



## WHEY PROTEINS AND IMMUNITY

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*The special composition of whey proteins provides a biological activity that exceeds the properties of just a high quality amino acids source. They are one of the very few ingredients shown to modulate immune function in both cell culture studies and animal models. New scientific evidence documents benefits for active adults.*

The ability to avoid many forms of illness and disease depends largely upon strong immunity. Exercise, a busy lifestyle and aging are all shown to compromise immune function. While nutritional status underlines the health of the immune system, research suggests that, from a nutrition perspective, more is needed than a balanced diet to optimize immune function for maintaining good health and preventing disease.

In comparison to most other protein sources, whey proteins are unique in their ability to optimize a number of key aspects of immune function.

Although the exact mechanisms are not yet fully understood, whey proteins appear to modulate immune function by boosting glutathione (GSH) production in various tissues and preserving the muscle glutamine reservoir. GSH is the centerpiece of the body's antioxidant defense system that regulates many aspects of immune function. Muscle glutamine is the essential fuel of the immune system. Therefore, the incorporation of whey proteins into the diet may help promote strong immunity and protect the health of active people of all ages as well as those with a compromised immune system.



## INTRODUCING THE IMMUNE SYSTEM

The immune system is a vast and complex network of cells, organs and molecules that work together to defend the body against foreign microorganisms such as bacteria, parasites and viruses. The immune system has the ability to recognize millions of foreign invaders, and anything that triggers an immune response to these invaders is referred to as an antigen.<sup>44</sup> The immune system has many different ways of preventing invasion of foreign particles including 1) atomic barriers, such as the skin and mucous membranes, which physically block the entry of microbes and 2) physiological barriers, such as body temperature and acidity, which inhibit the growth of, and kill microbes.<sup>50</sup> If invaders pass through these barriers, different cellular processes are triggered to attack and eliminate the antigen. To protect and maintain health, this essential process occurs virtually hundreds of times every day and never ceases.



The immune system is often divided into non-specific and specific defenses.<sup>15</sup> Non-specific cellular defenses such as phagocytic cells and natural killer (NK) cells attack and destroy foreign microbes without requiring specific antigen markers, whereas the hallmarks of specific immune defense are precision and memory.<sup>21</sup> Specific immune defense involves the recruitment of the B cells and T cells (lymphocytes); only these cells remember how to conquer past invaders and therefore form the basis of all our vaccines.<sup>29</sup> Specific immune defense involves two recognition strategies. The first is the humoral immune response that consists of soluble plasma proteins derived from the B cells, called antibodies or immunoglobulins, that are synthesized in response to a foreign substance.<sup>11</sup> The second form of specific immunity consists of the direct cellular immune response where T lymphocytes directly attack and eliminate virus-infected cells or cancer cells.<sup>64</sup> Some T cells, (called helper T cells) produce chemicals such as cytokines that communicate with and recruit other immune cells.<sup>61</sup> Often, all of these defense systems work synergistically together to conquer foreign organisms.<sup>15,29</sup>



## LIFESTYLE FACTORS THAT AFFECT IMMUNE FUNCTION

### *Aging*

Aging is associated with an increased generation of free radicals from everyday biochemical processes and this can lead to oxidative stress.<sup>38</sup> Oxidative stress damages cell membranes and proteins and is responsible for the onset of many of the diseases that are usually associated with aging.<sup>65</sup> An integral role of the immune system is to reduce oxidative stress.<sup>58</sup> Individuals who live a long and healthy life appear to be equipped with optimal cellular defense mechanisms that maintain a robust immune response.<sup>2</sup>



### Physical Fitness

An individual's level of physical fitness (cardiac efficiency) is now understood to be an important factor in preventing heart disease, particularly in men, and increasing longevity.<sup>17</sup> Physical fitness can only be improved by partaking in regular, vigorous exercise. However, scientists now understand that strenuous or prolonged exercise is a metabolic stress that suppresses immune cell function.<sup>47</sup> Exercise has a direct impact on the immune system; it affects the distribution of lymphocytes within the body and causes a large percentage of these immune cells to be removed from circulation. This transient decrease in host-defense activation is called an "open window of immune suppression."<sup>44,46</sup> This temporary suppression of the immune system lasts from 6 to 48 hours and predisposes active people to an increased risk of infection during fitness training.<sup>44,46</sup>

