desynchronization of the activities of these neurons caused by disruption in normal circadian rhythms due to imbalances in GABA, acetylcholine, serotonin, and glutamate (13-14, 20, 30-31, 33-42). Circadian information obtained from the activities of these neurotransmitters is processed in the SCN which then sends output to the pineal gland and the autonomic nervous system (23-24). The pineal gland responds to signals from the SCN by either ramping up or shutting down melatonin production with the corresponding results of either increasing or decreasing drowsiness (21-22). Circulating melatonin levels increased 10-fold during sleep (12).

**Neurotransmitter-mediated autonomic nervous system activity.** The SCN organizes opposing signals from anatomically-separate sympathetic and parasympathetic neurons arising in the brainstem and paraventricular nucleus of the hypothalamus to determine autonomic output from the brain (23-24). Acetylcholine is the primary neurotransmitter responsible for propagation of signals by the autonomic nervous system and the only neurotransmitter involved in signal transmission by the parasympathetic nervous system, (8, 23, 43-44). Cholinergic activity follows a circadian pattern which reflects the sensitivity of acetylcholine to changes in light with levels increasing in response to light exposure and decreasing with diminishing light. Consequently, cholinergic neurons would be expected to play a prominent role in mediating the diurnal pattern of autonomic activity observed during sleep and wakefulness (12, 19).

At sleep onset, parasympathetic functions are activated while sympathetic functions are suppressed. The elevated level of parasympathetic activity observed during sleep begins to subside prior to waking in parallel to the increase in sympathetic activity (45). Parasympathetic activity slows heart rate resulting in decreased metabolic activity which conserves energy for restorative functions. The role of the SCN in regulation of autonomic functions during sleep suggests an association between imbalances in neurotransmitters that modulate circadian rhythms and the pathology of sleep disorders which are characterized by poorly coordinated autonomic nervous system activity (23, 46-49).

## **Scientific Support for Use of GABAdone in Management of Sleep Disorders**

The use of **GABAdone** in management of sleep disorders is supported by experimental and clinical data which have identified specific roles for each ingredient in the initiation and maintenance of sleep. An optimal balance between the activities of excitatory and inhibitory neurotransmitters involved in the sleep process is essential to achieving restorative sleep (11-12, 20, 25, 50). If sufficient amounts of any one of the neurotransmitters involved are not available, or their release is not well-synchronized with circadian rhythms, then restorative sleep will not occur. Many sleep disorders arise from the disruption of normal circadian patterns due to imbalances in serotonin as well as GABA, acetylcholine, and glutamate which are involved in transmission of circadian input to the SCN (13-14, 20, 30, 34-35, 42 36-37, 39-40).

Commonly used drugs which modify sleep patterns through effects on neurotransmitter release and receptor activity, but do not restore neurotransmitter balance will alter other aspects of the sleep cycle that can interfere with restorative sleep (7). Benzodiazepine drugs reduce sleep latency by increasing the efficiency of synaptic transmission of GABA, but they also abolish REM sleep and stages IV and V of NREM sleep which decreases the period of restorative sleep (18). Selective serotonin reuptake inhibitors (SSRIs), the class of antidepressants which includes fluoxetine and sertraline, increase sleep latency but also decrease REM and slow-wave sleep (51). Most hypnotic drugs act mainly by increasing the sensitivity of GABA receptors and drugs that promote wakefulness act mainly by stimulating release or inhibiting reuptake of serotonin and other monoamines (18, 52-54).

**Neurotransmitter activity during the sleep/wake cycle.** The cycling between periods of sleep and wakefulness is controlled by the synchronized activity between sleep-active neurotransmitters (GABA) and wake-active neurotransmitters (serotonin, acetylcholine, and glutamate). Desynchronization of the wake-promoting effects of glutamate and acetylcholine with the sleep-promoting effects of GABA disrupts the normal circadian rhythms which modulate the sleep-wake cycle and the balance in parasympathetic and sympathetic nervous systems activities during sleep and waking periods (12-13,19-20 34, 36). Coordination of the activity between sleep-active and wake-active neurotransmitters is essential to regulation of the sleep-wake cycle by the SCN. A decrease in light exposure stimulates the withdrawal of acetylcholine, serotonin, and glutamate from the reticular formation accompanied by increased GABAergic activity in the cerebral cortex. These changes shift brain electrical activity to wave patterns associated with drowsiness and initiation of NREM sleep. Experimental manipulation of the sleep-wake cycle in healthy volunteers has revealed a dependence of sleep latency, sleep efficiency, and REM sleep propensity on circadian phase (41).

During sleep, specific patterns of brain electrical activity are modulated by changes in neurotransmitter levels which regulate the duration of each stage and the timing of transitions between stages (8, 16, 55). Acetylcholine concentrations fluctuate from high levels while awake to lower levels during slow-wave Stage IV sleep and begin to increase again during REM sleep (8). Cholinergic activity stimulates delta waves in the transition from deep slow-wave sleep to REM sleep, increases the duration of Stage IV sleep, and increases the frequency and duration of REM sleep (14, 55-59). Release of acetylcholine is also associated with increased theta wave activity during the transition from the early to the later stages of the sleep cycle (56). A more rapid onset of REM sleep and a reduction in restorative sleep are characteristic

of sleep abnormalities in depressed mood states suggesting abnormalities in cholinergic activity. In fibromyalgia, the intrusion of alpha wave patterns into delta wave sleep indicates an abnormal transition between sleep stages related to the availability of acetylcholine (31).

