REVIEW

Folia Microbiol. **53** (5), 378–394 (2008) `http://www.biomed.cas.cz/mbu/folia/`

Beneficial Health Effects of Milk and Fermented Dairy Products – *Review*

L. Ebringer[a], M. Ferenčík[b,c], J. Krajčovič[a]

[a]*Institute of Cell Biology, Faculty of Science, Comenius University, 842 15 Bratislava, Slovakia*
[b]*Institute of Neuroimmunology, Slovak Academy of Sciences, 845 10 Bratislava, Slovakia*
[c]*Institute of Immunology, Faculty of Medicine, Comenius University, 811 08 Bratislava, Slovakia*
e-mail ebringer@fns.uniba.sk

Received 4 December 2007
Revised version 9 April 2008

ABSTRACT. Milk is a complex physiological liquid that simultaneously provides nutrients and bioactive components that facilitate the successful postnatal adaptation of the newborn infant by stimulating cellular growth and digestive maturation, the establishment of symbiotic microflora, and the development of gut-associated lymphoid tissues. The number, the potency, and the importance of bioactive compounds in milk and especially in fermented milk products are probably greater than previously thought. They include certain vitamins, specific proteins, bioactive peptides, oligosaccharides, organic (including fatty) acids. Some of them are normal milk components, others emerge during digestive or fermentation processes. Fermented dairy products and probiotic bacteria decrease the absorption of cholesterol. Whey proteins, medium-chain fatty acids and in particular calcium and other minerals may contribute to the beneficial effect of dairy food on body fat and body mass. There has been growing evidence of the role that dairy proteins play in the regulation of satiety, food intake and obesity-related metabolic disorders. Milk proteins, peptides, probiotic lactic acid bacteria, calcium and other minerals can significantly reduce blood pressure. Milk fat contains a number of components having functional properties. Sphingolipids and their active metabolites may exert antimicrobial effects either directly or upon digestion.

Abbreviations

AA	arachidonic acid	LA	lactalbumin
ACE	angiotensin I-converting enzyme	LCPUFA	long-chain oligounsaturated fatty acid(s)
CLA	conjugated linoleic acid	LDL	low-density lipoprotein
DHA	docosahexaenoic acid	LG	lactoglobulin
EGF	epidermal growth factor	MCP	monocyte chemotactic protein
EPA	eicosapentaenic acid	M-CSF	macrophage colony-stimulating factor
FA(s)	fatty acid(s)	MCT	medium-chain triglycerides
FFA(s)	free fatty acid(s)	MFGM	milk fat globule membrane
GALT	gut-associated lymphoid tissue	MIP	macrophage inflammatory protein
G-CSF	granulocyte colony-stimulating factor	NGF	nerve growth factor
GIT	gastrointestinal tract	NK	natural killer
HAMLET	human α-lactalbumin made lethal to tumor cells	sIgA	secretory immunoglobulin A
HDL	high-density lipoprotein	RANTES	Regulated upon Activated Normal T-Expressed and presumably Secreted chemokine
IFN	interferon		
Ig	immunoglobulin	TGF	transforming growth factor
IGF	insulin-like growth factor	TLR	Toll-like receptor
IL	interleukin	TNF	tumor necrosis factor

CONTENTS

1 INTRODUCTION

The health benefits of milk and fermented milk products have been known since medieval times. Drinking milk has taken the advantage of the extensive nutritional value not only to the child, but also to the adult and the elderly. The health benefits of dairy products are the result of biologically active components that are present in native milk and also, due to their suitably modulated activities produced through the action of probiotic bacteria, in the fermented or sour milk products. In addition to modification of several milk components the probiotics may act also directly as preventive agents, or in therapy of some contagious, atopic, tumor or other severe diseases (Ferenčík and Ebringer 2003; Gill and Guarner 2004; Santosa *et al.* 2006). The health-promoting effect of the prefermented functional foodstuffs materializes directly through interaction with consumed microorganisms (probiotic effect) or, indirectly, as a result of action of microbial metabolites generated during the fermentation process (biogenic effect). The most important biogenic metabolites include vitamins, proteins, peptides, oligosaccharides, and organic acids, including FAs.

In many cases, the health-promoting mechanisms of probiotic action are not sufficiently known. However, the majority of them are based on the positive effect they exert on the immune response, *i.e.* on their immunomodulatory activity (Isolauri *et al.* 2001; Biancone *et al.* 2002). In most cases, this is due to stimulation of natural immunity (Newburg 2005; Galdeano and Perdigon 2006). In doing so they modulate primarily the production of cytokines and antimicrobial peptides (Trebichavský and Šplíchal 2006). This is the mode of action of not just typical sour milk functional foodstuffs, such as sour milk, kefir or yoghurt (Meydani and Ha 2000; Farnworth 2005) produced by the food-processing industry, but also that of the diet supplements containing the probiotic bacteria in pure form. However, the latter are the products of pharmaceutical industry and, in contrast to functional foodstuffs, they have a standard composition, and known immunomodulatory characteristics, verified both experimentally and in controlled clinical studies. In terms of their quality and efficiency they are also under regular pharmaceutical control. It will be therefore more precise to call them **immunobiotics**, in order to distinguish them from classical probiotics in functional foodstuffs (Clancy 2003).

