

Trepadone™ Product Information

Indications

Trepadone is intended for use in the management of joint disorders associated with pain and inflammation. ***Trepadone*** is a medical food that must be used in patients who are under the active and 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 joint degeneration and/or injuries associated with pain and inflammation.¹

Pain is a complex process mediated by neurotransmitters which transmit signals originating from a pain-inducing stimulus to specific centers in the brain where it is perceived. Although joint pain may originate from different sources, the most common are destruction of articular (joint) cartilage and inflammation. Loss of the cushioning effect of the articular cartilage increases the impact of weight-bearing activity on the ends of the bones in the joint while inflammation heightens pain by sensitizing the joint pain receptors to mechanical stimuli. ***Trepadone*** provides a balance of neurotransmitters with well-defined roles in the modulation of pain and inflammation complemented by a blend of antioxidants, anti-inflammatory agents, and immunomodulators that moderate the effects of inflammation on the pain response through effects on eicosanoid production and glucocorticoid release. ***Trepadone*** also contains glucosamine and chondroitin sulfate which maintain the structural integrity and functional properties of joints.

Ingredients

Trepadone is a proprietary blend of neurotransmitter precursors (L-arginine, L-glutamine, L-histidine, choline bitartrate, 5-hydroxytryptophan, L-serine) and neurotransmitters (gamma-aminobutyric acid [GABA]); polyphenolic antioxidants (grape seed extract, cinnamon bark, cocoa); anti-inflammatory compounds (omega-3 fatty acids and histidine); immunomodulatory peptides (whey protein hydrolysate); precursors of functional components of joint connective tissue (glucosamine and chondroitin sulfate); and an adenosine antagonist (cocoa powder). Each of these ingredients has been specifically selected based on scientific support for their roles in the physiological processes involved in reduction of pain and inflammation associated with joint disorders. These roles are summarized in this monograph in the section *Scientific Support for Use of Trepadone in Management of Joint Disorders*.

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

All of the ingredients included in **Trepadone** 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 it is safe when consumed in amounts obtained from these foods as they are typically ingested or prescribed.

Targeted Cellular Technology™

Trepadone 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 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 amino acid 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 neurotransmitters, which is especially important for processes associated with circadian rhythms in which timing is critical such as utilization of arginine for the production of nitric oxide and release of histamine in the hypothalamus (1, 2).

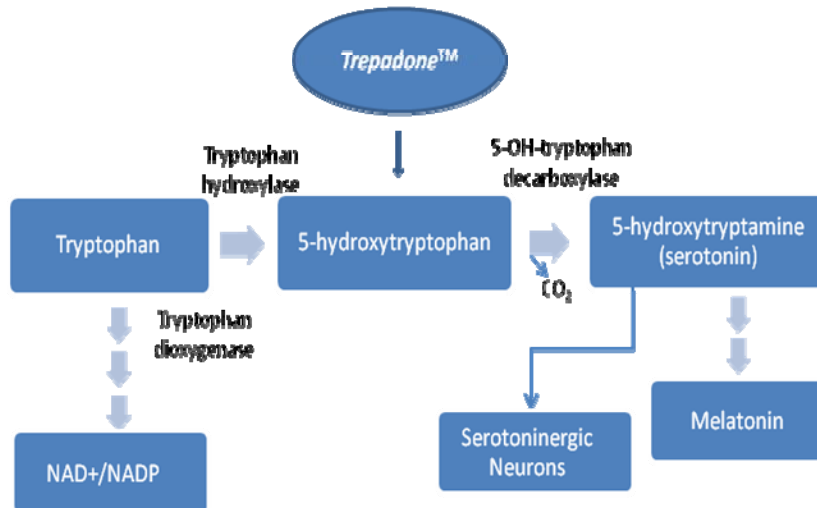
Previous attempts to provide an exogenous source of precursor amino acids in the quantities required to support neurotransmitter synthesis for individuals with specific needs necessitated that large amounts of these amino acids be added to the formulations. For patients whose requirements were considerably higher than normal, the amounts of exogenous amino acids that needed to be added were not practical to consume on a daily basis. Ingestion of these large quantities of amino acids contributed to increased risk of adverse effects as well as saturation of tissue uptake receptors resulting in attenuation of the response to these supplemental amounts. 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, thus making daily dosing more feasible and reducing the potential for tolerance. Unlike pharmaceutical products which are not innate components of the pain process, and thus may lose their effectiveness in a relatively short period of time, the effectiveness of **Trepadone** is not attenuated.

Metabolism

Trepadone is a source of amino acids and other nutrients for patients with pain and inflammation associated with joint disorders. These patients require additional amounts of arginine, glutamine, 5-hydroxytryptophan, choline, histidine, and L-serine to support the synthesis of the neurotransmitters, nitric oxide, GABA and glutamate, serotonin (5-hydroxytryptamine), acetylcholine, histamine, and D-serine, respectively. Under normal physiological conditions, glutamate, arginine, and serine are metabolized as nonessential amino acids because endogenous synthesis is sufficient to satisfy metabolic demand. When needs are altered as in joint disorders, the usual rate of synthesis is no longer sufficient and these amino acids become conditionally essential, requiring that a supplemental amount be consumed. Histidine has been considered a nonessential amino acid for adults because it can be obtained from breakdown of skeletal muscle and hemoglobin; however, there is no evidence of de novo histidine synthesis in mammalian tissues and therefore an exogenous supply is important, especially during times of increased needs, to preserve muscle mass and plasma hemoglobin concentration. Choline, carnitine, and omega-3 fatty acids (eicosapentanoic acid) are also considered nonessential nutrients under normal conditions, but become conditionally essential with chronic pain and inflammation when metabolic demand is increased. The need for dietary sources of chondroitin sulfate and glucosamine may also be increased in patients with joint disorders.

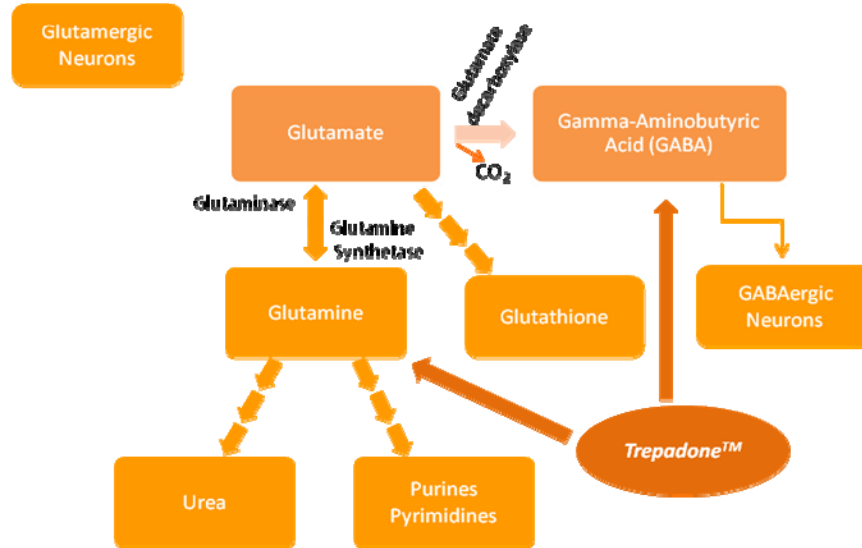
In contrast to the amino acids which are nonessential under normal conditions, tryptophan is an essential amino acid that must always be consumed from exogenous sources, as the enzymes required for its synthesis are absent in humans. Because it is an essential amino acid, the amount of tryptophan consumed determines the amount available to be divided among multiple pathways of utilization. Tryptophan is a precursor not only of serotonin, but also of the coenzymes nicotinamide adenine dinucleotide (NAD⁺) and nicotinamide adenine dinucleotide phosphate (NADP) (Figure 1). The competition between these and other metabolic pathways for the limited supply of tryptophan restricts the amount of serotonin that can be produced from supplemental amounts of the amino acid. To overcome this limitation, **Trepadone** provides 5-hydroxytryptophan, which is the immediate precursor of serotonin in the pathway of conversion from tryptophan (Figure 1). The availability of this intermediate circumvents the limiting step in serotonin synthesis and lessens the dependence of serotonin levels on the amount of tryptophan consumed. By facilitating production of serotonin without requiring large amounts of tryptophan as a precursor, **Trepadone** conserves the existing supply of the amino acid for other uses, thus improving metabolic efficiency.

Figure 1. Competing Pathways of Tryptophan Metabolism



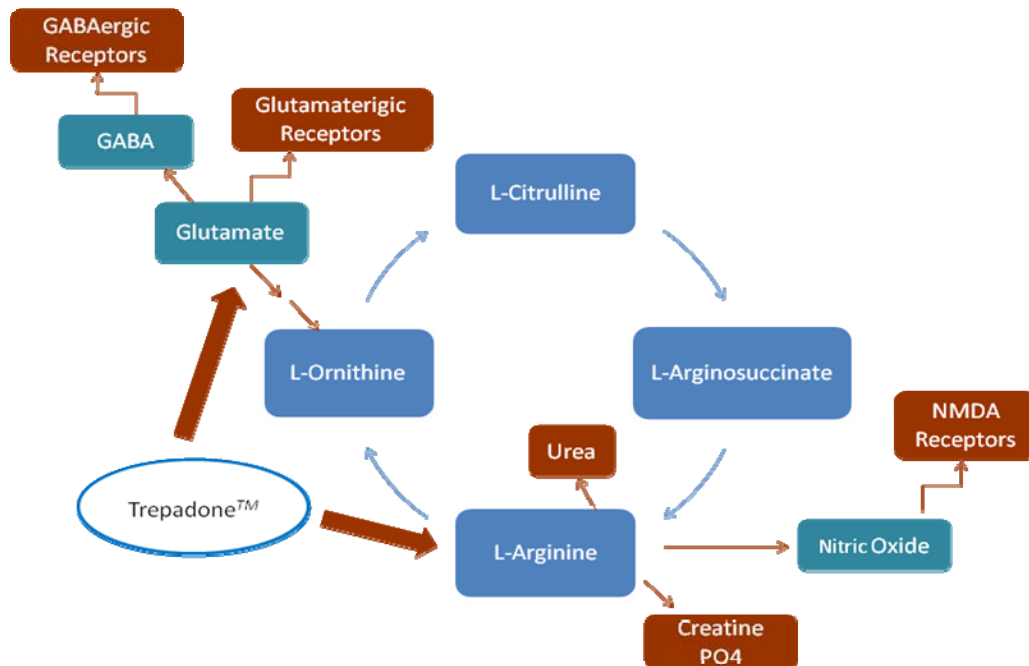
As nonessential amino acids, glutamate, arginine and serine are not normally dependent on exogenous sources, thus metabolic competition for these amino acids develops only under conditions of increased demand. For individuals with joint disorders, the requirement for glutamate increases because additional amounts are needed to support GABA synthesis as well as to maintain activity of glutamatergic neurons. Under normal physiological conditions, glutamate can be supplied by several sources including 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, and thus the amount of GABA available to function as neurotransmitters. As a source of both GABA and glutamate, *Trepadone* improves metabolic efficiency by insuring that there are adequate amounts of both neurotransmitters available while conserving the supply of glutamine for other uses.