There is growing evidence that lifestyle factors and dietary behavior are important co-factors in the immune response to exercise.<sup>31</sup> Exercise training without optimal nutritional intervention can result in compromised immune function.<sup>31</sup>

Many active people do not realize that their muscles and their immune system are intimately connected. The amino acid glutamine is the essential fuel that powers immune function and it is synthesized predominately within muscle tissue.<sup>53</sup> This amino acid cannot be manufactured by immune cells and must be provided by muscle.<sup>61</sup> The immune system requires large amounts of glutamine on a continuous basis. Problems arise if metabolic demands exceed synthesis rates. When lifestyle factors that create stress (such as inadequate nutrition and lack of sleep) are combined with intense exercise training, the body's demand for glutamine can easily exceed synthesis capabilities.<sup>53</sup> At the very least, this can result in a reduction in athletic performance. But more serious repercussions are reoccurring infections and persistent illnesses such as chronic fatigue syndrome.<sup>50,53</sup>



*Studies have shown that whey proteins can improve glutathione status. Glutathione is the essential fuel that powers immune function.*

Linkages between diet habits and quality of life continue to surface in numerous areas of research! Collectively, many of these epidemiological, pre-clinical and clinical studies provide rather compelling evidence that many essential and non-essential dietary components are capable of improving immune function to help prevent disease. However, the diets of many adults in the United States and numerous other countries do not contain enough of the nutrients that optimize the immune system.<sup>1,2,48,51</sup>

To initiate and maintain an immune response, rapid protein synthesis is required and this is why amino acids (the building blocks of protein) are critical to immune function.<sup>31</sup> Inadequate protein intake impairs immunity, with a particularly detrimental impact on the T-cell system, resulting in an increased incidence of opportunistic infections.<sup>29</sup> However, a substantial amount of research now shows that the type of protein in nutritionally adequate diets can influence the efficacy of the immune response.<sup>9,11-14,64</sup>

In describing the influence of nutrients that regulate immunity, it is important to understand that the preferred term to describe these influences is modulation.<sup>22</sup> This phrase encompasses both enhancement and suppressive responses from nutrients that result in a favorable impact on immunity.<sup>22</sup>

**THE IMMUNE-ENHANCING CAPABILITIES OF WHEY PROTEIN**

One of very few ingredients (or dietary supplements) shown to modulate immune function using proven in vitro and in vivo models are whey proteins. Often the improvements have correlated with a measurable improvement in immune mediated health.<sup>7,14,30,40,55</sup>

Whey proteins are the soluble class of dairy proteins that make up approximately 20% of the total bovine milk proteins.<sup>35,46</sup> Whey protein is a collective term that encompasses a range of fractions including the major bovine proteins alpha-lactalbumin and beta-lactoglobulin, and minor fractions such as serum proteins, lactoferrin, immunoglobulins and tissue growth factors.<sup>23</sup> Individually, these fractions are established immune-enhancing constituents that modulate a range of immune functions.<sup>22</sup> Whey protein fractions are linked to a range of bioactive functions such as prebiotic effects, promotion of tissue repair, maintenance of intestinal integrity, destruction of pathogens and elimination of toxins.<sup>19,62</sup> Commercially available whey concentrates (WPC) and isolates (WPI) are a rich, heterogenous mixture of these proteins.<sup>29,37</sup> Therefore, this review focuses on the data obtained from the incorporation of WPC (most often WPC80) and WPI ingredients into the diet.