However, one major part of milk – whey, has traditionally not been paid as much attention as happened with sour milk, because it is a by-product of cheese making. During the Middle Age whey was considered not only as a medicine but also as a skin balm and an aphrodisiac. Whey proteins, namely α-lactalbumin, β-lactoglobulin, lactoferrin, lactoperoxidase and serum albumin, possess important nutritional and biological properties particularly with regard to prevention of diseases. Antimicrobial, anticarcinogenic, immunostimulatory and other health-promoting activities of whey proteins have been recently reviewed by Madureira *et al.* (2007). Dairy products and their components, mainly whey, contribute to the regulation of body mass by providing satiety signals. Therefore, whey proteins have a potential as physiologically dairy components for a person with obesity and metabolic syndrome. High-protein dairy products and, in particular those that contain whey proteins, may reduce fat deposition and improve insulin sensitivity (Pfeiffer and Schrezenmeir 2006; Luhovyy *et al.* 2007; Dunshea *et al.* 2007). Dairy proteins and peptides also improve the bioavailability of minerals and trace elements, such as calcium, magnesium, manganese, zinc, selenium and iron (Vegarud *et al.* 2000).

2 MILK AND ITS FUNCTION

Human milk and milk from other mammals is a complex physiological liquid with multifaceted functionality in gastrointestinal tract (GIT), where it facilitates postnatal adaptation of a newborn child through stimulating the cell growth and the digestive tube maturation, formation of the symbiotic microflora, and the development of gut associated lymphoid tissue (GALT). It is a complex mixture of specific bioactive proteins, lipids and saccharides, which represent important signals regulating the development of GIT (Clare and Swaisgood 2000; Donovan 2006). Human milk is a means of communication between the mother and the newborn child, which reduces the risk of infection, and stimulates the function of mucosal immunity (Morrow *et al.* 2005; Newburg *et al.* 2005).

Aside from the nutritious value consisting of basic proteins, lipids and saccharides, milk contains also numerous biologically active substances, such as immunoglobulins, enzymes, antimicrobial peptides, oligosaccharides, hormones, cytokines and growth factors (Clare and Swaisgood 2000; Donovan 2006; Pouliot and Gauthier 2006). An overview of the components present in human milk is shown in Table I. Several of them are direct components of the newborn immune system, and they help to induce and maintain the immune homeostasis of a newborn infant. The milk contains a heterogeneous population of cells, especially macrophages, T-lymphocytes and neutrophiles, which comprise an important part of the defense against pathogenic bacteria.

Table I. Bioactive compounds in human milk[a]

Components of the immune system	Hormones and growth factors	Lipids and fatty acids	Saccharides
Immunoglobulins (sIgA, IgG, IgM)	pituitary hormones	LCPUFA	oligosaccharides
Lactoferrin	steroid hormones	AA	mucins
Lactoperoxidase	adipokines (leptin)	DHA	lactose
Lysozyme	neuropeptides	EPA	
Lactoadherin	thyroid hormones	CLA	
Cytokines	EGF	cholesterol	
Nucleotides	IGF	MFGM	
Macrophages	NGF		
Neutrophils	TGF		
T-lymphocytes			
Toll-like receptors			

[a] Arranged according to Donovan (2006); AA – arachidonic acid, CLA – conjugated linoleic acid, DHA – docosahexaenoic acid, EGF –epidermal growth factor, IGF – insulin-like growth factor, LCPUFA – long-chain oligounsaturated fatty acids, MFGM – milk fat globule membrane, NGF – nerve growth factor, TGF – transforming growth factor, EPA – eicosapentaenoic acid.

So far, in the milk of mammals more than 60 different enzymes were identified (Fox 2003), the majority of which become inactive during heat processing. The heat processing at higher temperatures causes not just denaturation of digestion enzymes (proteinases, lipases, amylases, phosphatases), but also enzymes with antioxidant and antimicrobial characteristics that are important not just in terms of milk stability but also in terms of protection of mammals against pathogenic agents: lysozyme, catalase, superoxide dismutase, lactoperoxidase, myeloperoxidase, xanthine oxidoreductase, ribonuclease, *etc*.

Fresh milk contains also a mixture of antimicrobial agents that exhibit bacteriostatic and even bactericidal activities. They are used for the protection of the milk gland and they are transferred from mother to progeny, whereby they support the protection of progeny against contagious diseases. In addition to immunoglobulins (antibodies) the best understood protective factors include lactoperoxidase (De Witt and van Hooydonk 1996), xanthine oxidoreductase (Harrison 2002) and lysozyme (Maga *et al*. 1998).

Lactoperoxidase, or lactoperoxidase system (lactoperoxidase–thiocyanate–hydrogen peroxide) makes possible the prolongation of milk storage, since it also suppresses propagation of psychrotropic bacteria. It is effective especially against Gram-negative catalase-positive bacteria, such as pseudomonads, coliform bacteria, salmonellae and shigellae (Reiter and Perraudin 1991). The lactoperoxidase system is used as a natural conservation agent in milk industry in tropical regions, with a deficit of refrigeration installations due to technical or economic reasons (Seifu *et al.* 2005). The xanthine oxidoreductase reduces nitrates and nitrites, which both have bactericidal effects, down even to a cytotoxic nitric oxide (NO). This enzyme produces also hydrogen peroxide, which is a substrate for lactoperoxidase, and NADPH oxidase – parts of efficient antimicrobial systems.

3 MILK PROTEINS AND PEPTIDES

The most important milk proteins include caseins, β-lactoglobulin, α-lactalbumin, immunoglobulins, lactoferrin and serum albumin. They exert their biological activities either directly, or after degradation to different peptides. Their biological activities are diverse (Table II) but the majority of them interfere in a specific way with the functionality of the immune system.

In the reaction with proteolytic enzymes the casein generates different bioactive peptides that, through their action, affect not just the immune system, but also digestive, cardiovascular and nervous systems (Korhonen and Pihlanto 2006). They can be released from an inactive protein sequence during digestion of milk in GIT, through microbial fermentation of milk, or through its hydrolysis by proteolytic enzymes. Certain technologies have been developed recently that allow the isolation of some of these bioactive peptides in the form of nutritional supplements. In natural form they are present also in fermented milk products, such as yoghurt, sour milk, bryndza (Liptauer cheese) and some other cheeses. The structure and exact biological function of many of these peptides is not yet known.

