Therapeutic Applications of Whey Protein

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Abstract

Whey, a protein complex derived from milk, is being touted as a functional food with a number of health benefits. The biological components of whey, including lactoferrin, beta-lactoglobulin, alpha-lactalbumin, glycomacropeptide, and immunoglobulins, demonstrate a range of immune-enhancing properties. In addition, whey has the ability to act as an antioxidant, antihypertensive, antitumor, hypolipidemic, antiviral, antibacterial, and chelating agent. The primary mechanism by which whey is thought to exert its effects is by intracellular conversion of the amino acid cysteine to glutathione, a potent intracellular antioxidant. A number of clinical trials have successfully been performed using whey in the treatment of cancer, HIV, hepatitis B, cardiovascular disease, osteoporosis, and as an antimicrobial agent. Whey protein has also exhibited benefit in the arena of exercise performance and enhancement.
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Introduction

In recent years, milk constituents have become recognized as functional foods, suggesting their use has a direct and measurable effect on health outcomes.[1] Whey, a by-product of cheese and curd manufacturing, was once considered a waste product. The discovery of whey as a functional food with nutritional applications elevated whey to a co-product in the manufacturing of cheese.[2] Milk contains two primary sources of protein, the caseins and whey. After processing occurs, the caseins are the proteins responsible for making curds, while whey remains in an aqueous environment. The components of whey include beta-lactoglobulin, alpha-lactalbumin, bovine serum albumin, lactoferrin, immunoglobulins, lactoperoxidase enzymes, glycomacropeptides, lactose, and minerals.[2] In additional, whey derived from buttermilk versus cheese contains the lipid sphingomyelin.

Several cultures consider fermented foods part of a healthful diet. Historically, whey was considered a cure-all used to heal ailments ranging from gastrointestinal complaints to joint and ligament problems. Nanna Rognvaldardottir, an Icelandic food expert, describes how whey, called syra by the Icelandic people, is fermented and stored in barrels. Syra is diluted with water and ingested or used as a marinade or preservative for meat and other food. Syra was the most common beverage of Icelandic people and is thought to have replaced ale, due to lack of grains in the region.[3]

Today, whey is a popular dietary protein supplement purported to provide antimicrobial activity, immune modulation, improved muscle strength and body composition, and to prevent cardiovascular disease and osteoporosis. Advances in processing technology, including ultrafiltration, microfiltration, reverse osmosis, and ion-exchange, have resulted in development of several different finished whey products. Whey protein concentrates (ranging from 80-95 percent protein), reduced lactose whey, whey protein isolate, demineralized whey, and hydrolyzed whey are now

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Table 1. Types of Commercially Available Whey Proteins

Product Description	Protein Concentration	Fat, Lactose, and Mineral Content
Whey Protein Isolate	90-95%	Little if any
Whey Protein Concentrate	Ranges from 25-89% Most commonly available as 80%	Some fat, lactose, and minerals As protein concentration increases, fat, lactose, and mineral content decreases.
Hydrolyzed Whey Protein	Variable Hydrolysis used to cleave peptide bonds Larger proteins become smaller peptide fractions Reduces allergic potential compared with non-hydrolyzed	Varies with protein concentration
Undenatured Whey Concentrate	Variable Usually ranges from 25-89%	Some fat, lactose, and minerals As protein concentration increases, fat, lactose, and mineral content decreases. Processed to preserve native protein structures; typically have higher amounts of immunoglobulins and lactoferrin

available commercially. Each whey product varies in the amount of protein, carbohydrates, immunoglobulins, lactose, minerals, and fat in the finished product. These variables are important factors in the selection of whey fractions for specific nutritional applications. Table 1 describes the various whey protein products available.

Whey Protein Manufacturing

Protein from bovine whole milk consists of approximately 20-percent whey protein. When casein is removed from whole milk, liquid whey remains, having a protein concentration of about 65 percent. The following is a summary of the Ohio State University method of manufacturing whey protein powder. Milk is high-temperature, short-time pasteurized (163 degrees F for 30 seconds) and held overnight at 40 degrees C. The following morning the mixture is cooled to 30 degrees C, inoculated with a lactic acid culture, and incubated for 30 minutes. Rennet extract is added and the mixture is stirred, resulting in coagulation of curd.

Rennet is derived from the abomasum (fourth stomach) of newly born calves. Chymosin, the active enzyme ingredient of rennet, aids in the coagulation of milk by separating it into curds and whey. In a newly born calf, chymosin aides in the digestion and absorption of milk. Adult cows do not have this enzyme.

The liquid whey is drained through a stainless steel screen and the remaining curd is cut and cooked at 30 degrees C. Whey liquid is then filtered at 45 degrees C and brought to a pH of 3 by adding citric acid. The liquid is filtered to one-fifth its original volume, resulting in whey concentrate that is approximately 80-percent protein. This can be additionally micro-filtered to increase protein concentration to as high as 95 percent.

Table 2. Amino Acid Residues in Bovine versus Human Lactoferrin

	Bovine Lactoferrin	Human Lactoferrin
Alanine	67	63
Proline	30	35
Arginine	39	43
Lysine	54	46
Asparagine	29	33
Valine	47	48
Tryptophan	13	10
Cysteine	34	32
Threonine	36	31
Isoleucine	15	16
Serine	45	50
Glutamine	29	27
Glutamic acid	40	42
Phenylalanine	27	30
Methionine	4	5
Leucine	65	58
Glycine	48	54
Tyrosine	22	21
Aspartic Acid	36	38
Histidine	9	9
Total number of residues	689	691

From: Pierce A, Colavizza D, Benaissa M, et al. Molecular cloning and sequence analysis of bovine lactoferrin. Eur J Biochem 1991;196:177-184.

