

## Review

# Emerging Health Properties of Whey Proteins and Their Clinical Implications

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**Key words: whey proteins, bioactives, nutraceuticals,  $\beta$ -lactoglobulin,  $\alpha$ -lactalbumin, glycomacropeptide, lactoferrin, lactoperoxidase, growth factors, review**

The nursery rhyme “Little Miss Muffet sat on a tuffet (small stool) eating her curds and whey. . . .” is recognition of the fact that over the centuries “curds and whey”, the two major components of cow’s milk, have been widely accepted as part of a healthy diet. Milk provides complete nourishment for the neonate for six months from birth, containing factors that help develop various organ systems including the brain, immune system, and the intestine. Importantly it provides immune protection at a time when the neonates own immune system, though fully developed, is albeit immature. Many adult consumers include cow’s milk as part of a healthy diet as it provides protein and essential nutrients, vitamins, and minerals, in particular calcium for strong bones. There is a growing appreciation that milk, and in particular whey, contains components that not only provide nutrition, but can also prevent and attenuate disease, or augment conventional therapies, when delivered in amounts that exceed normal dietary intakes. This paper reviews the emerging health properties of whey proteins and their clinical implications.

### Key teaching points:

- The composition of milk, including the major and minor whey proteins.
- The health promoting properties of milk components, and their ability to augment conventional therapies.
- $\beta$ -Lactoglobulin can be employed to inhibit rather than promote allergy, and can inhibit carcinogenesis in animals.
- $\alpha$ -Lactalbumin protects against infection, directly kills cancer cells when complexed with oleic acid, improves morning alertness, and induces anxiolytic and rewarding effects in animals.
- Glycomacropeptide inhibits colitis, and enhances cognitive development when fed to animals.
- Lactoferrin is a chameleon that enhances immunity to prevent cancer, and yet suppresses immunity to block inflammatory disease; promotes bone growth, and has anti-microbial activity.
- Naturally occurring antibodies exist in milk which bind to cholesterol in the human digestive tract and prevent its absorption into the bloodstream.
- Whey proteins inhibit angiotensin-I-converting enzyme (ACE), and may have utility in the management of high blood pressure.
- Whey proteins are being commercialized or marketed to augment cancer treatments, fight inflammatory disease, heal wounds, promote bone repair, lower blood pressure and cholesterol, and treat acne.

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Paper was presented at the Institute of Food Technologists Annual Meeting, June 24–28, Orlando, Florida, 2006.

Disclosures: Support was received from LactoPharma, a joint venture between Fonterra and Auckland Uniservices, cofunded by the Foundation for Research, Science, and Technology. The presentation was sponsored by the National Dairy Council.

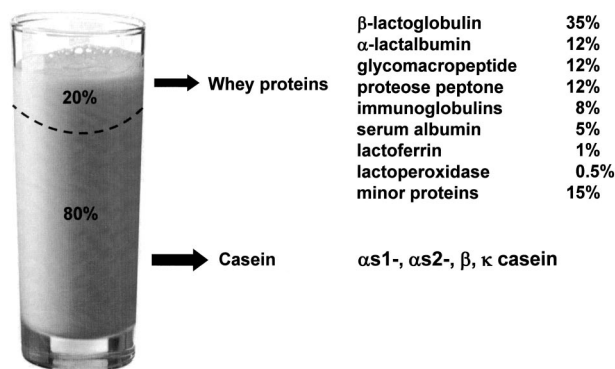
Journal of the American College of Nutrition, Vol. 26, No. 6, 713S–723S (2007)

