**Lister V™ Product Information**

**Indications**

*Lister V* is intended for use in the management of viral infections and impaired immune function. *Lister V* 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 metabolic disorders, chronic diseases, injuries, premature birth associated with inflammation, and other medical conditions, as well as from pharmaceutical therapies.¹

Viral infections are the most common diseases that affect humans. Communication between the immune system and the nervous system is key to an effective defense against these infections and to adequate protection against cellular damage caused by them. The central and peripheral nervous systems communicate with the immune system through the activity of neurotransmitters. Neurotransmitters facilitate the exchange of sensory and metabolic information between the brain, the spinal cord, and peripheral nervous system by propagation of electrical impulses over specific neural pathways. The immunoregulatory role of neurotransmitters involves modulation of immune functions and the effects of the immune system on nervous system functions. *Lister V* is designed to provide a balance of neurotransmitters, antioxidants, and other ingredients that support the activities of the immune system to protect against viral infections.

**Ingredients**

*Lister V* is a patented blend of neurotransmitter precursors (L-arginine, choline bitartrate, L-glutamine, L-lysine, L-cysteine, and L-histidine); stimulators of precursor uptake (cinnamon); modulator of precursor utilization (L-lysine, L-cysteine); polyphenolic antioxidants (grape seed extract, green tea extract, cinnamon bark, cocoa extract); anti-inflammatory amino acids and precursors of anti-inflammatory molecules (L-histidine, L-lysine, L-cysteine, L-glutamine); immunomodulatory peptides (whey protein hydrolysate), nutrients (L-glutamine), and herbs (echinacea); and adenosine antagonists (cafféine, cocoa extract). *Lister V* is also a source of zinc that is a neural messenger, influences neurotransmitter activity, serves as an antioxidant and immunomodulator and as cofactor in numerous enzymatic reactions critical to immune function and is involved in the inhibition of viral uptake in the nasal passages.

All of the ingredients in *Lister V* have been carefully selected based on scientific support for their roles in the synthesis and activity of the specific neurotransmitters involved in regulating the immune response. These roles are summarized in this monograph in the section *Scientific Support for Use of Lister V in Management of Viral Infection and Impaired Immune Function*. The other ingredients in the formulation are functional components of the *Targeted Cellular Technology™* system.

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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 Lister V 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™**

Lister V has been formulated using Targeted Cellular Technology (TCT), an integrated molecular system that facilitates the uptake and utilization of neurotransmitter precursors by target cells within the nervous system. This 5-component patented system consists of (1) specific neurotransmitter precursors; (2) a stimulus for the neuronal uptake of these precursors by specific neurons; (3) an adenosine antagonist that blocks the inhibitory effect of adenosine on neuronal activity (adenosine brake); (4) a stimulus to trigger the release of the required neurotransmitters from targeted neurons; and (5) a mechanism to prevent attenuation of the precursor response, a well known phenomenon associated with precursor administration. This process is described in the United States Patents: 7,601,369, 7,595,067, 7,585,523, 7,582,315.

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, nitric oxide (NO), and histamine (1-3).

Previous attempts to provide an exogenous source of precursor amino acids and other biogenic amines in the quantities required to support neurotransmitter synthesis for individuals with specific needs necessitated that large amounts of these nutrients be added to the formulations. For patients whose precursor requirements are considerably higher than normal, the amounts of exogenous amino acids needed are not practical to consume on a daily basis. 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 using Targeted Cellular Technology allows ingestion of smaller amounts of amino acids to elicit the same response as larger amounts, making daily dosing more feasible and reducing the potential for tolerance. Unlike pharmaceutical products that are not innate components of processes that regulate immune function and thus may lose their effectiveness in a relatively short period of time, the effectiveness of Lister V is not attenuated.
Lister V™ Product Information

**Metabolism**

*Lister V* is a source of amino acids, biogenic amines, and other nutrients for patients with viral infections and impaired immune function. These patients require additional amounts of arginine, choline, glutamine and histidine to support synthesis of the neurotransmitters NO, acetylcholine, glutamate and histamine, respectively. They also require increased amounts of cysteine and glutamine for synthesis of the intracellular antioxidant glutathione and lysine to moderate NO production from arginine. Additional zinc is also needed to support a wide range of activities critical to immune function and interruption of viral replication.

Under normal physiological conditions, arginine, glutamine, and cysteine are metabolized as nonessential amino acids because endogenous synthesis is sufficient to satisfy metabolic demand. When needs are altered such as with an immune challenge, the usual rate of synthesis is no longer sufficient and these amino acids become conditionally essential, requiring that supplemental amounts be consumed. Lysine is an essential amino acid that must always be provided in the diet, but the amounts needed are increased when the requirement for arginine is increased. Histidine has been considered a nonessential amino acid for adults because blood levels can be maintained by 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 need to preserve muscle mass and plasma hemoglobin concentration.

Under normal conditions, glutamine is synthesized from glutamate in virtually all tissues by the addition of an amino group (Figure 1). Glutamine is the most abundant amino acid in circulation and in the intracellular free amino acid pool, and makes up approximately 15-25% of skeletal muscle protein. In catabolic states such as infection and injury, glutamine stores are rapidly depleted. The primary function of glutamine is as a carrier of amino groups that are utilized in the synthesis of numerous compounds such as urea, which is produced in large amounts when protein catabolism is increased to dispose of excess nitrogen waste. Glutamine is also required for the synthesis of purines and pyrimidines and therefore demand is increased by the rapid rate of lymphocyte proliferation in response to an immune challenge. Glutamine has neurotransmitter activity and also supports glutamatergic activity by contributing to the pool of glutamate, which is the major excitatory neurotransmitter of the central nervous system.

The need for glutamine may be increased as much as 2- to 4-fold (>20 g) during infection which requires that a supplemental amount be obtained from the diet. If glutamine intake is not sufficient, then the endogenous supply will be depleted and synthesis of glutathione and nucleic acids will be compromised. A decrease in the supply of glutamine will draw on the available supply of glutamate and thus will compromise glutamate-dependent functions. *Lister V* improves metabolic efficiency by providing supplemental glutamine to ensure that there is a sufficient supply of this amino acid thus sparing glutamate for its other important metabolic roles which include contributing to the pool of arginine and supporting synthesis of glutathione (γ-glutamylcysteinylglycine). Glutathione is a potent intracellular antioxidant that prevents cellular damage from reactive oxygen species that are produced by cytotoxic immune cells to facilitate viral destruction.
Because arginine can be synthesized de novo from glutamine and glutamate, it is not considered an essential nutrient (Figure 2). Arginine functions as a precursor of NO, polyamines, urea, and the high energy storage compounds creatine and creatine phosphate. Infection increases the demand for NO which diverts the supply of arginine from its other metabolic pathways. To compensate for the decrease in arginine available for these competing pathways, glutamate and glutamine contribute to the precursor pool through generation of ornithine and citrulline in which are metabolized in the urea cycle to synthesis arginine. *Lister V* improves metabolic efficiency by insuring that there is a sufficient amount of arginine available to satisfy the competitive demands for this amino acid thereby conserving the supply of glutamine and glutamate for their other roles in cellular metabolism.
The need for cysteine can be satisfied in adults by de novo synthesis with methionine and serine as precursors. Cysteine is utilized with glutamate to produce glutathione; however, the availability of cysteine is the rate-limiting factor for glutathione synthesis. Cysteine also has specific effects that are important to cell-mediated immunity. *Lister V* provides cysteine to ensure the synthesis of an adequate supply of glutathione.