The final whey protein concentrate is warmed and spray-dried to achieve whey protein powder. Whey protein concentrates can then be put through an ion-exchange process to remove fat and lactose. In addition, some manufacturers hydrolyze (cleaving peptide bonds via enzymes or heat) the whey to provide more peptides and free amino acids in the final product.[4]

The commercial success of whey protein has led to the development of high quality whey protein supplements manufactured as primary products and not as a by-product of cheese manufacturing. Manufacturers take special care to preserve the biological activity, native protein structure, and protein-bound-fats in the finished product. Proteins are processed under low temperatures and not exposed to fluctuating pH changes to avoid denaturing the native structures. In addition, the source of milk and the health of the milking cows is thought to contribute to immune-enhancing activity of whey products.[5,6]

Biological Components

Amino Acid Content

Collectively, whey proteins have all the essential amino acids and in higher concentrations compared to various vegetable protein sources such as soy, corn, and wheat gluten.[2] In addition to having a full spectrum of amino acids, the amino acids found in whey are efficiently absorbed and utilized, relative to free amino acid solutions.[7]

Relative to other protein sources, whey has a high concentration of branched-chain amino acids (BCAAs) – leucine, isoleucine, and valine. BCAAs, particularly leucine, are important factors in tissue growth and repair. Leucine has been identified as a key amino acid in protein metabolism during the translation-initiation pathway of protein synthesis.[8]

Whey proteins are also rich in the sulfur-containing amino acids cysteine and methionine. With a high concentration of these amino acids, immune function is enhanced through intracellular conversion to glutathione.

Lactoferrin

Lactoferrin, an iron-binding glycoprotein, is a non-enzymatic antioxidant found in the whey fraction of milk as well as in colostrums. The lactoferrin component of whey consists of approximately 689 amino acid residues, while human lactoferrin consists of 691 residues.[9] Whey lactoferrin is composed of a single polypeptide chain with two binding sites for ferric ions. Before processing, bovine lactoferrin is only 15-20 percent saturated with iron. Iron-depleted lactoferrin, defined as containing less than five-percent iron, is referred to as apolactoferrin. Human breast milk contains apolactoferrin.[10] The concentration of lactoferrin in human milk and colostrums is approximately 2 mg/mL and 7 mg/mL, respectively, while in bovine milk and colostrums it is approximately 0.2 mg/mL and 1.5 mg/mL, respectively.[11] Lactoferrin is a dominant component of whey protein in human breast milk; however, the concentration in most commercial whey protein powders is only 0.35-2.0 percent of total proteins. Table 2 illustrates the difference in amino acid profiles between bovine and human lactoferrin.

Immunoglobulins

An immunoglobulin (Ig) is an antibody or gamma-globulin. There are five classes of antibodies – IgA, IgD, IgE, IgG, and IgM. IgG constitutes approximately 75 percent of the antibodies in an adult. IgG is transferred from mother to child *in utero* via cord blood and by breast-feeding, and serves as a child's first line of immune defense – referred to as "passive immunity." IgA is secreted in breast milk and ultimately transferred to the digestive tract in the newborn infant, providing better immunity than a bottle-fed child.[12] Colostrums contain significantly greater concentrations of immunoglobulins than mature milk. Immunoglobulins reach maximum concentration the first 24-48 hours post-parturition and decline in a time-dependent manner following peak concentration.[13]

Similarly, the whey fraction of milk appears to contain a significant amount of immunoglobulins, approximately 10-15 percent of total whey proteins. An *in vitro* study demonstrated bovine milk-derived IgG suppresses human lymphocyte proliferative response to T cells at levels as low as 0.3 mg/mL of IgG. The authors further conclude bovine milk IgG typically ranges between 0.6-0.9 mg/mL and is therefore likely to confer immunity that could be carried to humans.[14] Studies show raw milk from non-immunized cows contain specific antibodies to human rotavirus, as well as antibodies to bacteria such as *E. coli*, *Salmonella enteriditis*, *S. typhimurium*, and *Shigella flexneri*.[15,16]

beta-Lactoglobulin

beta-Lactoglobulin represents approximately half of the total protein in bovine whey, while human milk contains no beta-lactoglobulin. Besides being a source of essential and branched chain amino acids, a retinol-binding protein has been identified within the beta-lactoglobulin structure. This protein, a carrier of small hydrophobic molecules including retinoic acid, has the potential to modulate lymphatic responses.[17]

alpha-Lactalbumin

alpha-Lactalbumin is one of the main proteins found in human and bovine milk. It comprises approximately 20-25 percent of whey proteins and contains a wide variety of amino acids, including a readily available supply of essential and branched chain amino acids. Purified alpha-lactalbumin is most readily used in infant

Table 3. Components Found in Whey Protein

Whey Components	% of Whey Protein	Benefits
beta-Lactoglobulin	50-55%	Source of essential and branched chain amino acids
alpha-Lactalbumin	20-25%	Primary protein found in human breast milk Source of essential and branched chain amino acids
Immunoglobulins	10-15%	Primary protein found in colostrum Immune modulating benefits
Lactoferrin	1-2%	Antioxidant Antibacterial, antiviral , and antifungal Promotes growth of beneficial bacteria Naturally occurs in breast milk, tears, saliva, bile, blood, and mucus
Lactoperoxidase	0.50%	Inhibits growth of bacteria
Bovine Serum Albumin	5-10%	Source of essential amino acids Large protein
Glycomacropeptide	10-15%	Source of branched chain amino acids Lacks the aromatic amino acids phenylalanine, tryptophan, and tyrosine

formula manufacturing, as it has the most structurally similar protein profile compared to breast milk. However, due to cost effective measures, most dairy-based infant formulas contain ingredients such as demineralized whey with higher levels of beta-lactoglobulin, making them less similar to human milk.