Published by the American College of Nutrition

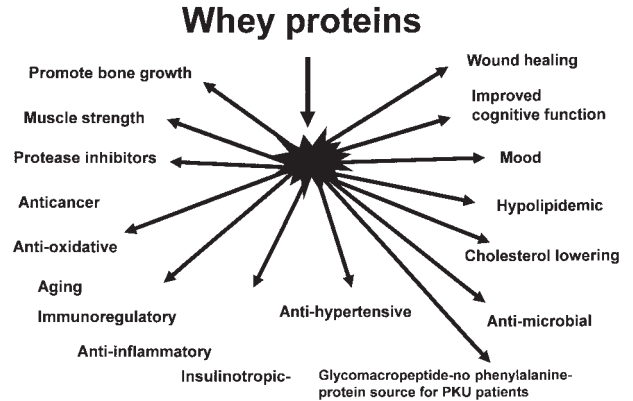
## INTRODUCTION AND BACKGROUND

Whey is separated from the curd during the cheese-making process. It is the liquid remaining after milk has been curdled and strained to remove the caseins (curds). It contains proteins, lactose, vitamins, minerals, and traces of fat. Whey protein, which represents 20% of the total protein content of milk, is sold as a nutritional supplement, and is particularly popular in the sport of bodybuilding. Whey contains only five major proteins, namely  $\beta$ -lactoglobulin,  $\alpha$ -lactalbumin, glycomacropeptide (depending on the method of whey manufacture), proteose peptone 3, immunoglobulins, and serum albumin, which together make up ~85% of whey protein (Fig. 1). All of the major milk proteins except for serum albumin and immunoglobulins are synthesized by epithelial cells in the mammary gland. In addition, both milk [1,2] and colostrum [3] contain literally hundreds of low abundance proteins, the most abundant being lactoferrin (Lf) and lactoperoxidase (LPO). There is increasing interest in milk, and particularly whey, as a potentially rich natural source of bioactive compounds to reduce disease risk and/or to prevent disease development [4], but the reality is that only the six or so most abundant whey proteins can be readily isolated from milk in amounts that meet commercial needs. There are a few exceptions, as discussed below, where a bioactive(s) is sufficiently potent that it only needs to be enriched within a medical food (food that is formulated to be consumed or administered enterally under the supervision of a physician, and that is intended for specific dietary management of a disease or condition) to exert effects that can change the course of disease. That said, science is finding new ways of harnessing the bioactivities of the major whey proteins.

Whey proteins have been reported to have utility in many different applications ranging from effects on bone, muscle, blood, brain, pancreas, immune, cancer, infection, metabolism, wound healing, learning, and aging (Fig. 2). A whey protein diet inhibited the development of dimethylhydrazine-induced malignancy in mice [5], enhanced liver and heart glutathione



**Fig. 1.** Approximate percent contributions of the major proteins in whey. Milk protein is comprised of approximately 20% whey and 80% casein. Illustrated is the percent contribution of each major whey component.



**Fig. 2.** Whey proteins exhibit a diverse array of functional properties that affect different biological processes and organ systems.

concentrations in aging mice and increased longevity [6], and increased biliary levels of secretory IgA in mice [7]. Milk whey protein, including its basic protein fraction (milk basic protein [MBP]), promotes bone formation and suppresses bone resorption in healthy adult woman and men [8]. Similarly, an acidic whey protein fraction supported the recovery of bone loss when fed to ovariectomised rats [9]. High amounts of lactalbumin in the diet increased the humoral response of mice, and splenic mitogen responses [10]. Whey is enriched in glutamine, which is a fuel for rapidly dividing cells and has been considered to be “conditionally-essential” during times of metabolic stress (eg as experience by endurance athletes) or illness. A whey protein concentrate inhibited the formation of ulcerative lesions in the stomach caused by ethanol ingestion [11], and subcutaneous administration of indomethacin [12]. Whey proteins have insulinotropic effects and reduce the postprandial glycemia in healthy subjects, and Type 2 diabetic patients [13]. A whey protein concentrate reduced the severity of rotavirus-induced diarrhea in a mice model [14].

While the main mineral binders or chelators of calcium are the caseins, whey proteins bind specific minerals including calcium, magnesium, zinc, iron, sodium and potassium, and are viewed as a new generation of super foods [15]. Here, the emerging health benefits of specific whey proteins are reviewed with an emphasis on their immunomodulatory properties.