**GABA.** The asymmetry of the relationship between wakefulness and sleep is reflected in the fact that the period of wakefulness is more likely to be extended than it is to be shortened relative to the period of sleep, indicating that these states are mediated by different neurotransmitter systems (10). The transition from waking to sleep is mediated by the coordinated inhibition of multiple arousal systems in response to activation of GABAergic neurons (7, 11, 16, 34, 60-61). Almost all of the sleep-active or sleep-promoting neurons in the brain are GABAergic and concentrated in the median preoptic nucleus and ventrolateral preoptic (VLPO) area of the rostral hypothalamus (9, 17, 24-25, 29, 56). NREM sleep is promoted by GABAergic neurons in the VLPO region whereas REM sleep is promoted in the areas adjacent to the VLPO. Lesions in the GABAergic-rich anterior hypothalamus have been associated with severe insomnia and fragmented sleep (12, 25). Sleep deficits caused by damage to these areas of the brain can be reversed by electrical, thermal, or chemical stimulation indicating that decreased GABAergic activity contributes to disruptions in sleep patterns (35, 59-60). The activation of GABAergic neurons by decreased light exposure and sleep deprivation also suggests a dependence of sleep homeostasis on GABA production and release (11, 28).

**Neurotransmitter activity in sleep to waking transition.** The transition from sleep to waking is initiated by an increase in activity of the wake-active serotonergic neurons in the dorsal raphe nucleus, cholinergic neurons in the brainstem and basal forebrain, and monoaminergic (norepinephrine and dopamine secretors) neurons in the rostral pons, midbrain, and posterior hypothalamus (9, 12, 14, 16-17, 24-25, 28-29, 32, 35-36, 42, 56-57, 59-63). In the SCN, glutamate mediates the synchronization of circadian clocks to environmental cues through the process of entrainment which involves activation of its N-methyl-D-aspartate (NMDA) receptor (25). Glutamatergic neurons which are widely distributed in the brain are also active in the initiation and maintenance of the waking state (8, 56, 64).

**Glutamate.** The modulation of circadian rhythms by acetylcholine and glutamate is an important component of the regulation of the autonomic nervous system by these neurotransmitters (65). Autonomic activity follows diurnal patterns which are closely coordinated with the sleep/wake cycle (25). Imbalances in acetylcholine and glutamate upset these rhythms and thus disrupt autonomic functions (20, 31, 41, 66). Desynchronization of the wake-promoting effects of glutamate and acetylcholine with the sleep-promoting effects of GABA and serotonin also disrupts the normal circadian rhythms which modulate the sleep-wake cycle and the balance in autonomic nervous system activity during periods of sleep and waking (20, 31, 41, 56-57).

Glutamate interacts with acetylcholine to stimulate wakefulness through synaptic transmission of light-induced signals to the SCN (7, 11, 16). Interactions between glutamate and acetylcholine have been identified in modulation of sleep, arousal, and vigilance (43, 59, 65, 67-70). In the central nervous system, glutamate receptors are concentrated in areas of high cholinergic activity. Both neurotransmitters initiate and maintain arousal in the perifornical lateral hypothalamus and both promote vigilance in the basal forebrain through effects on hypocretin- or orexin-secreting neurons which mediate neuroendocrine

control of arousal (59, 65, 69-71). Stimulation of arousal is mediated by glutamate and acetylcholine through depolarizing effects that increase release of hypocretin or orexin (34, 56, 60-61, 65) and opposed by GABA and serotonin through hyperpolarizing effects that inhibit release. Acetylcholine also enhances glutamatergic activity as a coagonist in activation of the glutamate-dependent NMDA receptor (43).

**Acetylcholine.** Acetylcholine concentrations normally vary over the sleep/wake cycle from high levels observed during periods of waking to low levels during slow-wave Stage IV sleep and return to higher levels during REM sleep (56). Increased cholinergic activity is associated with an increase in theta wave patterns which characterizes the transition from early to later stages of the sleep cycle (60). It also increases delta wave patterns that mark the transition from deep slow-wave sleep to REM sleep, extends the time spent in Stage IV and Stage V sleep, and increases the frequency and duration of REM sleep (7, 34, 45, 62, 66, 72).

**Serotonin.** Increased serotonergic activity is associated with wakefulness fluctuating in parallel with circadian patterns and inversely with neurotransmitter systems that inhibit arousal (22, 35, 37, 72). Sleep is initiated and sleep latency is decreased at times of low serotonergic activity (73). Activity is highest during periods of waking, slow during NREM sleep, and virtually silent in REM sleep (25, 30, 56, 58, 74). During sleep, peak serotonin levels are observed in the first hours following onset and the lowest levels are reached in the transition from NREM to REM sleep. Altered patterns of serotonin production and release have been linked to disturbances in the sleep process which contribute to sleep apnea and snoring, and may also be linked to disruption in the balance of autonomic nervous system activities associated with these sleep disorders (24, 30, 45, 72, 74). Genetic evidence of an abnormal serotonin transporter which has been reported in sleep disorders as well as depression and fibromyalgia may explain the relatively lower levels of brain serotonin observed in patients with these conditions (45, 73).

**Neurotransmitter interactions.** The pattern of changes in the amounts and activities of different neurotransmitter systems over the sleep cycle reflects the complexity of the interactions between them (4, 42, 64-65). Local release of GABA in areas of the brain where serotonergic activity is concentrated inhibits the serotonin-mediated effects which maintain brain activity during waking periods and underlies the low levels of serotonin activity observed during REM sleep (9). The decrease in serotonergic activity induced by increased GABA concentration releases cholinergic neurons from the inhibitory effects of serotonin thereby facilitating the transition from NREM to REM sleep (25). Cholinergic activity during waking periods is mediated by the inhibitory effects of acetylcholine in the midbrain reticular formation which promote a restful state and the excitatory effects in the basal forebrain which promote vigilance.

**Acetyl-L-carnitine.** Acetyl-L-carnitine enhances cholinergic activity by promoting the synthesis of acetylcholine and by its own cholinomimetic effects (75-76). By increasing the availability of acetylcholine, acetyl-L-carnitine acts similarly to cholinesterase inhibitors except that it promotes acetylcholine synthesis instead of inhibiting its hydrolysis. Although the exact mechanism by which acetyl-L-carnitine enhances acetylcholine synthesis by cholinergic neurons has not been identified, it may be similar to the mechanism that stimulates uptake of acetyl CoA by the mitochondria. Cholinergic activity may be further enhanced by acetyl-L-carnitine through effects that block the postsynaptic

inhibitor potential of cholinergic receptors. Acetyl-L-carnitine also directly influences synaptic transmission by a mechanism which is independent of acetylcholine involving neurotrophic factors, neurohormones, synaptic morphology, and the coordination of the activities of multiple neurotransmitters (77).

