# Effects of milk-derived bioactives: an overview

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Milk contains various components with physiological functionality. Peptides derived from caseins and whey proteins including opioid peptides, antihypertensive peptides, casein phosphopeptides, α- and β-lactorphins and albutensin have been shown to possess various bioactive properties. This review considers an overview of the bioactive components in milk proteins and whey and their physiological function.

**Casein-based bioactive peptides: Casein phosphopeptides: Whey-based bioactive peptides: Opioid: Angiotensin I converting enzyme: α-Lactorphin: β-Lactorphin**

## Introduction

The benefit of milk in preventing infection has been recognised for thousands of years. Much of this activity has been attributed to antibodies, but the role of other minor proteins such as lactoferrin and lactoperoxidase and of complex sugars in milk as bioactive agents is only now being recognised. Milk is a complete food for newborn mammals. It is the sole food during the early stages of rapid development. Milk contains approximately 5 % lactose, 3·2 % protein, 4 % lipid and 0·7 % mineral salts. The nutritional value of milk and milk products is due to these constituents.

Milk contains various components with physiological functionality. Milk contains high levels of immunoglobulins and other physiologically active compounds for warding off infection in the newborn. Similarly, colostrum is important for newly born mammals as it provides necessary immunity against infections. This review provides an overview of the bioactive components in milk and colostrum, their physiological functions and established and potential health implications and benefits.

## Physiological role of casein-based bioactive substances

Casein is the main protein component of milk constituting about 80 % of the total milk protein fraction. The concentration of casein, whey proteins and minor whey proteins in milk is shown in Table 1. Although, neither casein nor individual casein fractions have any established physiological role, peptides derived from casein have been shown to possess various bioactive properties. These bioactive peptides are hidden in an active state inside the polypeptide chain of caseins. Bovine casein consists of four components: $\alpha_{s1}$-, $\alpha_{s2}$-, β- and κ-caseins (Walstra & Jenness, 1984; Maubois & Leonil, 1989). On the other hand, human casein consists of primarily β- and κ-caseins. Bioactive peptides are produced by *in vitro* and *in vivo* enzymatic proteolysis of bovine or human casein and whey proteins as reviewed by several workers (Maubois & Leonil, 1989; Yamauchi, 1992; Schlimme & Meisel, 1993; Tirelli *et al.* 1997; Jelen & Lutz, 1998).

A brief outline of bioactive peptides, their precursors and possible bioactive roles is shown in Table 2. Opioid peptides are those having pharmacological similarities to opium (morphine) and are derived from casein called casomorphins. The major opioid peptides as shown in Tables 2 and 3 derived from bovine milk are fragments of β-casein (Meisel & Schlimme, 1990). The opioid peptides fragments of β-casein are called β-casomorphins due to their morphine-like behaviour. All κ-casein fragments known as casoxins, behave as opioid antagonists. Further opioid peptides are two $\alpha_{s1}$-casein exorphins (α-casomorphins) which were found in pepsin hydrolysates of α-casein (Fiat *et al.* 1993). A tetrapeptide amide, morphiceptin (Table 3) is the most active opioid agonist in the bovine β-casomorphin group. Two opioid antagonists, casoxin C and D also belong to this group (Loukas *et al.* 1983). All bioactive peptides derived from bovine α-casein behave as opioid antagonists. Several immunomodulating peptides resulting from casein have been detected including immunopeptides from $\alpha_s$-casein and β-casein. Casein phosphopeptides (CPPs) can be released *in vitro* and *in vivo* by gastrointestinal trypsin from $\alpha_{s1}$-, $\alpha_{s2}$- or β-caseins (Naito *et al.* 1972; Kitts & Yuan, 1992; Tirelli *et al.* 1997).

Some peptides show antihypertensive activity (Maruyama & Suzuki 1982; Maruyama *et al.* 1985). These peptides referred to casokinins (Table 4).