## DESCRIPTION OF SUBJECT

### $\beta$ -Lactoglobulin

$\beta$ -Lactoglobulin, a member of the lipocalin family, is the most abundant protein in milk. The endogenous function of  $\beta$ -lactoglobulin, apart from being an important source of amino acids, is not known. It has been proposed to participate in the digestion of milk lipids in the neonate by enhancing the activity of pregastric lipase through removal of the fatty acids that

inhibit this enzyme [16]. It binds to retinol, triglyceride, and long-chain fatty acids and enhances their intestinal uptake in preruminant calves [17,18]. It is the major allergen in cow's milk, responsible for causing milk allergy.  $\beta$ -lactoglobulin has been conjugated with acidic oligosaccharides to reduce its antigenicity. Immunization of mice with the conjugates led to a reduced T cell response that became predominantly Th1-mediated, suggesting the conjugates may actually have utility in preventing Th2-mediated allergy [19]. Oral administration of recombinant *Lactococcus lactis* expressing bovine  $\beta$ -lactoglobulin induced a specific Th1 response, suggesting probiotics expressing  $\beta$ -lactoglobulin could be useful in the management of food allergy [20]. Intranasal coadministration of live lactococci expressing IL-12 and  $\beta$ -lactoglobulin produced a protective Th1 response that inhibited allergic airway disease in mice [21]. Acidic  $\beta$ -lactoglobulin-derived peptides hydrolyzed with *Lactobacillus paracasei* peptidases repressed lymphocyte stimulation, upregulated IL-10 production, and downregulated IFN- $\gamma$  and IL-4 secretion [22]. The above findings provide evidence that not only can milk's major allergen be rendered non-allergenic, it can also be modified or administered in a fashion that paradoxically inhibits rather than stimulates the allergic process.

Peptides Tyr-Leu (f102–103) and Ala-Leu-Pro-Met-His-Ile-Arg (f142–148) derived from  $\beta$ -lactoglobulin, termed lactokinin, are inhibitors of angiotensin-I-converting enzyme (ACE) [reviewed in 23,24], and represent potential nutraceuticals/functional food ingredients for the prevention and/or treatment of high blood pressure.  $\beta$ -lactorphin (f102–105), another peptide derived from  $\beta$ -lactoglobulin with ACE-inhibitory activity, improved vascular relaxation in spontaneously hypertensive rats, and is also an opioid receptor agonist suggesting it can modulate absorption processes in the intestinal tract [25]. Conversely,  $\beta$ -lactotensin (His-Ile-Arg-Leu) (f146–149) is an ileum-contracting peptide derived from  $\beta$ -lactoglobulin, which exhibits hypertensive activity. It is a natural ligand for neurotensin NT2 receptors, has an anti-stress effect, promotes the abolition of fear memory [26], reduces sensitivity to painful stimuli [27], and consolidates memory [28]. It is able to reduce blood cholesterol levels, but only when given parenterally, and not orally [29]. In terms of cancer,  $\beta$ -lactoglobulin reduced dimethylhydrazine-induced colonic aberrant crypt foci formation in rats [30].

### $\alpha$ -Lactalbumin

Human  $\alpha$ -lactalbumin inhibits the proliferation of certain stromal cell types, including mammary epithelial cells and normal rat kidney cells in culture, but not fibroblasts [31].  $\alpha$ -lactalbumin preparations from several mammalian species were all found to be growth inhibitory for cultured mammary epithelial cells, raising the suggestion that  $\alpha$ -lactalbumin could act as a feed-back inhibitor of mammary cell growth during lactation, and perhaps of other cell types as well.

$\alpha$ -Lactalbumin is an immunostimulator. It stimulates the production of IL-1 $\beta$  by ovine bronchoalveolar lavage macrophages in culture [32], and lactoimmunopeptides Tyr-Gly (f50–51, f18–19) and Tyr-Gly-Gly (f18–20) from the N-terminus of  $\alpha$ -lactalbumin stimulate the proliferation of human blood lymphocytes in culture [33]. There is redundancy here as milk  $\kappa$ -casein generates Tyr-Gly (f38–39) as a caseinopeptide. Gly-Leu-Phe (f51–53), an immunostimulating peptide derived from bovine  $\alpha$ -lactalbumin, binds to specific sites on human neutrophils and monocytes [34], stimulates superoxide anion production by neutrophils [35], and human monocyte-macrophage adherence and phagocytosis of human senescent red blood cells [36]. It protects mice against *Klebsiella pneumoniae* infection [37]. It was argued that Gly-Leu-Phe released after digestion of milk peptides may play a physiological role in promoting the development of immature polymorphonuclear cells in the intestines of neonates and adults. Several  $\alpha$ -lactalbumin-derived peptides including  $\alpha$ -lactorphin (Tyr-Gly-Leu-Phe) (f50–53), and Tyr-Gly have ACE-inhibitory activity and can lower blood pressure in spontaneously hypertensive rats [25].  $\alpha$ -lactorphin is also an opioid agonist [reviewed in 23].