The proteolytic system of milk bacteria consists of proteinase bound to the cell wall, and several intracellular peptidases. The milk lactic acid bacteria proteinases, such as those of *Lactococcus lactis* release biologically active oligopeptides from α- and β-caseins, which contain amino acid sequences that are present in casomorphines, lactorphines, casokinines and immunopeptides. They are peptides with two or more biological activities. **Casomorphines** and **lactorphines** have pharmacological characteristics similar to morphine (opiate-like effects). They act as analgetics; they stimulate excretion of some hormones, especially insulin and somatostatin; they prolong gastrointestinal resorption of nutrients; they modulate transport of amino acids in the intestine; and they act also as antidiarrhea agents (Brandsch *et al.* 1994; Meisel 1997, 2005). These atypical opiate peptides differ from endogenous opiates, such as enkephalines and endorphines, only in their N-end sequences (Teschemacher and Brantl 1994).

Table II. Biological activities of milk proteins and peptides[a]

Effect	Active substance/effector
Opioid antagonist	lactoferroxins
Opioid agonist	α-lactorphins, β-lactorphins
Antimicrobial	lactoferrin, lactoferricin
Immunomodulatory	immunopeptides, *e.g.* immunocasokinin
Hypotensive (ACE inhibitors)	lactokinins, casokinins
Antioxidant	peptides derived from α-LA and β-LG, *e.g.* Met-His-Ile-Arg-Leu, Tyr-Val-Glu-Glu-Leu
Hypocholesterolemic	peptides derived from β-LG, *e.g.* Ile-Ile-Ala-Glu-Lys
Antithrombotic	casoplatelin
Mineral binding	caseinophosphopeptides

[a] According to Mizushima *et al.* 2004; Silva and Malcata 2005; McCann *et al.* 2006; Walker *et al*. 2006; Hartmann and Meisel 2007.

On the other hand, the fermentation of milk generates also opiate antagonists – **casoxins** and **lactoferrins** (Yoshikawa *et al.* 1988). For the biological effect it is often important to consider the synergic activity of bioactive peptides with nonpeptidic milk components, such as oligosaccharides, glycolipids and fats (Schanbacher *et al*. 1997).

The fermentation of milk generates substances with immunostimulatory and antitumor effects. In order for them to emerge, it is necessary that a probiotic strain has proteolytic activity. This indicates that the effective agents are peptides as was also shown in experiments with the probiotic strain *Lactobacillus helveticus* in mice. A long-term application of milk fermented by *L. helveticus* reduced the growth of experimental tumor of the milk gland. In cases when the strain *L. helveticus* without proteolytic activity was used in the experiments, the immunostimulatory and antitumor activity was not present (Matar *et al.* 2001). The essential cause of such antitumor and immunostimulatory activity was the higher apoptosis of tumor cells and decreased production of proinflammatory cytokines, especially IL-6, in mouse immunocytes (Rachid *et al.* 2006).

The hydrolysis of milk proteins caused by probiotic bacteria produces also other oligopeptides with various biological activities. These include small peptides, such as Val-Pro-Pro and Ile-Pro-Pro, with a hypotensive effect (Sappo *et al*. 2003; Huth *et al*. 2006). Hypocholesterolemic effects are also exhibited by some peptides derived from β-lactoglobulin, such as, *e.g*., the pentapeptide Ile-Ile-Ala-Glu-Lys (Nagaoka *et al.* 2001).

The way they affect the blood pressure is based on inhibition of ACE activity (angiotensin I-converting enzyme), the enzyme that converts angiotensin I to angiotensin II. Angiotensin II causes vasoconstriction with simultaneous inactivation of bradykinin with vasodilatation activity, which results in hypertension. The proteolytic activity of milk bacteria during milk fermentation generates also hypotensive peptides with a larger number of amino acid units – **casokinins** and **lactokinins** (Sipola *et al.* 2002).

In addition to hypotensive peptides, the fermentation of milk using different starting milk bacterial strains can produce also peptides with antithrombotic (**casoplatelins**), antimicrobial (**casocidin I**, **casecidin**) and immunostimulatory (immunopeptides) effects (Meisel and Bockelmann 1999; Claire and Swaisgood 2000). Some peptides, such as **isracidin** and **lactoferricin**, have also antifungal activity (Bellamy *et al.* 1994; Lahow and Regelson 1996). Immunopeptides increase the activity of the immune system cells, such as proliferation of lymphocytes, activity of NK cells, synthesis of antibodies and production of cytokines (Trebichavský and Šplíchal 2006).

3.1 β-Lactoglobulin

In milk and especially in whey there is a relatively high content of **β-lactoglobulin**, which represents more than half of the present proteins. It acts as an effective emulgator and immunomodulator. Its molecule contains a hydrophobic part which can bind vitamin A, vitamin D, calcium and FAs, simplifying their resorption (Brown 1984; Cho *et al.* 1994; Wang *et al.* 1997; Beaulieu *et al.* 2006). In addition to the bioactivities exerted by the native molecule, β-lactoglobulin can exhibit further physiological functions because of numerous bioactive peptides that are contained within the protein. These peptides are inactive within the sequence of the precursor protein but they can be released by *in vivo* or *in vitro* enzymic proteolysis. Once released, these peptides play important roles in human health. Their antihypertensive, antithrombotic, opioid, antimicrobial, immunomodulant, and hypocholesterolemic properties have been reported. All β-lactoglobulin-derived peptides also exhibit radical-scavenging activity (Hernández-Ledesma *et al.* 2007). The peptides that are produced through proteolytic degradation of β-lactoglobulin have not yet been sufficiently described. An interesting peptide is **β-lactotensin**, which stimulates contraction of smooth muscles (Yoshida and Owens 2005). β-Lactoglobulin is responsible for milk allergies, which affect 2–3 % of children. In the majority of cases these allergies disappear by the time the child reaches three years of age.