Figure 2. Competing Pathways for Utilization of Glutamate



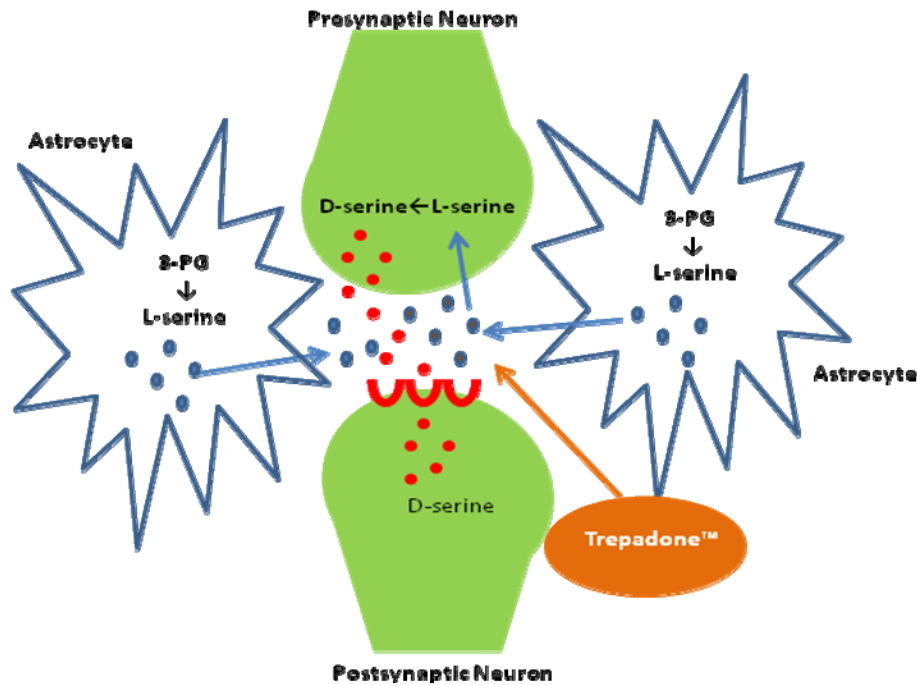
The metabolic pathways which generate arginine are also normally sufficient to ensure an adequate supply of this amino acid. Arginine is utilized as a precursor of nitric oxide as well as creatine phosphate and urea (Figure 3). When demand for nitric oxide is increased, arginine is diverted from the pathways for synthesis of these other compounds. To compensate for the resulting decrease in arginine available to these competing metabolic pathways, glutamate is mobilized as a substrate for synthesis of the additional amounts of arginine needed. **Trepadone** improves metabolic efficiency by insuring that there is a sufficient amount of arginine available to satisfy the competitive demands for this amino acid which will otherwise deplete the glutamate body pool and upset neurotransmitter balance (nitric oxide, glutamate, and GABA). Supplemental arginine will also ensure that there is sufficient glutamine available to conserve the existing supply of glutamate.

Figure 3. Competing Pathways for Utilization of Arginine



Serine is a nonessential amino acid which functions as a neurotransmitter/neuromodulator in the brain and spinal cord in its D-isomeric form (3). Since amino acids can only be incorporated into human proteins as L-isomers, the only role for D-serine is to function as a neurotransmitter. As a nonessential amino acid, L-serine is synthesized in adequate amounts under normal conditions from 3-phosphoglycerate, an intermediate of glucose metabolism. To produce the neurotransmitter, L-serine is converted to D-serine by serine racemase localized primarily in specific neurons in the central nervous system (Figure 4). These neurons normally obtain L-serine from plasma because they lack the enzymes for de novo synthesis of this amino acid. When demand for D-serine is high, these neurons rely more nearby astrocytes to produce additional L-serine. As a result, astrocyte production of L-serine is limiting to the amount of D-serine synthesized by CNS neurons. **Trepadone** provides additional L-serine to overcome this limitation and ensures that there are adequate levels of D-serine available to support serine-regulated neuronal activity.

Figure 4. Pathway for Synthesis of D-Serine



Choline is considered a nonessential nutrient under normal physiological conditions because it can be synthesized in sufficient amounts to meet usual metabolic demand. A steady supply of choline is also available from breakdown of phosphatidylcholine (lecithin), an abundant phospholipid found in cell membranes which can serve as a reservoir of choline to meet short-term needs. If cholinergic activity is sustained at high levels over time, the demand for acetylcholine will eventually exceed the amount of choline that can be supplied by de novo synthesis or from the membrane phospholipid pool, and additional choline must be consumed from exogenous sources. **Trepadone** provides supplemental choline to prevent an acetylcholine deficiency when demands for the neurotransmitter are increased.

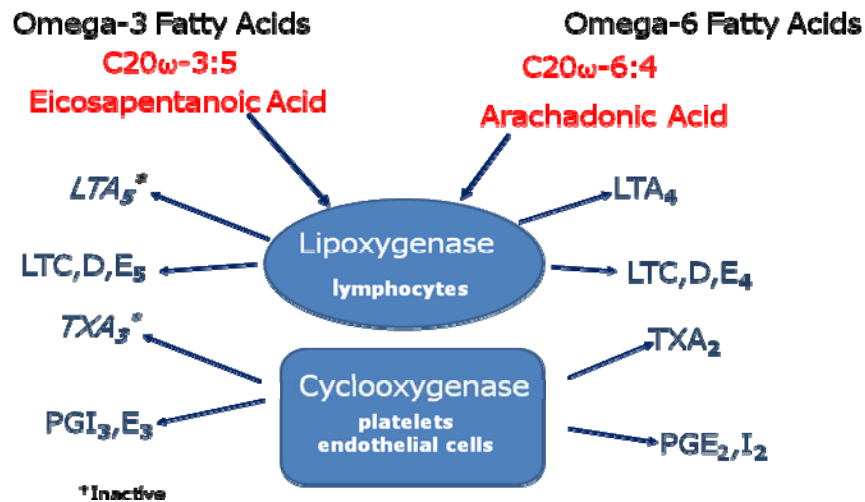
In addition to the amino acid precursors of neurotransmitters involved in pain and inflammation, **Trepadone** is also a source of the omega-3 fatty acid precursors of anti-inflammatory eicosanoids, a family of biologically active lipids that include prostaglandins, thromboxanes, and leukotrienes. The omega-3 fatty acid-derived eicosanoids have effects that oppose their omega-6 fatty acid-derived counterparts (Figure 5). In the lymphocytes, eicosapentanoic acid, the primary omega-3 fatty acid substrate, is converted to anti-inflammatory leukotrienes (LTC₅, LTD₅, LTE₅) whereas arachidonic acid, the primary omega-6 fatty acid substrate, is converted to the corresponding proinflammatory leukotrienes (LTB₄, LTC₄, LTD₄, LTE₄). LTB₄ is a potent chemotactic agent for leukocytes which also stimulates the release of lysosomal enzymes and

enhances generation of reactive oxygen species and production of the cytokines, tumor necrosis factor α , interleukin-1, and interleukin-6. High concentrations of these cytokines are particularly destructive to tissues and if sustained at high levels, are contributing factors to chronic inflammatory diseases such as rheumatoid arthritis (RA).

In the platelets and endothelial cells, eicosapentanoic acid is converted to prostaglandins (e.g., PGE₃) which have anti-inflammatory effects in addition to biologically weak thromboxanes, while arachidonic acid is converted to the family of prostaglandins (e.g., PGE₂) which induce fever, increase vascular permeability and vasodilation, and enhance pain and edema caused by other agents. Since both types of fatty acids compete for the same enzymes in the lymphocytes and platelets (lipoxygenase and cyclooxygenase, respectively), an imbalance in dietary intake that favors omega-6 fatty acids will give these fatty acids a competitive advantage resulting in increased production of omega-6 fatty acid-derived eicosanoids and pro-inflammatory effects will dominate. If intake of omega-3 fatty acids is increased relative to omega-6 fatty acid intake, the balance of eicosanoids will tip in favor of the anti-inflammatory effects of the omega-3-fatty acid-derived eicosanoids which will mitigate the pro-inflammatory effects of the omega-6 fatty acid-derived eicosanoids. Lipoxygenase and cyclooxygenase have a greater affinity for omega-3 fatty acids than for omega-6 fatty acids and therefore, omega-3 fatty acid intake does not have increase by a large amount to competitively inhibit the synthesis of omega-6 fatty acid-derived eicosanoids.

Although both omega-3 and omega-6 fatty acids are ≥ 20 carbons polyunsaturated acids with multiple double bonds, omega-3 fatty acids are structurally different from omega-6 fatty acids in metabolically significant ways involving both the number of double bonds and the positioning of these double bonds relative to the carboxyl group (omega carbon) of the fatty acid chain. Both the omega-3- and the omega-6-derived eicosanoids are synthesized from their respective 20-carbon fatty acid precursors, eicosapentanoic acid (omega-3) and arachidonic acid (omega-6), by the same enzymes in the same metabolic pathways.