The angiotensin I converting enzyme (ACE) hydrolyses largely inactive angiotension I to the octapeptide angiotenson II, which increases blood pressure. This enzyme also hydrolyses bradykinin, which is a hypotensive. Thus ACE

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**Table 1.** Concentration and biological functions of major milk proteins

| Protein | Concentration (g/l) Cow | Human | Function |
|---|---|---|---|
| Total caseins | 26·0 | 2·7 | Ion carrier (Ca, $PO_4$, Fe, Zn, Cu) Precursors of bioactive peptides |
| α-Casein | 13·0 | | |
| β-Casein | 9·3 | | |
| κ-Casein | 3·3 | | |
| Total whey protein | 6·3 | 67·3 | |
| β-Lactoglobulin | 3·2 | | Retinol carrier, binding fatty acids, possible antioxidant |
| α-Lactalbumin | 1·2 | 1·9 | Lactose- synthesis in mammary gland, Ca carrier, immunomodulation, anticarcinogenic |
| Immunoglobulins (A, M, and G) | 0·7 | 1·3 | Immune protection |
| Serum albumin | 0·4 | 0·4 | |
| Lactoferrin | 0·1 | 1·5 | Antimicrobial, antioxidative, immunomodulation, iron absorption, anticarcinogenic |
| Lactoperoxidase | 0·03 | | Antimicrobial |
| Lysozyme | 0·0004 | 0·1 | Antimicrobial, synergistic effect with immunoglobulins and lactoferrin |
| Miscellaneous | 0·8 | 1·1 | |
| Proteose–peptone | 1·2 | | Not characterised |
| Glycomacropeptide | 1·2 | | Antiviral, bifidogenic |

Adapted from Walstra & Jenness (1984), Yamauchi (1992), Korhonen *et al.* (1998).

inhibitors are antihypertensive peptides. Other antihypersensitive peptides are located in the primary sequence of bovine β-lactoglobulin (β-lactorphins) and human β- and κ-casein (Mullally *et al.* 1996).

The κ-casein fragment named casopiastrin obtained from tryptic hydrolysates, shows antithrombotic activity by inhibiting fibrinogen binding on platelets (Fiat *et al.* 1993).

Opioid peptides released from casein during digestion showed gastrointestinal motility (Daniel *et al.* 1990). Some of these peptides have been found to affect gastrointestinal transit time. Casomorphins have been found to prolong gastrointestinal transit time and to exert antidiarrhoeal action. The effect of β-casomorphins on the motility of rat intestinal tract was studied using the non-absorbable marker $^{141}$Ce.

The opioid peptides have shown morphin-like activity. When opioid peptides are injected into the bloodstream they induce an analgesic and sedative effect due to their action on the nervous system. α-Lactorphin has been shown to exert a weak opioid activity to smooth muscles. β-Lactorphin has been shown to have a smooth muscle-contracting effect (Antila *et al.* 1991). Teschemacher (1987) and Paroli (1988) also reported analgesic and sedative effects of opioid peptides when injected in the bloodstream.

Studies have suggested that opioid agonist and opioid antagonists are formed in the gut as a result of *in vivo* hydrolysis of milk casein. β-Casomorphins have been detected in the plasma of pregnant or lactating women (Bicknell, 1985; Yen *et al.* 1985). Similarly, casomorphins have been detected in the duodenal chyme of minipigs and in the human small intestine (Svedberg *et al.* 1985) as a result of *in vivo* digestion.

*In vitro* activity of immunomodulating peptides resulting from tryptic and chymotryptic hydrolysates of $\alpha_{s1}$- and β-casein has been reported to stimulate the macrophage activity against red blood cells (Parker *et al.* 1984). Casein-derived immunopeptides have been shown to stimulate the phagocytic activities of murine and human macrophages and to protect against *Klebsiella pneumoniae* infection in mice. The peptides may stimulate the proliferation and maturation of T cells and natural killer cells for the defence of the newborn against a large number of bacteria, particularly enteric bacteria. Injection of casein or α-lactalbumin peptides has been found to have direct immunomodulating activity against *Klebsiella pneumoniae* in rats (Migliore-Samour *et al.* 1989). Lahov & Regelson (1996) have reported antibacterial activity of isracidin, the 1–23 fragment of $\alpha_{s1}$-casein obtained from the action of chymosin, against *Staphylococcus aureus* and *Candida*

**Table 2.** Bioactive peptides derived from bovine milk proteins

| Bioactive peptide | Protein precursor | Bioactivity |
|---|---|---|
| Casomorphins | α-, β-Casein | Opioid agonists |
| Casokinins | α-, β-Casein | Antihypertensive |
| Casoxins | κ-Casein | Opioid antagonists |
| Casoplatelins | κ-Casein, transferrin | Antithrombotic |
| α-Lactorphin | α-Lactalbumin | Opioid agonist |
| β-Lactorphin | β-Lactoglobulin | Opioid agonist |
| Lactoferroxins | Lactoferrin | Opioid antagonists |
| Immunopeptides | α-, β-Casein | Immunostimulants |
| Caseinophosphopeptides | α-, β-Casein | Mineral carriers |

Adapted from: Meisel & Schlimme (1990); Schlimme & Meisel (1993).