The peptides originating from β-lactoglobulin may cause oral tolerance, and subsequently reduce the production of IgE which is specific for this protein (Pecquer *et al.* 2000), an effect that can be used in the prevention of milk allergies. Bioactive peptides derived from β-lactoglobulin are currently a subject of intensive research.

3.2 α-Lactalbumin

In contrast to β-lactoglobulin, **α-lactalbumin** has low immunogenicity and thus also a low allergy-inducing potential, which make it a good candidate to become a valuable nutrient for children. It is capable of inducing apoptosis of tumor and immature cells (Svensson *et al.* 2000) and thus it can exert anticancer activity. Its hydrolysis produces peptides with immunomodulatory effects. One of them is the tripeptide Gly-Leu-Phe, which stimulates phagocytosis of macrophages through specific receptors as well as respiratory burst of neutrophiles (Jaziri *et al.* 1992). It is therefore considered to be a substance with an important antimicrobial activity (Pihlanto-Leppälä *et al.* 2000; Pellegrini 2003). Its stress reduction also belongs among the bioactivities of α-lactalbumin (Markus *et al.* 2002). It was shown to raise brain serotonin, reduce cortisol concentration and improve mood under stress. α-Lactalbumin may also exert antiulcerative properties. It protects the rat stomach mucosa against ulcerative lesions caused by indomethacin (Mezzaroba *et al.* 2006).

3.3 Immunoglobulins

Immunoglobulins contain antibodies that directly participate in the anti-infectious defense of both the milk gland and the newborn infant. The main immunoglobulin in milk is IgG_1, and then also IgM, IgA and IgG_2. In addition to the protection against microbial pathogens, they are responsible for activation of complement, stimulation of phagocytosis, preventing adhesion of microbes, and neutralization of viruses and toxins. They also increase the intracellular levels of glutathione, which is the key cell antioxidant (Bounous and Gold 1991). The secretory **IgA** (sIgA) plays a special role, which works in a nonspecific way, covering infectious agents and preventing their adhesion to the mucosal epithelium. The concentration of immunoglobulins is higher in colostrum. It can be increased by the immunization of pregnant mothers, which takes place in practice among farm animals. Using such cow colostrum one can prepare food supplements not just for veals, but also for humans. Their enhanced preventive activity against different microbial infections has been documented in several studies (Mehra *et al.* 2006).

3.4 Cytokines and chemokines

Several different cytokines and chemokines have been discovered in human milk and the list is growing very rapidly (Böttcher *et al.* 2003; Ustundag *et al.* 2005; Kverka *et al.* 2007). Cytokines operate in networks and produce various effects that contribute to the orchestration, development, and functions of the immune system. The production of cytokines is delayed in neonates and infants, and cytokines in human milk may be able to interact with mucosal tissues in the upper parts of the respiratory and alimentary tracts, providing anti-inflammatory and immunomodulatory effects upon the recipient infants.

In human colostrum and milk about 40 different cytokines and chemokines have been detected so far. They include interleukins (IL-1β, IL-2, IL-4, IL-5, IL-6, IL-10, IL-13, IL-16), interferon-γ, growth factors (EGF, G-CSF, M-CSF, TGF-α, TGF-β), and chemokines (IL-8, MCP-1, MIP-1, RANTES and eotaxin) (Garofalo and Goldman 1998; Böttcher *et al.* 2003; Kverka *et al.* 2007).

There are individual differences in the cytokine composition of breast milk. Their levels depend on the lactation stages and health condition of the mother. Higher levels of many cytokines have been found in colostrum than in transitional or mature milk (Ustundag *et al.* 2005). The physiological changes of cytokine profile at different periods of lactation may reflect the changes in the immune system of the breast and alteration in the needs of the newborn for these cytokines. Milk of allergic mothers contains higher levels of IL-4, IL-8 and RANTES than milk of nonallergic mothers (Böttcher *et al.* 2003). The cytokine profile in milk may be also influenced by infection. For instance, in milk of healthy cows there are no cytokines IL-1, IL-6, IL-10 and IL-12 but their levels are detectable in milk of cows with intramammary infections (Riollet *et al.* 2001).

3.5 Lactoferrin

Lactoferrin is a multifunctional glycoprotein to which several physiological roles have been attributed. These include regulation of iron homeostasis, host defense against a broad range of microbial infections, anti-inflammatory activity and cancer protection. Lactoferrin is present in milk in smaller concentration than other proteins. In addition to milk, it can be found also in colostrum, mucosa secretions, tears, saliva, nasal exudates, gastrointestinal fluids and in neutrophil granules. Due to its antimicrobial and immunomodulatory activity it belongs to the best described proteins. Depending on the conditions and place of its target action, it can act either as immunosupressive, anti-inflammatory, or immunostimulatory agent. The cause of its immunosupressive and anti-inflammatory potential is the inhibition of cytokines TNF, IL-1 and IFN-γ, on the one hand, and, the stimulation of IL-10 production, on the other. This results in the suppression of activity of several cells of the immune system, including lymphocytes and macrophages (Machnicki *et al.* 1993; Hayashida *et al*. 2004). The immunostimulatory effect is achieved by the stimulation of T- and B-lymphocyte proliferation (Beaulieu *et al*. 2006), a higher number of circulating NK cells, cytotoxic and helper T-lymphocytes. This can lead to protection against the growth of tumor metastases (Iigo *et al*. 1999). The activation of Peyer's plaques stimulates the mucosal immunity (Wang *et al.* 1998). The phagocytic activity of human neutrophils is also increased by the presence of lactoferrin (Miyauchi *et al*. 1998). The main peptide that is produced in the course of lactoferrin degradation is lactoferricin. It contains 25 amino acids from the N-terminus of lactoferrin molecule, and it is responsible for the majority of its immunomodulatory effects. Lactoferrin and lactoferricin have been the milk proteins most comprehensively studied for their antiviral activity. They exhibit antiviral activity against hepatitis C (Isawa *et al*. 2002), human papillomavirus (Mistry *et al*. 2007), herpes simplex virus (Jenssen 2005), and in combination with interferon and ribavirin in patients with chronic hepatitis C (Kaito *et al*. 2007).