**Whey protein hydrolysate.** Whey protein hydrolysate comprises several proteins and peptides with anti-inflammatory, immunomodulatory, and antioxidant properties (78-79). In addition,  $\alpha$ -lactalbumin and  $\beta$ -lactoglobulin, interact with opioid receptors indicating that these proteins also have anti-nociceptive effects (78-80). Whey is a high biological value protein derived from milk which contains all 22 amino acids necessary for human protein synthesis and metabolism including the neurotransmitter precursors, tryptophan, arginine, and histidine.

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

**Table 3. Roles of GABAdone Ingredients in the Sleep Process**

<b>Ingredient</b>	<b>Effector Molecules</b>	<b>Effects</b>	<b>Roles</b>
<b>GABA</b>	GABA	Inhibitory neurotransmitter	Primary sleep-active neurotransmitter; promotes NREM and REM sleep; coordinates inhibition of multiple arousal systems; modulates circadian rhythms through transmission of light signals from photoreceptor cells in the retina to the SCN; promotes homeostasis of the sleep cycle; inhibits serotonergic activity; inhibits arousal through hyperpolarizing hypocretin (orexin) neurons
<b>5-OH-tryptophan</b>	Serotonin	Excitatory neurotransmitter	Promotes wakefulness; initiates sleep and decreases sleep latency; primary modulator of circadian rhythms; transmits nonphotic signals to the SCN; precursor of melatonin; regulates autonomic function through effects on circadian rhythms; inhibits activity of cholinergic neurons which facilitate the transition from NREM to REM sleep
<b>Choline</b>	Acetylcholine	Inhibitory neurotransmitter (midbrain reticular formation)  Excitatory neurotransmitter (basal forebrain)	Decreases in the reticular formation in response to decreased light exposure; elicits delta wave patterns which increases the frequency and duration of REM sleep; elicits theta waves which initiates sleep; promotes REM sleep in the midbrain reticular formation and vigilance in the basal forebrain ; primary neurotransmitter of the autonomic nervous system
<b>Glutamate</b>	Glutamate	Excitatory neurotransmitter	Active in initiation and maintenance of the waking state; modulates circadian rhythms by transmission of light signals from photoreceptor cells in the retina to the SCN; mediates synchronization of circadian clocks to environmental cues through the NMDA receptor; interacts with acetylcholine; stimulates arousal through depolarizing hypocretin (orexin) neurons

Ingredient	Effector Molecules	Effects	Roles
<b>Acetyl-L-carnitine</b>	Acetyl-L-carnitine	Precursor uptake stimulator; cholinomimetic agent	Enhances production of acetylcholine; blocks the postsynaptic inhibitor potential of cholinergic receptors; directly stimulates cholinergic synaptic effects; influences synaptic transmission by effects on neurotrophic factors, neurohormones and synaptic morphology; coordinates the activities of multiple neurotransmitters
<b>Whey Protein Hydrolysate</b>	$\alpha$ -lactalbumin, $\beta$ -Lactoglobulin, Glycomacropeptide, Lactoferrin	Antinociceptive; Opioid Agonist; Immunomodulator; Antioxidant; Anti-inflammatory	$\alpha$ -lactalbumin and $\beta$ -lactoglobulin reduce pain through interactions with opioid receptors; other peptides reduce the effects of inflammation on pain (78-80)
<b>Gingko biloba</b>		Stimulates amino acid uptake by neurons	Modulates presynaptic choline uptake and acetylcholine release (81-82)
<b>Valerian Root Extract</b>		Potentiates GABA effects	Inhibits breakdown of GABA (83); induces sedation and decreases central nervous system activity (84)
<b>Cocoa Powder</b>	Caffeine	Adenosine antagonist	Binds to adenosine receptors to disinhibit the adenosine brake which promotes the inhibitory effect of adenosine on neuronal activity. (85-86)
<b>Grape seed extract</b>	Polyphenols	Antioxidant	Preserves receptor membrane integrity and prevents attenuation of responses to neurotransmitter precursors (87-89)

### **Nutritional Requirements of Sleep Disorders**

The nutritional requirements of most interest to patients with sleep disorders are nutrients and dietary factors which support the synthesis and activities of neurotransmitters involved in regulation of the sleep/wake cycle. **GABAdone** is formulated with balanced amounts of GABA, 5-hydroxytryptophan, choline and glutamate which modulate the sleep cycle, and the neuromodulator acetyl-L-carnitine to optimize circadian-dependent processes, using *Targeted Cellular Technology* to control the timing of the release of these ingredients.

**Neurotransmitter balance.** The balance of the activities of these neurotransmitters is important because the interactions among them influence the quantity and quality of sleep. Balance in the production and release of neurotransmitters is also important because the net input received by the brain is determined by the highly integrated functions of these neurotransmitters and the complexity of the multiple feedback loops between them. These interactions explain why an imbalance in the intake of a nutrient or dietary factor which supports the synthesis or activity of any one neurotransmitter can influence the activities of the others, potentially inducing absolute and relative deficiencies (29, 34, 42, 60, 90-91).

The therapeutic effects of many drugs approved for treatment of sleep disorders and depression involve manipulation of brain levels of serotonin indicating that imbalances in this neurotransmitter and the neurotransmitters that respond to changes in serotonergic activity contribute to alterations in sleep

patterns manifested in these conditions, and supports the benefits of consuming additional amounts of these neurotransmitters and nutrient precursors to restore homeostasis (4, 18, 34, 40, 50, 52-54, 92-97).

**Nutrient requirements in disease.** The concept that nutrient requirements are modified by disease has been recognized for more than 30 years, and is supported by numerous studies which have shown changes in plasma, urinary, and tissue levels of nutrients with modified intakes of these nutrients that correspond to changes in physiological endpoints reflective of specific pathologies (98). These requirements can be estimated by determining the level of intake at which a physiological response is improved indicating that the balance between intake and metabolic demand has been favorably modified. The nature of the pathological characteristics of a disease will determine the relative amounts of nutrients needed to restore balance between intake and demand. The degree of coordination between the activities of different neurotransmitters is an important consideration in assessing the amounts of dietary precursors needed (50, 92-96, 99).