The requirement for choline is dependent on the demand for acetylcholine and is thus increased with an increase in cholinergic activity. Acetylcholine is produced from choline in an acetylation reaction catalyzed by choline acetyltransferase with acetyl coenzyme A (CoA) as the acetyl group donor (Figure 3). Under usual metabolic conditions, the primary source of choline for acetylcholine synthesis is the hydrolysis of the membrane phospholipid phosphatidylcholine (lecithin) which serves as a reservoir of choline to meet short-term needs. When the demand for acetylcholine exceeds the amount of choline that can be supplied by the membrane pool, dietary choline becomes an increasingly more important source. *Lister V* provides additional amounts of choline to meet the increased needs for acetylcholine when demand is elevated over an extended period of time. By supplying an exogenous source of choline, *Lister V* prevents the depletion of membrane phosphatidylcholine and thus preserves the structural integrity of the cell.

![Biosynthesis of Acetylcholine](image)

Lysine and histidine are both essential amino acids that must be consumed in increased amounts when there is an increased demand by the immune system. Because these amino acids cannot be synthesized endogenously, the amounts consumed determine the amounts available for the competing pathways of utilization. Lysine is incorporated into collagen and elastin fibers where it undergoes posttranslational hydroxylation to form the cross-linkages that give connective tissue its tensile strength. Lysine also regulates intracellular levels of arginine by competing for sites on a common membrane transporter for cellular uptake. Lysine is also a precursor of carnitine, a substrate utilized in the synthesis of acetyl-L-carnitine that enhances the synthesis and activity of acetylcholine. Lysine is provided by *Lister V* to support tissue repair and wound healing, enhance acetylcholine-dependent cellular activities, and maintain intracellular arginine levels. Histidine is also provided by *Lister V* as precursor of histamine.
Histamine is synthesized and released by human lymphocytes, neurons, basophils and mast cells. The immunoregulatory activity of histamine depends on the particular tissue receptor subtype.

Zinc is also an essential nutrient that supports the activity of more than 300 enzymes either as a structural component or as a cofactor in reactions catalyzed by these enzymes. Rapidly proliferating cells such as those of the immune system are especially sensitive to zinc status. Since most transcription and replication factors are zinc-dependent, cellular proliferation does not occur in the absence of zinc. Zinc also interferes with viral uptake across nasal membranes. *Lister V* provides supplemental zinc to ensure that adequate amounts are available to support the increase in zinc-dependent metabolic activity required for the immune response.

**Dosage**

The recommended dose of *Lister V* is 2 capsules taken 2 to 3 times daily. *Lister V* should be taken at the first signs of viral infection and continued for 5 days. *Lister V* should be taken at the onset of herpes-induced cold sores or incipient viral infection and continued until symptoms have disappeared. As with any medical food, the best dosing protocol should be determined by a physician based on assessment of individual needs.

No interactions with drugs or herbal supplements have been reported for patients taking *Lister V* at the recommended doses. Patients taking pharmaceutical agents to treat other conditions may continue to take these medications with *Lister V*. These patients should be monitored by a physician and therapeutic doses modified based on clinical response. *Lister V* can be used with antiviral agents such as amantadine. Patients taking antiviral agents with *Lister V* should maintain the dosage of these drugs as directed by a physician.

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

**Table 1.  *Lister V* Composition**

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>mg/kg body weight¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>L-arginine</td>
<td>0.6 – 4.6</td>
</tr>
<tr>
<td>L-glutamine</td>
<td>0.1 – 3.0</td>
</tr>
<tr>
<td>L-histidine</td>
<td>0.4 – 3.1</td>
</tr>
<tr>
<td>L-leucine</td>
<td>0.1 – 3.0</td>
</tr>
<tr>
<td>L-cysteine</td>
<td>0.1 – 0.9</td>
</tr>
<tr>
<td>Lecithin</td>
<td>0.5 – 3.0</td>
</tr>
<tr>
<td>Echinacea</td>
<td>0.1 – 3.0</td>
</tr>
<tr>
<td>Grape seed extract</td>
<td>0.2 – 1.5</td>
</tr>
<tr>
<td>Green tea extract</td>
<td>0.2 – 0.9</td>
</tr>
</tbody>
</table>
**Lister V™ Product Information**

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>mg/kg body weight¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cinnamon bark</td>
<td>0.2 – 1.5</td>
</tr>
<tr>
<td>Metabromine</td>
<td>0.1 – 1.0</td>
</tr>
<tr>
<td>Whey protein hydrolysate</td>
<td>0.3 – 2.6</td>
</tr>
<tr>
<td>Zinc (as Zinc Oxide)</td>
<td>0.05 – 2.0</td>
</tr>
</tbody>
</table>

¹ Dosing range of 2 capsules taken 2-3 times daily

**Side Effects**

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

*Lister V* contains L-arginine which has been associated with side effects when consumed alone as a supplement. Side effects specific to oral supplementation with L-arginine have been reported at doses of 3-100 g/d; however, doses of up to 15 g/d are generally well tolerated. A 2-capsule dose of *Lister V* contains 126 mg of L-arginine. Adverse effects were dependent on the dosage regimen and were not observed when divided doses were ingested (4). The most common adverse reactions noted with L-arginine supplementation have been observed at intakes of 15-30 g/d and included nausea, abdominal cramps, diarrhea, and vomiting. Some patients may experience these symptoms at lower doses. Most of the side effects associated with arginine and N-acetyl-cysteine supplements have been observed at single doses of >9 g in adults (>140 mg/kg), most often when part of a daily regime of approximately>30 g/d (>174 mmol/d). Single doses of 3-6 g rarely provoked side effects.

*Lister V* is contraindicated in patients who may be hypersensitive to any component of an arginine-containing preparation. Arginine must not be taken alone by patients testing positive for HIV-1 infection but may be consumed by these patients in combination with lysine as provided in *Lister V*. Long-term safety studies have not been conducted with L-arginine. Because it may stimulate growth hormone production, pregnant women and nursing mothers should avoid L-arginine supplementation. Individuals with renal or hepatic failure should exercise caution in the use of supplemental L-arginine. Supplements of arginine and citrulline taken orally can increase local NO production in the small intestine which may be harmful under certain circumstances.