In terms of cancer,  $\alpha$ -lactalbumin inhibits the growth of human colon adenocarcinoma cell lines in culture [38]. HAMLET (human  $\alpha$ -lactalbumin made lethal to tumor cells) is a protein-lipid complex of  $\alpha$ -lactalbumin and oleic acid that induces apoptosis-like death in tumor cells, but leaves fully differentiated cells unaffected [39]. It reduced progression of human glioblastoma xenografts when infused into the rat brain [40], and inhibited the growth of skin papillomas in humans when topically applied [41].

$\alpha$ -lactalbumin is a protein source that is particularly rich in tryptophan. Evening intake of  $\alpha$ -lactalbumin by human volunteers increased plasma tryptophan availability and improved morning alertness and brain measures of attention [42]. In rats,  $\alpha$ -lactalbumin ingestion enhanced serotonin release and induced anxiolytic and rewarding effects, suggesting it has beneficial effects on mood [43].  $\alpha$ -lactalbumin chelates heavy metals, hence reduces oxidative stress, and when orally administered protects against ethanol- and stress-induced gastric mucosal injury in rats, suggesting it may have utility as an agent to prevent ulcers [44]. When intraperitoneally injected, it prevented alopecia induced by the anticancer agent etoposide in a neonatal rat model [45].  $\alpha$ -lactalbumin appears to be effective in inhibiting associations of the pathogens enteropathogenic *E. Coli*, *Salmonella typhimurium*, and *Shigella flexneri* with intestinal cells [46].

### Glycomacropeptide

Glycomacropeptide (GMP) is present in whey due to the action of chymosin on casein during the cheesemaking process. It is high in branched chain amino acids and lacks the aromatic amino acids phenylalanine, tryptophan, and tyrosine. A lack of phenylalanine makes it one of the few naturally occurring

proteins that can be safely ingested by individuals with phenylketonuria (PKU). GMP has an inhibitory effect on acid gastric secretions, and modifies the blood concentration of regulatory digestive peptides [47]. It has been proposed to induce satiety, due to the fact that it induces release of cholecystokinin [48,49], but this notion was not realised in humans fed GMP [50].

GMP exerts both immunosuppressive and immunostimulatory properties. Thus, it inhibits mouse lymphocyte (T and B) proliferation in a sialic acid-dependent fashion [51]. It stimulates monocytes to upregulate the anti-inflammatory factor IL-1 receptor antagonist (IL-1RA) [52]. In accord with its *in vitro* properties, it attenuated trinitrobenzenesulfonic acid-induced colitis in rats with an efficacy comparable to that of sulfasalazine, an established drug used in the treatment of inflammatory bowel disease [53]. Conversely, Snow Brand Milk Products was granted a patent on the use of bovine GMP for accelerating human B lymphocyte growth [Snow Brand Milk Products Co (1996) Human normal B lymphocyte accelerating agent. Japanese Patent, 96018997]. Bovine GMP significantly enhanced the proliferation and phagocytic activities of human macrophage cell lines in culture in a sialic acid-dependent fashion [54].

GMP supplementation increases zinc absorption when fed to infant rhesus monkeys [55], and is a potentially promising agent for preventing intestinal infections [reviewed in 56]. Thus, GMP binds cholera and *Escherichia coli* enterotoxins and inhibits bacterial and viral adhesion [57]. Feeding GMP as a protein-bound source of sialic acid to piglets during early development enhanced learning, suggesting sialic acid in mammalian milks could play a role in cognitive development [58].

### Proteose Peptone 3

The proteose peptone fraction of milk has been defined as those proteins that remain in solution after milk has been heated at 95°C for 20 minutes and then acidified to pH 4.7. There are four major components that comprise proteose peptone, with proteose-peptone component 3 (PP3) fragment being the major component at 25% by weight [59]. PP3 is found only in whey (but not that of humans), and is produced by the fermentation of fat-free bovine milk. It enhances monoclonal antibody production by human hybridoma cells [60]. Lactophorin, a synthetic 23 amino acid residue fragment of bovine PP3, inhibits the growth of both Gram positive and negative bacteria [61].