Diseases with pathologies that involve imbalances in neurotransmitters will increase the requirements for certain amino acids and other dietary precursors to restore homeostasis (4 50, 84-86, 99-104). For most amino acids and other dietary precursors of neurotransmitters, uptake by target neurons is a concentration-driven process; therefore, intakes of precursors must be sufficient to increase the extracellular to intracellular concentrations to levels high enough to drive a rapid rate of uptake (101, 104-107). The rate of precursor uptake by target neurons is important to neurotransmitter synthesis because the enzymes involved are found only in these neurons and thus the amount of substrate available is the limiting factor in neurotransmitter production (108-109). As blood levels of these dietary precursors rise in response to increased intakes, the concentration-driven rate of precursor uptake by target neurons is increased, making more substrate available for neurotransmitter production and subsequent release (93, 103, 106-107, 110-111). Changes in intakes of dietary precursors of these neurotransmitters will therefore influence physiological responses by affecting neurotransmitter availability (34, 60, 94, 97, 101, 103, 106-108, 111-115).

A large body of peer-reviewed published data supports the basis for increased requirements of tryptophan (37, 100, 104-105, 107, 111, 116, 118-123), glutamate (124-125), and choline (104, 126-129) in conditions which depend on neurotransmitter balance (50, 90, 92-96, 99, 102-104, 107, 119, 130-131). Patients who show decreased blood levels of certain amino acids despite maintaining their usual protein intake may have needs for these amino acids that are selectively increased as a result of specific physiological requirements associated with their disease (132). This observation may be explained by the competitive demands for certain amino acids by different metabolic pathways which decrease the supply of neurotransmitters available to function in the sleep process (Refer to the section *Metabolism* in this monograph).

**Nutrient effects on neurotransmitter availability.** Certain physiologic and biochemical mechanisms must exist in order for nutrient consumption to affect neurotransmitter synthesis (108). These conditions are listed below. The extent to which neurotransmitter synthesis in any particular aminergic neuron is affected by changes in precursor availability will vary directly with the firing frequency of the neuron. Consequently, precursor administration can produce selective physiologic effects by enhancing

neurotransmitter release from some but not all of the neurons potentially capable of utilizing the precursor for the particular effect. It is also useful in predicting when administering the precursor might be useful for amplifying a physiologic process, or for treating a pathologic state.

### **Requirements for Effects of Dietary Precursors on Neurotransmitter Synthesis**

1. Absence of significant feedback control of plasma precursor levels
2. Ability of plasma precursor levels to control influx into or efflux from the central nervous system
3. Presence of a low-affinity (unsaturated) transport system mediating the flux of precursor between blood and brain
4. Low-affinity kinetics of enzyme that initiates conversion of precursor to neurotransmitter
5. Lack of in vivo end-product enzyme inhibition by the neurotransmitter

**Requirement for tryptophan and 5-hydroxytryptophan.** Low blood tryptophan levels have been associated with decreased brain serotonin concentration and disturbances in the sleep/wake cycle indicating an increased need for tryptophan to correct the serotonin deficiency associated with these disturbances (22, 38, 53-54, 74, 89-90, 95, 99, 101, 108, 112, 119-121, 124, 132-137). The low levels of serotonin accompanied by low 5-hydroxytryptophan levels in patients with sleep disorders also implicate the presence of a tryptophan deficiency secondary to increased metabolic demand which may also be contributing to disturbances in sleep patterns (120, 124, 134, 136-137). Imbalances in serotonin production and release may be further complicated if tryptophan metabolism is also altered in the disorder (134, 136).

In conditions where tryptophan metabolism is altered, intake of 5-hydroxytryptophan, the metabolic intermediate in the conversion pathway of tryptophan to serotonin, would be a more effective approach for restoring balance in serotonin levels than administration of tryptophan (Figure 2). (See section on Metabolism in this monograph). Therapeutic administration of 5-hydroxytryptophan has been shown to be effective in treating a wide variety of conditions that involve serotonergic activity including depression and insomnia (6). Intakes of 5-hydroxytryptophan are well-absorbed with approximately 70% of the dose measured in the blood levels following oral administration. This molecule crosses the blood-brain barrier and effectively increases central nervous system synthesis of serotonin. The appearance of increased amounts of 5-hydroxyindolacetic acid, the primary metabolite of serotonin in cerebrospinal fluid, following administration of 5-hydroxytryptophan confirms that supplemental intake of the metabolic intermediate not only increases production of serotonin but that it is released by serotonergic neurons (42, 104-106, 111). By affecting both the production and release of various neurotransmitters, changes in intakes of precursor nutrients and dietary factors can influence the physiological functions dependent on these neurotransmitters (7, 90, 92-94, 97, 99, 102-103, 107-108, 111-112, 131, 138).

**Requirement for choline.** In addition to tryptophan, low blood levels of choline and GABA have been noted in patients with sleep disorders indicating that the needs for choline and glutamate are not being met at the current levels of intakes of these patients (4,112, 120, 124, 134, 139-140). Low blood levels of choline and GABA have also been reported in patients with sleep disorders indicating that the requirements for these precursors were not being met at current levels of intake (128, 135, 139, 141). A dietary deficiency of choline has been associated with sleep apnea syndromes and disorders of restorative sleep (50, 94, 101-102, 142). Plasma levels of glutamate have been found to be a highly significant discriminatory variable for identifying patients with major depression (143). The insensitivity of acetylcholine and serotonin to circulating levels of GABA observed in patients with sleep disorders suggests impaired control of the normal sleep/wake cycle which may be related to imbalances among these neurotransmitters resulting from inadequate intakes of their nutrient precursors (4, 6, 25).