**Abbreviations and Definition of Terms**

The definitions for the abbreviations and terms referenced in this monograph are summarized in Table 2.
# Abbreviations and Definitions of Terms

<table>
<thead>
<tr>
<th>Term/Abbreviation</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Antigen-Presenting Cells</td>
<td>Prepare antigens for recognition by T-cells and B-cells; include monocytes/macrophages, Te, NK cells</td>
</tr>
<tr>
<td>Antioxidants</td>
<td>Protect against cell damage from exposure to reactive oxygen species</td>
</tr>
<tr>
<td>Cholinergic</td>
<td>Neurons that synthesize and release acetylcholine</td>
</tr>
<tr>
<td>COX-2</td>
<td>Inducible form of cyclooxygenase, the controlling enzyme in the synthesis of proinflammatory prostaglandins; activated by macrophages at the site of inflammation</td>
</tr>
<tr>
<td>Cytokines</td>
<td>Intracellular immunoactive proteins that bind to multi-unit receptors on surfaces of target cells to elicit a specific response; produced by all nucleated cells with the largest quantities activated immunocytes and</td>
</tr>
<tr>
<td>Excitatory Neurotransmitters</td>
<td>Mediators of neural signals that accelerate the rate of transmission through depolarizing postsynaptic neuronal membranes resulting in increased responsiveness to a stimulus or reduced responsiveness through activation of inhibitory mechanisms</td>
</tr>
<tr>
<td>Glucocorticoids</td>
<td>Stress hormones secreted by the adrenal gland as the end product of the activation of the HPA axis; inhibits activation of the HPA axis by negative feedback</td>
</tr>
<tr>
<td>Glutamatergic</td>
<td>Neurons that stimulate and release glutamate; contain storage vesicles for zinc</td>
</tr>
<tr>
<td>Glutathione</td>
<td>Potent cellular antioxidant synthesized from glutamate, cysteine, and glycine</td>
</tr>
<tr>
<td>HPA axis</td>
<td>Hypothalamus-pituitary-adrenal axis; hormone system that facilitates bi-directional communication between the neuroendocrine and immune systems</td>
</tr>
<tr>
<td>Inflammatory mediators</td>
<td>Modulate the inflammatory response; includes cytokines and prostaglandins</td>
</tr>
<tr>
<td>Inhibitory Neurotransmitters</td>
<td>Mediators of neural signals that slow the rate of transmission through hyperpolarization of postsynaptic membranes; inhibit responsiveness to a stimulus or activate responsiveness by inhibition of inhibitory mechanisms</td>
</tr>
<tr>
<td>Neuropeptides</td>
<td>Stimulate neurohormone secretion and regulate cytokine release from immunocytes; include corticotrophin releasing hormone, adrenal corticotropic hormone, arginine vasopressin, thyrotropin, growth hormone, and prolactin</td>
</tr>
<tr>
<td>Neurotransmitters</td>
<td>Secreted by presynaptic neurons in response to an action potential generated by a stimulus, bind to postsynaptic neurons which alters their membrane properties resulting in transmission of a signal down neural pathways to a specific center in the brain where signals are interpreted to initiate a response</td>
</tr>
<tr>
<td>NMDA receptor</td>
<td>N-methyl-D-aspartate receptors which are present in glutamatergic synapses in the central nervous system</td>
</tr>
<tr>
<td>NO</td>
<td>Nitric oxide; neurotransmitter derived from arginine; cytotoxic effector molecule</td>
</tr>
<tr>
<td>iNOS</td>
<td>Inducible enzyme isoform of NO synthase; catalyzes the synthesis of NO from arginine; induced by IFN-γ (gamma interferon) and TNF (tumor necrosis factor) released from activated immunocytes in response to viral infections</td>
</tr>
<tr>
<td>Prostaglandins</td>
<td>Inflammatory mediators produced from the omega-6 polyunsaturated fatty acid, arachidonic acid by the cyclooxygenase pathways</td>
</tr>
<tr>
<td>Targeted Cellular Technology</td>
<td>A patent-pending process that facilitates endogenous production, uptake, and utilization of neurotransmitter precursors</td>
</tr>
</tbody>
</table>
Mechanism of Action

Understanding the mechanism of action of Lister V in the management of viral infections and impaired immune function requires a brief overview of the immune system and its interactions with the brain and the peripheral nervous system. Environmental factors that disrupt the bi-directional communication between the nervous system and immune system or interfere with intercellular communication within the immune system weaken immune defenses resulting in disease. Homeostasis can be restored by supplemental amino acids and biogenic amines that support the activity of neurotransmitters that mediate the exchange of information between these systems. An intact communication network is essential for a healthy immune response.

The immune system communicates with the brain and peripheral neurons through pathways involving the sympathetic nervous system and endocrine organs (5-7). The brain is connected to lymphoid tissues by autonomic innervation of the thymus, bone marrow, lymph nodes, and spleen and is able to affect lymphocyte development through peripheral nerve endings distributed within specific cellular compartments of the spleen and lymph nodes, particularly in zones of T-cells and macrophages (7-9). The immunomodulatory activities of the endocrine system are dependent on the sympathetic nervous system that mediates the expression of hormone-releasing factors from the hypothalamus and pituitary (Lawrence). These factors include corticotropin releasing hormone (CRH) and arginine vasopressin (AVP) from the hypothalamus, and adrenocorticotropin hormone (ACTH) and thyroid-stimulating hormone (TSH) from the pituitary. The binding of these neuropeptides to target endocrine organs such as the adrenal cortex, thyroid, and thymus stimulate the production and release of corticosteroids, thyroid hormone, and zinc-thymulin, respectively.

The effects of the sympathetic nervous system and endocrine system on immune functions are mediated by neurotransmitters. Neurotransmitters are amino acids, biogenic amines, or amino acid derivatives that function as mediators of physiological responses to physical, chemical, or electrical stimuli. The interaction between one or more of these stimuli with neuronal membrane receptors generates an action potential that is transmitted between presynaptic neurons and postsynaptic neurons by a series of reactions mediated by neurotransmitters. These action potentials alter the resting membrane potentials of presynaptic neurons which trigger the release of neurotransmitters from storage vesicles into the synaptic cleft where they then bind to receptors on postsynaptic neurons. Neurotransmitter binding perturbs the resting membrane potential of postsynaptic neurons setting off a sequence of electrochemical events that propagate the initial signal down the axons of afferent nerve fibers to the terminal endings of presynaptic neurons. This sequence of events is repeated until the signal reaches specific centers in the brain where it is processed. The same series of neurotransmitter-mediated electrochemical events is also enacted to propagate output from the brain down efferent neural pathways to innervated effector organs and to transmit signals originating within the brain over internal circuits between different regions.

The rate of signal transmission between presynaptic and postsynaptic neurons within the central and peripheral nervous systems is dependent upon the electrochemical properties of the neurotransmitter and the nature of the postsynaptic membrane receptors. Excitatory neurotransmitters depolarize the 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 membrane which raises the stimulus threshold and reduces the frequency and rate of transmission. Although neurotransmitters are classified as excitatory or inhibitory based on their primary effects on membrane electrochemical potential, these classifications do not always predict the effector organ response. For example, excitatory neurotransmitters can suppress a response by activation of inhibitory mechanisms whereas inhibitory neurotransmitters can activate a response by suppression of these mechanisms.