Figure 5. Competing Pathways of Eicosanoid Synthesis

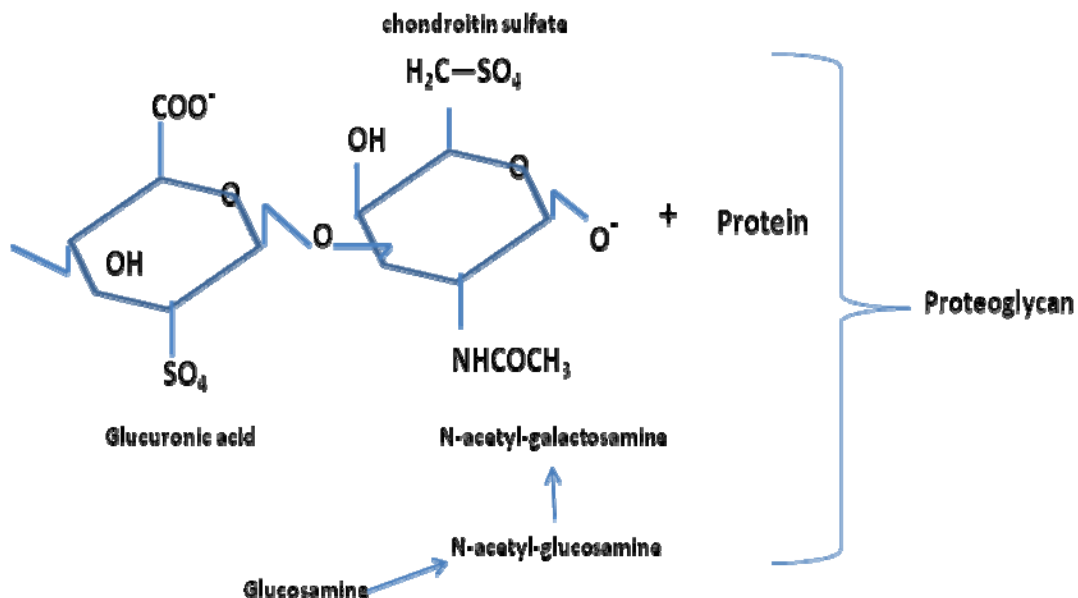


LT=leukotrienes, TX=thromboxanes, and PG= prostaglandins

Trepadone is a source of glucosamine and chondroitin sulfate which are precursors of the proteoglycans found in the extracellular matrix of joint cartilage that are responsible for the tensile strength and elasticity of this tissue and its resistance to compression. Proteoglycans are formed by the covalent linking of glycosaminoglycans such as chondroitin sulfate to a protein core (Figure 6). Chondroitin sulfate is a polymeric carbohydrate comprising a repeating disaccharide unit of glucuronic acid and N-acetyl-galactosamine. It is the most abundant glycosaminoglycan in the human body and is concentrated primarily in cartilage, tendons, ligaments, and blood vessels.

Glucosamine is an amino-sugar which serves as a precursor for synthesis of all glycosaminoglycans in the human body. Supplemental glucosamine, obtained from **Trepadone**, ensures that an adequate supply of substrate for production of glycosaminoglycans such as chondroitin sulfate, while supplemental chondroitin sulfate ensures that a sufficient amount of this particular glycosaminoglycan is available to support increased synthesis of proteoglycans in joint cartilage.

Figure 6. Glucosamine and Chondroitin Sulfate in Proteoglycan Synthesis



Dosage

The recommended dose of *Trepadone* is 2 capsules taken 1 to 4 times daily as directed by a physician. *Trepadone* should be taken with water at least 30 minutes before or after eating. *Trepadone* can also be used with low dose aspirin or NSAIDS once daily. *Trepadone* is formulated to reduce inflammation and support the function of drug anti-inflammatory agents thereby reducing the drug side effects. As with any medical food, the best dosing protocol should be determined by assessment of individual needs. At the doses of *Trepadone* recommended to relieve pain and inflammation, the amounts of each ingredient consumed based on body weight are presented in Table 1.

Table 1. *Trepadone* Composition

Ingredient	mg/kg body weight ¹
δ-aminobutyric acid (GABA)	1.5 – 12.0
L-arginine	0.6 - 4.6
Whey protein hydrolysate	0.6 - 4.6
L-histidine	0.4 – 3.1
L-serine	0.4 – 3.1
L-glutamine	0.4 – 3.1
5-hydroxytryptophan	0.2– 1.9

Ingredient	mg/kg body weight ¹
(griffonia seed, 95% w/w)	
grape seed extract	0.2 – 1.5
chondroitin sulfate	
glucosamine	
omega-3 fatty acids (tuna oil)	
cocoa powder	0.2 – 1.5

¹Dosing range of 1 to 2 capsules

Patients who are taking pharmaceutical agents to relieve pain may continue to take these medications with **Trepadone**. **Trepadone** is formulated with omega-3 fatty acid, glucosamine and chondroitin sulfate to reduce inflammation, and thus to act synergistically with aspirin and nonsteroidal anti-inflammatory drugs. If use of a drug in conjunction with **Trepadone** is effective in relieving pain, then the drug dosage may be further tapered to lower levels under medical supervision.

Side Effects

As with any amino acid therapy, headache, nausea, or dry mouth may be experienced in some people after beginning treatment with **Trepadone**. These symptoms are mild and temporary, and readily managed by increasing fluid intake. The development of side effects with use of **Trepadone** can be minimized by careful titration of the dosage. All of the ingredients in **Trepadone** are regularly consumed in amounts normally found in foods or dietary supplements; therefore development of an adverse reaction to **Trepadone** 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 cell damage from exposure to oxygen free radicals
Anti-inflammatory	Inhibition of the synthesis and release of chemicals that initiate and sustain an inflammatory response
Antinocioption	Reduction of pain through inhibition of nociceptor activity
Articular (joint) capsule	A layer of connective tissue covering the ends of the bones that connect at the joint

Term/Abbreviation	Definition
Articular cartilage	A pad of hyaline cartilage that covers the articulating surfaces of the bones in the shoulder, hand, elbow, and knee and reduces friction and distributes forces of weight-bearing
Articulating surface	End of bones that move against one another in the joint
Chondrocytes	Cells that synthesize and extrude collagen within the extracellular matrix of joint connective tissue
Collagen	A component of connective tissue that gives it tensile strength and elasticity
Chondroitin sulfate	A polymeric carbohydrate comprising a repeating disaccharide unit of glucuronic acid and N-acetyl-galactosamine which is the most abundant glycosaminoglycan in the human body and is concentrated in cartilage, tendons, ligaments, and blood vessels.
Connective tissue	Tissue involved in structure and support that is found predominately in tendons, ligaments, and joints
CRP	C-reactive protein, a summary indicator of inflammation
Eicosanoids	Biologically active lipids derived from the 20-carbon polyunsaturated fatty-acids, eicosapentanoic and arachidonic acids
Excitatory Neurotransmitters	Mediators of neural signals that accelerate the rate of transmission through depolarizing postsynaptic neuronal membranes resulting in increased responsive to a stimulus or reduced responsiveness to a stimulus through stimulation of inhibitory mechanisms
GABAergic	Neurons that secrete gamma-aminobutyric acid
Glucosamine	Precursor of glycosaminoglycans
Glycosaminoglycans (GAG)	A polymeric carbohydrate with repeating amino groups which is a structural component of joint cartilage
Hyaline cartilage	Semi-transparent cartilage that is strong, flexible and elastic
Inhibitory Neurotransmitters	Mediators of neural signals that slow the rate of transmission through hyperpolarization of postsynaptic membranes; inhibit responsiveness to a stimulus
Leukotrienes	Class of eicosanoids synthesized in lymphocytes by lipoxygenase
Neuromodulators	Moderate responsiveness of neurons to stimulants
Neurotransmitter	Secreted by presynaptic neurons in response to an action potential generated by a stimulus, binds to postsynaptic neurons which alters their membrane properties resulting in transmission of a signal down the neural pathways to a specific center in the brain which interprets the signals to initiate a response
NMDA	N-methyl-D-aspartate receptors which release glutamate thereby stimulating release of substance P
Nociceptors	Receptors at terminal ends of nerve fibers that initiate pain signaling in response to noxious stimuli
OA	Osteoarthritis
Omega-3 fatty acids	Polyunsaturated fatty acids with an odd number of double bonds; the 20-carbon eicosapentanoic acid is a precursor of anti-inflammatory eicosanoids
Omega-6 fatty acids	Polyunsaturated fatty acids with an even number of double bonds; the 20-carbon arachidonic acid is a precursor of pro-inflammatory eicosanoids
Prostaglandins	Compounds derived from (omega-6) or (omega-3) that modulate the inflammatory response
Proteoglycans	Class of glycoproteins formed by covalent linkage of glycosaminoglycans to a protein core; regulates the flow of synovial fluid through articular cartilage
RA	Rheumatoid arthritis

Term/Abbreviation	Definition
Serotonergic	Neurons that secrete serotonin
Synovial membrane	Inner lining of the articular capsule
Synovial fluid	Clear viscous superfiltreated plasma that lubricates the joints and nourishes the articular cartilage
<i>Targeted Cellular Technology</i>	A patent pending process that facilitates endogenous production, uptake, and utilization of neurotransmitter precursors.

Mechanism of Action

Understanding the mechanism of action of **Trepadone** in the management of joint disorders requires a brief overview of the pathophysiology of joint pain and the role of neurotransmitters and inflammation in the pain process. Pain is a complex series of reactions that originates with an interaction between local pain receptors (nociceptors) and noxious stimuli and terminates in pain perception centers in the brain (4-6). Pain reduction is accomplished by moderating the responsiveness of the nociceptors to noxious stimuli, regulating the transmission of pain signals over the neural pathways of the peripheral and central nervous system, and controlling inflammation which sensitizes the nociceptors to noxious stimuli. Pain associated with joint disorders is typically induced by a mechanical stimulus and is always accompanied by inflammation. The neurotransmitters, neurotransmitter precursors, immunomodulators, antioxidants, and anti-inflammatory agents provided in **Trepadone** have been chosen to function in a complementary manner to inhibit the neuronal activity which exacerbates the transmission of pain signals and to mitigate the sensitizing effects of inflammation on neuronal responsiveness (4, 7-21).

Joints are structurally designed to provide stability and mobility to the skeleton. The surfaces of the bones that connect at the joints are protected from the stress of mechanical forces by the flexibility and resilience of the connective tissue in the joint. The three types of joints in the human skeleton are differentiated by the composition of the connective tissue and the degree of mobility it permits. The rigid fibrous connective tissue that makes up the fibrous joints connecting the bones in the skull, and the tibia to the fibula renders these joints almost completely or entirely immovable. The cartilaginous joints that connect the vertebrae, pubic bones, and the ribs to the sternum are characterized by a thick pad of fibrocartilage which permits only slight to moderate movement. In the highly mobile synovial joints, a pad of hyaline cartilage covers the articulating surfaces (moving ends) of the bones in the shoulder, elbow, hand, and knee. Because of the high degree of mobility of synovial joints, disorders that affect these joints are the most common source of pain and also the most incapacitating.

Pain originates in the synovial joints with the pain receptors localized in the articular (joint) capsule that covers the ends of the bones. The nerves in the articular capsule are sensitive to the rate and direction of motion, compression, tension, and vibration as well as pain. The inner lining

of the articular capsule (synovial membrane) is richly supplied with blood and lymphatic vessels that allow rapid repair and regeneration of the tissue. Layered over the joint capsule is a pad of articular cartilage which reduces joint friction and distributes the forces of weight-bearing activity. The enclosed space where the bones of the joint move against one another is the joint cavity which is filled with clear viscous plasma superfiltrate (synovial fluid) of the synovial membrane that functions as a lubricant and as a source of nourishment for the articular cartilage. Since the articular cartilage is devoid of blood vessels, lymphatic vessels and nerves, it regenerates slowly and is insensitive to pain.