### Immunoglobulins

Milk immunoglobulins normally provide passive immunity for the neonate, but they are also potentially powerful agents that could be incorporated into diets to remove toxic, or undesirable dietary factors. Origo Biosciences researchers have isolated a naturally occurring antibody in milk which binds to cholesterol in the human digestive tract and prevents its absorption into the bloodstream (<http://www.origobiosciences.com>).

They aim to introduce this anti-cholesterol antibody, which appears to have greater efficacy than phytosterols and azetidinone-based cholesterol absorption inhibitors, into the functional food market as a food supplement.

The concentration of colostrum whey antibodies against a particular pathogen can be raised by immunising cows with the pathogen or its antigens. The hyperimmune whey that results can potentially provide prophylactic protection against various infectious gut microbes including rotavirus and *Helicobacter pylori* [reviewed in 62]. Microactin, a milk protein concentrate of hyperimmune milk from Stolle Milk Biologics, inhibits joint inflammation in osteoarthritis patients [63].

### Bovine Serum Albumin

Bovine serum albumin (BSA) is a source of essential amino acids, whose therapeutic potential is largely unexplored. It inhibited the growth of the estrogen-responsive breast cancer cell line MCF-7 in culture [64], and 4-nitroquinoline-1-oxide-induced genotoxicity [65]. Serorphin (Tyr-Gly-Phe-Gln-Asn-Ala) (f399–404) is a BSA-derived peptide with opioid agonist activity [reviewed in 23]. Another BSA peptide albutensin A or serokinin (Ala-Leu-Lys-Ala-Trp-Ser-Val-Ala-Arg) (f208–216) is an ACE inhibitor, and is reported to have ileum contracting and relaxing activities [reviewed in 23]. Albutensin A (Ala-Phe-Lys-Ala-Trp-Ala-Val-Ala-Arg) from human serum albumin dose-dependently decreased food intake after intracerebroventricular or peripheral administration in fasted mice via a mechanism involving the C3a receptor [66].

## LOW ABUNDANCE PROTEINS

Here, low abundance proteins are meant to include those proteins representing 1% or less of the protein content of whey. The amounts of Lf (~0.2 mg/ml) and LPO (~0.03 mg/ml) in milk are close to the limit in terms of the recovery of whey proteins for sale as nutraceuticals or food supplements. Growth factors in trace amounts such as TGF- $\beta$  (4 ng/ml) [67] remain commercially viable as they can be extracted as enriched fractions, and still retain substantial activity. Bovine colostrum is potentially a rich source of low abundance proteins, containing higher amounts of immunoglobulins, growth factors, cytokines, and nucleosides than are found in milk [68].

### Lactoferrin

Lactoferrin (Lf), a single-chain iron-binding glycoprotein, is arguably the most valuable biomedical protein present in whey, due to the numerous and diverse array of therapeutic properties it exhibits. Lf is a natural defence protein present in most secretions commonly exposed to normal flora including milk, colostrum, tears, nasal secretions, saliva, bile, pancreatic juice, intestinal mucus, and genital secretions [69]. It is secreted by neutrophils and present at high levels at sites of bacterial

infection. It is a multifunctional protein that may regulate iron absorption in the intestine, promotes intestinal cell growth, protects against microbial infection, regulates myelopoiesis, and regulates systemic immune responses. It is a chameleon in that it can enhance immunity to prevent cancer, and yet suppress immunity to block inflammatory disease.

Lf when topically applied is a potent regulator of bone cell activity and increases bone formation [70]. It stimulates osteoblast cell growth, and decreases osteoclastogenesis. When administered over the calvariae of adult mice it stimulated bone formation, and may have application in bone repair, and bone prosthetics technology.