The primary neurotransmitters involved in immunomodulation are acetylcholine, NO, glutamate, and histamine. Glutamate is the major excitatory neurotransmitter of the central nervous system. Histamine is also an excitatory neurotransmitter that functions in both the central and peripheral nervous systems. Acetylcholine and NO exhibit both excitatory and inhibitory effects on neuronal membranes depending upon the specific type and location of their receptors. Although not considered a neurotransmitter by convention, zinc satisfies several of the criteria that define a neural messenger in that it is stored in synaptic vesicles, released upon membrane depolarization, and acts at various receptors (10). Zinc is localized to glutamate-containing synaptic vesicles in the hippocampus suggesting that it might be a co-transmitter at glutamate receptors (11-12).

The immune response is triggered by detection of a virus or other antigen (e.g., malignant cells, foreign grafts) by macrophages or other phagocytic cells. The interaction between these immune cells and viruses trigger the release of inflammatory mediators that bind to receptors on cells within the neuroendocrine system which then completes the neuroendocrine-immune circuit. These inflammatory mediators also bind to receptors on immune cells which establishes a pathway for intercellular communication. Cytokines and prostaglandins are the primary inflammatory mediators involved in the immune response. Cytokines, which include interleukins (IL), tumor necrosis factors (TNF $\alpha$ and $\beta$) and interferon (INF-$\gamma$), are intracellular signaling proteins that bind to multi-unit receptors on the surface of target cell and although they are synthesized by all nucleated cells, the largest quantities are produced by activated immunocytes, specifically T-helper cells, monocyte/macrophages, and glial and dendritic cells (6). Prostaglandins, which are derivatives of the omega-6 fatty acid arachidonic acid (C20:n4), function in a wide range of physiological responses which includes modulation of the inflammatory response (5). The proinflammatory prostaglandins are synthesized by the inducible form of cyclooxygenase (COX-2) which is activated by macrophages at the site of inflammation (13-14). Some evidence suggests that COX-2 may also be expressed constitutively in small amounts in the central nervous system by glutamatergic neurons where it may have a role in glutamate-mediated neurotransmission (15).

Cytokines influence the release of immunomodulatory neuropeptides from the hypothalamus and pituitary through both neural and circulatory routes. The neural routes include interactions with peripheral neurons adjacent to the site of infection and direct stimulation of vagus afferent fibers that signal cholinergic receptors in the hypothalamus (5, 9). Blood-borne cytokines can traffic across the blood brain barrier by means of specific saturable transporters and directly affect neurotransmitter activity (8). Damage to the blood brain barrier caused by viral or bacterial infections also provides an entry into the brain for blood-borne cytokines (1). Since the pituitary is not protected by the blood brain barrier, it is readily accessible to blood-borne cytokines that can then directly stimulate release of ACTH and other hormone-releasing factors (9).
Experimental and clinical evidence indicates that the hypothalamus-pituitary-adrenal (HPA) axis is the primary hormonal link between the nervous system and immune system (8-9, 16). Dysregulation of the HPA axis is associated with impaired immune function and blunted responsiveness of the immune system in patients with sepsis (8-9). The HPA axis is activated by inflammatory mediators through acetylcholine-mediated stimulation of CRH and AVP in the paraventricular nucleus of the hypothalamus. Both of these neuropeptides bind to receptors on the corticotroph cells of the anterior pituitary to stimulate the release of ACTH for transport to receptors in the adrenal cortex where it binds to stimulate glucocorticoid release. The HPA axis is also activated by histamine and NO (13-17). Activation of the HPA axis is terminated by glucocorticoid binding to receptors in the hypothalamus that suppresses CRH release, and to receptors in the pituitary that suppresses ACTH release in response to CRH (18). CRH and ACTH are also secreted by peripheral neurons, but do not have the same effects on immune function as when secreted in the hypothalamus (19).

The location of the pituitary at the junction between the autonomic nervous system and peripheral endocrine organs gives it a pivotal role in neuroendocrine homeostasis (16). The pituitary secretes most of the neuropeptides and hormones that modulate the immune response including GH, PRL, TSH, and ACTH (9). The pituitary also activates the HPA axis through production of IL-1 that increases cholinergic activity in the hypothalamus (20-21). The pituitary also secretes other cytokines such as TNF which stimulates the inducible isoform of nitric oxide synthetase (iNOS) resulting in increased production of NO from arginine.

Other endocrine organs that figure prominently in the immune response are the thymus and pineal gland. The thymus is the primary endocrine organ of the cellular immune system and the only source of thymulin, a zinc-dependent hormone that regulates the formation and differentiation of T-lymphocytes as well as for the homing of stem cells into the thymus (16, 22-24). The thymus is linked to the autonomic nervous system by innervation with vagus fibers and the thoracic sympathetic chain (8, 16). The production of thymulin is regulated by IL-1, glucocorticoids, GH, PRL, thyroid hormone, insulin-like growth factor, and nerve growth factor (8). ACTH, GH, PRL, follicle stimulating hormone (FSH), luteinizing hormone (LH), and TSH are also synthesized by the thymus.

Thymic involution, which is associated with aging and a number of diseases of impaired immune function including HIV and AIDS, is in part a result of a general decline of the HPA axis and loss of direct innervation of the thymus and lymphoid tissues by the sympathetic nervous system (25). Thymic involution is also associated with altered pineal gland function. The pineal gland influences cellular immunity through production of melatonin (N-acetyl-5-methoxytryptamine) which regulates the synthesis of zinc-thymulin by direct effects on zinc turnover (8). The circadian patterns of immune system activity are regulated by changes in melatonin secretion in response to light exposure (26). Melatonin also influences immune function by modulating the diurnal fluctuations in HPA activity and secretion of GH and PRL. It also has peripheral effects that directly influence immunocyte activity (8).

The interactions between various immune cells in the detection and destruction of viruses are illustrated in Figure 4.
Figure 4. Viral Destruction by the Immune System

1. The immune response is initiated with detection of a virus by a macrophage that captures it and destroys it by phagocytosis. This process involves internalization and digestion of the virus. Viruses that escape these macrophages are available to infect nearby cells. Once viruses are localized intracellularly, they are accessible only to tissue macrophages and T-cells.

2. After the virus is digested, the macrophage functions as an antigen-presenting cell by displaying pieces of the virus on its surface as antigens.

3. The surface antigens on the macrophage are then recognized by specific T-helper cells that bind at the antigen site.

4. Formation of this bond stimulates production of cytokines that include interleukin-1 (IL-1), tumor necrosis factor-alpha (TNF-α) by the macrophage, and interleukin-2 (IL-2) and gamma interferon (IFN-γ) by the T-helper cell. These cytokines stimulate other immunocytes to initiate additional immune system activities that continue the process of antigen destruction.
5. IL-2 promotes the proliferation of T-helper cells and cytotoxic natural killer cells. The proliferating T-cells in turn promote the release of additional cytokines that stimulate proliferation of B-cells and production of immunoglobulins or antibodies by these B-cells.

6. The antibodies recognize the antigens on the surfaces of free-floating viruses and bind to them. The antibody-antigen complex facilitates destruction of the viruses by macrophages. This complex also activates the complement cascade that attracts leukocytes to the site of infection by chemotaxis. Complement also stimulates degranulation of mast cells to release histamine.