The transmission of pain signals from the joint capsule to the pain centers in the brain is regulated by neurotransmitters, amino acids or amino acid derivatives that relay these signals as electrical impulses over neural pathways to the pain centers in the brain where they are processed (22-24). The neurotransmitters are secreted from the terminal endings of the presynaptic neurons into the synaptic cleft where they bind to receptors on the membranes of postsynaptic neurons. Neurotransmitter binding changes the receptor membrane potential, and depending upon the electrochemical properties of the neurotransmitter, will either accelerate or inhibit transmission of the electrical impulse. Excitatory neurotransmitters such as glutamate, serotonin, and D-serine depolarize the membrane which lowers the stimulus threshold for neuronal firing, and increases the frequency and rate of signal transmission (25-27). Inhibitory neurotransmitters such as GABA hyperpolarize the membrane which raises the stimulus threshold resulting in a reduction in the frequency and rate of signal transmission (28). Acetylcholine can exhibit both excitatory and inhibitory effects on neuronal membranes depending upon the area of the brain where the receptors are located.

The responsiveness of neurons to pain signals is amplified by the presence of chemical or electrical phenomena that sensitize them to the incoming signals (29-31). Sensitized neurons discharge spontaneously with greater frequency over extended periods of time establishing the physiological basis for persistent or ongoing pain. Sensitized neurons also release increased amounts of neurotransmitters which augment the responsiveness of spinal cord neurons to all inputs leading to central sensitization (7, 24, 29-31). Activation of pain receptors by sensitizing agents not only amplifies cellular responsiveness to pain stimuli, but also attenuates neuronal sensitivity to antinociceptive receptor stimulants such as endogenous opioids (endorphins, dynorphins, and enkephalins), or exogenously administered opiates such as morphine (32-33).

Persistent pain is an outcome of hyperexcitability of the dorsal horn neurons in the spinal cord originating with severe or prolonged tissue or nerve injury (31, 34-35). This hyperexcitable state potentiates the responsiveness of the dorsal horn neurons to noxious mechanical and chemical stimuli (hyperalgesia) and reduces the pain threshold (allodynia) (35-36). These effects are mediated by pre-synaptic N-methyl-D-aspartate (NMDA)-type glutamate receptors in the spinal cord which transmit pain signals from the periphery to the brain, and by the neuropeptide

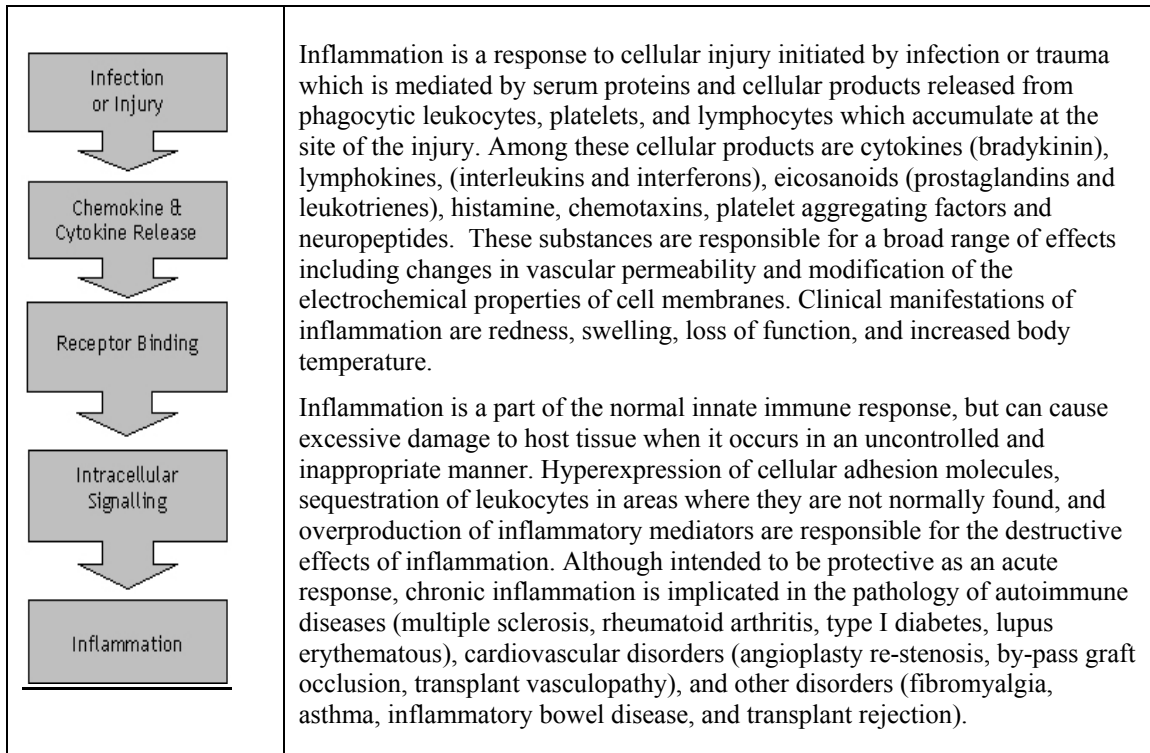
substance P which functions in a manner similar to a neurotransmitter except that it diffuses more widely and has longer lasting effects (37-40). The activated NMDA receptors release glutamate, an excitatory neurotransmitter that increases the neuronal discharge rate and promotes the release of substance P (41-42). D-serine regulates the activity of NMDA receptors in the brain.

The erosion of articular cartilage by enzymatic destruction of collagen fibers within the intracellular matrix is the primary lesion of joint disorders such as osteoarthritis (OA) (43). The loss of collagen disrupts the structural framework of the cartilage and loosens its attachment to the bone. The weakened collagen network is less effective in preventing the loss of fluid and escape of proteoglycans from the cartilage. As a result, the resiliency and cushioning properties are diminished. The shock-absorbing properties of articular cartilage are due to proteoglycans within the extracellular matrix that exert a pumping action which regulates the movement of synovial fluid through this tissue. The pumping action is controlled by the application and release of weight-bearing forces. Loss of proteoglycans from the articular cartilage decreases the flow of synovial fluid through the cartilage which deprives the chondrocytes of their source of nourishment thus reducing the synthesis and extrusion of collagen by these cells.

With this loss of structural integrity, articular cartilage becomes soft, frayed and thinned, and the underlying (subchondral) bone becomes sclerotic (hard and dense). Outgrowths of marginal osteophytes irritate the synovial membrane leading to synovitis and joint effusion. The joint capsule thickens and adheres to the underlying bone causing stiffness of the joint and limiting movement. As the joint capsule is distended and stretched, the pain receptors within the tissue are stimulated. Joint pain can also be initiated by inflammation of the connective tissue as a result of systemic autoimmune disease (rheumatoid arthritis) or of deposition of uric acid crystals that have precipitated from supersaturated synovial fluid (gout). Inflammation initially affects the synovial membrane, eventually spreading to the articular cartilage, joint capsule, and surrounding ligaments and tendons resulting in pain, joint deformity, and loss of function.

The presence of inflammation in the joint contributes to exacerbation of the pain response by increasing neuronal sensitivity to noxious stimuli (12-13, 31, 44-45). As part of the inflammatory response, cytokines, prostaglandins (PGE₂), leukotrienes (LTB₄), and other proinflammatory substances are released and accumulate at the site of tissue injury where they depolarize the peripheral terminals of local nociceptors. In the spinal cord, an elevated concentration of proinflammatory PGE₂ increases the amounts of neurotransmitters released, depolarizes spinal cord neurons, and blocks the effects of inhibitory neurotransmitters. An increase in electrical activity in nociceptors sensitized by proinflammatory substances stimulates the local release of chemicals which promote vasodilation, swelling, and the release of histamine from mast cells, thus sustaining inflammation-mediated neuronal sensitivity and prolonging pain.

The Inflammatory Cascade



Scientific Support for Use of Trepadone in Joint Disorders

The effectiveness of *Trepadone* in the management of pain associated with joint disorders is supported by an extensive body of experimental and clinical data which has identified specific roles for each of the ingredients in reduction of joint pain. *Trepadone* is formulated to ensure the availability of an appropriate balance of neurotransmitters and anti-inflammatory eicosanoids to modulate joint pain and inflammation, and of proteoglycans to preserve the integrity and functional properties of joint connective tissue. Because amino acid uptake by neurons is concentration-dependent, intakes must be sufficient to maintain blood concentrations at high enough levels to drive a rapid rate of uptake (46-50). Moreover, the enzymes that synthesize neurotransmitters are found only in neurons, thus the concentration-dependent rate of precursor uptake by these tissues is the limiting factor in neurotransmitter production. The balance of neurotransmitters released is important because neurotransmitter functions are highly interrelated and regulated by multiple feedback loops; therefore, increased physiological requirements in any one may influence the activities of the others and thus alter the response to a pain-inducing stimulus, inducing absolute and relative deficiencies. (51-53).

Supplemental glucosamine and chondroitin sulfate have been used for more than 40 years to alleviate joint pain. A number of studies have confirmed that both compounds are effective in reducing joint pain in patients with OA, although results have varied across studies (20, 54-56).

The lack of consistency in findings has been attributed to the heterogeneity of the structural composition of chondroitin sulfate indicating that the compounds studied were not identical and that slight differences in structure could have contributed to differences in responses to the supplement. The Glucosamine/Chondroitin Arthritis Intervention Trial (GAIT), a randomized, double-blind, placebo-control, multicenter trial which enrolled over 1500 patients, both glucosamine and chondroitin sulfate taken in combination for 24 weeks significantly reduced joint pain in patients with moderate to severe OA (55-57). In this study, glucosamine and chondroitin sulfate were found to have statistically significant effects on 14 of the 42 outcome measures compared with 6 of 42 observed for celecoxib (56).

The effectiveness of supplemental omega-3 fatty acids as anti-inflammatory agents in chronic inflammatory diseases including RA has been demonstrated by favorable changes in circulating concentrations or ex vivo production of inflammatory modulators (58-61). In vitro studies have shown that both EPA and DHA inhibit production of IL-6, the only cytokine that stimulates the synthesis of all the acute phase reactants involved in inflammation (61). Clinical trials in patients with RA, psoriasis, asthma, inflammatory bowel disorders, and systemic lupus erythematosus have suggested that omega-3 fatty acids have clinically important effects on chronic inflammation (12-13, 62). Significant reductions in C-reactive protein (CRP), a summary index of inflammation, have also been reported in patients with RA (62-63). The anti-inflammatory effects of omega-3 fatty acids are thought to be mediated, at least in part, by reduced synthesis of inflammatory molecules from omega-6 fatty acids.

Whey protein hydrolysate comprises several proteins and peptides with anti-inflammatory, immunomodulatory, and antioxidant properties. In addition, the whey proteins, α -lactalbumin and β -lactoglobulin, also interact with opioid receptors to reduce pain (64-66). Whey is a high biological value protein derived from milk that contains all 22 amino acids necessary for human protein synthesis and metabolism including tryptophan, arginine, and histidine.