Tsudu *et al*. (2006) demonstrated preventive effects of lactoferrin and lactoferricin on chemically induced colon carcinogenesis in the rat and transplanted carcinoma cell metastasis in the mouse. Lactoferrin possesses multifunctional potential to suppress carcinogenesis and it is a good candidate for practical application in humans. Bovine lactoferrin has been indicated in clinical studies on patients with *Helicobacter pylori* infection, chronic hepatitis C, tinea pedis, and other diseases (Yamauchi *et al*. 2006).

Recombinant human and bovine lactoferrin is now available for development into nutraceutical and pharmaceutical products (Weinberg 2007).

3.6 Phosphopeptides

From the viewpoint of mineral nutrition important are phosphopeptides that are formed through proteolytic degradation of casein. The phosphate residues are present in them in the form of serine monoesters. They create organophosphate salts that function as carriers of cations, including calcium and other minerals, as well as trace elements in the intestine. In most cases they form complexes with calcium phosphate that are resistant to enzyme hydrolysis in GIT. Such complexes maintain calcium in a soluble form and they protect the calcium phosphate against precipitation, allowing thus absorption of calcium from the received casein in the distal part of the intestine. This passive transportation system allows primary absorption of calcium that is required for the calcification of bones. **Caseinophosphopeptides** form the organophosphate salts combined with trace elements, such as Fe, Mn, Cu and Se, so they function as their carriers. Some caseinophosphopeptides have already been used for the treatment of rachitis (Kitts and Yuan 1992). The phosphopeptides that bind calcium inhibit the formation of caries lesions through recalcification of the tooth enamel (Reynolds 1999; Aimutis 2004), whereas the glycomacropeptide derived from κ-casein seems to contribute to the anticarries effect by inhibiting adhesion and growth of plaque-forming bacteria on oral mucosa (Brody 2000; Malkoski *et al*. 2001). The anticariogenic effect of caseinophosphopeptides has been well documented in both human intervention and animal model studies (Meisel 2001).

4 MILK LIPIDS

Milk and milk products, especially those with a high content of fats, saturated FAs and cholesterol have been considered during recent three–four decades to be risky food in terms of occurrence of various civilization diseases. This criticism has arisen especially because rich fat contains a high fraction of saturated FAs assumed to contribute to heart diseases, body-mass gain and obesity. Among the 12 major milk FAs, only three saturated acids, lauric, myristic, and palmitic acids, have been associated with raising total cholesterol in plasma (German and Dillard 2006). Due to persistent myths consumers are mostly informed about the adverse characteristics of milk and milk fats, although current science provides more and more evidence on the significance of some animal fats for health (MacRae *et al.* 2005). The milk fat contains many components that have certain functions: sphingomyelin and other sphingolipids, etheric lipids, butyric acid, vitamins A, D, E, including β-carotene (provitamin A) and essential FAs, including conjugated linoleic acid. Sphingolipids are estimated to constitute 0.01–0.02 % of the human diet with a yearly intake *per capita* of about 115 g, with dairy products being the primary source of uptake (Vesper *et al.* 1999). Despite this low consumption, sphingolipids and their metabolites are highly bioactive molecules with multiple beneficial effects on human health, *e.g.*, cancer inhibition, antimicrobial and immunomodulatory activities, as well as inhibition of cholesterol adsorption (Vesper *et al.* 1999; Schmelz *et al.* 2000; Eckhardt *et al.* 2001; Possemiers *et al.* 2005). Milk fat is not only a source of bioactive lipid components, it also serves as an important delivery medium for nutrients, including the fat-soluble vitamins (Parodi 1997). The vitamins dissolved in the fat are appreciated because of their antioxidant effects.

Butyric acid ($C_{4:0}$) is found only in the fat of ruminants, and it is believed to be an important anticarcinogen which, together with etheric lipids, vitamins A, D, E and the conjugated linoleic acid, forms a protective barrier mainly against different nontransmissible diseases (Parodi 1999, 2004; German 1999). Caprylic and capric acid ($C_{8:0}$ and $C_{10:0}$) may have antiviral activities (Thormar *et al.* 1994). Lauric acid ($C_{12:0}$) may have antiviral and antibacterial functions (Sun *et al.* 2002; Thormar and Hilmarsson 2007) as well as anticaries and antiplaque activity (Schuster *et al.* 1980). Among the 12 major milk FAs, only three saturated FAs – lauric ($C_{12:0}$), myristic ($C_{14:0}$) and palmitic ($C_{16:0}$) – have been associated with raising total cholesterol levels in plasma, but their individual effects are variable – both toward raising low-density lipoproteins and raising the level of beneficial high-density lipoproteins. The increase in HDL cholesterol caused by saturated FAs has beneficial effects. HDL acts as an antioxidant and prevents oxidation of LDL cholesterol in the blood (German and Dillard 2004). Diet rich in saturated fat has been regarded to contribute to the development of obesity, heart diseases and metabolic syndrome (Mensink and Katan 1992). Saturated stearic acid ($C_{18:0}$) does not seem to increase serum cholesterol concentration (Grundy 1994) and clinical trials demonstrated that it is not atherogenic (Mensink *et al.* 2003).