7. Host cells infected by viruses that have escaped capture by macrophages express antigens on their surfaces that are detected by natural killer cells. These cells release of cytotoxic substances such as nitric oxide and other reactive oxygen species that damage cell membranes and disrupt mitochondrial function resulting in necrosis and cell death.

8. Finally, as the infection is brought under control, T- and B-cells are inactivated by T suppressor cells. A few of these cells remain behind as "memory cells" which enable a more rapid response to subsequent exposure to the same virus.

Scientific Support for Use of Lister V in Management of Viral Infections and Impaired Immune Function

The effectiveness of Lister V in management of viral infections and impaired immune function is supported by an extensive body of experimental and clinical data demonstrating specific roles for each
ingredient in the immune response. Protection against cellular damage from viral infections requires increased and balanced production of acetylcholine, NO, and glutamate and glutathione which is dependent on availability of the precursors, choline, arginine, lysine, glutamine, and cysteine. Supplemental zinc is also a critical need to facilitate the accelerated rates of lymphocyte proliferation and differentiation that are essential to a robust immune response. *Lister V* is formulated to provide the optimum balance of amino acids, antioxidants, and zinc to support these roles and to maintain neuroendocrine-immune homeostasis that ensures that the protective effects of a strong immune response will prevent the cellular damage from a weak response.

Additional amounts of arginine are required by the immune system for increased production of NO, which has multiple roles in the nervous system including neurotransmitter activity and regulation, proliferation, survival, and differentiation of neurons (10). NO also functions as an intercellular and intracellular messenger in many tissues and regulates DNA binding and transcription of nF-κB, a transcription factor that controls cytokine synthesis (27). Diffusion of NO into the parvocellular cells of the hypothalamus activates COX-2 that stimulates the production of proinflammatory prostaglandins that enhance NO activity. Prostaglandins and NO together affect cholinergic, adrenergic, and histaminergic systems and stimulate the release of CRH and AVP in the hypothalamus which activates the HPA axis (9, 13, 16-17).

Synthesis of NO is increased by activation of the inducible isoform of nitric oxide synthase (iNOS) by IFN-γ and TNF released from activated immunocytes in response to viral infections such as Herpes virus (28-29), adenovirus (30-32), HIV-1 virus (33-35), rhinovirus (36-37), rotavirus (38-39), and Coxsackie B3 virus (40-41). It has been reported that the increased replication of parainfluenza virus which occurs in epithelial cells from patients with cystic fibrosis is caused by an inability of affected cells to produce NOS, and is supported by observations that the overexpression of NOS or the addition of a NO donor such as arginine provides protection against increased viral replication in these patients (42). Although the constitutive isoforms of NOS that are expressed in neurons (nNOS) and endothelial cells (eNOS) have a role in the immune response, only the inducible enzyme is involved in inflammatory processes (17, 43-45).

**NO is a potent cytotoxic effector molecule which controls virus and bacterial growth, destruction and uptake, and has direct static and cidal effects on viruses, bacteria, fungi, and parasites** (25). The cytotoxic properties of NO are attributed to its high reactivity which damages cellular DNA thus inhibiting cellular replication. Although NO is a potent inducer of apoptosis and necrosis in some cells, in controlled amounts, it offers powerful protection from cell death in many instances (43, 46-47). The production of cytotoxic NO is under the control of both inducible nitric oxide synthase and intrinsic constitutive nitric oxide synthase. Both forms of the isoform contribute to the direct cytotoxic effects of NO.

Clinical outcome during infection is therefore determined by the balance in NO production (48-49). Excess NO accumulates in tissues combining with superoxide to form peroxynitrite and other reactive oxygen species that inactivate cellular proteins and have other detrimental effects (44-47). Since NO is
also a powerful vasodilator, excess amounts can lead to severe hemodynamic instability (45-47). Chronic continuous exposure of the pituitary to blood-borne cytokines may lead to accumulation of NO in surrounding neurons leading to apoptosis and decreased hormone secretion (16).

NO production is tightly regulated by competition for the available supply of arginine between the isoforms of NO synthase that increases NO production and arginase that decreases available NO (47). This competition for arginine appears to be at the center of the regulation of the inflammatory process (44, 50). A relative increase in arginase activity will divert arginine away from NO synthesis to pathways of urea and ornithine production (Figure 2). Patients with infection and sepsis show increased activity of the arginase pathway that results in lower NO levels for a given blood level of arginine (51-52).

Supplementation with arginine and antioxidants restores NO production to levels consistent with a normal immune response (45). Patients with viral infections and compromised immune function will therefore benefit from Lister V that is designed to enhance production of NO by decreasing arginase activity.

Lysine is also added to Lister V to control NO production with supplemental arginine by competing for sites on a common membrane transporter that reduces cellular uptake of arginine.

Choline is also needed in additional amounts by the immune system for increased production of acetylcholine that has multiple functions in the immune response. The cholinergic anti-inflammatory pathway is a physiological neuro-immune mechanism that regulates innate immune function and controls inflammation (53). Acetylcholine is the primary neurotransmitter of the sympathetic nervous system (53), is responsible for activation of the HPA axis by CRH and AVP (14), and potentiates NO activity (13, 51, 54-55). Activation of the sympathetic nervous system by inflammatory mediators has effects on specific immunocytes that localize the inflammatory response to prevent the possible detrimental systemic effects of inflammation (6, 19). Recent studies indicate that acetylcholine also functions as an immune cytokine that prevents macrophage activation through a receptor-mediated anti-inflammatory pathway (56). Acetylcholine also has a role as an immunomodulator that controls cytokine production by inhibition of the transcription factor nF-κB (57).

Supplemental histidine benefits the immune system by increasing the production of histamine by lymphocytes and leukocytes (1). Histamine has immunoregulatory effects on T-cells and enhances secretion of proinflammatory cytokines that include IL-1 and IL-6. The diversity of the immunoregulatory functions of histamine can be explained by the specific receptor subtypes expressed in different tissues. The H3 receptor, which is expressed by all immune cells except T and B lymphocytes, regulates antigen-presenting capacity and proinflammatory activity and also controls neurogenic inflammation through local feedback loops between neurons and mast cells. Histamine also plays a role in activation of the HPA axis (13, 16).

Glutamine is the primary respiratory fuel utilized by lymphocytes, macrophages, and neutrophils and thus the supplemental amounts are needed in inflammatory states when immune cell proliferation and activities are increased (58-63). Glutamine also modulates the inflammatory response by attenuation of inflammatory cytokine production (60). In addition, it functions as a neurotransmitter at NMDA receptor sites in the brain. Glutamine is utilized as a substrate for the synthesis of glutathione that is a major intercellular antioxidant that protects against DNA damage from exposure to the large amounts of reactive
oxygen species produced during cytotoxic activities of immune cells (59). Under certain conditions, glutathione in its nitrosylated form (S-nitrosoglutathione) can also serve as a reservoir of intracellular NO. The concentration of intracellular glutathione is correlated with absolute counts of T-helper cells (CD4+). Intracellular glutathione levels are dependent on the supply of cysteine available which is rate-limiting to glutathione synthesis (64-65). Cysteine also influences immune function by enhancing IL-2-dependent T-cell proliferation and by decreasing transcription factors for synthesis of nF-κB (65).