**Table 3.** Bioactive peptides derived from milk protein components: opioid peptides

| Peptides | | Origin | Structure |
|---|---|---|---|
| Agonist | | | |
| β-Casomorphin 5 | | β-CN | Tyr-Pro-Phe-Pro-Gly |
| β-Casomorphin 5 | h | β-CN | Tyr-Pro-Phe-Val-Glu |
| Morphiceptin | | β-CN | Tyr-Pro-Phe-Pro-$NH_2$ |
| α-Casein exorphin | | $\alpha_{s1}$-CN | Arg-Gly-Phe-Gln-Asn-Ala |
| Antagonist | | | |
| Casoxin 4 | | κ-CN | Tyr-Pro-Ser-Tyr (O-$CH_3$) |
| Casoxin A | | κ-CN | Tyr-Pro-Ser-Tyr-Gly-Leu-Asn-Tyr |
| Casoxin B | h | κ-CN | Tyr-Pro-Tyr-Tyr (O-$CH_3$) |
| Casoxin C | | κ-CN | Tyr-Ile-Pro-Ile-Gln-Tyr-Val-Leu-Ser-Arg |
| Casoxin D | h | $\alpha_{s1}$-CN | Tyr-Val-Pro-Phe-Pro-Pro-Phe |
| Lactoferroxin A | | LF | Tyr-Leu-Gly-Ser-Gly-Tyr (-$OCH_3$) |

h, human.
Source: Yamauchi (1992).

**Table 4.** Bioactive peptides derived from milk protein components: other groups

| Peptides | | Origin | Structure |
|---|---|---|---|
| Peptides acting on smooth muscles | | | |
| Albutensin A | | SA | Ala-Leu-Lys-Ala-Trp-Ser-Val-Ala-Arg |
| β-Lactotensin | | β-LG | His-Ile-Arg-Leu |
| Casoxin C | | κ-CN | Tyr-Ile-Pro-Ile-Gln-Tyr-Val-Leu-Ser-Arg |
| Casoxin D | h | $\alpha_{s1}$-CN | Tyr-Val-Pro-Phe-Pro-Pro-Phe |
| Angiotensin-converting enzyme inhibitor (ACEI) | | $\alpha_{s1}$-CN | Phe-Phe-Val-Ala-Pro-Phe-Pro-Glu-Val-Phe-Gly-Lys |
| | | $\alpha_{s1}$-CN | Thr-Thr-Met-Pro-Leu-Trp |
| | | β-CN | Ala-Val-Pro-Try-Pro-Gln-Arg |
| | h | β-CN | Ser-Phe-Gln-Pro-Gln-Pro-Leu-Ile-Tyr-Pro |
| | | β-CN | Val-Val-Pro-Tyr-Pro-Gln-Arg |
| | | β-CN | Pro-Thr-His-Ile-Lys-Trp-Gly-Asp |
| Casoxin A | | κ-CN | Tyr-Pro-Ser-Tyr-Gly-Leu-Asn-Tyr |
| Casoxin C | | κ-CN | Tyr-Ile-Pro-Ile-Gln-Tyr-Val-Leu-Ser-Arg |
| Albutensin A | | SA | |
| Phagocytosis-stimulating peptides h | | β-CN | Val-Glu-Pro-Ile-Pro-Try |
| | | β-CN | Leu-Leu-Tyr |
| Peptides inhibiting platelet function | | κ-CN | Pro-His-Leu-Ser-Phe |
| | | κ-CN | Met-Ala-Ile-Pro-Pro-Lys-Lys-Asn-Gln-Asp-Lys |
| | | LF | Lys-Arg-Asp-Ser |
| DNA-synthesis stimulating peptide (BALB/c3T3 cell) | | β-CN | Ala-Val-Pro-Tyr-Pro-Gln-Arg (f 177–183) |
| | h | β-CN | β-CN (f 1–18), β-CN (f 105–117) |
| Glycomacropeptide | | κ-CN | (f 106–169) |
| Casein phosphopeptides | | $\alpha_{s1}$-CN | (f 43–79) (f 66–74) |
| | | β-CN | (f 1–25) |

h, human.
Source: Yamauchi (1992).