In a murine study, alpha-lactalbumin, in both the native and hydrolyzed state, enhanced antibody response to systematic antigen stimulation.[18] The same group proved alpha-lactalbumin has a direct effect on B-lymphocyte function, as well as suppressing T cell-dependent and -independent responses.[19]

Lactoperoxidase

Whey contains many types of enzymes, including hydrolases, transferases, lyases, proteases, and lipases. Lactoperoxidase, an important enzyme in the whey fraction of milk, is the most abundant enzyme and the majority of it ends up in whey following the curding process. Lactoperoxidase accounts for 0.25-0.5 percent of total protein found in whey. It has the ability to catalyze certain molecules, including the reduction of hydrogen peroxide.[20] This enzyme system catalyzes peroxidation of thiocyanate and some halides (such as iodine and bromium), which ultimately generates products that inhibit and/or kill a range of bacterial species.[21] During the pasteurization process, lactoperoxidase is not inactivated, suggesting its stability as a preservative.

Glycomacropeptide

Glycomacropeptide (GMP) is also referred to as casein macropeptide. GMP is a protein present in whey at 10-15 percent, due to the action of chymosin on casein during the cheese-making process. GMP is only present when chymosin is used during processing; therefore, cheeses such as cottage cheese not made with chymosin do not produce GMP in the curding process.[22] GMP is high in branched chain amino acids and lacks the aromatic amino acids including phenylalanine, tryptophan, and tyrosine. It is one of the few naturally occurring proteins that lacks phenylalanine, making it safe for individuals with phenylketonuria (PKU).

Bovine Serum Albumin

Bovine serum albumin (BSA) is a large protein that makes up approximately 10-15 percent of total whey protein. BSA is a source of essential amino acids, but there is very little available information regarding its potential therapeutic activity.

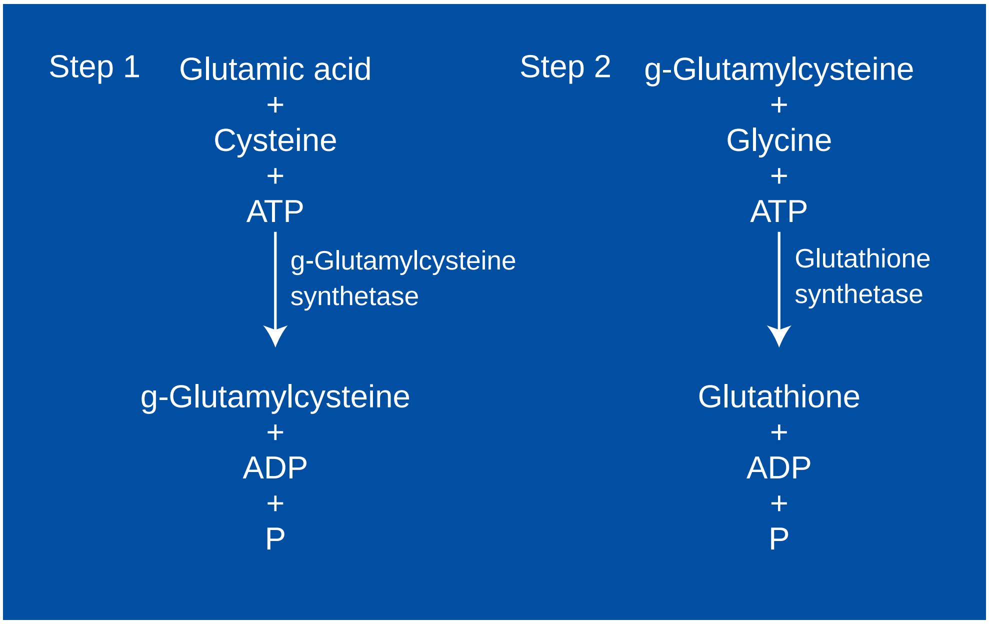
Table 3 summarizes the components found in whey.

Mechanism of Action

Whey has potent antioxidant activity, likely by contributing cysteine-rich proteins that aid in the synthesis of glutathione (GSH), a potent intracellular antioxidant.[2] GSH is comprised of glycine, glutamate, and cysteine (Figure 1). Cysteine contains a thiol (sulfhydryl) group that serves as an active reducing agent in preventing oxidation and tissue damage. As an antioxidant, glutathione is most effective in its reduced form. Riboflavin, niacinamide, and glutathione reductase are essential cofactors in the reduction of glutathione.[23] As a result of the glutathione/antioxidant component of whey, it is being investigated as an anti-aging agent.[24]

As a detoxifying agent, glutathione peroxidase (GSHPx), which is derived from selenium and cysteine, is an endogenous antioxidant enzyme with the ability to convert lipid peroxides into less harmful hydroxy acids. The peroxidases interact with hydrogen peroxide to reduce it to water, negating its oxidative potential. Both glutathione peroxidase activity and selenium concentrations have been shown to decrease as lactation continues, peaking at approximately one month after initiation. Practitioners use whey protein products as a source of cysteine to increase intracellular glutathione levels[25,26] and it has been reported that GSHPx activity in cow's milk, and presumably whey, is the same as in human milk.[27]

Figure 1. *Synthesis of Glutathione from Cysteine, Glutamate, and Glycine*



Studies on lactoferrin have demonstrated its ability to activate natural killer (NK) cells and neutrophils, induce colony-stimulating factor activity, and enhance macrophage cytotoxicity.[28-31]

Lactoferrin also appears to have antiviral, antifungal, and antibacterial properties. The antimicrobial effect is likely more potent in organisms that require iron to replicate, as lactoferrin has the unique ability to chelate iron in a way that deprives microorganisms of this essential nutrient for growth.[32] In additional, lactoferrin has the ability to release the outer membrane of gram-negative bacteria, the lipopolysaccharide component, thus acting as an antibiotic.[33]

Lactoferrin demonstrates anti-inflammatory properties. A mouse study revealed lactoferrin had the ability to regulate levels of tumor necrosis factor (TNF) and interleukin 6 (IL-6), thus decreasing inflammation and, ultimately, mortality.[34]