The effects of zinc on immune function are multifaceted ranging from the activation of zinc-dependent enzymes, to cell proliferation and apoptosis, cytokine expression, and the activation of thymulin (22-23, 66-68). Zinc promotes efficient communication between the neuroendocrine and immune systems and supports the interactions between the thymus and other components of the neuroendocrine system (23). NO synthesis from arginine, and thus the synthesis of proinflammatory prostaglandins, is dependent upon an adequate supply of zinc. Zinc depletion impairs all functions of monocytes, decreases cytotoxicity in NK cells, and reduces phagocytosis in neutrophil granulocytes. T-cell functions are also impaired by zinc deficiency and B-cells undergo apoptosis (69).

Zinc is vital for the stabilization of DNA and also participates in antioxidant enzymatic mechanisms that protect cell membranes against damage from reactive oxygen species (23-24, 66). Zinc functions as a transcription cofactor that stimulates lymphocyte proliferation, replication and differentiation and as a cofactor for enzymes and hormones important to cell-mediated immunity (22, 67). Most of the genes that are regulated by zinc are involved in signal transduction, responses to various stressors including oxidative stress, and effects on growth and energy utilization. Among the zinc-dependent genes involved in the immune response that have been identified are those which regulate the synthesis of lymphocyte-specific protein tyrosine kinase, T-cell cytokine receptors, and the DNA damage repair and recombination protein-23B (70).

The strongest evidence for zinc as a neuromodulator is based on its association with glutamate (11). Zinc ions are concentrated in the vesicles of certain glutamatergic terminals in the mammalian forebrain. The selective association of zinc with glutamate-containing synaptic vesicles suggests that it might be a co-transmitter at glutamate receptors (10). It is believed that during synaptic transmission, zinc is released and binds to receptors on the pre- or postsynaptic membranes (11). The finding that zinc chelators can double the amplitude of baseline NMDA responses suggests that zinc acts as an antagonist co-transmitter at the NMDA receptors (10, 12).

A brief summary of the roles of the ingredients provided in Lister V in management of viral infections and impaired immune response is presented in Table 3.

### Table 3. Roles of Lister V Ingredients in Management of Viral Infections and Impaired Immune Function

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Effector Molecule</th>
<th>Function</th>
<th>Role</th>
</tr>
</thead>
</table>

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<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Effector Molecule</th>
<th>Function</th>
<th>Role</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arginine</td>
<td>Nitric Oxide</td>
<td>Inhibitory and excitatory neurotransmitter; immunomodulator; cytotoxic effector</td>
<td>Intercellular and intracellular messenger; regulates DNA binding and transcription of nF-κB, a transcription factor for cytokine synthesis; stimulates production of anti-inflammatory prostaglandins by activation of COX-2; activates HPA axis</td>
</tr>
<tr>
<td>Glutamine</td>
<td>Glutamine</td>
<td>Respiratory fuel Amino group transporter</td>
<td>Attenuates inflammatory cytokine production; precursor for glutathione synthesis; substrate for nucleic acid and urea synthesis; contributes to synthesis of arginine and glutamine; reservoir of NO under some conditions; increases CD4+ T-cell counts</td>
</tr>
<tr>
<td>Glutamine</td>
<td>Glutamine</td>
<td>Amino group transporter</td>
<td>Protection against cell membrane damage from cytotoxic activities</td>
</tr>
<tr>
<td>Glutaminate</td>
<td>Excitatory neurotransmitter</td>
<td></td>
<td>Interacts with acetylcholine and zinc at glutamatergic receptors</td>
</tr>
<tr>
<td>Glutathione</td>
<td>Antioxidant; Immunomodulator</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Histidine</td>
<td>Histamine</td>
<td>Excitatory neurotransmitter; Inflammatory mediator</td>
<td>Activates the HPA axis; regulates effects on antigen-presenting cells; influences T-cell regulation; enhances secretion IL-1 and IL-6; controls neurogenic inflammation at the H3 receptor.</td>
</tr>
<tr>
<td>Lysine</td>
<td>Lysine</td>
<td>Arginine uptake inhibitor</td>
<td>Modulates intracellular arginine concentration; regulates NO production; contributes to connective tissue elasticity; precursor of carnitine which enhances synthesis and activity of acetylcholine</td>
</tr>
<tr>
<td>Cysteine</td>
<td>Cysteine</td>
<td>Rate-limiting substrate for glutathione synthesis</td>
<td>Affects intracellular IL-2-dependent T-cell proliferation; decreases transcription factors for nF-κB.</td>
</tr>
<tr>
<td>Choline Bitartrate</td>
<td>Acetylcholine</td>
<td>Inhibitory and excitatory neurotransmitter</td>
<td>Primary neurotransmitter of the autonomic nervous system; activates the HPA axis, modulates circadian rhythms, potentiates NO activity; inhibits transcription factor nF-κB.</td>
</tr>
<tr>
<td>Zinc</td>
<td>Zinc</td>
<td>Neural messenger; cotransmitter at glutamatergic receptors; enzyme cofactor; component of thymulin</td>
<td>Supports NO synthesis, lymphocyte proliferation, cytokine expression, monocyte function, NK cytotoxicity, and phagocytosis; promotes thymic T-cell differentiation and maturation through activation of thymulin</td>
</tr>
<tr>
<td>Cocoa Powder</td>
<td>Caffeine</td>
<td>Adenosine antagonist</td>
<td>Increases neuronal activity by competitively binding to adenosine receptors which disinhibits the “adenosine brake”</td>
</tr>
<tr>
<td>Ingredient</td>
<td>Effector Molecule</td>
<td>Function</td>
<td>Role</td>
</tr>
<tr>
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</tr>
<tr>
<td>Echinacea</td>
<td>Echinacea</td>
<td>Immunomodulator</td>
<td>Influences phagocytosis and macrophage-derived cytokine concentrations; activates polymorph nuclear leukocytes and NK cells; affects numbers and activities of T- and B-cells; reduces the incidence and duration of the common cold and prevents symptoms after clinical inoculation (131-132)</td>
</tr>
<tr>
<td>Grape seed extract</td>
<td>Polyphenols</td>
<td>Antioxidant</td>
<td>Preserves receptor membrane integrity and prevents attenuation of responses to neurotransmitter precursors (133)</td>
</tr>
<tr>
<td>Green Tea Extract</td>
<td>Polyphenols</td>
<td>Immunomodulator</td>
<td>Decreases production of NO and TNF-α; modulates gene expression of COX-2 (134)</td>
</tr>
<tr>
<td>Cinnamon Bark</td>
<td>Cinnamaldehyde</td>
<td>Inhibition of osteoclastogenesis</td>
<td>Reduction in osteoclast-like cell formation and inhibiting NFATc1(nuclear factor of activated T-cell 1) (135)</td>
</tr>
<tr>
<td>Cocoa Extract</td>
<td>Caffeine</td>
<td>Adenosine antagonists</td>
<td>Bind to adenosine receptors to disinhibit the adenosine brake; adenosine has an inhibitory effect on neuronal activity (136)</td>
</tr>
<tr>
<td>Whey Protein Hydrolysate</td>
<td>Lactoferrin, Lactoglobulin, Lactalbumin, Cysteine, Glutamine</td>
<td>Immunomodulator, Antioxidant, Anti-inflammatory</td>
<td>Source of glutamine and cysteine, increases production of glutathione, protects against cellular damage from reactive oxygen species (137)</td>
</tr>
</tbody>
</table>