*albicans*. The injection of isracidin into the udder of sheep and cow gave protection against mastitis.

In order to function physiologically in the human body, the active peptides must be absorbed from the intestine in an active form. However, there is no evidence that these peptides can be absorbed from the intestine in adults and the proposed properties remain to be proven. Di- and tripeptides can be easily absorbed in the intestine, however, it is not clear that larger bioactive peptides containing excess of three amino acids are absorbed from the intestine and reach the target organ. Yamamoto (1997) reported absorption of antihypertensive peptides, Val-Pro-Pro and Ile-Pro-Pro from sour milk.

The addition of CPPs to toothpaste formulas has been suggested to have anticariogenic effects and to prevent enamel demineralisation (Reynolds, 1994). CPPs are not unpalatable and can be used as an anticariogenic additive. Severe heat treatment of milk may cause dephosphorylation of phosphoseryl residues and may affect the bioavailability of CPP.

The casein phosphopeptides prevent the precipitation of calcium phosphate and increase the concentration of soluble calcium *in vitro* and also in the lumen of the small intestine of rat (Naito *et al.* 1972). Sato *et al.* (1986) reported that CPPs injected into the lumen of a ligated loop of small intestine enhanced absorption of calcium from the loop. On the other hand, Brommage *et al.* (1991) did not find any stimulating effect of CPPs in intestinal calcium absorption in rats eating a normal meal. Thus, the physiological role of CPPs may be due to inhibition of precipitation of calcium phosphate. A study by Yuan and Kitts (1991) in an animal system model could not demonstrate any improvement in calcium catabolism from diets containing CPP. CPPs-Ca complexes may enhance calcium absorption in the small intestine. However, further research is needed to confirm this.

Some peptides show antihypertensive activity. These peptides are called casokinins, which are inhibitors of the ACE. Angiotensin II decreases the renal output and increases water retention. ACE plays an important role in blood pressure regulation. This enzyme catalyses production of angiotensin II (which is a vasoconstrictor) and inactivation of bradykinin (which is a vasodilator). These peptides exhibit ACE inhibitory activity. Some specific inhibitors of ACE have been proven to be useful as antihypertensive drugs (Meisel & Schlimme 1994).

The antihypertensive effect of orally administered doses of Calpis sour milk or peptides (Val-Pro-Pro or Ile-Pro-Pro) on spontaneous hypertensive rats was studied by Nakamura *et al.* (1995). The sour milk or peptides decreased systolic blood pressure 6–8 h postadministration. Antihypertensive effect of sour milk containing two peptides Val-Pro-Pro and Ile-Pro-Pro was tested in hypertensive patients. Systolic blood pressure was decreased significantly at 4 and 8 weeks after the beginning of ingestion, suggesting a mild pharmacological effect of antihypertensive peptides. Sekiya *et al.* (1992) also studied the application of casein hydrolysate against hypertension in human volunteers.

Some peptides have shown antithrombotic activities. There are a number of similarities between clotting of blood and that of milk. Rennin or chymosin hydrolyses the peptide bond between residues 105 and 106 (Phe-Met) of κ-casein. There are structural and functional homologies between the dodecapeptide of fibrinogen and the 106–110 sequence of κ-casein (called casopiastrin). Casopiastrin has been shown to have antithrombotic activity as this peptide inhibits binding of fibrinogen on platelets (Fiat *et al.* 1993).