A summary of the roles of these and other ingredients in *Trepadone* in management of joint disorders is presented in Table 3.

Table 3. Roles of the *Trepadone* Ingredients in Joint Disorders

Ingredient	Effector Molecule	Function	Role in Neurotransmitter Metabolism
GABA	GABA	Inhibitory neurotransmitter	Dampens pain signals in the spinal cord and brain; activates glutaminergic nerve terminals which inhibit NMDA receptor activity and release of glutamate and substance P (67-69)
5-OH-tryptophan	Serotonin	Excitatory neurotransmitter	Decreases and modulates pain signals from nerve cells in the spinal cord and brain;

Ingredient	Effector Molecule	Function	Role in Neurotransmitter Metabolism
			increases adenosine production by substance P neurons which inhibits release of substance P; inhibits NMDA receptors to further decrease levels of substance P (70-72)
Choline	Acetylcholine	Inhibitory and Excitatory neurotransmitter	Promotes the synthesis and potentiates the effects of nitric oxide and serotonin; reduces neuronal sensitivity and firing; inhibits NMDA receptor activity and production of substance P; suppresses proinflammatory cytokines by activation of the parasympathetic nervous system (27, 73-75)
Glutamine	Glutamine	Facilitator of neurotransmitter precursor uptake	Promotes synthesis of neurotransmitters (17, 76-77)
	Glutamate	Excitatory neurotransmitter	Inhibits NMDA receptors through activation of GABAergic receptors (78)
	GABA	Inhibitory neurotransmitter	Dampens pain signals in the spinal cord and brain; activates glutaminergic nerve terminals which inhibit NMDA receptor activity and release of glutamate and substance P (67-69, 78-79)
	Glutathione	Antioxidant; Immunomodulator	Regulates synthesis of leukotrienes (80-81)
L-Serine	D-Serine	Neurotransmitter/ Neuromodulator	Regulates NMDA-receptor activity; Sensitizes opioid receptor systems to opioids, opioid-like agents, and other analgesics (natural opioids include endorphins, enkephalins, and dynorphins and synthetic opioids include morphine) (3,82-83)
L-Arginine	Nitric Oxide	Inhibitory and excitatory neurotransmitter; immunomodulator; anti-inflammatory	Inhibits pain at low doses and exacerbates pain at high doses by activation of neuronal nitric oxide synthase NOS; inhibits transmission of afferent pain signals in the spinal cord; acts on some peripheral neurons; activates natural opioids; stimulates production of anti-inflammatory prostaglandins. inhibits NMDA receptor activity (84-87)
L-Histidine	Brain histamine	Excitatory neurotransmitter; Anti-inflammatory	Acts in the spinal cord and brain; stimulates production of glucocorticoids which inhibit prostaglandin-mediated inflammation and act synergistically with nitric oxide; inhibits NMDA receptors (88-91)

Ingredient	Effector Molecule	Function	Role in Neurotransmitter Metabolism
Chondroitin Sulfate	Proteoglycan	Anti-catabolic Anti-inflammatory	Contributes to the joint connective tissue properties of tensile strength, elasticity, and resistance to compression (20, 54-55, 92)
Glucosamine	Glycosaminoglycans	Anti-catabolic Anti-inflammatory	Contributes to synthesis of proteoglycans (21, 54-56, 93)
Cocoa Powder	Caffeine	Adenosine antagonist	Increases neuronal activity by competitively binding to adenosine receptors which disinhibits the “adenosine brake” (14, 94-95)
Grape seed extract	Polyphenols	Antioxidant	Preserves receptor membrane integrity and prevents attenuation of responses to neurotransmitter precursors (96-98)
Whey Protein Hydrolysate	α -lactalbumin, β -Lactoglobulin, Glycomacropeptide, Lactoferrin	Opioid Agonist Immunomodulator Antioxidant Anti-inflammatory	α -lactalbumin and β -lactoglobulin reduce pain through interactions with opioid receptors; other peptides reduce the effects of inflammation on pain (64-66)
Omega-3 Fatty Acids	Eicosanoids: PGI ₃ , PGE ₃ and LTC ₅ , LTD ₅ , LTE ₅	Anti-inflammatory	Competitively inhibits omega-6 fatty acid-derived proinflammatory eicosanoids; inhibit production of IL-6 (12-13, 62, 99)
Cinnamon	Cinnamaldehyde 2-methoxy-cinnamaldehyde	Inhibition of osteoclastogenesis	Reduction in osteoclast-like cell formation and inhibiting NFATc1(nuclear factor of activated T cell 1) (100)

Nutritional Requirements of Joint Disorders

The nutrient requirements of most interest for patients with joint pain syndromes are those which function as neurotransmitters in the transmission of pain signals or are utilized for synthesis of neurotransmitters involved in this process (17, 19, 26, 47, 76, 75, 101-105). These nutrients are tryptophan, arginine, glutamate, serine, and choline which are precursors of serotonin, nitric oxide, GABA, D-serine, and acetylcholine, respectively. The need for increased intakes of omega-3 fatty acids and antioxidants is also increased in patients with joint disorders to moderate the effects of inflammation which protects the tissues from oxidative damage associated with the products of the inflammatory response. In addition, increased intakes of dietary factors that support the functional integrity of joint connective tissue are also beneficial to patients with joint disorders. Pain syndromes, particularly arthritis and back pain, are responsive to nutritional management (4, 106-111). The improvement in pain syndromes observed with increased intakes of precursors of neurotransmitters and connective tissue proteoglycans as well as of dietary antioxidants and anti-inflammatory agents supports the need for the higher requirements for these compounds in patients with joint disorders.

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 a particular pathology (112-113). These requirements can be estimated by determining the level of intake at which a physiological response is normalized indicating that the balance between intake and metabolic demand has been restored. Specific disease states such as OA and RA will determine the relative balance between intake and utilization, for example, improvement in perceived intensity of back pain following consumption of supplemental amounts of 5-hydroxytryptophan, arginine, and glutamine from *Trepadone* suggests that additional quantities of tryptophan, arginine, and glutamate are needed by individuals with pain syndromes. The degree of coordination of activity among various neurotransmitters underscores the importance of modulation of the amino acid precursor required for synthesis of these neurotransmitters because of the feedback loops involved (51, 53, 114).

The presence of a disease with underlying pathology that involves imbalances in neurotransmitters will increase the requirements for certain amino acids and other nutrient precursors to restore homeostasis (18, 74, 112, 115). As blood levels of these nutrients 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 (76, 115-117). The appearance of increased amounts of the primary metabolite of serotonin, 5-hydroxyindolacetic acid, in cerebrospinal fluid following administration of 5-hydroxytryptophan confirms that increased amounts of serotonin are not only produced but are also released by serotonergic neurons (115, 117). Increased availability of choline stimulates acetylcholine release (75). By affecting both the production and release of neurotransmitters, changes in dietary intakes of precursor nutrients can influence the physiological functions that are dependent on these neurotransmitters (44-50, 76-77, 102-103, 118-121).

A large body of peer-reviewed published data supports the basis for increased requirements of arginine (122), tryptophan (123-126), choline (127), glutamine (128-129), serine (130-131), and histidine (132-133) in pain syndromes. Patients suffering from different types of pain syndromes show decreased blood levels of the amino acids despite having a sufficient intake of protein indicating that the needs for these specific amino acids are selectively increased in these patients. This observation may be explained by the competitive demands for these amino acids by different metabolic pathways which decrease the supply of neurotransmitters available to function in the pain process (Refer to the section *Metabolism* in this monograph). Low blood levels of tryptophan accompanied by altered tryptophan metabolism have been frequently reported in patients with pain disorders and have also been associated with decreased brain

serotonin concentration. (101, 115, 117, 123, 134-136). These patients also commonly exhibit reduced blood levels of 5-hydroxytryptophan, arginine, choline, GABA, histidine, and serine. Moreover, they respond to oral administration of amino acid formulations with favorable changes in physiologic endpoints and improvements in clinical symptoms associated with pain, thus supporting the increased requirements for specific amino acids to normalize blood levels in patients with pain disorders (74, 119, 122-140).

The anti-inflammatory effects of omega-3 fatty acids have been well documented in animal and human studies (12-13, 58-62). Epidemiological data have also shown that an imbalanced intake of omega-3 fatty acids relative to omega-6 fatty acids is associated with increased risk of the most common causes of morbidity and mortality in the US (141-142). Neither omega-3 nor omega-6 fatty acids are synthesized by the human body and must be obtained through diet or with supplements. Omega-6 fatty acids are the predominant polyunsaturated fatty acids in the diet of Western countries. The dietary imbalance in fatty acids that favors omega-6 fatty acids can be attributed to the limited number of foods that are rich sources of omega-3 fatty acids (fatty fish, flaxseed oil) in contrast to omega-6 fatty acids which are widely distributed in the diet (corn oil, soybean oil, safflower oil, and sunflower oil). Furthermore, fish oils are virtually the only sources of the biologically active eicosapentaenoic acid.

Studies in patients with RA have shown that a dietary supplement of fish oil can significantly reduce morning stiffness and the number of painful joints by markedly reducing interleukin-1 β production (13). These observations suggest that patients with inflammatory disease have an increased need for omega-3 fatty acids. Supplemental amounts of these fatty acids will correct the imbalance in intake relative to omega-6 fatty acids and therefore mitigate the effects of chronic inflammation sustained by the excess of the proinflammatory eicosanoids that dominate when the balance of intakes is tipped in favor of omega-6 fatty acids.

A summary of support for increased requirements of amino acids and omega-3 fatty acids in patients with pain and inflammation due to joint disorders is found in Table 4.

Table 4. Observations Supporting Increased Nutrient Requirements in Joint Disorders

Nutrient	Biochemical and Physiologic Observations	Clinical Observations
Tryptophan (123, 124-126, 136, 146, 143-145)	Reduced blood levels of tryptophan and serotonin	Depression, behavioral changes
Choline (127)	Reduced parasympathetic autonomic nervous system function	Decreased function of NMDA receptors; diminished responses to GABA and serotonin.