**Nutritional Requirements of Immune Function**

The nutritional requirements of most interest to patients with viral infections and impaired immune function are the nutrients and dietary factors that support immunomodulatory neurotransmitter synthesis and activity (arginine, glutamine, histidine, lysine, choline), promote cellular proliferation (glutamine, zinc), modulate the inflammatory process (arginine, glutamine, choline, histidine, zinc), protect against cellular damage from free radicals (arginine, lysine, glutamine, cysteine, choline), and facilitate intercellular communication (arginine, choline, zinc). Because the scope of nutrient-dependent immune activities is so broad, the immune system is highly sensitive to nutrient intakes and tests of immunocompetence are widely used in clinical settings to assess nutritional status. Improvements in key indicators of immune function and reductions in morbidity and mortality have been demonstrated in patients with malnutrition and infectious disease who received supplemental amounts of arginine, glutamine, and cysteine (71); however, the most pronounced effects of supplemental intakes of any nutrient on immune status are observed in patients who show evidence of absolute or relative deficiencies of the particular nutrients (72-74).
The concept that nutrient requirements are modified by disease has been recognized for more than 30 years and is supported by studies that have shown changes in plasma, urinary, and tissue levels of nutrients associated with abnormalities in physiological endpoints reflective of specific pathologies (75). Nutrient requirements can be estimated by identifying the level of intake at which alterations in related physiological responses are improved, indicating that the balance between intake and metabolic demand has been favorably modified. The presence of a disease with an underlying pathology that increases demand for particular neurotransmitters will increase the requirements for dietary precursors and other dietary factors involved in the metabolism of these neurotransmitters to restore homeostasis (75-81).

For most amino acids and other dietary precursors of neurotransmitters, neuronal uptake is a concentration-driven process; therefore, intakes of precursors must be high enough to increase the extracellular to intracellular ratio to a level that will drive a rapid rate of uptake (82-84). As blood levels of these dietary precursors rise in response to increased intakes, the concentration-dependent rate of precursor uptake by target neurons is increased, making more substrate available for neurotransmitter production and subsequent release (85). Changes in intakes of dietary precursors of these neurotransmitters will therefore influence physiological responses by affecting neurotransmitter production and release (77-78, 82-88). Uptake of dietary precursors such as arginine that depend on membrane transporters are less affected by extracellular concentrations. For these nutrients, competitive interactions with other nutrients will have a greater effect such as observed between arginine and lysine that bind to the same sites on a common membrane transport carrier (89-90).

The rate of precursor uptake by target neurons is important for neurotransmitter activity because the enzymes involved in synthesis are confined to these neurons and thus the amount of substrate available is the limiting factor for neurotransmitter production. The balance of neurotransmitters released from these neurons is also important to ensure effectiveness of signal transmission because the highly interrelated functions of neurotransmitters and the complexity of multiple feedback loops will determine the net input received by the brain. These interactions explain why an imbalance in the intake of a nutrient or dietary factor that supports the synthesis or activity of any single neurotransmitter or immunomodulatory substance can influence the activities of the others, potentially inducing absolute and relative deficiencies (91-93). It also explains why both excess and deficient intakes of a nutrient or dietary factor can have similar adverse effects on immune function (69, 93-94). For example, alterations in zinc-dependent functions observed in a zinc deficiency are similar to those observed with excess zinc intake (69, 94).

Several key clinical studies in immunosuppressed individuals (e.g., burn patients, individuals with cancer and HIV infection, and those undergoing surgery or who have experienced major traumas) have tested the hypothesis that supplemental arginine and/or glutamine is beneficial to immune function and clinical outcome (95). Plasma levels of arginine and choline that are reduced in patients with infection and sepsis returned towards normal as the infection subsided indicating that the demand for these nutrients had been increased (96-99). The importance of an adequate intake of arginine for patients with infection is supported by the observation that a marked reduction in serum levels was predictive of mortality in these patients (45). The finding that patients surviving septic shock had higher plasma nitrate levels than nonsurvivors indicates that there is a critical need for NO in these patients and suggests that the
incremental increase in demand for arginine imposed by this need would need to be satisfied by supplemental arginine to raise plasma levels and increase NO production (100-101).

Dietary intake is the primary determinant of plasma arginine levels since the rate of endogenous synthesis does not increase to compensate for depletion, increased turnover, increased requirement, or inadequate supply, but depends instead on the fluxes of glutamine, glutamate, ornithine, citrulline, and lysine (45, 50, 100-103). Metabolic utilization of arginine and the factors that affect rates of de novo synthesis have the greatest impact on the dietary requirement for this amino acid and thus the need to support immune function during infection and sepsis would be expected to considerably increase this requirement (104). The decline in circulating arginine levels which is seen in patients with infection is similar to what has been observed in healthy humans consuming arginine-reduced diets indicating that the increased demands of infection induce a relative deficiency of arginine which can be reversed by increasing intake (2, 105-106). It has been demonstrated that arginine utilization and turnover is increased by infection and that formulations that provide supplemental arginine and restore blood arginine levels improve the clinical status of these patients (99). A body of evidence also suggests that increased intake of arginine upregulates immune function and reduces the incidence of postoperative infection (45, 100, 107).

Dietary choline is also the primary contributor to plasma choline accounting for a greater proportion of plasma concentration than de novo synthesis (107-108). An increase in serum choline levels of as much as 52% has been observed with dietary choline supplementation (22). High levels of plasma choline promote the expression of high affinity choline transporters on cholinergic neurons that regulate the synaptic availability of choline and facilitate the release of acetylcholine from these neurons (109). Synaptic acetylcholine levels regulate choline uptake by cholinergic neurons through a negative feedback mechanism that inhibits transporter activity. The need for increased choline intake by patients with infection is indicated by the reduced levels of plasma acetylcholine in these patients which are accompanied by a suppression of the immune response and a reduction in lysosomal enzymes and phagocytic activity (110). These low levels of acetylcholine have been attributed to the increased amounts of acetylcholinesterase released into the plasma by parasites and other pathogens.

Supplemental intakes of nutrients with antioxidant capacity such as glutamine and cysteine have also been shown to improve immune function in numerous clinical trials (57). Both amino acids are precursors of the glutathione that supports the activity of the glutathione peroxidase antioxidant enzyme system (99). Supplemental intakes of glutamine and of cysteine provided as N-acetyl cysteine also protect against cellular damage from oxidative stress through suppression of inflammation (17).