Most of the claimed physiological properties of the

**Table 5.** Bioactive peptides derived from whey proteins

| Precursor protein | Fragment | Peptide sequence | Name | Function |
|---|---|---|---|---|
| α-Lactalbumin | 50–53 | Tyr-Gly-Leu-Phe | α-Lactorphin | Opioid agonist ACE inhibition |
| α-Lactoglobulin | 102–105 | Tyr-Leu-Leu-Phe | β-Lactorphin | Non-opioid stimulatory effect on ileum |
| | 142–148 | Ala-Leu-Pro-Met His-Ile-Arg | – | ACE inhibition |
| | 146–149 | His-Ile-Arg-Leu | β-Lactotensin | Ileum contraction |
| Bovine serum albumin | 399–404 | Tyr-Gly-Phe-Gln-Asp-Ala | Serorphin | Opioid |
| | 208–216 | Ala-Leu-Lys-Ala-Trp-Ser-Val-Ala-Arg | Albutensin A | Ileum contraction ACE inhibition |
| Lactoferrin | 17–42 | Lys-Cys-Arg-Arg-Trp-Glu-Trp-Arg-Met-Lys-Lys-Leu-Gly-Ala-Pro-Ser-Ile-Pro-Ser-Ile-Thr-Cys-Val-Arg-Arg-Ala-Phe | Lactoferricin | Antimicrobial |

Adapted from: Korhonen *et al.* (1998).

casein-based bioactive peptides have been carried out *in vitro* or in animal model systems and these hypothesised properties remain to be proven in humans.

## Physiological role of whey protein-based bioactive substances

Whey proteins comprise approximately 20 % of total milk proteins (Table 1). The whey proteins are not coagulated by acid and are resistant to the action of chymosin. As a result these proteins remain present in acid and rennet wheys. α-Lactalbumin is one of the main proteins in human milk. α-Lactalbumin contains readily digestible amino acids. β-Lactoglobulin represents about half the total protein in whey of cows' milk. However, it is absent from human milk (Table 1).

Many whey proteins are claimed to possess physiological properties. Bovine whey contains metal binding proteins, imunoglobulins, growth factors and hormones. Bioactive peptides obtained from whey proteins and their physiological effects have been less extensively studied than those from caseins. Most of the functions of whey proteins are related to the immune or digestive systems. Wong and Watson (1995) have shown immunostimulatory functions of whey proteins. McIntosh *et al.* (1995) have reported anticarcinogenic effects of whey proteins in mice and rats. However, further research is warranted to substantiate these findings. Antimicrobial properties of these whey protein fractions are well established. Lactoperoxidase, lactoferrin, and imunoglobulins have already been commercialised.

Some of the bioactive peptides obtained from whey proteins include α-lactorphin, β-lactorphin, albutensin A and β-lactotensin. Serorphins obtained from bovine blood serum albumin have shown opioid activity (Table 5) (Tani *et al.* 1994).

Minor whey proteins such as lactoferrin, lactoperoxidase, lysozyme and immunoglobulins are considered antimicrobial proteins. Lactoferrin is a dominant whey protein in human milk and plays an important role in iron uptake in the intestine (Hutchens *et al.* 1994; Viljoen, 1995). Bovine lactoferrin is homologous to human lactoferrin. The concentration of lactoferrin in bovine colostrum and milk is about 1·5–5 mg/ml and 0·1mg/ml, respectively (Tsuji *et al.* 1990). In human milk and colostrum the reported levels are 2–4 g/l and 6–8 g/l, respectively. This indicates that lactoferrin is even more important for humans than for bovine species. The presence of high concentrations of lactoferrin in colostrum and the passing on to the newborn suggests the involvement of lactoferrin in the primary defence system against pathogenic bacteria (Kussendrager, 1993). Lactoferricin is a single peptide consisting of 25 amino acid residues. A similar active peptide consisting of 47 amino acid residues has been obtained from human lactoferrin. The molecule is folded into two globular units, each capable of binding one ferric ($Fe^{3+}$) ion.

Some whey proteins are known to contain bioactive peptides with weak opioid activity including serorphin and albutensin from the serum albumen fraction, lactoferroxin from lactoferrin and lactotensin from β-lactoglobulin.

Lysozyme is an antimicrobial enzyme found in colostrum and human milk. The enzyme hydrolyses $\beta - 1 \rightarrow 4$ linkages between *N*-acetylmuramic acid and 2-acetyl-amino-2-deoxy-D-glucose residues in bacterial cell walls, resulting in cell lysis. The concentration of lysozyme in colostrum and normal milk is about 0·14–0·7 and 0·07–0·6 mg/l, respectively (Korhonen, 1977). Lysozyme is a 15 kDa single-chain protein. The amino acid content of bovine milk lysozyme is different from that of human milk and egg white lysozyme.