In addition to the above-mentioned properties, alpha-lactalbumin can chelate heavy metals.[35] It reduces oxidative stress because of its iron-chelating properties.[36]

Whey has been recently touted as a healthful dietary supplement to reduce blood pressure. Antihypertensive peptides have been isolated in the primary sequence of bovine beta-lactoglobulin.[37] These peptides give whey significant angiotensin I converting enzyme (ACE) inhibitory activity, which blocks the conversion of angiotensin I to angiotensin II, a highly potent vasoconstrictor molecule.[38] beta-Lactoglobulin has been described by Nagaoka et al as a cholesterol-lowering agent. In animal studies, beta-lactoglobulin inhibited cholesterol absorption by changing micellar cholesterol solubility in the intestine.[39]

Absorption and Digestion of Milk Proteins: Casein versus Whey and Lactoferrin

The physical manifestations of milk in the gut vary, based on the type of milk protein. Whey comprises approximately 20 percent of milk protein, while 80 percent of protein in milk is casein.[32] These two proteins possess quite different properties. Casein proteins form curds in the stomach, increasing hydrolysis and slowing entrance to the small intestine. Whey proteins, on the other hand, do not coagulate under acidic conditions. They are considered to be "fast proteins," as they reach the jejunum quickly after entering the gastrointestinal tract.[40] After reaching the small intestine, the hydrolysis of whey is slower than that of casein, allowing for greater absorption over the length of the small intestine.

A study by Arnal et al demonstrates that whey's rapid absorption patterns are superior for postprandial protein utilization and overall nitrogen balance in elderly women.[41] Most recently, a randomized, single-blind study examining protein satiety found whey protein to exhibit a higher postprandial level of plasma amino acids compared to casein.[42]

A study by Troost et al examined the effects of an orally administered recombinant human lactoferrin (rhLF) in the digestive tract. The study population consisted of eight female ileostomy patients who consumed 5 g rhLF and collected full ileostomy output for 24 hours. In a 24-hour period, only 4 μg of lactoferrin were excreted. The study concluded dietary rhLF does not reach the colon because it is digested in the stomach and small intestine.[43] A similar study by the same group was conducted on 12 healthy volunteers. On three separate days, volunteers were instructed to consume 4.5 g lactoferrin. After conclusion of the study, results revealed bovine lactoferrin survived passage through the stomach, demonstrating the ability of this protein to survive in an acidic environment.[44]

Clinical Indications for Whey

Cancer

Whey protein concentrates have been researched extensively in the prevention and treatment of cancer. Glutathione stimulation is thought to be the primary immune-modulating mechanism. In a review of whey protein concentrates in the treatment of cancer, Bounous discusses the anti-tumor and anticarcinogenic potential. The amino acid precursors to glutathione available in whey might: (1) increase glutathione concentration in relevant tissues, (2) stimulate immunity, and (3) detoxify potential carcinogens.[45] Other authors conclude the iron-binding capacity of whey may also contribute to anticancer potential, as iron may act as a mutagenic agent causing oxidative damage to tissues.[46]

Many animal studies have examined the effects of whey and its immune-enhancing components, including lactoferrin and beta-lactoglobulin.[47-54] In animal studies where colon cancer was induced, whey demonstrated significantly lower incidence of tumors, as well as fewer aberrant crypts.[47,49,52,54] When whey-based protein powders were compared to soy-based protein powders, similar effects were also observed.[48,52] Yoo et al demonstrated lactoferrin has the ability to inhibit metastasis of primary tumors in mice with cancer.[50] Bovine serum albumin (10-15 percent of total whey protein) has demonstrated inhibition of growth in human breast cancer cells *in vitro*.[55] A hamster study demonstrated fractionated whey has the ability to prevent and treat 5-fluorouracil chemotherapy-induced oral mucositis.[56] This protection is thought to occur via induction of tumor growth factor-beta (TGF-β), which reduces basal epithelial cell proliferation.

A recent *in vitro* study by Kent et al demonstrated that an isolate of whey protein, when compared to a casein-based protein, increased glutathione synthesis and protected human prostate cells against oxidant-induced cell death.[57] An *in vitro* study on a human hepatoma cell line was conducted using a high lactoferrin whey concentrate (Immunocal®), a baicalein medium, or a combination of the two.[58] Baicalein, a potential anticancer drug, is a flavonoid extracted from *Scutellaria revularis* that is thought to have antitumor activity. Immunocal alone did not have a significant impact on the hepatoma cell line. However, when Immunocal was combined with baicelein, cytotoxicity was enhanced by inducing a higher rate of apoptosis than in the group treated with baicalein alone.

To date, few clinical trials on whey and cancer have been conducted. It has been proposed that GSH concentrations are high in tumor cells, giving them resistance to chemotherapeutic agents.[59] In 1995, a small trial was conducted on five patients with metastatic carcinoma of the breast, one patient with pancreatic cancer, and one with liver cancer.[59] Patients were given 30 g whey protein concentrate (Immunocal) for six months. In six patients, blood lymphocyte GSH was elevated initially, suggesting high tumor GSH levels. After completion of the study, two of the patients showed signs of tumor regression and a return of lymphocyte GSH levels to normal, while two patients showed signs of tumor stabilization without normalization of glutathione levels. Three patients had progression of disease and lymphocyte GSH levels increased from initial measurements. The conflicting results and small size of this study indicate the need for a larger clinical trial to investigate the potential of a whey protein concentrate singly and as an adjunct to chemotherapy.

In a recent clinical trial, 20 patients with stage IV malignancies (one bladder, five breast, two prostate, one neuroblastoma, one ovarian, one gastric, three colon, one mesothelioma, two lymphoma, two non-small cell lung, and one osteosarcoma) received a combination of 40 g/day non-denatured whey protein concentrate, 50-100 g/day intravenous Transfer Factor Plus® (a supplement containing several immunoactive components), 1-2 g/day oral ascorbic acid, *Agaricus blazei* tea, a multiple vitamin/mineral complex, 500 mg *Andrographis paniculata* twice daily, and a soy extract for six months.[60] After six months there were 16 survivors, all of whom had significantly higher NK function and higher mean hemoglobin and hematocrit levels. All patients noted having an improved quality of life during the course of the study. No comparison was made between this combination therapy and whey protein alone.