Despite being the most abundant amino acid in the blood, glutamine levels are rapidly depleted by infection. Endogenous glutamine synthesis and release from skeletal muscle is increased by as much as 2-fold during infection yet the intracellular pool is still depleted indicating that the rate of release exceeds the rate of synthesis (111). Plasma glutamine levels do not appear to be affected by the increased release of glutamine from skeletal muscle during infection that suggests that glutamine availability is reduced by competition for tissue uptake and thus becomes rate limiting for lymphocyte proliferation, phagocytosis, and antibody production (60, 112). A decrease in availability of glutamine has been shown to impair antigen-presenting capability of monocytes, reduce phagocytic activity, decrease lymphocyte proliferative
response to mitogens, and shift the Th1/Th2 ratio of T-helper cells to lower levels associated with a decrease in the cell-mediated immune response (113-114).

Treatment with exogenous glutamine has been shown to be highly effective in decreasing the incidence of infection in trauma and surgery patients, specifically in lowering the risk of post-surgical infections (58, 115). Although a clearly defined glutamine deficiency syndrome has not been described, endogenous production is not sufficient to meet the increased and altered tissue demands imposed by trauma, sepsis, infection, and inflammation (111). Since most naturally-occurring food proteins contain 4% to 8% of their amino acid residues as glutamine, an average of less than 10 g of dietary glutamine is likely to be consumed daily. Studies in stressed patients indicate that considerably larger amounts of glutamine (20-40 g/day) may be necessary to maintain glutamine homeostasis (116).

Abnormal immune function and increased rates of infectious diseases have been widely reported in humans with zinc deficiency (117). In non-critically ill patients, zinc supplementation has been associated with an improvement in markers of immune function (118). Severe zinc deficiency can cause substantial impairment of cellular immunity resulting in infection and even death (69). Although the effects of mild to moderate zinc deficiency on immunity are considerably less severe, almost all immune cells show some impairment. Immunologic abnormalities characteristic of zinc deficiency are evident in HIV disease, most notably a reduction in the number of circulating T lymphocytes (119). The impairments in immune function associated with a zinc deficiency can be reversed by zinc supplementation when the amounts are adapted to the actual requirements of individual patients (69).

A summary of support for increased requirements of specific amino acids in patients with viral infections and impaired immune function is found in Table 4.

**Table 4. Observations Supporting Increased Nutrient Requirements for Immune Function**

<table>
<thead>
<tr>
<th>Nutrient</th>
<th>Biochemical and Physiologic Observations</th>
<th>Clinical Observations</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arginine</td>
<td>Plasma levels reduced by infection and increased with dietary supplementation</td>
<td>Reduced plasma levels observed with infection and sepsis returned towards normal when infection subsided; restoration of blood levels associated with improved clinical status; elevated plasma nitrate levels in survivors of septic shock</td>
</tr>
<tr>
<td>Choline</td>
<td>Plasma levels reduced by infection and increased with dietary supplementation; associated with increased plasma acetylcholinesterase released from pathogens</td>
<td>Reduced plasma levels with infection and sepsis returned towards normal when infection subsided; associated with suppression of immune response and a reduction in lysosomal enzymes and phagocytic activity</td>
</tr>
<tr>
<td>Cysteine</td>
<td>Plasma levels reduced by infection and increased with dietary supplementation</td>
<td>Inflammation suppressed and glutathione levels increased with supplementation</td>
</tr>
<tr>
<td>Glutamine</td>
<td>Plasma levels reduced by infection and increased with dietary supplementation</td>
<td>Decreased the incidence of infection in trauma and surgery patients, and specifically post-surgical infections</td>
</tr>
<tr>
<td>Nutrient</td>
<td>Biochemical and Physiologic Observations</td>
<td>Clinical Observations</td>
</tr>
<tr>
<td>----------</td>
<td>----------------------------------------</td>
<td>-----------------------</td>
</tr>
<tr>
<td>Histidine</td>
<td>Anemia observed with deficiency</td>
<td>Low serum levels in patients with wounds associated with impaired healing; decreased hemoglobin levels within 20 days after beginning a histidine-free diet (Cooperman).</td>
</tr>
<tr>
<td>Lysine</td>
<td>Inversely related to NO levels</td>
<td>Arginine/lysine ratio lower in patients with sepsis than in ICU patients with noninflammatory conditions</td>
</tr>
<tr>
<td>Zinc</td>
<td>Reduction in T-cells with deficiency</td>
<td>Immunological impairments in HIV associated with deficiency reversed by zinc supplementation</td>
</tr>
</tbody>
</table>

**Clinical Validation of Lister V for Use in Management of Viral Infections and Impaired Immune Function**

The relationship between intakes of precursor amino acids and production of the corresponding neurotransmitters has been validated by observations of improvements in neurotransmitter-mediated clinical outcomes with supplemental intakes of these amino acids (84, 120-125). Changes in levels of a neurotransmitter in the blood and/or its metabolites in cerebrospinal fluid following ingestion of a dietary precursor from a medical food reflect uptake and utilization of the nutrient or dietary factor by target cells, thus demonstrating the biological availability of dietary precursors and the clinical utility of the medical food as a source of these precursors (80-81, 126-130).

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. For example, a medical food which provides supplemental arginine is clinically validated in individuals with low blood arginine levels if blood levels are increased following ingestion (biological availability) and are accompanied by an increase in NO production (physiological change) and subsequent improvement in an associated functional parameter (onset, duration and severity of infection) (clinical response).

Several open label trials have been conducted with the Lister V formulation. The use of Lister V reduced the incidence of viral infections and improved the onset, duration, and severity in patients with these infections. The data presented in Figure 5 demonstrate a reduction in the duration of herpes simplex-induced cold sores in subjects treated with Lister V compared with placebo. Duration was defined as the number of days from onset of symptoms to loss of scab. In the Lister V group, recovery was complete for all subjects after 12 days compared with a 60% recovery rate over the same time period in the placebo group. Recovery was first noted after 2 days in the Lister V group, but no signs of recovery were apparent in the placebo group until after 4 days.
Figure 5. Effect of Lister V on Clearance of Cold Sores

![Graph showing clearance of cold sores with Lister V](image)

Figure 6 presents the differences in changes in severity of cold symptoms between patients receiving Lister V (n=13) and controls who received a placebo (n=10). After 7 days, a statistically significant difference in severity was noted between groups (p<0.01). The between-group differences were first observed by the second day of use.

Figure 6. Severity of Cold Symptoms

![Graph showing severity of cold symptoms](image)

The prevalence of symptoms following onset of a common cold was also compared in this study (Figure 7). Residual symptoms of nasal congestion and sore throat were assessed. At Day 7, the difference in the percentage of patients with these symptoms was statistically significant (p=0.02). At this time, 40% of subjects in the Lister V group reported residual symptoms compared with 60% in the Placebo group.
Differences between groups were first noted between the third and fourth day after beginning use of *Lister V*.

**Figure 7. Prevalence of Symptoms Following Onset of Common Cold**

![Graph showing the prevalence of symptoms following the onset of a common cold. The graph compares Placebo and Lister V treatments, with a p-value of 0.02 for residual symptoms of nasal congestion and sore throat.](image-url)

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