Lactoperoxidase is a major antibacterial agent in colostrum. The enzyme, in the presence of $H_2O_2$ catalyses the oxidation of thiocyanate ($SCN^-$) and produces an intermediate product with antimicrobial properties. The concentration of lactoperoxidase in colostrum and milk is about 11–45 mg/ml and 13–30 mg/ml, respectively (Korhonnen, 1977).

Immunoglobulins are not transferred across the placenta to the mammalian fetus and hence babies and calves are born with very low concentrations of serum immunoglobulins. Immunoglobulins occur in high concentration in bovine or human colostrum and the absorption of immunoglobulins occurs from colostrum to provide passive immunity after birth. The antibodies protect the newborn against infections. The major immunoglobulins in human milk are IgA. IgG1 is the main immunoglobulin type in colostrum, whereas IgM, IgA and IgG2 are present at lower concentrations. Colostrum contains approximately 100 times higher concentrations of immunoglobulins than milk.

α-Lactorphin produced from α-lactoglobulin showed weak opioid activity, whereas the effects of β-lactorphin produced from β-lactoglobulin are less clear. In studies by McIntosh *et al.* (1993, 1995) and Regester *et al.* (1996) whey-protein fed rats showed the lowest incidence of colon cancer (30 %) compared to 55 % for meat diet and 60 %

**Table 6.** Protective factors in human milk

| Protective factor | Pathogen | Mechanism |
|---|---|---|
| Oligosaccharides | Clostridia, *Escherichia coli*, various pathogens | Prebiotics/bifidobacteria inhibit growth of pathogens |
| Oligosaccharides | Rotavirus | Prebiotics/bifidobacteria improve immune response |
| Oligosaccharides | *Campylobacter jejuni* | Binds bacterium |
| Oligosaccharides | *Streptococcus pneumoniae* | Binds bacterium |
| Fucosylated oligosaccharides | Enterotoxin *E.coli* | Binds stable toxin |
| Mucin | *E. coli* | Binds bacterium |
| GM1 ganglioside | Vibrio cholera | Binds toxin |
| GM1 gangliosides | *C. jejuni* | Binds toxin |
| GM1 gangliosides | Enterotoxigenic *E. coli* | Binds labile toxin |
| Mannosylated glycoprotein | Enterohaemorrhagic *E. coli* | Binds toxin |
| Lactoferrin | Gram −ve and Gram +ve bacteria | Iron sequestration/membrane disruption |

for soy diet. α-Lactalbumin and β-lactoglobulin have physiological properties of whey proteins including immunoenhancing effects. The possible role of α-lactalbumin as an antitumour agent is being investigated (Hakansson *et al.* 1995) (Table 6).

The two peptides, α-lactorphin and β-lactorphin have been shown to cause contraction of smooth muscles similar to morphine. Albutensin and β-lactotensin cause contraction of guinea-pig ileum longitudinal muscle. Whey proteins including α-and β-lactorphin and albutensin appear to have ACE inhibitory activity.

The specific roles of various immunoglobulins, lactoferrin and lactoperoxidase in protecting the newborn calf are well established. β-Lactoglobulin can bind retinol and can transport this into the small intestine (McLeod *et al.* 1996)

Lactoferrin exhibits both bacteriostatic and bacteriocidal activity against a range of microorganisms. Lactoferrin also causes release of lipopolysaccharide molecules from the outer membrane of the Gram-negative bacteria and acts directly as an antibiotic. Lactoferrin has been found to inhibit the growth of *Escherichia coli*, *Salmonella typhimurium*, *Shigella dysenteriae*, *Listeria monocytogenes*, *Bacillus stearothermophilus* and *Bacillus subtilis* (Batish *et al.* 1988; Payne *et al.* 1990; Saito *et al.* 1991). The antimicrobial effect is mainly on the organisms that require iron as lactoferrin chelates iron thereby depriving the organisms of a source of this nutrient. Lactoferrin interacts with the bacterial cell membrane leading to permeability changes and causes release of lipopolysaccharide from the outer membrane of the Gram-negative bacteria. A direct interaction between lysozyme and lactoferrin was observed with *Micrococcus luteus* (Yamauchi, 1992). Lactoferricin, a peptide derived from bovine lactoferrin due to action of pepsin has been found to have antimicrobial activity against various bacteria and *Candida albicans* (Jones *et al.* 1994).