Glutamine (128-129)	Reduced blood levels; reduced blood and tissue glutathione	Increased muscle protein catabolism from metabolic stress
GABA (146)	Reduced blood and brain GABA levels	Loss of synaptic inhibition; seizures
Arginine (1, 122, 138, 147-150)	Reduced plasma arginine and nitric oxide levels increase with dietary supplementation; rate-limiting for nitric oxide production; reduced production of anti-inflammatory prostaglandins	Increased plasma nitrates and exhaled nitric oxide with arginine supplements; circadian effects on utilization impacts timing of intake
Serine (130-131, 151-152)	Reduced blood levels of glycine	Loss of sensitivity to both natural and synthetic inhibitors of pain
Histidine (142-143, 161-164)	Reduced blood levels; decreased hemoglobin (source of histidine); increased cortisol	Increased cortisol requirements
Omega-3 Fatty Acids (13, 140-141)	Reduced blood levels of eicosapentanoic acid and docosahexanoic acid	Increased CRP, interleukin-1 β , and interleukin-6
Polyphenolic Antioxidants (10, 98, 155-158)	Reduced nitric oxide; increased levels of proinflammatory prostaglandins	Altered platelet function; Decreased oxidative damage from administration of pro-oxidant compounds

Clinical Validation of Trepadone for Use in Joint Disorders

The relationship between intakes of nutrient 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 (17, 19, 26, 49-50, 77, 86-87, 115-117, 134). Changes in the levels of a neurotransmitter in the blood and/or its metabolites in cerebrospinal fluid following ingestion of the precursor reflect its uptake and utilization by target neurons in the central nervous system, thus confirming biological availability and clinical utility of the supplemental nutrient when ingested from a medical food (86, 116, 120-121).

The clinical benefit of a medical food can be validated by changes in biological, physiological, and clinical endpoints following administration to individuals with a specific disease or disorder. For example, a medical food which provides supplemental arginine is clinically validated in individuals with low blood arginine levels when blood arginine 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 a joint

disorder shows an increase in serotonin levels after administration of a medical food containing tryptophan or 5-hydroxytryptophan (biological availability) and increased serotonin metabolites in cerebrospinal fluid (physiological change) associated with improvement in joint pain (clinical response), then the clinical benefit of the medical food is validated.

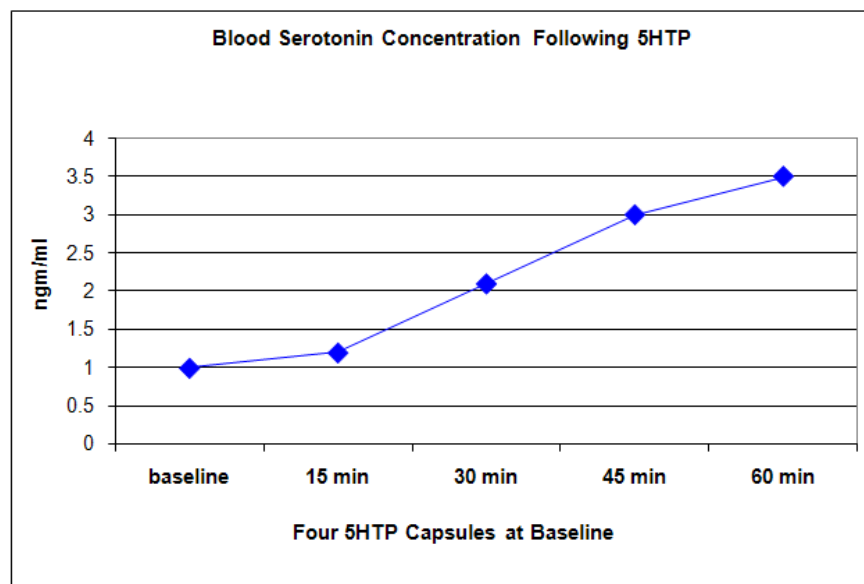
Trepadone provides balanced amounts of amino acid precursors of neurotransmitters involved in the pain response in a formulation using *Targeted Cellular Technology* to control the timing of their release.

Independent published clinical trials show that low doses of the ingredients arginine, choline and GABA given alone reduce the perception of pain (Internal unpublished data).

Biological Availability

The biological availability of 5-hydroxytryptophan, the source of serotonin in **Trepadone**, has been demonstrated by observed changes in blood serotonin levels within 15 minutes of ingestion of 2000 mg of 5-hydroxytryptophan (Figure 7). These levels continued to increase and were more than 3-fold higher than baseline levels at 60 minutes, confirming that 5-hydroxytryptophan was being utilized to increase production of serotonin.

Figure 7. Effect of 5-Hydroxytryptophan Supplementation on Blood Serotonin Levels



These data indicate that oral administration of 5-hydroxytryptophan results in the production of serotonin.

Selected References

1. Borgonio A, Witte K, Stahrenberg R, Lemmer B. Influence of circadian time, ageing, and hypertension on the urinary excretion of nitric oxide metabolites in rats. *Mech Ageing Dev* 1999 November 2;111(1):23-37.
2. Haas HL, Sergeeva OA, Selbach O. Histamine in the nervous system. *Physiol Rev* 2008;88:1183-1241.
3. Wolosker H, Dumin E, Balan L, Foltyn VN. D-amino acids in the brain: D-serine in neurotransmission and neurodegeneration. *FEBS J* 2008;275:3514-3526.
4. McAlindon TE, Biggee BA. Nutritional factors and osteoarthritis: recent developments. *Curr Opin Rheumatol* 2005 September;17(5):647
5. Cesaro P, Ollat H. Pain and its treatments. *Eur Neurol* 1997;38(3):209-15.
6. Aghabeigi B. The pathophysiology of pain. *Br Dent J* 1992 August 8;173(3):91-7.
7. Zimmermann M. Pathobiology of neuropathic pain. *Eur J Pharmacol* 2001 October 19;429(1-3):23-37.
8. Sawynok J, Reid A. Interactions of descending serotonergic systems with other neurotransmitters in the modulation of nociception. *Behav Brain Res* 1996;73(1-2):63-8.
9. Lewis DA. Anti-inflammatory drugs from plant and marine sources. *Agents Actions Suppl* 1989;27:3-373.
10. Manthey JA. Biological properties of flavonoids pertaining to inflammation. *Microcirculation* 2000;7(6 Pt 2):S29-S34.
11. Friedrich MJ. Loss of nerve: a molecular approach to better treatment of chronic pain. *JAMA* 2000 January 12;283(2):187-8.
12. Mori TA, Beilin LJ. Omega-3 fatty acids and inflammation. *Curr Atheroscler Rep* 2004;6:461-467.
13. Simopoulos AP. Omega-3 fatty acids in inflammation and autoimmune diseases. *J Am Coll Nutr* 2002;21:495-505.
14. Sawynok J. Adenosine receptor activation and nociception. *Eur J Pharmacol* 1998 April 17;347(1):1-11.
15. Dunwiddie TV, Masino SA. The role and regulation of adenosine in the central nervous system. *Annu Rev Neurosci* 2001;24:31-55.

16. Bach-Rojecky L. Analgesic effect of caffeine and clomipramine: a possible interaction between adenosine and serotonin systems. *Acta Pharm* 2003 March;53(1):33-9.
17. Conlay LA, Zeisel SH. Neurotransmitter precursors and brain function. *Neurosurgery* 1982 April;10(4):524-9.
18. Fernstrom JD. Effects on the diet on brain neurotransmitters. *Metabolism* 1977 February;26(2):207-23.
19. Zeisel SH. Dietary influences on neurotransmission. *Adv Pediatr* 1986;33:23-47.
20. Hochberg MC, Clegg DO. Potential effects of chondroitin sulfate on joint swelling: a GAIT report. *Osteoarthritis Cartilage* 2008;16 Suppl 3:S22-S24.
21. Reginster JY, Bruyere O, Neuprez A. Current role of glucosamine in the treatment of osteoarthritis. *Rheumatology (Oxford)* 2007;46:731-735.
22. Fields HL, Heinricher MM, Mason P. Neurotransmitters in nociceptive modulatory circuits. *Annu Rev Neurosci* 1991;14:219-45.
23. Willis WD. Role of neurotransmitters in sensitization of pain responses. *Ann N Y Acad Sci* 2001 March;933:142-56.
24. Furst S. Transmitters involved in antinociception in the spinal cord. *Brain Res Bull* 1999 January 15;48(2):129-41.
25. Farber L, Haus U, Spath M, Drechsler S. Physiology and pathophysiology of the 5-HT₃ receptor. *Scand J Rheumatol Suppl* 2004;(119):2-8.
26. Thomas RJ. Excitatory amino acids in health and disease. *J Am Geriatr Soc* 1995 November;43(11):1279-89.
27. Belousov AB, O'Hara BF, Denisova JV. Acetylcholine becomes the major excitatory neurotransmitter in the hypothalamus in vitro in the absence of glutamate excitation. *J Neurosci* 2001 March 15;21(6):2015-27.
28. Dickenson AH, Chapman V, Green GM. The pharmacology of excitatory and inhibitory amino acid-mediated events in the transmission and modulation of pain in the spinal cord. *Gen Pharmacol* 1997;28:633-638.
29. Ebersberger A. Physiology of meningeal innervation: aspects and consequences of chemosensitivity of meningeal nociceptors. *Microsc Res Tech* 2001 April 15;53(2):138-46.
30. Aley KO, McCarter G, Levine JD. Nitric oxide signaling in pain and nociceptor sensitization in the rat. *J Neurosci* 1998 September 1;18(17):7008-14.

31. Sutherland SP, Cook SP, McCleskey EW. Chemical mediators of pain due to tissue damage and ischemia. *Prog Brain Res* 2000;129:21-38.
32. Ono T, Inoue M, Rashid MH, Sumikawa K, Ueda H. Stimulation of peripheral nociceptor endings by low dose morphine and its signaling mechanism. *Neurochem Int* 2002 December;41(6):399-407.
33. Chevlen E. Opioids: a review. *Curr Pain Headache Rep* 2003 February;7(1):15-23.
34. Herrero JF, Laird JM, Lopez-Garcia JA. Wind-up of spinal cord neurones and pain sensation: much ado about something? *Prog Neurobiol* 2000 June;61(2):169-203.
35. Gracely RH. Pain measurement. *Acta Anaesthesiol Scand* 1999 October;43(9):897-908.
36. Ernberg M, Lundeberg T, Kopp S. Pain and allodynia/hyperalgesia induced by intramuscular injection of serotonin in patients with fibromyalgia and healthy individuals. *Pain* 2000 March;85(1-2):31-9.
37. Almay BG, Johansson F, Von Knorring L, Le Greves P, Terenius L. Substance P in CSF of patients with chronic pain syndromes. *Pain* 1988 April;33(1):3-9.
38. Abbadie C, Brown JL, Mantyh PW, Basbaum AI. Spinal cord substance P receptor immunoreactivity increases in both inflammatory and nerve injury models of persistent pain. *Neuroscience* 1996 January;70(1):201-9.
39. DeVane CL. Substance P: a new era, a new role. *Pharmacotherapy* 2001 September;21(9):1061-9.
40. Harrison S, Geppetti P. Substance p. *Int J Biochem Cell Biol* 2001 June;33(6):555-76.
41. Liu H, Mantyh PW, Basbaum AI. NMDA-receptor regulation of substance P release from primary afferent nociceptors. *Nature* 1997 April 17;386(6626):721-4.
42. Afrah AW, Stiller CO, Olgart L, Brodin E, Gustafsson H. Involvement of spinal N-methyl-D-aspartate receptors in capsaicin-induced in vivo release of substance P in the rat dorsal horn. *Neurosci Lett* 2001 December;316(2):83-6.
43. Pathophysiology or other reference re joint disorders
44. International Symposium on Substance P and Related Peptides: Pain, Inflammation, Visceral and CNS Functions. Proceedings. Shizuoka, Japan, November 3-6, 1992. *Regul Pept* 1993 July 2;46(1-2):1-471.
45. Calder PC, Zurier RB. Polyunsaturated fatty acids and rheumatoid arthritis. *Curr Opin Clin Nutr Metab Care* 2001;4:115-121.