Oleic acid ($C_{18:1}$) is the single unsaturated FA with the highest concentration in milk (≈8 g/L). Monounsaturated oleic acid is considered to be favorable for health because it lowers both plasma cholesterol, LDL cholesterol and triacylglycerols, and replacement of saturated FAs by *cis*-unsaturated FAs reduces the risk of coronary artery disease (Mensink *et al.* 2003). The concentration of oligounsaturated (‘polyunsaturated’) FAs in bovine milk – linoleic ($C_{18:2\omega6}$) and α-linolenic ($C_{18:3\omega3}$) acids is ≈2 g/L. These FAs may be converted to FAs with 20 carbon atoms, *e.g.*, arachidonic acid ($C_{20:4\omega6}$) and eicosapentaenoic acid (EPA) ($C_{20:5\omega3}$). Clinical trials have shown that a diet rich in monounsaturated and/or oligounsaturated FAs gives better protection against cardiovascular diseases than a diet rich in oligounsaturated FAs only (De Lorgeril *et al.* 1994).

Lipids, especially unsaturated FAs and medium-chain saturated FAs and their monoglycerides, are generally most active against bacteria (Thormar and Hilmarsson 2007). Several studies suggest that lipids play a role in the natural defense against infections in skin and mucosal membranes and that they, together with lipids in mother’s milk, take part in the innate immune response. Unsaturated FAs, particularly oleic and linoleic, and their monoacylglycerols are the most active antiviral lipids in human milk, whereas di- and triacylglycerols are inactive (Welsh and May 1979). The effect of antimicrobial milk lipids on intestinal infections is supported by the results showing that feeding with high milk fat diets diminished intestinal colonization of *Listeria* but not of *Salmonella*. *Salmonella* is much less susceptible to the inhibitory activity of lipids (Sprong *et al.* 1999).

The milk fat from sheep and goats is rich in medium-chain triacylglycerols (MCT) made up of FAs with a $C_{6–10}$ carbon chain. FAs $C_{6:0}$, $C_{8:0}$ and $C_{10:0}$ can be found in the milk of goats up to 15–18 %, and in sheep milk up to 12–14 %, while only 5–9 % in the milk of cows. The nature of fat content in sheep and goat milk, in comparison with cow milk, presents advantages for consumer health (San Sampelayo *et al.* 2007). The low molar mass of medium-chain FAs and their solubility in water facilitate the action of digestive enzymes, making hydrolysis faster and more complete than that of long-chain FAs. Reduced chain length means that MCT are more rapidly absorbed by the body and more quickly metabolized. Because of that MCT are of special interest from the therapeutic point of view. They are used in the treatment of patients with absorption problems, problems of pancreatic insufficiency, patients with deficit of biliary salts, as well as in cases of intestinal resection. MCT are used also in the nutrition of undernourished patients, prematurely born children, children with epilepsy and other diseases (Babayan 1981; Hachelaf *et al.* 1993; Razafindrakoto *et al*. 1993; Garcia Unciti 1996; Alférez *et al.* 2001).

The most recently discovered aspect deals with the composition of milk fats in ruminants, focusing on isomers of conjugated linoleic acid (CLA). CLA is a collective term to describe one or more of the C_{18} dienoic positional and geometric FA isomers of the essential conjugated octadecedienoic acid. Human and animal studies have shown that CLA can reduce adiposity, exert anticarcinogenic activity, improve plasma lipoprotein profiles and significantly modulate humoral and cellular immunity (Khanal and Olson 2004; Rainer and Heiss 2004; Fricon *et al.* 2004; MacRae *et al*. 2005). Approximately 75–90 % of the total CLA content in the milk fat and >75 % of the fat from beef meat is composed of the isomeric *cis*-9,*trans*-11-$C_{18:2}$, which shows anticarcinogenic and anti-atherogenic effects. The minimum effective dose of CLA to prevent the occurrence of cancer identified in animal models is 0.05 % of the daily nutrition needs (Dhiman *et al.* 2005). Linoleic acid is the principal FA that is a precursor of the synthesis of *cis*-9,*trans*-11-CLA, as a consequence of the biohydrogeneration that takes place in the rumen, due to the action of *Butyrivibrio fibrisolves* (Kepler and Tove 1967; Koppová *et al*. 2006). The milk products and meat from ruminants are the most abundant source of CLA in the human diet.

According to the knowledge of CLA effects in metabolism and antiproliferative and pro-apoptotic activities on various types of cancer cells it can be assumed that the *cis*-9,*trans*-11 isomer of conjugated linoleic acid belongs among candidates for nutritional cancer therapy (Ochoa *et al.* 2004). Based on studies of the health-promoting activities of lipids, both *in vitro* and *in vivo*, the possibility of using such lipids as active ingredients in prophylatic and therapeutic dosage is considered (Haug *et al*. 2007; Thormar and Hilmarsson 2007).

5 MILK OLIGOSACCHARIDES

Lactose and other oligosaccharides comprise the third most abundant constituent of milk. The disaccharide lactose (milk sugar) is hydrolyzed by the enzyme β-galactosidase (lactase) to D-glucose and D-galactose, which are absorbed in the intestine. Insufficient activity of β-galactosidase causes intolerance to the milk sugar with all its consequences, including diarrhea. The intolerance to lactose occurs in the Central European population at a 5 % rate but, in Asia and Latin America it reaches 90 %. The individuals that are intolerant to lactose show better tolerance to fermented milks than to native milks. The best for them are the yoghurts with viable milk bacteria. Through heat processing the yoghurts lose their ability to metabolize milk sugar. This has been shown in the clinical experiments where lactase-deficient individuals were unable to metabolize lactose after being fed with pasteurized yoghurt (containing dead milk bacteria) (Lerebours *et al*. 1989).