Milk lysozyme is active against a number of Gram-positive and some Gram-negative bacteria. There seems to be a synergistic action of lysozyme and lactoferrin against *E. coli* as the latter damages the outer membrane of Gram-negative bacteria and the organism becomes susceptible to lysozyme. Combinations of lysozyme and lactoferrin are more bacteriostatic than either of the proteins alone (Suzuki *et al.* 1989). The enzyme is toxic to Gram-positive and Gram-negative bacteria such as *Pseudomonas aeruginosa*, *Salmonella typhimurium*, and *Listeria monocytogenes*. Gram-negative organisms may be inhibited or killed. Gram-positive bacteria are more resistant and are generally only inhibited in their growth.

Several clinical studies (reviewed by Goldman, 1989; Davidson, 1996, Pakkanen & Aalto, 1997 have demonstrated efficacy of immune milk preparations in the therapy of gastrointestinal diseases. Immune milk preparations are protective against rotavirus infections in children, enteropathogenic or enterotoxigenic *E. coli* infections, and *Helicobacter pylori*.

## Milk sugars and other milk-based bioactive components and their physiological effects

Human milk contains a number of protective factors including oligosaccharides, mucin and gangliosides. These oligosaccharides are complex sugar structures attached to lactose. The concentration of oligosaccharides in human milk can range from 10 to 20 g/l. Human milk contains more types and higher amounts of oligosaccharides than cow milk. Oligosaccharide structures can also comprise the carbohydrate portion of glycoconjugates, such as glycolipids and glycoproteins. Glycoproteins have oligosaccharides attached to a protein. Lactoferrin is one of the major glycoproteins in human milk whey. Glycoprotein in human milk inhibits the binding of enterohaemorrhagic *E. coli* (Newburg & Newbauer, 1995).

Lactose has been reported to enhance calcium absorption. Lactose can be used to produce lactulose and lactooligosaccharides. Lactulose is used as a promoter of probiotic bacteria and its use is now widespread in infant formulas. Lacto-oligosaccharides are also used as probiotic growth promoters. Lacto-oligosaccharides are produced from lactose by enzymatic processes involving the reverse of the lactose hydrolysis reaction by β-D-galactosidase enzyme. The oligosaccharides in human milk inhibit the binding of host cells by enteropathogenic *E. coli*, *Campylobacter jejuni* and *Streptococcus pneumoniae* to the target cells. Fucosylated oligosaccharide fraction in human milk protects against enterotoxigenic *E. coli*.

Mucin is a long macromolecule in human milk and links with oligosaccharides. Human milk mucin complex binds to rotavirus and inhibition of rotavirus has been reported. Mucin-associated glycoprotein (lactadherin) is responsible for binding with rotavirus (Yolken *et al.* 1992).

Although the protein fraction of milk has been widely

studied in terms of bioactive compounds, there are other compounds that have shown physiological significance. Calcium is thought to play a role in the regulation of blood pressure. There is some epidemiological evidence that higher intakes of calcium, especially from dairy products are associated with maintenance of blood pressure. However, more research is needed to substantiate this. The possible protective role of calcium in prevention of colon cancer has been investigated by researchers at the Dutch Dairy Research Institute (NIZO) in Ede. It has been hypothesised that the main factor involved in promotion of colon cancer is the presence of bile salts. Milk appears to play some role in providing calcium phosphate which binds bile salts in order to prevent their toxic effect (van der Meer & Lapre, 1991).

Lipid-based bioactive compounds in milk include fatty acids. The role of conjugated linoleic acid for inhibition of cancer and atherosclerosis has been studied (Pariza, 1997; Schmelz & Merrill, 1997). Traditional dairy products such as yogurt, kefir and koumiss have been consumed for centuries throughout the world. A number of health benefits have been claimed to be associated with the consumption of fermented milk products. The recent trend is to incorporate probiotic organisms such as *Lactobacillus acidophilus* and bifidobacteria known as AB cultures in fermented dairy foods (Dave & Shah, 1997). The generally accepted definition of 'probiotics' is that they are live microbial food or feed supplements that provide a beneficial effect on hosts (human or animal) by improving the microbial balance in the intestine. Probiotic products are viewed as healthy foods by consumers. The intake of these bacteria is reported to help restore the balance in the intestinal microflora, which may have been lost due to stress, antibiotic use or due to illness.