46. Turner EH, Loftis JM, Blackwell AD. Serotonin a la carte: supplementation with the serotonin precursor 5-hydroxytryptophan. *Pharmacol Ther* 2006;109:325-338.
47. Wurtman RJ. Dietary treatments that affect brain neurotransmitters. Effects on calorie and nutrient intake. *Ann N Y Acad Sci* 1987;499:179-90.
48. Wurtman RJ, Hefti F, Melamed E. Precursor control of neurotransmitter synthesis. *Pharmacol Rev* 1980;32:315-335.
49. Fernstrom JD. Effects of precursors on brain neurotransmitter synthesis and brain functions. *Diabetologia* 1981;20 Suppl:281-289.
50. Fernstrom JD, Fernstrom MH. Tyrosine, phenylalanine, and catecholamine synthesis and function in the brain. *J Nutr* 2007;137:1539S-1547S.
51. Carlsson A. Interaction between dopaminergic and serotonergic systems. *Clin Neuropharmacol* 1992;15 Suppl 1 Pt A:616A-617A.
52. Dickenson AH. Plasticity: implications for opioid and other pharmacological interventions in specific pain states. *Behav Brain Sci* 1997 September;20(3):392-403.
53. Brown DW. Abnormal fluctuations of acetylcholine and serotonin. *Med Hypotheses* 1993 May;40(5):309-10.
54. Vangsness CT, Jr., Spiker W, Erickson J. A review of evidence-based medicine for glucosamine and chondroitin sulfate use in knee osteoarthritis. *Arthroscopy* 2009;25:86-94.
55. Clegg DO, Reda DJ, Harris CL et al. Glucosamine, chondroitin sulfate, and the two in combination for painful knee osteoarthritis. *N Engl J Med* 2006;354:795-808.
56. The NIH Glucosamine/Chondroitin Arthritis Intervention Trial (GAIT). *J Pain Palliat Care Pharmacother* 2008;22:39-43.
57. Read SJ, Dray A. Osteoarthritic pain: a review of current, theoretical and emerging therapeutics. *Expert Opin Investig Drugs* 2008;17:619-640.
58. Calder PC. Omega 3 polyunsaturated fatty acids, inflammation and immunity. *World Rev Nutr Diet* 2001;88:109-116.
59. Calder PC. N-3 polyunsaturated fatty acids and inflammation: from molecular biology to the clinic. *Lipids* 2003;38:343-352.
60. Calder PC. Polyunsaturated fatty acids and inflammation. *Prostaglandins Leukot Essent Fatty Acids* 2006;75:197-202.

61. Sundrarjun T, Komindr S, Archararit N et al. Effects of n-3 fatty acids on serum interleukin-6, tumour necrosis factor-alpha and soluble tumour necrosis factor receptor p55 in active rheumatoid arthritis. *J Int Med Res* 2004;32:443-454.
62. Duffy EM, Meenagh GK, McMillan SA, Strain JJ, Hannigan BM, Bell AL. The clinical effect of dietary supplementation with omega-3 fish oils and/or copper in systemic lupus erythematosus. *J Rheumatol* 2004;31:1551-1556.
63. Hong H, Xu ZM, Pang BS et al. Effects of simvastain combined with omega-3 fatty acids on high sensitive C-reactive protein, lipidemia, and fibrinolysis in patients with mixed dyslipidemia. *Chin Med Sci J* 2004;19:145-149.
64. Ha E, Zemel MB. Functional properties of whey, whey components, and essential amino acids: mechanisms underlying health benefits for active people (review). *J Nutr Biochem* 2003;14:251-258.
65. Yalcin AS. Emerging therapeutic potential of whey proteins and peptides. *Curr Pharm Des* 2006;12:1637-1643.
66. Teschemacher H. Opioid receptor ligands derived from food proteins. *Curr Pharm Des* 2003;9:1331-1344.
67. Malan TP, Mata HP, Porreca F. Spinal GABA(A) and GABA(B) receptor pharmacology in a rat model of neuropathic pain. *Anesthesiology* 2002 May;96(5):1161-7.
68. Gu Y, Huang LY. Gabapentin potentiates N-methyl-D-aspartate receptor mediated currents in rat GABAergic dorsal horn neurons. *Neurosci Lett* 2002 May 24;324(3):177-80.
69. Aanonsen LM, Wilcox GL. Muscimol, gamma-aminobutyric acidA receptors and excitatory amino acids in the mouse spinal cord. *J Pharmacol Exp Ther* 1989 March;248(3):1034-8.
70. Eide PK, Hole K. Subsensitivity of serotonin and substance P receptors involved in nociception after repeated administration of a serotonin receptor agonist. *J Neural Transm* 1989;77(1):1-10.
71. Costall B, Naylor RJ. 5-HT3 receptors. *Curr Drug Targets CNS Neurol Disord* 2004 February;3(1):27-37.
72. Eide PK, Hole K. Interactions between serotonin and substance P in the spinal regulation of nociception. *Brain Res* 1991 June 7;550(2):225-30.
73. Decker MW, Meyer MD, Sullivan JP. The therapeutic potential of nicotinic acetylcholine receptor agonists for pain control. *Expert Opin Investig Drugs* 2001 October;10(10):1819-30.
74. Brown DW. Abnormal fluctuations of acetylcholine and serotonin. *Med Hypotheses* 1993;40:309-310.

75. Ulus IH, Wurtman RJ. Choline increases acetylcholine release. *Lancet* 1987;1:624.
76. Fernstrom JD. Dietary precursors and brain neurotransmitter formation. *Annu Rev Med* 1981;32:413-25.
77. Fernstrom JD. Dietary amino acids and brain function. *J Am Diet Assoc* 1994;94:71-77.
78. Berretta N, Paolucci E, Bernardi G, Mercuri NB. Glutamate receptor stimulation induces a persistent rhythmicity of the GABAergic inputs to rat midbrain dopaminergic neurons. *Eur J Neurosci* 2001 September;14(5):777-84.
79. Johnston GA. Medicinal chemistry and molecular pharmacology of GABA(C) receptors. *Curr Top Med Chem* 2002;2:903-913.
80. Ford-Hutchinson AW. Regulation of leukotriene biosynthesis. *Cancer Metastasis Rev* 1994;13:257-267.
81. Cantin AM, Begin R. Glutathione and inflammatory disorders of the lung. *Lung* 1991;169:123-138.
82. Wolosker H. NMDA receptor regulation by D-serine: new findings and perspectives. *Mol Neurobiol* 2007;36:152-164.
83. Ahmadi S, Muth-Selbach U, Lauterbach A, Lipfert P, Neuhuber WL, Zeilhofer HU. Facilitation of spinal NMDA receptor currents by spillover of synaptically released glycine. *Science* 2003 June 27;300(5628):2094-7.
84. Mackenzie IS, Rutherford D, MacDonald TM. Nitric oxide and cardiovascular effects: new insights in the role of nitric oxide for the management of osteoarthritis. *Arthritis Res Ther* 2008;10 Suppl 2:S3.
85. Budzinski M, Misterek K, Gumulka W, Dorociak A. Inhibition of inducible nitric oxide synthase in persistent pain. *Life Sci* 2000;66(4):301-5.
86. Holthusen H, Arndt JO. Nitric oxide evokes pain at nociceptors of the paravascular tissue and veins in humans. *J Physiol* 1995 August 15;487 (Pt 1):253-8.
87. Mizutani T, Layon AJ. Clinical applications of nitric oxide. *Chest* 1996 August;110(2):506-24.
88. Hirasawa N, Ohuchi K, Kawarasaki K, Watanabe M, Tsurufuji S. Occurrence of histamine-production-increasing factor in the postanaphylactic phase of allergic inflammation. *Int Arch Allergy Appl Immunol* 1989;88(4):386-93.
89. Galeotti N, Ghelardini C, Bartolini A. Antihistamine antinociception is mediated by Gi-protein activation. *Neuroscience* 2002;109(4):811-8.

90. Brown RE, Stevens DR, Haas HL. The physiology of brain histamine. *Prog Neurobiol* 2001;63:637-672.
91. Bugajski J, Gadek-Michalska A, Bugajski AJ. Nitric oxide and prostaglandin systems in the stimulation of hypothalamic-pituitary-adrenal axis by neurotransmitters and neurohormones. *J Physiol Pharmacol* 2004;55:679-703
92. Lauder RM. Chondroitin sulphate: a complex molecule with potential impacts on a wide range of biological systems. *Complement Ther Med* 2009;17:56-62.
93. Dahmer S, Schiller RM. Glucosamine. *Am Fam Physician* 2008;78:471-476.
94. Jacobson KA, Moro S, Manthey JA, West PL, Ji XD. Interactions of flavones and other phytochemicals with adenosine receptors. *Adv Exp Med Biol* 2002;505:163-71.
95. Ribeiro JA, Sebastiao AM, de Mendonca A. Adenosine receptors in the nervous system: pathophysiological implications. *Prog Neurobiol* 2002 December;68(6):377-92.
96. Luceri C, Caderni G, Sanna A, Dolara P. Red wine and black tea polyphenols modulate the expression of cyclooxygenase-2, inducible nitric oxide synthase and glutathione- related enzymes in azoxymethane-induced f344 rat colon tumors. *J Nutr* 2002 June;132(6):1376-9.
97. Sovak M. Grape Extract, resveratrol, and its analogs: A Review. *J Med Food* 2001;4(2):93-105
98. Scalbert A, Williamson G. Dietary intake and bioavailability of polyphenols. *J Nutr* 2000 August;130(8S Suppl):2073S-85S.
99. Jegerschold C, Pawelzik SC, Purhonen P et al. Structural basis for induced formation of the inflammatory mediator prostaglandin E2. *Proc Natl Acad Sci U S A* 2008;105:11110-11115
100. Tsuji-Naito K. Aldehydic components of cinnamon bark extract suppresses RANKL-induced osteoclastogenesis through NFATc1 downregulation. *Bioorg Med Chem* 2008;16:9176-9183.
101. Seltzer S, Marcus R, Stoch R. Perspectives in the control of chronic pain by nutritional manipulation. *Pain* 1981 October;11(2):141-8.
102. Fernstrom JD. Can nutrient supplements modify brain function? *Am J Clin Nutr* 2000 June;71(6 Suppl):1669S-75S.
103. Lehnert H, Wurtman RJ. Amino acid control of neurotransmitter synthesis and release: physiological and clinical implications. *Psychother Psychosom* 1993;60(1):18-32.
104. Wurtman RJ. Nutrients affecting brain composition and behavior. *Integr Psychiatry* 1987 December;5(4):226-38.