Hepatitis

Whey protein supplementation demonstrates variable effects in patients infected with Hepatitis B or C. Initially it was found that bovine lactoferrin prevented hepatitis C virus (HCV) infection *in vitro* in a human hepatocyte line.[61] These results prompted further clinical trials.

A pilot study was conducted on 11 patients with chronic HCV. Each patient received either 1.8 or 3.6 g bovine lactoferrin daily for eight

Table 4. Comparison of Whey Products Immunocal and Protectamin

Amino Acid	Immunocal (g/100 g)	Protectamin (g/100 g)
Aspartic	9.80	10.50
Glutamic	16.37	17.48
Serine	3.56	5.58
Glycine	1.64	1.84
Histidine	2.04	1.73
Arginine	2.30	2.24
Threonine	4.63	6.97
Alanine	4.80	4.87
Proline	3.76	6.01
Tyrosine	3.76	1.97
Valine	4.53	6.06
Methionine	2.13	2.27
Isoleucine	6.41	6.32
Leucine	12.56	9.96
Phenylalanine	4.00	3.15
Lysine	10.68	9.19
Tryptophan	2.86	1.53
Cysteine/Cystine	4.17	2.28
Minerals		
Sodium	0.3	0.1
Potassium	0.5	0.5
Calcium	0.4	0.3
Chloride	0.4	0.1
Phosphate	0.3	0.3
Magnesium	0.3	0.1

From: Micke P, Beeh KM, Buhl R. Effects of long-term supplementation with whey proteins on plasma glutathione levels of HIV-infected patients. Eur J Nutr 2002;41:12-18.

weeks. In patients with low pretreatment viral loads of HCV, decreases in HCV RNA and serum alanine transaminase were observed. In patients with higher pretreatment HCV viral loads, levels did not change significantly.[62]

A dose-response trial of 45 individuals with HCV was conducted at doses of 1.8, 3.6, and 7.2 g lactoferrin daily for eight weeks.[63] A virological response was observed in only four patients, although HCV RNA was still detectable. Eight patients had a virological response – a 50 percent or greater decrease in HCV RNA – eight weeks after the treatment ended. There were no significant variations in dose-dependant responses. This trial left many unanswered questions for future studies regarding whey supplementation and HCV, including optimum dose, duration, and the potential effects of combining supplementation with conventional treatments such as interferon therapy.

In an open study on 25 patients diagnosed with either Hepatitis B or C, patients were given 12 g whey (Immunocal) twice daily for 12 weeks.[64] Prior to the start of treatment with whey, patients were given 12 g casein protein daily for two weeks. Patients were also given casein following Immunocal for a four-week period. In the 17 patients with HCV, no significant changes were noted. In the group with hepatitis B virus (HBV), serum lipid peroxidase levels decreased, while IL-2 and NK activity increased. In six of eight HBV patients serum alanine transferase levels were reduced, while plasma glutathione levels increased in five of the same eight. This trial shows promise for the use of whey protein in the treatment of HBV.

Human Immunodeficiency Virus (HIV)

Glutathione deficiency is a common problem for individuals infected with HIV. In an effort to increase cysteine, and ultimately glutathione, several studies have been conducted on the use of whey proteins in HIV-positive individuals.[65,66] In a study by Micke et al, 30 subjects with HIV were randomized to receive a daily dose of 45 g whey protein from one of two sources – Protectamin® or Immunocal. The two products have different amino acid profiles and Immunocal is produced at a lower isolation temperature (<72 degrees C). After two weeks of oral supplementation, the Protectamin-supplemented group demonstrated significantly elevated glutathione levels, while the Immunocal group had statistically non-significant elevations. Table 4 compares the amino acid profiles of the two products.

The same researchers conducted a subsequent six-month study, using the same dose and products.[65] Similarly, in the Protectamin group glutathione levels increased within a two-week period, while levels in the Immunocal group did not. All participants were then crossed over to receive Protectamin. After six months, all patients had a significantly increased glutathione level when compared to baseline numbers.

Two small studies have been conducted on HIV-positive women examining exercise, whey supplementation, and body composition.[67,68] In a 2000 study the author notes an increase in body mass composition with whey supplementation, whereas the 2001 study notes no effect. Both studies note an improvement in quality of life with increased exercise and whey protein intake. Treatment groups received 1.0 g/kg daily of undenatured bovine-derived whey protein powder.

Antimicrobial

Plasma levels of lactoferrin have been found to be elevated due to release from neutrophils during infection, inflammation, tumor development, and iron overload.[11] Several studies have revealed lactoferrin plays a direct role in the body's defense against pathogens, including findings that individuals more susceptible to infection have lower levels of neutrophil lactoferrin.[69,70] Several studies have examined the effects of whey protein concentrates on the eradication of *Helicobacter pylori*.[71-73] *H. pylori* is widely accepted as the causative agent in over 90 percent of duodenal ulcer cases.[74] To date, the most effective form of eradication of *H. pylori* is one week of a triple antibiotic therapy. Due to the expense of treatment and concern for antibiotic resistance, new treatments are being sought.

In an open, randomized, single-center study of 150 individuals with diagnosed *H. pylori* infection, patients were given antibiotics at varying doses and durations (range 7-10 days) in conjunction with 200 mg encapsulated lactoferrin.[73] Analysis of the study revealed 100-percent eradication of *H. pylori* in the group using the seven-day antibiotic course with the addition of lactoferrin. On the other hand, the "classic" seven-day triple antibiotic group had a success rate of 76.9 percent, while the 10-day treatment group demonstrated a 70.8-percent success rate.