Lf has marked effects on immune cells in culture, being an immunostimulator and immunoregulator. Both bovine and human Lf inhibit the proliferative response and cytokine production of Th1, but not Th2 cell lines [71]. Human Lf differentiates mouse B cells to enable them to better present antigen to T cells *in vitro* [72]. Lf increases the phagocytic activity of bovine blood monocytes [73], and lactoferrin B (FKCRRWQWRMKKLGAPSITCVRRAF) (f17–41) obtained by hydrolysis of Lf with pepsin promotes phagocytosis by neutrophils [74] and stimulates the release of IL-8 [75]. Lf treatment of sheep neutrophils was found to have a dose-dependent enhancing effect on superoxide production [76]. Lf is directly tumoricidal in that pepsin-digested bovine Lf induces apoptotic cell death of oral cancer cells [77], and bovine lactoferrin selectively induces apoptosis in human leukemia and carcinoma cell lines [78].

**Oral Ingestion of Bovine Lf Stimulates the Immune System of the Gut, and Enhances and Reconstitutes the Peripheral Immune System.** When ingested, Lf increases intestinal expression of NOD2, IFN- $\beta$ , and IL-12p40 in healthy mice [79]. It increased the output of neutrophil precursors and attenuated the spontaneous production of TNF- $\alpha$  and IL-6 by peripheral blood cells of human volunteers [80,81]. It enhanced the production of superoxide anion and nitric oxide by peritoneal macrophages in *Candida* treated mice, and IL-12 and IFN- $\gamma$  production [82]. Lf attenuates immobilization-stress-induced suppression of cellular and humoral immune responses in mice [83].

**Oral Ingestion of Lf Exerts Anti-Cancer Activity.** When ingested, bovine Lf suppressed the development of tumors in the colon, lung, and oesophagus of rats exposed to chemical carcinogens [84]. It inhibited colon carcinoma 26 lung metastasis in mice [85]. There are over 30 other studies demonstrating that orally ingested bovine Lf reduces tumor growth. In addition to effects on carcinogenesis and tumor growth, it accelerates reconstitution of the humoral and cellular immune response during chemotherapy-induced immunosuppression in mice [86–89]. Oral ingestion of Lf enhanced the production of IL-18 in intestinal epithelial cells, and IFN- $\gamma$  in the lamina propria, and increased the number of CD4+, CD8+, and NK cells in the intestinal mucosa of tumor-bearing mice [90,91]. Oral ingestion of bovine Lf that has been saturated with iron,

designated Lf+, appears to display enhanced anti-tumor activity in combination with chemotherapy, the combination being capable of completely eradicating tumors that are otherwise completely insensitive to chemotherapy [WO 2006/054908]. Oral ingestion of recombinant human Lf inhibited the growth of small mouse mammary and squamous carcinoma tumors established in immunocompetent mice [92]. It synergized with cis-platinum and radiotherapy to inhibit tumor growth, and increased the expression of IL-18 in the intestine, boosted spleen NK cell activity, and increased the number of CD8+ T cells in the blood.

**Lf Attenuates Inflammatory Disease.** Lf protects against lethal endotoxin shock in germ-free piglets as it blocks the binding of LPS to monocytes [93], and has myelosuppressive effects in mice when injected intravenously [94]. Human Lf injected into the joints of mice suppressed inflammation in autoimmune and infectious arthritis [95], and oral treatment of rats with bovine Lf inhibited carrageenan-induced joint inflammation, which correlated with a slight decrease in the ability of splenocytes to produce proinflammatory cytokines [96]. Topical Lf inhibits allergen-induced Langerhan's cell migration and cutaneous inflammation in humans [97]. It reduces obstructive jaundice-induced injury to the liver and normalizes inflammatory responses of jaundiced rats [98]. Oral administration of human Lf attenuated inflammation in the dextran-sulfate-induced model of colitis [99]. Lf is a protease inhibitor which inhibits trypsin, and cysteine proteases. Aerosolized Lf inhibited airway hypersensitivity in sheep asthma model [100]. Orally ingested and inhaled human Lf reduced asthmatic symptoms in primates (www.agenix.com) [101].

**Lf Exerts Anti-Microbial Immunity.** Oral ingestion of Lf protected mice against cytomegalovirus infection by T-cell-dependent augmentation of NK cell activity [102]. Oral administration of Lf (or LPO) has the potential to attenuate pneumonia, as evidenced by its ability to affect pneumonia in influenza-virus-infected mice through the suppression of infiltration of inflammatory cells in the lung [103]. Lf inhibits enterovirus 71 infection by binding to VP1 protein and host cells [104]. Bovine Lf prevents dendritic cell-mediated human immunodeficiency virus type 1 transmission by blocking the DC-SIGN-gp120 interaction, and is capable of blocking HIV-1 replication in T cells [105]. Orally administered bovine Lf inhibits hepatitis C virus viremia in chronic hepatitis C patients with high viral loads [106]. It enhances systemic or peripheral immune responses to other pathogens, partly due to increased killing by macrophages [107]. Oral ingestion of Lf upregulates immune responses in post-surgical patients, suggesting it could protect against post-surgical complications [108].