During fermentation of the cloddish sheep cheese in the sheep farm or bryndza (Liptauer cheese) producing plant lactose is transformed through fermentation into lactic acid, and thus compared with the milk the share of milk sugar in bryndza is significantly lower. This is the reason why mature bryndza is tolerated also by individuals suffering from lactose intolerance.

Lactulose is a disaccharide composed of D-galactopyranose and D-fructofuranose. It originates during heat processing of milk and has important prebiotic properties. Lactulose has beneficial health effect mainly by selective stimulation of the growth and/or activity of probiotic bacteria including bifidobacteria and lactobacilli (Gibson *et al.* 2004). Randomized-controlled trials of its effect in a clinical context are few, although animal studies show anti-inflammatory effects in inflammatory bowel disease, Crohn's disease and ulcerative colitis (MacFarlane *et al.* 2006). Synthetic lactulose is used in the treatment of constipation; it was originally thought that other milk oligosaccharides have no biological function and that they represent only a by-product of synthesis of glycoproteins and glycolipids during the production of milk. Nowadays it is clear that milk oligosaccharides are a large family of different molecules, some of which participate in the protection against infectious agents. Oligosaccharides are present in milk either in the form of free molecules or conjugated with other compounds (*e.g.*, as lipid- or protein-conjugated); some of them actually represent dissolved receptors which can bind pathogens and thus prevent their binding to the respective target receptor in GIT mucosa, and the follow-up initiation of infection.

The majority of oligosaccharides with this function are fucosylated. They are produced through the action of enzymes encoded by genes associated with expression of the Lewis blood system antigens. Therefore the specific antimicrobial activity of milk fucosylated oligosaccharides is directly linked with the maternal Lewis blood group type. The specific oligosaccharides are thus one of the main innate immunological mechanisms of human milk, through which the mother protects an infant against enteric as well as other pathogens. The knowledge of the structure and function of these protective oligosaccharides could become a basis for the development of new preventive and therapeutical drugs against GIT pathogens (Morrow *et al*. 2005; Newburg *et al.* 2005).

In addition to natural oligosaccharides the sour milk products contain also polysaccharides produced by the present probiotic bacteria, as well as their hydrolyzed products. In fermented functional foods they contribute to their stability and organoleptic properties. An example of such exopolysaccharide is **kefiran,** comprised of D-galactose and D-glucose in the ratio 1 : 1. It is the basic bioactive component of kefir (Farnworth 2005).

6 HEALTH-PROMOTING EFFECTS OF FERMENTED MILKS

6.1 Hypocholesterolemic effects

The results of several clinical studies indicate that a regular administration of selected probiotics may reduce the concentration of serum cholesterol, especially of LDL (Mikeš *et al*. 1995; Bertolami *et al*. 1999; Agerholm-Larsen *et al*. 2000). So far the mechanism of this effect has not been explained. However, it is assumed that probiotic bacteria can metabolize cholesterol and thus reduce its resorption in GIT. This has been shown in experiments which indicate that both *in vitro* and *in vivo* lactobacilli, bifidobacteria and other milk bacteria assimilate cholesterol, incorporate it into membranes, deconjugate and precipitate the bile acids (Gilliland *et al*. 1995; Tahri *et al*. 1996; Pereira and Gibson 2002). Deconjugated bile acids are less soluble and therefore are less absorbed from the intestinal lumen than their conjugated forms. As a result there is a higher excretion of free bile acids through stool. In addition to that free bile acids are less efficient in solubilization and re-absorption of fats in the intestine.

However, not all species and strains of milk bacteria and sour milk products fermented with them exhibit a hypocholesterolemic effect. This was shown in clinical testing of Danish yoghurt Gaio fermented with *Streptococcus thermophilus* and *Enterococcus faecium.* The clinical study after 8 weeks of administration of 450 mL per d showed that the Gaio yoghurt had significantly reduced the level of cholesterol in the serum of a volunteer (by 8.4 %). In the same experiments yoghurt produced with *Streptococcus thermophilus* and *Lactobacillus acidophilus* or *L. rhamnosus* was simultaneously tested; however, these three bacteria did not exhibit the hypocholesterolemic effect (Agerholm-Larsen *et al.* 2000).

It was shown that a long-term (six-month) daily consumption of fermented milk products increases the serum HDL cholesterol, and improves the ratio LDL/HDL (Kiessling *et al.* 2002).

These results correspond also with the first analyses of the traditional Slovak Liptauer cheese (bryndza). Analysis of 166 strains of the families *Enterococcus* and *Lactobacillus* isolated from nonpasteurized bryndza revealed that individual isolates metabolize cholesterol by 12 to 56 % (*our unpublished results*). The clinical study with 8 weeks of bryndza consumption (100 g a day) among volunteers has shown a statistically significant lowering of the level of total cholesterol as well as LDL cholesterol. The lowering was most significant among persons with higher starting values of cholesterol. A surprising finding was the lowering of glycemia level, and the level of serum creatinine, C-reactive protein and blood pressure (Mikeš *et al*. 2005).

6.2 Antioxidant effects

Free radicals are produced in the body in the course of regular metabolism but when exposed to xenobiotic agents from foods and environment the risk of radical production significantly increases. The most important are the free radicals derived from oxygen. Provided the antioxidant system of the organism does not manage to neutralize them rapidly enough, they can cause destructive or lethal changes (such as, *e.g*., apoptosis) through oxidation of membrane lipids, proteins, enzymes and DNA. The cellular and subcellular damage caused by free radicals plays an important role in the pathogenesis of cancer, cardiovascular diseases, allergies, atherosclerosis, and other civilization diseases (Haliwell and Guteridge 1989; Bergendi *et al*. 1999; Agerholm-Larsen *et al*. 2000). The more extensive damage of the vital molecules in the brain through oxidation was detected also in patients with Alzheimer's disease (Liu *et al*. 2003; Filipčík *et al.* 2006).