**Typical Amino Acid Composition of Selected Commercial Products  
Content per 100 g of edible portion**

Amino acid	Whey protein concentrate 80%	Whey protein isolate
Tryptophane	1.20 g	1.50 g
Threonine	5.36 g	6.25 g
Isoleucine	4.80 g	5.90 g
Leucine	8.08 g	13.00 g
Lysine	7.84 g	9.15 g
Methionine	1.60 g	2.05 g
Cysteine	2.72 g	3.10 g
Phenylalanine	2.48 g	2.30 g
Tyrosine	2.24 g	3.15 g
Valine	4.45 g	5.35 g
Arginine	2.00 g	2.65 g
Histidine	1.20 g	1.35 g
Alanine	4.08 g	6.00 g
Aspartic acid	8.00 g	9.00 g
Glutamic acid	13.28 g	13.00 g
Glycine	1.36 g	2.35 g
Proline	5.12 g	4.80 g
Serine	4.08 g	5.00 g

Source: Data obtained from product and specification sheets and nutritional analysis provided by US whey manufacturers. Differences exist between products, please consult your supplier for exact information.

The addition of WPC to the diet is shown to significantly improve primary and secondary intestinal tract antibody responses to a variety of different vaccine antigens that are currently in medical use.<sup>40</sup> A study demonstrated that rodents which consumed a diet containing 20% protein from WPC, showed significantly better immune response to influenza vaccine, diphtheria and tetanus toxoids, poliomyelitis vaccine, ovalbumin and cholera toxin, compared to mice fed regular chow.<sup>40</sup> The addition of WPC to the diet resulted in higher levels of antigen-specific antibodies to all these immune challenges during both short term (2 weeks) and longer term (12 weeks) administration.<sup>40,41</sup>



One of the most important steps in specific immune reactivity is clonal expansion (proliferation) to produce a pool of antigen-reactive lymphocytes.<sup>22</sup> For experimental purposes, proliferation of these immune cells in living animals is stimulated via the addition of cell cultures of mitogens which are targeted toward B cells or T cells. In studies that have compared a range of commercially available protein sources, the superiority of whey proteins to improve specific immune reactivity is clear.<sup>8-14,30</sup>

In response to parasitic infection in the gut, mice fed an alpha-lactalbumin-enriched whey protein (20% of daily protein intake) were shown to possess significantly greater total white blood cell (CD4+ and CD8+) and lymphocyte counts and higher cytokine production by spleen cells than those fed casein or soy protein isolate.<sup>30</sup> These results, combined with assessment of faecal oocyst production (a measure of the level of infection), demonstrated that dairy proteins have a much greater impact on immune responsiveness than soy protein and this directly results in a reduction of the severity of infection.<sup>30</sup>

Humoral immune response governs the rate of antibody production and recruitment to foreign microorganisms, and is therefore thought to be an integral component of specific immunity.<sup>11</sup> In studies that have compared the effects of protein sources such as whey proteins, soy, casein, wheat, corn, egg white, fish, beef and spirulina maxima (all as 20% of daily protein intake), humoral immune response and antibody production to foreign microorganisms is shown to be significantly better in animals fed whey proteins.<sup>8,9,11-14</sup> This immuno-enhancing effect of whey proteins has been observed in at least six unrelated strains of mice, and in some instances the immune response observed with whey proteins was nearly five times greater than the response observed with other dietary protein sources.<sup>8,11</sup> In mice not challenged with an immune stimulus, the type of protein was shown to have little or no effect on body growth, food consumption, and serum levels of protein or circulating leukocytes.

However, in response to an immune challenge, whey proteins were shown to enhance the development of humoral immunity (measured by plaque-forming cells in the spleens of mice) to T cell-dependent antigens beyond that which has traditionally been considered to represent a “normal” response.<sup>8,9,12</sup>

To further ascertain the role of whey proteins on humoral responsiveness, the same researchers completed another study that investigated the effects of consuming different proteins on resistance to pneumococcal infection.<sup>14</sup> The mice fed WPC (20g/100g protein diet) resisted fatal infection with *Streptococcus pneumoniae* to a significantly better extent than mice fed casein.<sup>14</sup> Acquired immunity to this infection is largely dependant on the humoral response. Based on the available literature, it appears that whey proteins are most effective at optimizing this key aspect of specific immunity.<sup>14</sup>