In a small study, 12 children suffering from chronic pharyngitis were administered a combination of 500 mg erythromycin three times daily and 100 mg bovine lactoferrin in a gargle. All children tested positive for Group A Streptococci. After 15 days of treatment, fewer intracellular Group A Streptococci were found compared to a group treated with antibiotics alone.[75]

In a concise review, Shah discusses the bacteriostatic and bacteriocidal activity lactoferrin exhibits against a number of organisms, including *Escherichia coli*, *Salmonella typhimurium*, *Shigella dysenteriae*, *Listeria monocytogenes*, *Bacillus stearothermophilus*, *Bacillus subtilis*, and *Micrococcus luteus*.[32,76-79] Shah further discusses that lactoferrin, when in combination with lysozyme, is a more potent bacteriostatic agent against *Pseudomonas aeruginosa, Listeria monocytogenes, and E. coli.*[32,80] In addition to the above-mentioned bacteria, lactoferrin has also demonstrated antifungal activity toward *Candida albicans*.[81]

Cardiovascular Disease

In recent years, studies have linked a high-fat diet to an increased risk of cardiovascular disease (CVD). Because CVD is linked to a number of other factors, including increased age, genetics, obesity, sedentary lifestyle, and alcohol intake, quality of dietary fat must be taken into consideration. Milk is composed of more than 12 different types of fat, including sphingolipids, free sterols, cholesterol, and oleic acid.[82] Several studies have found milk intake and milk products lower blood pressure and reduce the risk of hypertension.[83-85]

A study was conducted on a group of 20 healthy adult males to investigate whether a fermented milk supplement with an added whey protein concentrate would affect serum lipids and blood pressure.[86] The fermented milk contained both *Lactobacillus casei* and *Streptococcus thermophilus.* During the course of eight weeks, volunteers consumed 200 mL of fermented milk with whey protein concentrate or a placebo in the morning and evening. The placebo consisted of a non-fermented milk product without the addition of whey protein concentrate. After eight weeks, the fermented-milk group showed significantly higher HDLs and lower triglycerides and systolic blood pressure than did the placebo group. While total cholesterol and LDL levels were lower in the fermented-milk group, the difference was not statistically significant.

Exercise

Whey protein supplements, including purified alpha-lactalbumin sports drinks, have been readily utilized in the consumer market because of the high protein quality score and high percentage of BCAAs.[87] Whey proteins can contain up to 26-percent branched chain amino acids,[2,88] which are efficient substrates for synthesizing new proteins. For example, the BCAA leucine acts as a signaling molecule for initiation of protein synthesis.[8,89] It has been speculated that the quality of a particular protein for enhancing muscle hypertrophy and strength is related to its leucine content.[36] The amino acid profile in dietary proteins influences their nitrogen utilization, and poor quality dietary proteins have been shown to increase nitrogen losses and limit protein synthesis.[90,91]

Human studies documenting the beneficial effect of whey protein supplementation on muscle size and strength are limited. Burke et al demonstrated that men engaged in resistance training programs while supplementing with whey protein concentrates showed greater improvements in strength than men using resistance training alone.[92] Forty-two men, ages 18-31, familiar with weight training, followed the same high volume, heavy load, and free-weight resistance-training program for 12 weeks. The participants were divided into three groups in double-blind fashion: whey protein (1.2 g/kg body wt/day), a multi-ingredient whey protein sports supplement (1.3 g/kg body wt/day), or placebo (1.2 g/kg body wt/day of maltodextrin). At baseline there were no significant differences among the groups in lean tissue mass or strength as determined by four measurements, including bench press, squat, isokinetic knee extension, and isokinetic knee flexion. After 12 weeks men who received whey protein or the multi-ingredient whey protein sports supplement in combination with resistance training showed greater improvements in one of four muscle strength measurements. In addition, whey supplemented groups showed greater improvements in lean tissue mass over the placebo group. While this study does not demonstrate whey protein's superiority over other protein sources, it does show improvement in resistance training with the addition of protein supplementation.

Lands et al showed the effect of three months whey protein supplementation (10 g twice daily) versus the same amount of casein as placebo on muscular performance in 18 men.[93] At baseline there were no significant differences in age, height, weight, percent ideal body weight, whole leg isokinetic cycle testing, peak power, or 30-second work capacity. Peak power and 30-second work capacity improved significantly in the treatment group with no change in the placebo group. Body weight remained unchanged in both groups, but the supplemented group had a decrease in percent body fat.

While moderate exercise enhances immunity,[94] intense athletic training has been shown to stress the immune system.[95-97] Free radical production and increased inflammatory activity are thought to contribute to impaired immune activity in over-trained athletes. In highly trained individuals muscular performance and recovery can be hindered by oxidative stress.[98-100] The availability of glutathione has been shown to reduce oxidative stress.[101]

Acting as a cysteine donor, whey has been shown to raise intracellular GSH levels *in vitro*.[102] Lands et al proposed enhanced biosynthesis of intracellular glutathione, shown by an increase of lymphocyte GSH levels in test subjects, is the mechanism responsible for the improved muscular performance observed in their study.[93]

Whey's amino acid profile makes it ideal for body composition and to support protein synthesis and muscle growth. Other bioactive components found in whey might benefit additional aspects of health in active people and trained athletes by improving immune function and gastrointestinal health and exhibiting anti-inflammatory activity. Whey components, such as IgA, glutamine, and lactoferrin, can beneficially impact common complaints among athletes, including repeated infections and gastrointestinal disturbances.

Lower levels of sIgA and glutamine[103,104] have been found after intensive exercise and in over-trained individuals, and have been correlated with increased frequency of infection.[105] In addition, glutamine deficiency has been proposed to contribute to gastrointestinal complaints experienced by highly trained individuals.[106] Free radical damage is thought to delay muscle recovery and impair performance.[107] Whey prevents free radical damage through supporting intracellular glutathione levels and supplying lactoferrin for additional antioxidant activity.