Dietary choline is the primary contributor to plasma choline levels accounting for a greater proportion of the plasma concentration than de novo synthesis (124, 127-129, 136, 144). Under steady state conditions, the brain enzyme is not completely saturated, thus the rate of acetylcholine production is driven by the availability of choline and acetyl CoA (112, 145-146). The rate of choline transport across the blood brain barrier is increased by an amount proportional to the increase in serum concentration and is followed by an increase in the release of acetylcholine from cholinergic neurons (112). In the brain, most of the free choline is phosphorylated to phosphatidylcholine (lecithin) in order to moderate the rate of acetylcholine synthesis in the presence of increased availability of precursor; however, the appearance of choline in cerebrospinal fluid confirms that there is a pool of free choline in the brain (147-148).

Incorporation of choline into membrane phosphatidylcholine provides a ready reserve of precursor for acetylcholine synthesis over periods of short duration (147). In a normal physiological state, the major source of choline utilized for acetylcholine synthesis is membrane phosphatidylcholine (140, 147, 149). When demand for acetylcholine is increased, dietary choline becomes an increasingly more important source of precursor over prolonged periods of demand. If a supplemental source of choline is not provided to meet increased demands, loss of membrane phosphatidylcholine will eventually compromise cell membrane function and trigger apoptosis (129, 140, 147, 150-152).

Since changes in choline levels in the blood and urine correspond to changes in dietary choline intake, measurements of choline levels in these body fluids have been used to evaluate choline status following dietary deficiency or augmentation. Low blood levels of choline indicate that the requirements for the dietary precursors are not being met at current levels of intake (112, 127, 134, 147). Serum choline levels are more responsive to supplementation with dietary choline than to a choline deficiency with increases of as much as 52% observed with supplementation (107) compared with decreases of 20% observed with a choline-deficient diet (112, 147). The need for increased choline intake by patients with infection is indicated by the reduced levels of plasma acetylcholine observed in these patients accompanied by a suppression of the immune response and a reduction in lysosomal enzymes and phagocytic activity (135). These low levels of acetylcholine have been attributed to the increased amounts of acetylcholinesterase released into the plasma by parasites and other pathogens.

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 the amount of free choline available is limited (148). Brain phosphatidylcholine levels decrease in parallel with the decrease in serum choline which further suggests that brain choline concentration is maintained within narrow limits at the expense of larger tissue pools of phosphatidylcholine and other phospholipid precursors (serine and ethanolamine) (127, 147). Data from an experimental study in rats showed that brain choline concentration increased within 5 hours following oral administration of choline chloride (148). The consumption of a choline-free diet for 7 days lowered serum choline and brain phosphatidylcholine concentration suggesting that choline kinase, the controlling enzyme in phospholipid synthesis, is unsaturated with substrate in vivo and thus may serve as a modulator of the response of brain choline concentrations to alterations in the supply of circulating choline.

Other studies have confirmed that dietary choline can be utilized by central cholinergic neurons as a precursor of acetylcholine (148). An increase in plasma choline in response to choline supplementation promotes the expression of high affinity choline transporters on cholinergic neurons which regulate the synaptic availability of choline and facilitate the release of acetylcholine from these neurons (117, 129, 136, 147, 160). Synaptic acetylcholine levels are regulated by a negative feedback mechanism in which accumulation of the neurotransmitter inhibits transporter activity on cholinergic neurons to prevent further uptake of choline. Anticholinergic drugs such as chlorpromazine, atropine, and cholinesterase inhibitors decrease acetylcholine release by inhibition of these transporters (153-155).

Clinical evidence of a human choline deficiency was first reported in adults receiving total parenteral nutrition (TPN) (156-157). These patients exhibited hepatic morphologic and aminotransferase abnormalities which were reversed by choline-supplemented TPN. The effects of inadequate choline intakes on physiological endpoints are rapidly observed. Clinical signs of deficiency were documented in men with otherwise normal nutritional status after consuming a choline-deficient diet for a period of < 2 weeks (134, 158-159). Changes in blood and urine markers of organ dysfunction (muscle and liver enzymes) were also been reported in these men. Decreases in plasma levels of choline and phosphatidylcholine accompanied by elevated alanine aminotransferase, a biochemical marker of liver damage, and elevated creatine kinase, a biological marker of muscle damage, have also been observed with a dietary choline deficiency (5, 111, 115, 152, 158-160).

**Neurotransmitter deficiency syndromes.** Specific neurotransmitter deficiency syndromes related to inadequate intakes of dietary precursors have also been identified for GABA and serotonin further supporting a link between requirements for nutrient precursors and production of sufficient amounts of the corresponding neurotransmitters. A syndrome characterized by a basic depressive state, sleep disorders, and other clinical symptoms has been attributed to a GABA deficiency based on the observation of a rapid reversal of these symptoms following administration of an enzyme inhibitor of GABA catabolism (161). Several findings raise the possibility that inadequate intakes of tryptophan may be related to a brain serotonin deficiency in patients with fibromyalgia/fibrositis. An inverse relationship between blood tryptophan concentration and severity of musculoskeletal pain has been reported in patients with these conditions which was accompanied by significantly lower levels of serum tryptophan

( $p = 0.002$ ) compared with healthy adults suggesting that a functional deficiency of serotonin may be involved in the pathology of this syndrome (162).

A possible link between inadequate intake of tryptophan and a serotonin deficiency is also supported by evidence of altered tryptophan metabolism associated with low tryptophan blood levels (163). A trend towards lower levels of plasma tryptophan was associated with a significantly lower tryptophan membrane transport ratio ( $p < 0.01$ ) in patients with primary fibromyalgia/fibrositis compared with controls indicating that insufficient amounts of tryptophan were reaching target tissues when plasma levels were below normal (162-163). A specific neuroendocrine response (i.e., prolactin release with tryptophan infusion) suggestive of postsynaptic serotonin receptor supersensitivity was associated with a 15 to 20% reduction in fasting total plasma tryptophan levels in 22 healthy subjects consuming a tryptophan-restricted diet (135). A serotonin deficiency has also been linked to glutamate intakes based on the finding that plasma glutamate levels are a highly significant discriminatory variable in distinguishing patients with major depression from controls (143, 162).