Some studies that have examined the effects of different proteins on cell-mediated immunity have shown a beneficial impact from whey proteins.<sup>64</sup> In mice, WPC added to an adequately balanced diet enhanced humoral response and neutrophil function as well as footpad delayed-type hypersensitivity response (a widely used assessment of T-cell mediated immune responses).<sup>64</sup> These immuno-enhancing properties of WPC were seen in direct comparison to supplementation with soy protein isolate.<sup>64</sup>

Whey proteins also appear capable of enhancing other neutrophil responses. Supplementation with either WPC or a mixture of lactoferrin and lactoperoxidase was able to enhance the ability of neutrophils to neutralize free radical production and minimize oxidative stress.<sup>63</sup> Additionally, the incorporation of lactoferrin into the diet is shown to enhance T cell-mediated NK cell function which correlates to increased protection against cytomegalovirus.<sup>55</sup>





*The Role of Glutathione and Whey Proteins in Immunity*

The glutathione (GSH) antioxidant system is the principal mechanism that protects cells against oxidative stress caused by pollution, toxins, exercise and UV exposure.<sup>58</sup> An integral role of the immune system is to reduce oxidative stress.<sup>38</sup> Therefore, an adequate supply of GSH is a crucial factor in the maintenance of an effective immune system.<sup>6,58,65</sup>

GSH must be synthesized within cells using three amino acids: cysteine, glutamate and glycine.<sup>65</sup> However, cysteine is the rate-limiting amino acid in GSH production.<sup>43,58</sup> An adequate supply of cysteine in plasma and tissues is essential to maintaining a high ratio of GSH to GSSG in cells and ensure optimal defense against oxidative stress.<sup>6,37,58,65</sup>

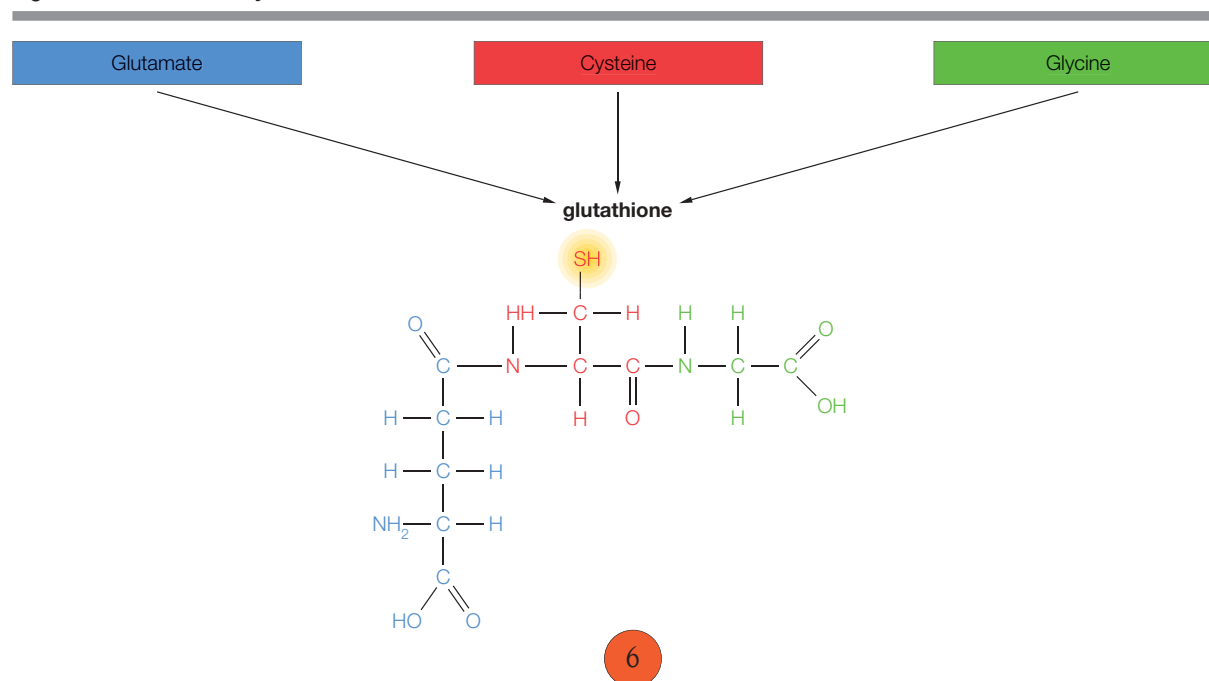
Whey proteins are a rich source of cysteine. WPC and WPI generally contain a concentration of cysteine that is at least 4-fold higher than other high quality proteins.<sup>16</sup> Both WPC and WPI are shown in research to be an effective cysteine donor that maintains a concentration of active GSH (a favorable GSH:GSSG ratio) in cells.<sup>8,9,37,39,43</sup> In comparison to other commercially available protein sources, optimization of the immune response via enhanced production of GSH in immune cells has been achieved with whey proteins.<sup>7-14,39,40,44</sup>