Obesity

Obesity has reached epidemic proportions in the United States. Low-fat, high-carbohydrate dietary trends are being eschewed in favor of higher-protein, lower-carbohydrate diets. For individuals eating high-protein diets, whey is an attractive source of dietary protein. Whey protein isolates can be as high as 95-percent protein, after the removal of fat and lactose, and contain valuable minerals and vitamins. Whey has made a significant commercial impact in the weight-loss industry for its protein content alone. The essential and non-essential amino acids in whey act as substrates for protein synthesis and may improve body mass index in individuals participating in exercise programs.[92]

Calcium is thought to influence energy metabolism because intracellular calcium regulates adipocyte lipid metabolism and triglyceride storage.[108] Zemel et al demonstrated a greater effect of dairy versus nondairy sources of calcium for improving body composition. Calcium-fortified cereal was compared to calcium-fortified cereal plus nonfat dried milk for accelerating weight and fat loss in mice. The addition of nonfat dried milk resulted in substantial amplification of these effects. The mechanism responsible for increased fat loss found with dairy-based calcium versus nondairy calcium has not yet been elucidated. The researchers speculate, "this additional activity resides in the whey fraction of milk."[108] The bioactive components in whey are thought to act synergistically with calcium to attenuate lipogenesis, accelerate lipolysis, and effect nutrient partitioning between adipose tissue and skeletal muscle.[108]

While there is increasing evidence dairy intake and whey protein may play a role in the prevention and/or attenuation of the rising epidemic of obesity, there is minimal data to suggest dairy, calcium from dairy, or whey can impact individuals with established obesity.

Infant Formula and Infantile Colic

Creating a substitute for mother's milk has proved to be challenging. It is estimated a nursing infant ingests about 3 g lactoferrin daily during the first week of life,[109] whereas a calf drinking two liters of milk a day ingests about 2 g lactoferrin daily. It is well accepted that nursing infants have a much richer gut flora than do bottle-fed infants, particularly with Bifidobacteria and Lactobacilli.[2] Such flora is normally associated with an increased resistance to colonization of the digestive tract with pathogenic bacteria.[110] In a study by Roberts et al it was determined that the addition of lactoferrin to a feeding formula increased levels of Bifidobacteria in bottle-fed babies. The levels of Bifidobacteria in formula-fed babies that were supplemented with lactoferrin were not as high as those found in breast-fed babies. In addition, Bifidobacteria in formula-fed babies took up to three months to develop, while Bifidobacteria developed more rapidly in nursing infants.[111]

In a double-blind, German study, 102 healthy infants less than two-weeks old were randomized to receive either a standard cow's milk formula or an infant formula containing partially hydrolyzed whey protein.[112] Results of this study indicate the whey-protein fed infants had significantly more Bifidobacteria in their stools, ultimately affording improved gastrointestinal immunity. It has been observed in previous studies that higher levels of Bifidobacteria in the digestive system decrease the potential for developing atopic disease for at-risk infants with family history.[113]

A randomized, double-blind, placebo-controlled study of 43 infants with diagnosed infantile colic was conducted to determine whether a hypoallergenic, hydrolyzed whey formula was superior to a standard cow's milk formula.[114] Infantile colic is described as at least three hours per day of crying for at least three days a week for a minimum of three weeks.[115] Parents kept a 24-hour diary for two weeks during the study. After a one-week qualification period, infants in the study were randomized to receive either whey or cow's milk formula for one week. A clinically relevant result was observed in the whey formula group, with crying time reduced to less that one hour per day – a one-hour greater reduction than found in the cow's milk formula group.

Osteoporosis

Milk has been proposed as a nutritional food that aids in the prevention of osteoporosis due to its bioavailable calcium content.[116] Researchers have begun to examine the different components of milk to determine if a particular isolate is responsible for the bone-protective effects. Initially, *in vitro* and animal studies determined milk basic protein (MBP), a component of whey, has the ability to stimulate proliferation and differentiation of osteoblastic cells as well as suppress bone resorption.[117-119] MBP is prepared from fractionated whey through cation exchange resin. The total protein concentration of MBP is 98 percent, containing lactoferrin, lactoperoxidase, and other minor components. Several *in vivo* studies on rats determined that both whey protein and fractionated whey protein had the ability to increase femoral bone strength in young ovariectomized rats.[120-122]

To date three clinical trials have been conducted to determine the effects of MBP in bone metabolism.[123-125] In a trial by Toba et al 30 healthy adult men were given a beverage containing 300 mg MBP.[123] Subjects were instructed to drink one beverage daily for 16 days while maintaining a normal diet. After 16 days, serum calcium and urinary calcium excretion were unchanged among all subjects. Both serum osteocalcin and procollagen I carboxy-terminal propeptide (PICP) levels increased after 16 days, indicating increased bone formation. Osteocalcin and PICP are biochemical markers released from osteoblasts to assess bone formation.

A similar study was conducted on a group of 33 healthy adult women to assess bone metabolism.[124] Women in the study were randomized to receive either a placebo or a 40 mg MBP beverage daily. Baseline left calcaneus bone-mineral density tests were performed on all women, and were repeated again at the completion of the study. Results indicated bone mineral density was significantly increased in the MBP group compared to placebo. Biochemical indices also revealed the MBP group exhibited a significant decrease in urinary cross-linked N-teleopeptides, indicating an inhibition of osteoclast-mediated bone resorption.