## Lactoperoxidase

The principal utility of lactoperoxidase (LPO) is as a protective factor against infectious microbes. Oral administration of LPO attenuated pneumonia in influenza virus-infected mice

by suppressing the infiltration of inflammatory cells in the lung [109]. LPO treatment of sheep neutrophils was found to have an enhancing, dose-dependent, effect on superoxide production [76].

### Natural Growth Factors in Whey

Mitogenic bovine whey extract (MBWE), which contains six known growth factors (TGF- $\beta$ , IGF-I and -II, PDGF, and FGF-1 and -2) promoted healing of incisional wounds in rats [110]. TGF- $\beta$  is present in milk in relatively low concentrations (1–4 mg/L), yet it can be enriched as in the oral supplement Modulen, which inhibits Crohn's disease in humans [111–113], and methotrexate-induced mucositis in rats [114].

## WHEY PROTEINS IN ADJUNCT THERAPY

Whey proteins hold promise in adjunct therapies for the treatment and/or prevention of cancer and infection. Immunocal, a whey protein concentrate, may have potential as an adjuvant in cancer treatments, as it enhanced the cytotoxicity of baicalein against the HepG2 cell line [115]. As mentioned above, orally ingested bovine and human Lfs augment cancer chemotherapy. Orally ingested iron-saturated Lf (Lf+) also augmented immunotherapy of cancer, including dendritic cell therapy [WO 2006/054908]. A greater than additive increase in DNA fragmentation was seen when lactoferrin B was used in combination with tamoxifen against a breast cancer cell line, suggesting that combination therapy with lactoferrin B and tamoxifen warrants investigation as a treatment for breast cancer [116]. As regards infection, feeding of encapsulated Lf in conjunction with triple antibiotic therapy, completely eradicated *H. Pylori* infection, whereas antibiotic therapy alone was not as effective [117]. Lf and lactoferrin inhibit Herpes simplex 1 and 2 infection *in vitro*, and exhibited synergy when combined with acyclovir [118]. It was proposed that Lf supplementation of the diet be combined with interferon therapy for the treatment of patients infected with hepatitis B or C [119]. Gargling with an Lf solution augmented the efficacy of erythromycin against chronic pharyngitis in children tested positive for Group A Streptococci [120]. Intramammary infusion of Lf in combination with penicillin was three to five times more effective in combatting beta-lactam resistant microorganisms than penicillin alone in the treatment of mastitis in cows [121]. Lf displayed synergy *in vitro* with cefpodoxime proxetil against a panel of bacteria [122]. In summary, whey proteins, in particular Lf, show strong promise for the augmentation of anti-cancer and anti-infection therapies. Feeding of whey protein has no known side-effects, and Lf has recently been notified as GRAS (Generally Regarded as Safe).