*Whey Proteins, Immune Function and Exercise Performance*

Supplementation with branched chain amino acids (BCAAs) before exercise is shown to prevent the decline in lymphocyte proliferation and prevent the post-exercise fall in plasma glutamine concentration after exercise.<sup>32</sup> Muscle glutamine is constantly exported into the blood as it is the essential metabolic fuel that powers immune function.<sup>53</sup> BCAAs are used exclusively by muscle to manufacture glutamine.<sup>32</sup> Whey protein is the richest (and most cost effective) natural source of BCAAs.<sup>62</sup> One investigation has shown that supplementation with WPI, (1.5 grams per kilogram of body weight per day) for 11 weeks is able to maintain plasma glutamine concentrations that can decline during intense training programs, as well as provide significantly better muscular strength gains than casein.<sup>20</sup>

In terms of endurance performance, one investigation showed that six weeks of WPI supplementation (at dose of 1 gram per kilogram of body weight per day) prevented a decline in both whole blood and mononuclear cell GSH concentrations that was seen in the control group during 6 weeks of intense training.<sup>45,57</sup>

**Figure 1. Glutathione is Synthesized from Three Amino Acids**



**NUTRITIONAL INTERVENTION TO ENHANCE IMMUNITY**

Inadequate protein (amino acids) intake may retard immune function. Current literature suggests that protein requirements for healthy, active people may be higher than previously recommended<sup>5,48</sup> Increasing the ratio of protein in the diet is now considered a safe, effective strategy to enhance health by lowering blood lipid concentrations, improving insulin/glucose metabolism and promotes the loss of excess weight.<sup>28</sup> To maintain health and optimum immune function, current research suggests that active individuals and older adults have increased dietary protein requirements than what has been traditionally recommended<sup>5,48</sup> Research suggests the type of protein in the diet can influence the intensity of the immune response, even in nutritionally adequate diets.<sup>9,63</sup> In comparison to other protein sources, whey proteins are shown in research to boost GSH concentrations as well as optimize certain aspects of immune function.

Whey proteins are quickly absorbed in the body to provide an abundant supply of essential amino acids to organs and tissues, and they stimulate muscle regenerative mechanisms.<sup>23</sup> Consuming 20 or 30 grams of WPI and WPC together with carbohydrates before and after exercise training may be an ideal strategy to help minimize exercise-induced immune suppression as well as enhance muscle recovery. By virtue of its excellent amino acid profile, absorption kinetics and immuno-enhancing capabilities, whey protein is a highly nutritious ingredient that may benefit a variety of populations. Although clear guidelines regarding a daily dosage of whey protein are yet to be established, research has shown benefits with dosages within the vicinity of 20 grams/day, up to 1.5 grams per kilogram of body weight per day.



**Essential Amino Acid Content of Selected Proteins (mg/g of protein)**

Amino acid	Total whey protein	FAO AA scoring pattern
Isoleucine	76	40
Leucine	118	70
Lysine	113	55
Methionine + cysteine	52	35
Phenylalanine + tyrosine	70	60
Threonine	84	40
Tryptophan	24	10
Valine	72	50
<b>Total</b>	<b>609</b>	<b>360</b>

Source: Reference Manual for US Whey and Lactose Products. USDEC.