**Requirement for carnitine.** Acetyl-L-carnitine supplements at doses of 1000-2000 g/d have also been shown to reduce fatigue associated with fibromyalgia and mild depression (dysthymia) after 8-24 weeks indicating that needs for carnitine were increased in these diseases (164-167). The results of a double-blind placebo-controlled trial of patients with a clinical diagnosis of fibromyalgia who received supplemental acetyl-L-carnitine for a 10-week period revealed a statistically significant decrease from baseline in self-assessment of fatigue, tiredness upon awakening and sleep experience (119). These parameters were also significantly improved compared with controls.

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

**Table 4. Observations Supporting Increased Nutrient Requirements in Sleep Disorders**

<b>Nutrient</b>	<b>Blood/Tissue/Urinary Levels</b>	<b>Clinical Observations and Associated Biochemical Findings</b>
<b>GABA</b>	Low blood and brain levels	Insomnia, fragmented sleep; deficiency syndrome characterized by a basic depressive state, sleep disorders, nuchal headache, and other clinical symptoms
<b>Tryptophan</b>	Low blood levels	Sleep apnea, snoring; low blood 5-hydroxytryptophan and serotonin levels; increased serotonin metabolites in cerebrospinal fluid with tryptophan supplementation; low membrane transport ratio; low blood and brain serotonin levels; postsynaptic serotonin receptor supersensitivity
<b>Choline</b>	Low plasma levels	Sleep apnea syndromes and disorders of restorative sleep; depression-associated sleep disorders; decreased transport across the blood brain barrier; decreased parasympathetic autonomic nervous system activity; documented human choline deficiency diseases; diminished responses to GABA and serotonin; increased creatine phosphokinase and alanine transaminase; myocyte and lymphocyte apoptosis

Nutrient	Blood/Tissue/Urinary Levels	Clinical Observations and Associated Biochemical Findings
<b>Carnitine</b>	Low plasma levels	Fatigue associated with mild depression (dysthymia) and fibromyalgia; acetylcholine deficiency; cholinergic deficits
<b>Glutamate</b>	Low blood levels	Insomnia, fragmented sleep; low blood and tissue glutathione levels; GABA deficiency characterized by a basic depressive state, sleep disorders, and other clinical symptoms; loss of synaptic inhibition; seizures

### **Clinical Validation of GABAdone**

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 dietary factors (8, 50, 53-54, 99, 103, 106-107, 110, 112, 119-120, 131, 134-137, 143). A change in the 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 the uptake and utilization of the nutrient or dietary factor by target cells for synthesis of the neurotransmitter, thus demonstrating the biological availability of dietary precursors and the clinical utility of the medical food as a source of these precursors (29, 50, 60, 93-95, 99, 103, 106-107, 111, 117, 120, 123-124, 127, 129, 131, 136, 140-141, 143-146, 148, 152, 162-169).

The clinical benefits that 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. If an individual with a sleep disorder ingesting a medical food containing 5-hydroxytryptophan shows an increase in blood levels following ingestion of the molecule (biological availability) which is associated with increased concentrations of serotonin metabolites in cerebrospinal fluid (physiological response) and an improvement in sleep patterns (clinical response), the clinical benefit of this medical food as a source of precursors for serotonin production has been validated. Improvement in sleep latency from 120 to 10 minutes following consumption of 2000 mg of 5-hydroxytryptophan would support the requirement for an additional allowance of tryptophan by individuals having difficulty falling asleep and maintaining sleep.

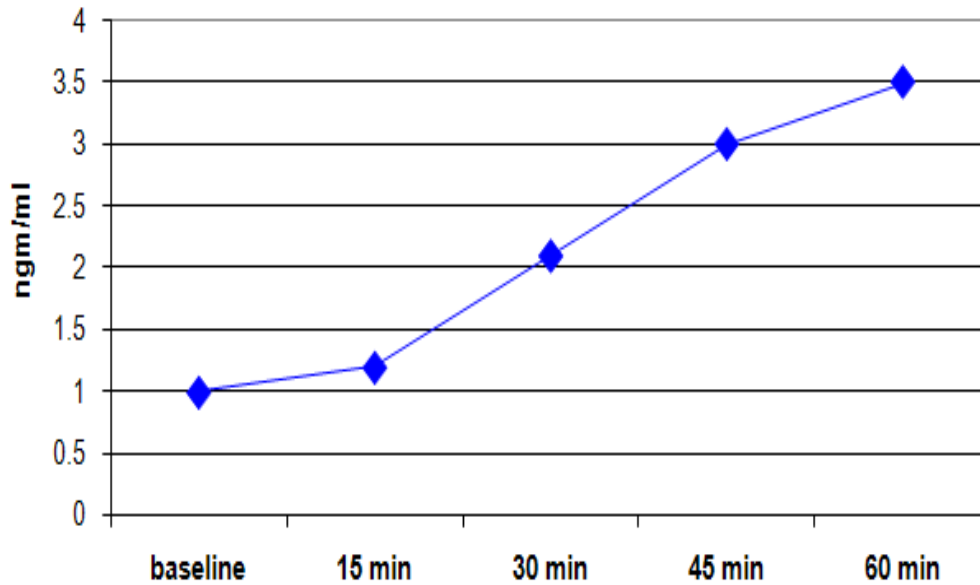
**GABAdone** has been formulated with specific ratios of choline, glutamate, 5-hydroxytryptophan, and acetyl-L-carnitine using *Targeted Cellular Technology* to control the timing of the release of each ingredient. If sufficient amounts of these nutrients are not available, or their availability is not well-synchronized with demand, imbalances in neurotransmitter activity may contribute to the development of sleep disorders (13-14, 20, 30, 34-35, 36-37, 39-40, 42).