The authors state 400-800 mL of milk is equivalent to the 40 mg dose of MBP. More recently, Yamura et al conducted a similar double-blind study examining 30 women over a six-month period. Results indicate a daily dose of 40 mg per day of MBP significantly increases radial bone density.[125]

Gastrointestinal Support

A small, randomized, double-blind, crossover study was conducted on 10 children with short bowel syndrome to examine the effect of a hydrolyzed versus a non-hydrolyzed whey protein on growth and development.[126] Energy intake, nitrogen balance, intestinal permeability, and weight gain were similar among all children, indicating the particular form of whey was not an essential component. In children experiencing bowel resection, food introduction and promotion of normal growth and development is of utmost importance. The increased transit time of whey proteins in the small intestine makes it an ideal protein source for this small subset of children.

Whey protein has demonstrated a protective effect on the gastric mucosa. This effect is thought to be related to the sulfhydryl component, particularly cysteine and its link with glutamic acid in the production of glutathione.[127,128] Several animal studies have demonstrated this protective effect.[129,130] In a study by Rosaneli et al, rats fed a whey protein concentrate showed a 41-percent reduction in ulcerative lesions caused by ethanol ingestion, while a 73-percent reduction rate was observed following repeat doses of whey.[129] A study by Matsumoto et al demonstrated an isolated alpha-lactalbumin formula had a four-fold effect on reducing ulcerative lesions compared to a whey protein formula containing only 25-percent alpha-lactalbumin.[130]

Other Uses of Whey Protein

The wide range of essential and non-essential amino acids, minerals, fats, and biologically active proteins in whey provide extensive application in clinical nutrition. Adequate protein intake is essential for post-surgical wound healing and protein depletion delays healing time.[131] Whey provides protein necessary for wound healing. Surgery stresses the body, altering natural defenses, leading to various post-surgical complications. Zimecki et al demonstrated lactoferrin regulated the immune response and provided protective measures in post-surgical patients.[132]

Human studies demonstrated whey protein improved cognitive function and coping ability in highly stressed individuals.[133,134] A rise in serotonin is thought to improve adaptation to stress,[135] and the authors proposed the tryptophan available in whey provides a substrate to increase brain serotonin levels. The treatment groups for both studies received alpha-lactalbumin-enriched whey protein because it has the highest tryptophan concentration of whey protein fractions.

Table 5 summarizes the clinical trials using whey.

Conclusion

Milk is one of the oldest functional foods available to mammals. From birth, mammals rely on mother's milk for nutrition and immune protection. Scientists are beginning to develop an understanding of the various components of milk, including whey, and how they may impact health and disease. Considerable positive research regarding whey and its biological components continues to be published. It is noteworthy some of the research has been funded by commercial dairy organizations, although a significant amount of clinical data from Asia was not supported by dairy-aligned interest groups.

To date, no severe adverse reactions have been noted following administration of whey protein products, although some patients note minor gastrointestinal disturbances. For individuals with frank milk allergies, whey products may not be suitable, although many individuals sensitive to dairy find that casein is the culprit and they can tolerate whey. Other dairy-sensitive individuals are lactose-intolerant. Most whey proteins are processed to remove lactose and finished whey products only contain trace amounts. De-lactosed whey, produced from crystallizing a majority of the lactose out and recovering the remaining whey, is appropriate for lactose-intolerant individuals.

Table 5. *Summary of Clinical Trials Using Whey Protein*

Condition	Dose of Whey Protein	Study Duration	Results
Cancer	30 g daily	6 months	2 of 5 patients had tumor regression Results suggested an increase in glutathione levels in healthy cells and decreased glutathione levels in cancer cells
	40 g daily Stage 4 malignancies Used other natural therapies.	6 months	16/20 survivors at 6 months Increased NK cell function Increased glutathione Increased hemoglobin and hematocrit Improved quality of life
Hepatitis B	12 g daily	12 weeks	Decreased serum lipid peroxidase levels Increased IL-2 and NK activity Decreased serum alanine transferase activity Increased plasma glutathione levels
HIV	45 g daily	2 weeks and 6 months	Increased glutathione levels in both trials
Cardiovascular Risk Factors	200 mL of fermented milk combined with liquid whey daily	8 weeks	Increased HDL Decreased triglycerides Decreased systolic BP Decreased total cholesterol
Exercise	1.2 g/kg body weight daily	12 weeks	Improved lean tissue mass Improvement in one of four muscle strength measurements
	10 g twice daily	3 months	Significant improvements in peak power Significant increase of 30-second work capacity Increased lymphocyte glutathione levels
Infant Formula	Standard Cow Formula vs Partially Hydrolyzed Whey Formula (doses varied)	12 weeks	Increased Bifidobacteria proportion Increased gastrointestinal immunity Decreased potential for developing atopic diseases
		1 week	Decreased incidence of infantile colic

A challenge test with a small portion of a particular whey supplement for an individual with dairy sensitivities would be indicated before beginning therapeutic amounts.

As researchers further investigate whey components it can be expected that new functional foods will develop. In addition to the isolated colostrum, lactoferrin, and alpha-lactalbumin products currently available, glycomacropeptide, lactoperoxidase, whey immunoglobulin, and bovine serum albumin isolated products may be investigated.

Currently the variety of whey proteins available allows for tailoring their use for specific clinical indications. Hydrolyzed whey provides readily available di- and tri-peptide fractions attractive to athletes and other individuals desiring a quickly absorbed, low allergenicity protein source. Undenatured whey provides the highest concentrations of intact native proteins such as lactoferrin and immunoglobulins for immune modulation. This is attractive to practitioners using whey as a functional food for immune-compromised patients and as an antimicrobial agent. Glycomacropeptide isolates do not contain the amino acids phenylalanine, tryptophan, or tyrosine, providing a valuable protein source for individuals with PKU.

Because of the wide variety of whey products, practitioners using whey could benefit from obtaining an analysis of whey proteins being recommended to patients. Helpful information for deciding whether a particular whey protein is appropriate for individuals includes total protein concentration and percent beta-lactoglobulin, alpha-lactalbumin, glycomacropeptides, immunoglobulins, bovine serum albumin, and lactoferrin. In addition, the presence of fat, lactose, and minerals should be considered.

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