## WHEY PROTEINS AND CANCER

Epidemiological data suggests that diet is a major factor in the origin of cancer.<sup>33,34,44,64</sup> The unique capability of whey proteins to enhance GSH production and modulate immune function inspired scientists to investigate whey proteins effects on experimentally-induced carcinoma.

In mice, supplementation with WPC (20% of protein intake) as compared to casein was shown to lower the incidence and size of colon tumors.<sup>10</sup> These findings were replicated in another study several years later by a different group of researchers.<sup>44</sup> This later study also showed that WPC had twice the protective effect against the development of intestinal tumors as compared to soy protein. The American Cancer Society estimates that colon cancer will remain the second leading cause of cancer deaths in the United States in 2004, followed by breast, pancreatic and prostate cancer. Therefore, the potential anticancer effects from whey protein supplementation are particularly relevant to humans.

Whey protein's anti-carcinogenic properties have also been demonstrated in additional types of malignancies such as mammary tumors in female rats.<sup>34</sup> In one study, whey protein supplementation was found to be at least twice as effective as soy protein isolate at reducing both tumor incidence and multiplicity.<sup>34</sup>

Whey protein's unique capability to increase GSH concentrations in cells and protect against cancer development has also been demonstrated in human prostate cells.<sup>37</sup> Treatment with a hydrolyzed WPI increased intracellular GSH by 64% and protected cells from oxidant-induced cell death, whereas treatment with hydrolyzed sodium caseinate did not significantly increase cellular GSH.<sup>37</sup>

In cancer research, whey protein supplementation is shown to maintain a high concentration of GSH in cells and boost cellular antioxidant defenses that promote carcinogen detoxification.<sup>10,33,34,44</sup> Due to the positive findings, whey protein supplementation is starting to be viewed as a non-pharmaceutical adjunct therapy in the treatment of cancer.<sup>7</sup>



Dr. Michael Murray, author of "How to Prevent and Treat Cancer with Natural Medicine," recommends that cancer patients take 20-30 grams of whey protein twice a day to obtain adequate amounts of glutamine. He states that both glutamine and branched-chain amino acids (whey protein is rich in BCAAs) are critical to cellular health and protein synthesis. Whey protein also may help support recovery from cancer treatments, such as chemotherapy and radiation, and surgery.



**BENEFITS OF WHEY PROTEINS FOR STRONG IMMUNITY**

- The branched chain amino acids (BCAAs) are metabolized exclusively by muscle to manufacture glutamine; the fuel that powers immune function. Whey proteins are the richest known source of BCAAs. Commercially available whey protein formulations contain approximately 26% BCAA and 6% glutamate.<sup>16</sup> Therefore, over one third of whey protein's entire amino acid profile is devoted entirely to muscle glutamine synthesis.
- Cysteine is the rate limiting amino acid in GHS formation. GHS is the centerpiece of all antioxidant defenses and controls many key processes of immune function. Whey proteins contain at least a 4-fold higher concentration of the amino acid cysteine (per 100gms of protein) when compared to other high quality protein sources.
- An increase in GHS status is thought to optimize immune function. Compared to other common protein sources, incorporating whey proteins into the diet is shown to boost GHS production and optimize immunity.
- Supplementation with whey proteins is also shown to maintain GHS status in healthy people during intense exercise training. In some instances, this has resulted in improved athletic performance and/or body composition (less body fat and more lean tissue).

**Whey & Peanut Butter Meal Replacement Bar\* (40% Carbohydrate/30% Protein/30% Fat)**

Ingredient	%
Honey	18.29
High Fructose Corn Syrup	16.17
Chocolate Coating	14.89
<b>Whey Protein Isolate</b>	<b>11.12</b>
<b>Hydrolyzed Whey Protein Isolate</b>	<b>9.79</b>
Peanut Butter	8.33
Peanut Flour	7.33
Chopped Peanuts	7.24
Maltodextrin	3.52
Vitamin/Mineral Blend	1.68
Vanilla Extract	1.03
Soy Fiber	0.61
Total	100.00

\*Formula courtesy of DAVISCO FOODS INTERNATIONAL, Inc.