### ***Biological Availability***

The biological availability of 5-hydroxytryptophan, the source of serotonin in **GABAdone**, has been demonstrated by changes in blood serotonin levels observed within 15 minutes of ingestion of 2000 mg of 5-hydroxytryptophan (Figure 9). These levels continued to increase to more than 3-fold higher than

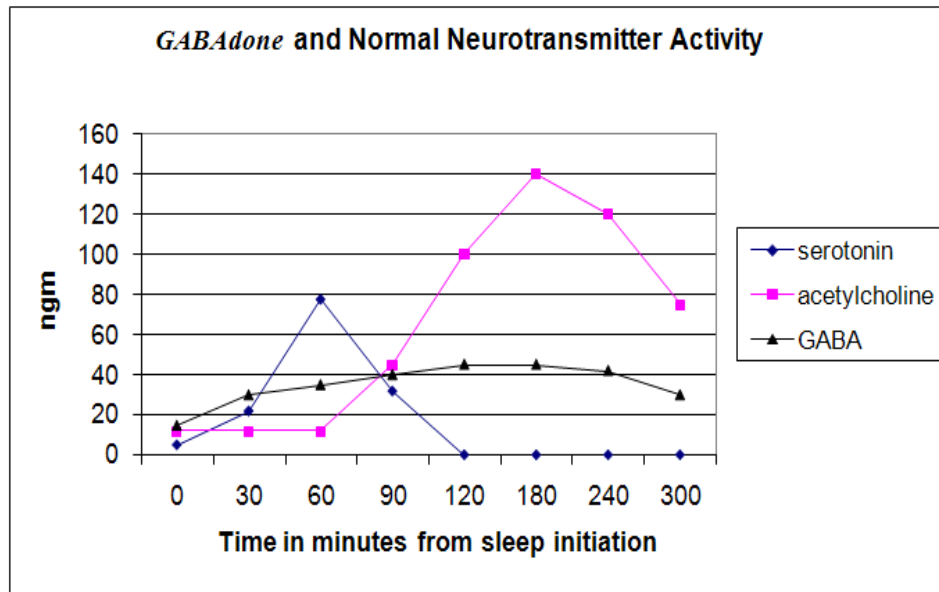
baseline levels at 60 minutes, confirming that 5-hydroxytryptophan was being utilized to increase production of serotonin.

**Figure 9. Effect of 5-Hydroxytryptophan Supplementation on Blood Serotonin Levels**



#### *Physiological Response*

Figure 10 depicts the pattern of changes observed in the blood levels of serotonin, acetylcholine, and GABA following consumption of **GABAdone** at the recommended 2 capsule dose. These data confirm that the levels of neurotransmitters obtained from **GABAdone** or from the precursors provided in **GABAdone** change over the sleep cycle in patterns expected based on observations with endogenous neurotransmitters.

Figure 10. Physiological Response to **GABA**done Administration

### Clinical Response

A number of clinical trials performed with **GABA**done have demonstrated favorable effects on initiation of sleep, frequency of snoring, and duration of restorative sleep. These trials include:

- 2 open-label trials of effects on induction and maintenance of sleep and frequency of snoring
- 8 open label trials of effects on awakening in the middle of the night
- 1 randomized, double-blind, placebo-controlled trial of effects on timing and quality of sleep.

### *A Randomized Placebo-Controlled Trial of an Amino Acid Preparation on Timing and Quality of Sleep*

The effects of **GABA**done on timing and quality of sleep were examined in a randomized, double-blind, placebo-controlled trial in 18 subjects > 18 years of age with a history of self-reported deficiency of restorative sleep. The primary exclusion criteria were current use of sleep medications, known endocrine disease, or previous treatment with **GABA**done. Subjects who met all eligibility criteria were randomized to treatment with 2000 mg/d (2 capsules) of **GABA**done or to placebo for a 1-week period and instructed to maintain their current sleep routine for the duration of the study. The primary clinical outcome variables were time to fall asleep and sleep quality scores based on both visual analogue and Likert numeric scales. In addition, activation of parasympathetic nervous system function was assessed by a repeat 24-hour ECG on the 6th day and again on the night of the 7th day of the treatment period using Heart Rate Variability analysis, a method that has been validated in patients with sleep disorders. Parasympathetic system activation which is associated with normal sleep patterns is considered to be an objective indicator of restorative sleep and reduced snoring.

The results from this study are displayed in Figures 9 through 12. Statistically significant improvements from baseline in time to fall asleep ( $p=0.01$ ) (Figure 11), hours of sleep (Figure 12) ( $p=0.01$ ), number of awakenings during the night ( $p<0.01$ ) (Figure 13), and morning grogginess measured as an indicator of restorative sleep were demonstrated accompanied by a statistically significant reduction in snoring ( $p=0.01$ ) in subjects treated with **GABAdone** compared with placebo (Figure 14). These findings of improvements in sleep patterns examined in this study were confirmed by a statistically significant increase in the objective measure of parasympathetic nervous system activity in subjects treated with **GABAdone** (Figure 15). None of these parameters were changed by statistically significant amounts in the placebo group.