105. Anderson GH, Johnston JL. Nutrient control of brain neurotransmitter synthesis and function. *Can J Physiol Pharmacol* 1983 March;61(3):271-81.
106. Richardson DC, Schoenherr WD, Zicker SC. Nutritional management of osteoarthritis. *Vet Clin North Am Small Anim Pract* 1997 July;27(4):883
107. Ryan S. Nutrition and the rheumatoid patient. *Br J Nurs* 1995 February 9;4(3):132
108. McAlindon TE, LaValley MP, Gulin JP, Felson DT. Glucosamine and chondroitin for treatment of osteoarthritis: a systematic quality assessment and meta-analysis. *JAMA* 2000;283:1469-1475.
109. Bell RF. Food and pain: should we be more interested in what our patients eat? *Pain* 2007 May;129(1)
110. Hayward G, Goodwin GM, Cowen PJ, Harmer CJ. Low-dose tryptophan depletion in recovered depressed patients induces changes in cognitive processing without depressive symptoms. *Biol Psychiatry* 2005 March 1;57(5):517-24.
111. Cymet TC. A practical approach to fibromyalgia. *J Natl Med Assoc* 2003 April;95(4):278
112. *Modern Nutrition in Health and Disease*. 10th ed. Philadelphia: Lipincott Williams & Wilkin, 2006.
113. Kris-Etherton PM, Lefevre M, Beecher GR, Gross MD, Keen CL, Etherton TD. Bioactive compounds in nutrition and health-research methodologies for establishing biological function: the antioxidant and anti-inflammatory effects of flavonoids on atherosclerosis. *Annu Rev Nutr* 2004;24:511-38.
114. Buzsaki G, Draguhn A. Neuronal oscillations in cortical networks. *Science* 2004 June 25;304(5679):1926-9.
115. Young SN, Gauthier S. Tryptophan availability and the control of 5-hydroxytryptamine and tryptamine synthesis in human CNS. *Adv Exp Med Biol* 1981;133:221-230.
116. Young SN. Behavioral effects of dietary neurotransmitter precursors: basic and clinical aspects. *Neurosci Biobehav Rev* 1996;20:313-323.
117. Young SN, Gauthier S. Effect of tryptophan administration on tryptophan, 5-hydroxyindoleacetic acid and indoleacetic acid in human lumbar and cisternal cerebrospinal fluid. *J Neurol Neurosurg Psychiatry* 1981;44:323-328.
118. Meyers S. Use of neurotransmitter precursors for treatment of depression. *Altern Med Rev* 2000;5:64-71.
119. Anderson GH, Johnston JL. Nutrient control of brain neurotransmitter synthesis and function. *Can J Physiol Pharmacol* 1983;61:271-281.

120. Fernstrom JD, Fernstrom MH. Monoamines and protein intake: are control mechanisms designed to monitor a threshold intake or a set point? *Nutr Rev* 2001;59:S60-S65.
121. Anderson IM, Mortimore C. 5-HT and human anxiety. Evidence from studies using acute tryptophan depletion. *Adv Exp Med Biol* 1999;467:43-55.
122. Tangphao O, Chalon S, Coulston AM et al. L-arginine and nitric oxide-related compounds in plasma: comparison of normal and arginine-free diets in a 24-h crossover study. *Vasc Med* 1999;4(1):27-32.
123. Fernstrom JD. Effects of the diet and other metabolic phenomena on brain tryptophan uptake and serotonin synthesis. *Adv Exp Med Biol* 1991;294:369-76.
124. Kahkonen S, Ahveninen J, Pennanen S, Liesivuori J, Ilmoniemi RJ, Jaaskelainen IP. Serotonin modulates early cortical auditory processing in healthy subjects: evidence from MEG with acute tryptophan depletion. *Neuropsychopharmacology* 2002 November;27(5):862-8.
125. Pant KC, Rogers QR, Harper AE. Plasma and tissue free amino acid concentrations in rats fed tryptophan-imbalanced diets with or without niacin. *J Nutr* 1974 December;104(12):1584-96.
126. Sahakian BJ, Wurtman RJ, Barr JK, Millington WR, Chiel HJ. Low tryptophan diet decreases brain serotonin and alters response to apomorphine. *Nature* 1979 June 21;279(5715):731-2.
127. Kuksis A, Mookerjee S. Choline. *Nutr Rev* 1978 July;36(7):201-7.
128. Alexander JW. Specific nutrients and the immune response. *Nutrition* 1995 March;11(2 Suppl):229-32.
129. Field CJ, Johnson I, Pratt VC. Glutamine and arginine: immunonutrients for improved health. *Med Sci Sports Exerc* 2000 July;32(7 Suppl):S377-S388.
130. Regulation of serine dehydratase and phosphoglycerate dehydrogenase by proteins and essential amino acids. *Nutr Rev* 1974 March;32(3):88-9.
131. de Koning TJ, Klomp LW. Serine-deficiency syndromes. *Curr Opin Neurol* 2004 April;17(2):197-204.
132. Histidine: An essential amino acid for normal adults. *Nutr Rev* 1975 July;33(7):200-2.
133. Antener I, Verwilghen AM, Van GC, Mauron J. Biochemical study of malnutrition. Part VI: Histidine and its metabolites. *Int J Vitam Nutr Res* 1983;53(2):199-209.
134. Young SN, Teff KL. Tryptophan availability, 5HT synthesis and 5HT function. *Prog Neuropsychopharmacol Biol Psychiatry* 1989;13:373-379.

135. Timiras PS, Hudson DB, Segall PE. Lifetime brain serotonin: regional effects of age and precursor availability. *Neurobiol Aging* 1984;5(3):235-42.
136. Fernstrom JD, Wurtman RJ. Control of brain serotonin levels by the diet. *Adv Biochem Psychopharmacol* 1974;11(0):133-42.
137. Boer J, Duyvendak M, Schuurman FE, Pouw FM, Zaagsma J, Meurs H. Role of L-arginine in the deficiency of nitric oxide and airway hyperreactivity after the allergen-induced early asthmatic reaction in guinea-pigs. *Br J Pharmacol* 1999 November;128(5):1114-20.
138. Cooke JP, Oka RK. Atherogenesis and the arginine hypothesis. *Curr Atheroscler Rep* 2001 May;3(3):252-9.
139. Cerra FB. Nutrient modulation of inflammatory and immune function. *Am J Surg* 1991 February;161(2):230-4.
140. Angel-Meza AR, Ramirez-Cortes L, Adame-Gonzalez IG, Gonzalez B, I, Beas-Zarate C. Cerebral GABA release and GAD activity in protein- and tryptophan- restricted rats during development. *Int J Dev Neurosci* 2002 February;20(1):47-54.
141. Tapiero H, Ba GN, Couvreur P, Tew KD. Polyunsaturated fatty acids (PUFA) and eicosanoids in human health and pathologies. *Biomed Pharmacother* 2002;56:215-222.
142. A.P. Simopoulos and L.G. Cleland, Omega-6/Omega-3 Essential Fatty Acid Ratio Vol 92, Karger, Farmington, CT (2003).
143. Fernstrom JD, Fernstrom MH. Diet, monoamine neurotransmitters and appetite control. *Nestle Nutr Workshop Ser Clin Perform Programme* 2001;(5):117-31.
144. Byerley WF, Risch SC. Depression and serotonin metabolism: rationale for neurotransmitter precursor treatment. *J Clin Psychopharmacol* 1985 August;5(4):191-206.
145. Delgado PL, Charney DS, Price LH, Landis H, Heninger GR. Neuroendocrine and behavioral effects of dietary tryptophan restriction in healthy subjects. *Life Sci* 1989;45(24):2323-32.
146. Cocchi R. A syndrome from a possible GABA deficiency. Clinical-therapeutic report on 15 cases. *Acta Psychiatr Belg* 1978 March;78(2):407-24.
147. Efron DT, Barbul A. Modulation of inflammation and immunity by arginine supplements. *Curr Opin Clin Nutr Metab Care* 1998 November;1(6):531-8.
148. Efron DT, Barbul A. Arginine and immunonutrition: a reevaluation. *Nutrition* 2000 January;16(1):73-4.

-
149. Evoy D, Lieberman MD, Fahey TJ, III, Daly JM. Immunonutrition: the role of arginine. *Nutrition* 1998 July;14(7-8):611-7.
 150. Pita AM, Fernandez-Bustos A, Rodes M et al. Orotic aciduria and plasma urea cycle-related amino acid alterations in short bowel syndrome, evoked by an arginine-free diet. *JPEN J Parenter Enteral Nutr* 2004 September;28(5):315-23.
 151. Russell IJ, Michalek JE, Vipraio GA, Fletcher EM, Wall K. Serum amino acids in fibrositis/fibromyalgia syndrome. *J Rheumatol Suppl* 1989 November;19:158-63.
 152. Adibi SA, Modesto TA, Morse EL, Amin PM. Amino acid levels in plasma, liver, and skeletal muscle during protein deprivation. *Am J Physiol* 1973 August;225(2):408-14.
 153. Cho ES, Anderson HL, Wixom RL, Hanson KC, Krause GF. Long-term effects of low histidine intake on men. *J Nutr* 1984 February;114(2):369-84.
 154. Pestana A. Dietary and hormonal control of enzymes of amino acid catabolism in liver. *Eur J Biochem* 1969 December;11(2):400-4.
 155. Damas J, Bourdon V, Remacle-Volon G, Lecomte J. Pro-inflammatory flavonoids which are inhibitors of prostaglandin biosynthesis. *Prostaglandins Leukot Med* 1985 July;19(1):11-24.
 156. Lindahl M, Tagesson C. Flavonoids as phospholipase A2 inhibitors: importance of their structure for selective inhibition of group II phospholipase A2. *Inflammation* 1997 June;21(3):347-56.
 157. Mandel S, Youdim MB. Catechin polyphenols: neurodegeneration and neuroprotection in neurodegenerative diseases. *Free Radic Biol Med* 2004 August 1;37(3):304-17.
 158. Rein D, Paglieroni TG, Pearson DA et al. Cocoa and wine polyphenols modulate platelet activation and function. *J Nutr* 2000 August;130(8S Suppl):2120S-6S.