## BENEFICIAL EFFECTS OF WHEY PROTEIN SUPPLEMENTATION IN HIV INFECTION

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*High quality whey protein is a reliable source of amino acids and biologically active proteins. As a nutritional supplement whey is valuable to augment protein-rich diets in HIV infected patients. There is growing evidence that high quality whey protein formulas possess therapeutic properties in different pathological conditions. The following summary presents highlights about potential beneficial effects of whey protein in HIV infection and focus on clinical trials using whey protein supplementation. To obtain a full copy of a comprehensive review on this topic, please contact the U.S. Dairy Export Council.*

### *HIV Infected Patients can Benefit from High Quality Protein Supplements*

Although clinical trials using whey protein or other high quality protein supplements are scant, there are preliminary indications that HIV infected patients might benefit from a high protein diet. One trial that included 30 HIV infected women, randomized to whey protein, progressive exercise or combined treatment, revealed after 14 weeks of therapy body weight gain in the whey protein-only group, whereas the clinically favorable body cell mass (mass of metabolic active cells) increased in the groups that included exercise [Agin et al., 2001]. A randomized study comparing a peptide-based high

protein diet with a conventional standard diet showed superior clinical outcome (lean body mass increase and hospitalization frequency decrease) for the preparation including peptides [Chlebowski et al., 1993]. An analysis of 476 weight stable patients revealed an independent association of protein intake and body cell mass [Williams et al., 2003]. However, although the optimal amount and source of protein is still a matter of intensive debate, these observations provide rationales for a high quality protein supplementation.

### *Whey Protein Supplementation can Restore Glutathione Levels in HIV Infection*

In a small pilot study 3 HIV sero-positive patients were orally supplemented with an increasing amount of up to 39 g whey protein per day. After 3 months of treatment GSH content of blood mononuclear cells increased in 2 of 3 patients and reached normal levels in one individual [Bounous et al., 1993]. Two controlled clinical studies were recently performed. Firstly, in a double blinded fashion, 30 patients with advanced HIV-infection were randomized to daily dose of 45 g of two different whey protein preparations. The protein powder was supposed to be taken in three equal portions of 15 g supplementary to their routine therapy (in 90% conventional antiretroviral therapy). Pretherapy plasma GSH levels were significantly reduced. After two weeks of treatment plasma GSH levels in both group increased notably to normal levels in both groups [Micke et al., 2001]. In an open follow-up trial 18 patients were treated with one of the supplements. Over a 6 month period the GSH levels remained persistently stable. However, no significant influence on clinical parameters like body weight and T lymphocyte counts could be observed, most likely due to the small patient number in this study [Micke et al., 2002]. The results of these reports indicate that oral treatment with a cysteine-rich nutritional supplement derived from whey proteins is able to significantly and consistently increase plasma GSH levels to the normal range over a period of up to 6 months in GSH-deficient patients with

advanced HIV-infection. The therapy was well tolerated and no clinically meaningful adverse effects were observed.

### *High Quality Whey Proteins Represent a Reliable Protein Supplement in HIV Infection*

Whey proteins present a high quality protein source, with a favorable amino acid composition. Clinical trials were able to demonstrate beneficial effects of whey protein supplementation in HIV infected patients. However, every nutritional intervention should be considered and performed individually, based on patients' requirements and history (current stage of disease, therapy, body composition, etc.). It affords special medical knowledge and should not be carried out without consultation of the responsible HIV medical team.

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The U.S. Dairy Export Council would like to extend its appreciation to all who contributed to the development of this monograph, and would like to recognize the contribution of Carla Sorensen of the Whey Protein Institute, 11000 West 78th Street, Suite 220, Eden Prairie, Minnesota, 55344, USA. [www.wheyoflife.org](http://www.wheyoflife.org)



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