*L. acidophilus* is found naturally in the upper gastrointestinal tract and bifidobacteria occur in the large intestine. The beneficial effects of the presence of bifidobacteria in the gastrointestinal tract are dependent on their viability and metabolic activity. The growth of bifidobacteria is dependent on the presence of complex carbohydrates known as bifidogenic factors such as oligosaccharides. Some of these bifidogenic factors can be obtained from lactose including lactulose, lactitol or lactosucrose. These complex oligosaccharides are not digested by enzymes or acid of the stomach and pass on to the colon undigested. Here they are fermented by bifidobacteria and stimulate the growth of bifidobacteria. These complex oligosaccharides which promote the growth of beneficial organisms are referred to as prebiotics. 'Prebiotic' is a term to describe factors such as those found in the human milk that promote the growth of desirable bacteria in the intestine (Gibson & Roberfroid, 1995). This term also includes what is referred to as 'bifidus factors'. The effects of three oligosaccharides (β-fructo-oligosaccharides (FOS), β-galacto-oligosaccharides (BOS) and α-gluco-oligosaccharides (GOS)) on metabolism of intestinal microflora in germ-free rats inoculated with human faecal flora have been studied (Djouzi & Andrieux, 1997). FOS and BOS were preferred by bifidobacteria the number of which increased by 2 log cycles as compared with the rats on control diets. Analysis of caecal content revealed a decrease in pH. The products that contain both prebiotics and probiotics are referred to as 'synbiotics'. Japan is the world leader in probiotic and prebiotic products.

Breast-fed infants have a much higher percentage of *Bifidobacterium bifidum* than formula-fed infants. The latter group contained more adult microflora including coliform bacteria. Bifidobacteria produce acetic acid, butyric acid, lactic acid and pyruvic acid. The lactic acid and acetic acid account for >90 % of organic acids produced (Lankaputhra, 1997; Lankaputhra & Shah, 1998). It is widely accepted that because of acid production by *L. acidophilus* and bifidobacteria, the enteropathogenic bacteria are unable to grow. The growth of clostridia and *E. coli*, when cocultured with bifidobacteria, has been found to be inhibited even at neutral pH suggesting that acid production may not be solely responsible for inhibition. Metabolites produced by bifidobacteria may be partly responsible.

Certain nitrogen containing oligosaccharides stimulate growth of bifidobacteria. Human milk glycopeptides and glycoproteins are also thought to be stimulating the growth of bifidobacteria. These factors are absent from cow milk. The bifidus factor associated with casein may be attributable to the oligosaccharide moiety of those molecules.

Bovine lactoferrin has been demonstrated to stimulate the *in vitro* growth of human *Bifidobacterium* species (Petschow & Talbott, 1991). However, there is a lack of *in vivo* data demonstrating that addition of lactoferrin to infant formulas decreases the presence of harmful bacteria (Roberts *et al.* 1992).

Lactoferricin is highly effective against a broad range of Gram-positive and Gram-negative bacteria including *Listeria*, *E. coli*, *Salmonella* and *Campylobacter*, but not against several strains of *Bifidobacterium*. Lactoferricin is also effective against *Candida albicans*, which is the most important fungal pathogen of humans.

Protective effects of fucosylated oligosaccharides and glycoproteins and glycolipids against enterotoxigenic *E. coli* have been reported (Newburg *et al.* 1990). This inhibition appears to be associated with acidic glycolipids that contain sialic acid gangliosides. Oligosaccharides in human milk interfere with the attachment of *Haemophilus influenzae* and *Streptococcus pneumoniae* (Goldman & Goldblum, 1995).

## Conclusions

Dairy products can be considered as physiological foods and precursors for several bioactive substances. However, further research is needed particularly in humans to fully substantiate the role of the bioactive substances. Once the information regarding health benefits is accepted by the public, incorporation of whey proteins and milk sugar in the promotion of health benefits becomes a strong marketing tool. A major avenue for increasing the beneficial physiological effects of dairy products is likely to be probiotic foods. Over the past decade, Australia, North America, Europe, and Japan have seen a tremendous increase in the consumption of probiotic products. Probiotic foods are becoming increasingly popular. The combination

of whey proteins as physiological foods and a precursor of bioactive substances and milk sugar as prebiotics along with probiotic bacteria to modify the gastrointestinal flora have the potential to provide a complete food for maximum health benefits.

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