## COMMERCIALIZATION OF WHEY PROTEINS

Whey proteins have been commercialized for the treatment of a diverse array of diseases or conditions. Immunocal (<http://www.immunocal.com>), a whey protein concentrate from Immunotec Research, is marketed for the treatment of glutathione deficiency, high oxidative stress, and immune deficiency. It also appears to deplete tumor cells of GSH and render them more vulnerable to chemotherapy [123]. It increases glutathione concentrations in relevant tissues which may combat oxidant-driven tumor growth. Whey protein itself is particularly rich in substrates for GSH synthesis and may exert effects on carcinogenesis by enhancing GSH concentration [reviewed in 124]. Immunocal enhances immunity and increases plasma glutathione concentrations in individuals infected with hepatitis B [125]. Nestle market Modulen ([www.modulenibd.com](http://www.modulenibd.com)) enriched with milk TGF- $\beta$  for the treatment of Crohn's disease. Agennix (<http://www.agennix.com>) is attempting to commercialize talactoferrin, a human recombinant form of Lf for the treatment of cancer, asthma, wounds, and ulcers. In phase I/II human trials 40% of patients with progressive advanced solid tumors, who had failed conventional chemotherapy, showed stable disease, and tumors were reduced in ~12% of patients [126]. Talactoferrin has been fast-tracked by the Food and Drug Administration (FDA) for the clinical development of a first-line non-small cell lung cancer treatment [127]. The FDA has also granted Orphan Drug designation to talactoferrin for the treatment of renal cell carcinoma. Topical talactoferrin improved the healing of diabetic neuropathic ulcers in a phase I/II clinical trial [128]. LactoPharma (<http://www.lactopharma.com>), a joint venture with Fonterra, is aiming to commercialize Lf+, an iron-saturated form of bovine Lf, to augment cancer chemotherapy. It is also commercializing Lf as an agent to promote bone repair. TGR BioSciences (<http://www.tgr-biosciences.com.au>) is developing the milk extract Lacterm<sup>TM</sup> to alleviate oral mucositis, a debilitating side effect of cancer chemotherapy and radiotherapy. Stolle Milk Biologics ([www.smbimilk.com](http://www.smbimilk.com)) markets Microlactin, a beverage, to alleviate the symptoms and dysfunction associated with osteoarthritis [129]. Origo Biosciences (<http://www.origobiosciences.com>) aim to introduce a whey anti-cholesterol antibody as a food supplement. Davisco Foods International Inc. (<http://www.daviscofoods.com>) market BioZate 1, a hydrolyzed whey protein rich in bioactive peptides, for lowering blood pressure and cholesterol [130]. Ventria Biosciences (<http://www.ventria.com>) have developed an oral rehydration solution containing recombinant human lactoferrin and lysozyme, both produced in rice, which helps children recover faster from diarrhea [131]. DMV (<http://www.campina.com>) market Praventin containing lactoferrin and active whey protein fractions, which when ingested can help reduce acne.

## CONCLUSION

Our appreciation of milk has grown substantially from a time when it was seen purely as an excellent source of protein and calcium. There have been many investigations of the effects of milk proteins on cells in culture, but whether the results are relevant for oral feeding outcomes remains to be established in many cases. Bovine milk proteins due to their foreignness are unlikely to be delivered by routes other than by ingestion or topical application, and even the latter route raises concerns over the possibility of stimulating allergic responses. There is evidence that some whey protein peptides are absorbed directly into the bloodstream, but largely only in young animals having immature and leaky intestines. However, some milk whey peptides such as GMP are apparently absorbed from the intestine into the plasma [132]. Progress in the commercial exploitation of whey proteins for health and medicine is dependent upon identifying new indications to treat using the existing whey bioactives, finding new commercially viable technologies aimed at fractionating and isolating minor proteins with potent biological activity, and modifying the major proteins to either enhance their activities or acquire new functions. HAMLET, Lf+, and oligosaccharide-conjugated  $\alpha$ -lactalbumin are all examples of the latter. One of the most promising applications of whey proteins is in the augmentation of conventional therapies, and while Lf-mediated augmentation of therapies to fight cancer and infection is showing great promise, it is likely that other whey protein augmented preventions and/or treatments will emerge. Therapeutic minor milk proteins may have to be commercialized using recombinant technologies to synthesize the human equivalents. Progress will also come from a more thorough investigation of the activities and functions of whey peptides derived from hydrolyzing the major milk proteins, including their fermentation with proteolytic enzymes. In this respect, studies of whey peptides have lagged behind those of the caseinopeptides. The industrial-scale production of such peptides is limited by a lack of suitable technologies, however as with the minor proteins, products enriched with specific fractions of whey protein hydrolysates still remains a potentially viable approach. One of the major challenges is to determine how to best formulate milk bioactives as food supplements so that they can withstand the harsh environment of the gastrointestinal tract. Another challenge will be to ensure that their effects are not neutralized by antagonistic bioactives simultaneously ingested in foodstuffs. In this respect, the effects of Lf, LPO and  $\alpha$ -lactalbumin may be eliminated by their combination in whey or by other minor components of whey [32]. It can be expected that dairy food scientists will be gainfully employed for quite some while.

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*Received September 17, 2007*