**Figure 11. Changes in Time to Fall Asleep with *GABAdone* vs Placebo**

**Figure 12. Changes in Hours Slept with *GABAdone* vs Placebo**

Figure 13. Changes in Number of Awakenings with *GABA*done vs Placebo

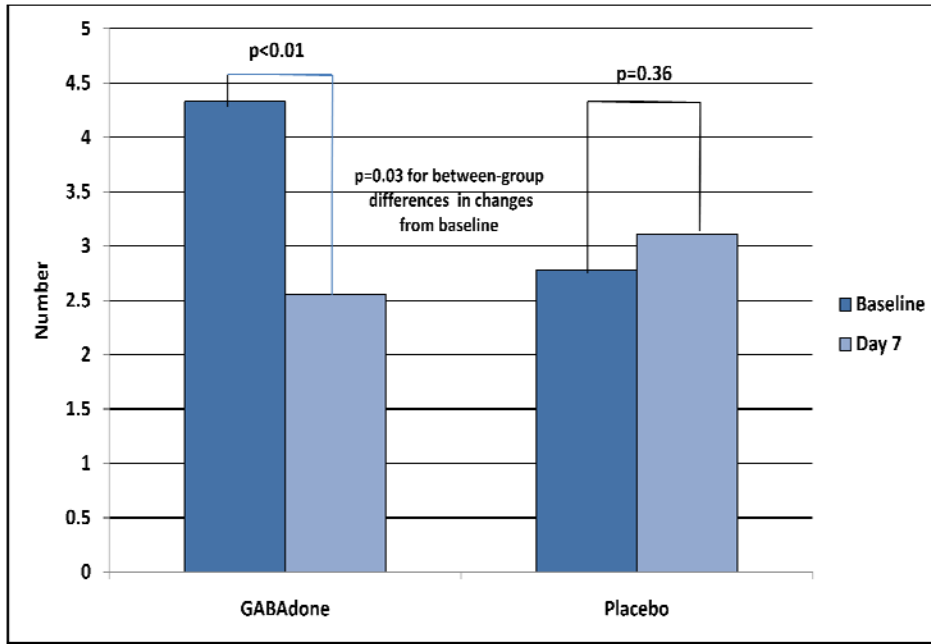


Figure 14. Changes in AM Grogginess with *GABA*done vs Placebo

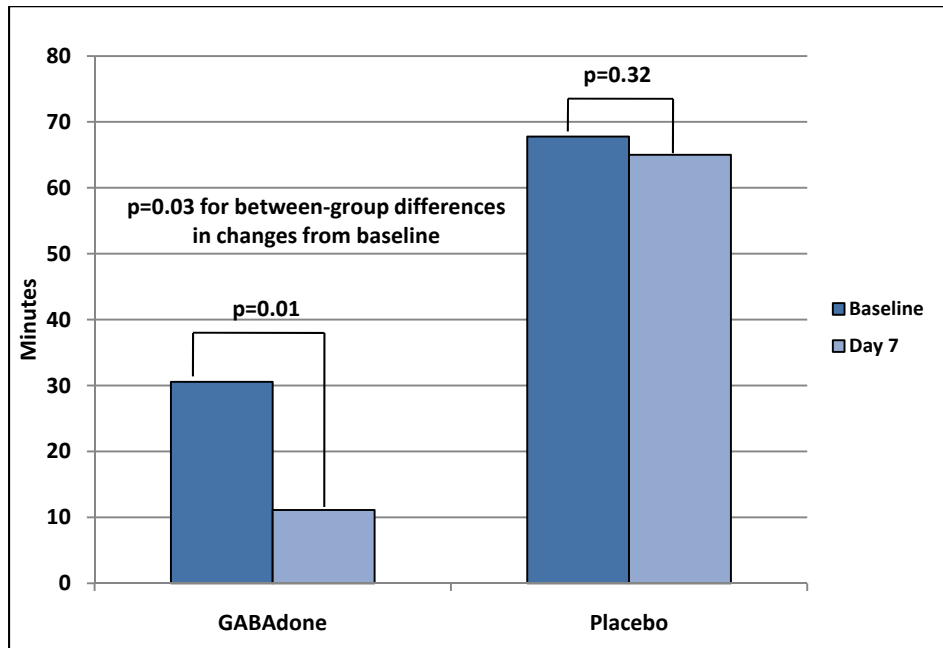
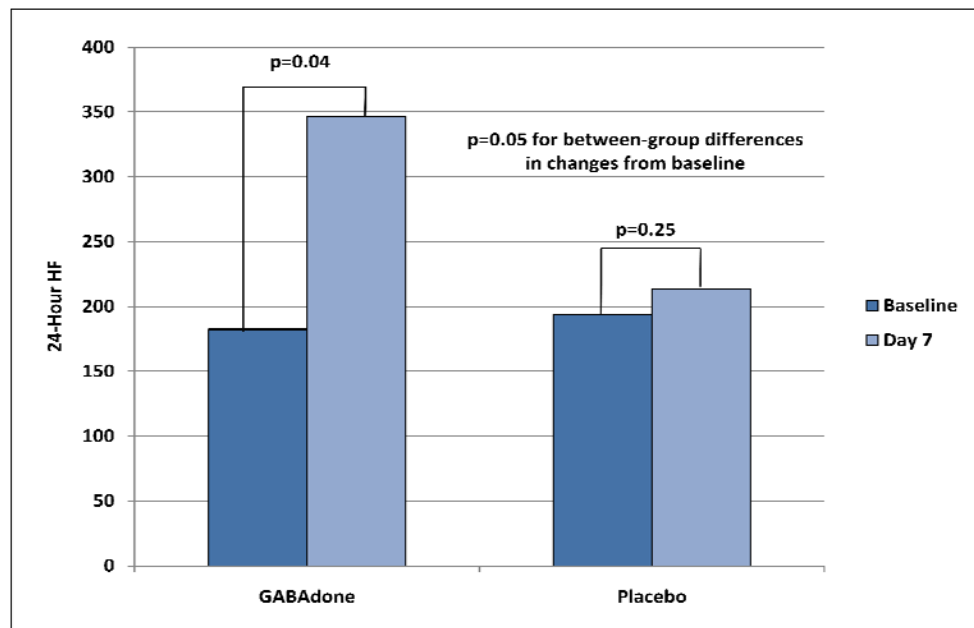


Figure 15. Changes in Parasympathetic Activity with **GABA**done vs Placebo

Compared with baseline, subjects in the **GABA**done treatment group showed a mean decrease of approximately 40% in time to fall asleep, a mean increase of approximately 2 hours in the hours slept, a mean decrease in frequency of awakenings from 4 times to 3, and a mean decrease of 64% in minutes of morning grogginess. None of the corresponding mean changes in the placebo group was increased by more than 5% of baseline values.

The between-group differences in changes from baseline to Day 7 in time to fall asleep ( $p=0.02$ ), hours slept ( $p=0.01$ ), number of awakenings ( $p=0.03$ ), and morning grogginess ( $p=0.01$ ) were also statistically significant. These changes were associated with a statistically significant increase of approximately 48% in parasympathetic activity in the **GABA**done treatment group ( $p=0.04$ ). The change in parasympathetic activity in the placebo group was <10% of baseline and not statistically significant.

The results of this study support a clinical benefit of **GABA**done for individuals with sleep disorders involving difficulty in falling asleep, maintaining sleep, resuming sleep after awakening, feeling tired upon awakening, and snoring.

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