The antioxidant defense mechanism in the body is composed not just of endogenous antioxidants but also of exogenous antioxidants from foods (vitamins C and E, carotenoids, pholates, flavonoids, phytoestrogens, selenium, *etc*.). It has been found only recently that also the probiotic microorganisms can effectively trap reactive forms of oxygen. The experiment with vitamin E-deficient rats has revealed that the intracellular extract from *Lactobacillus* sp. recovers this deficiency (Kaizu *et al*. 1993). The classical yoghurt bacteria *Lactobacillus delbrueckii* ssp. *bulgaricus* and *Streptococcus thermophilus* inhibit peroxidation of lipids through scavenging the reactive oxygen radicals, such as hydroxyl radical, or hydrogen peroxide (Ling and Yen 1999).

The antioxidant activity of several species and strains of milk bacteria contained in fermented milk can significantly affect human health. This has been confirmed also by clinical studies of goat milk fermented with a starter culture *Lactobacillus fermentum* ME-3 (Kullisaar *et al.* 2003; Songisepp *et al*. 2005). The healthy volunteers have consumed for 21 d each day 150 g of milk, either sour or nonfermented. Sour milk compared to nonfermented milk has shown important improvement of the overall antioxidant activity of blood, as well as antioxidant status, prolonged resistance of lipoprotein fraction to oxidation, reduced level of peroxide lipoproteins and oxidized LDL cholesterol, reduced level of glutathione redox ratio, and increased overall antioxidant activity.

The anti-atherogenic activity of fermented milk may be the result of concerted action of several factors. Some lactobacilli produce antioxidant factors also in the human gastrointestinal tract (Ljungh *et al.* 2002). The majority of milk bacteria show antioxidant behavior (eliminating the excess oxygen free radicals) producing superoxide dismutase, or glutathione. Taking this into account, several laboratories are working on the optional use of the milk bacteria in the form of food supplements that enhance also the antioxidant status of an individual. Perhaps the very first of such targeted products is the Estonian cheese Picante, in the manufacturing of which also *Lactobacillus fermentum* ME-3 with significant antioxidant and antimicrobial effects is added to the starting culture mix (Songisepp *et al.* 2004).

The antioxidant activity exerts also various peptides derived from α-lactalbumin, β-lactoglobulin and α-casein (Fitzgerald and Murray 2006).

7 THE IMPACT OF HEAT PROCESSING ON THE BIOLOGICAL ACTIVITY OF MILK

Heat treatment induces on the one hand the degradation, denaturation and inactivation of heat labile components and, on the other hand, formation of "new" substances, which are not present in the unprocessed product. Therefore, heat treatment conditions must be chosen in such a way that the desired results (hygienic safety) can be achieved while undesirable changes (reduction of nutritional value, changed organoleptic properties) are minimized.

Biologically active agents preserve their original activity only in raw milk. Higher temperature inactivates them. Heat damage of a dairy product depends on both the duration and the intensity of heating. Pasteurization does not disrupt the activity of vitamins dissolved in fats (vitamins A, E and D) and riboflavin. In case of the most gentle pasteurization (72–75 °C for several s) the majority of B-group vitamins dissolvable in water remain almost intact, their activity drops just between 1 and 10 %. The only exception is vitamin C, the content of which is lowered by 30–50 %. Higher temperatures affect the protein-like compounds, especially enzymes, hormones, growth factors, and different proteins, which bind mineral agents and some vitamins, thus participating in their absorption in the intestine. The higher temperatures adversely affect the absorption of some minerals, including calcium, iron and zinc, through denaturation of proteins that transfer them. Higher temperature will also damage the bond between the protein and folic acid, which will reduce its resorption. Folic acid is an important vitamin in terms of hematopoiesis and in pregnant women as a prevention of nerve system damage, such as, *e.g.*, spondyloschisis of newborn infants. In spite of the fact that milk contains a relatively small amount of folic acid (50 μg/L), its further processing to cheese and fermented milk products through milk bacteria substantially increases the content of this vitamin (Forssén *et al.* 2000). The whey proteins, *e.g.*, β-lactoglobulin, bind not just folic acid, but also vitamin B_{12}, riboflavin, vitamin D and retinol (β-carotene), and they significantly support its resorption. The bond of β-lactoglobulin with riboflavin, which acts as a cofactor for enzymes detoxicating azo compounds, indicates its significance in the prevention of carcinogenesis. Heat treatment and freeze-drying of bovine colostral whey decreased the content of immunoglobulins. It seems that IgM is most sensitive, while the contents of insulin-like growth factor 1 (IGF-1) and transforming growth factor β-2 (TGF-β2) were unaffected (Elfstrand *et al.* 2002). In contrast, other authors observed that heat treatment of bovine raw milk significantly changed the IGF-1 content (Kang *et al*. 2006). Traditional heat pasteurization of milk decreased the concentrations of serum albumin, β-lactoglobulin, and α-lactalbumin (Odriozola-Serrano *et al.* 2006). Short heat treatments can be potentially responsible for a depletion in the overall antioxidant properties of milk (Calligaris *et al.* 2004). Taylor and Richardson (1980) showed that the heat treatment decreased the antioxidant capacity of casein. Pasteurization of goat milk induced a decrease of the lipolysis level of cheeses. The use of pasteurization led to a limitation of the appearance of organoleptic defects in ripened cheeses. The flavor and odor of ripened cheeses made from pasteurized milk is also reduced compared with cheeses made from raw milk (Grappin and Beuvier 1997; Morgan *et al*. 2001). Processing of milk at higher temperatures denaturate not just the digestive enzymes but also enzymes and other biologically active compounds with antimicrobial and antioxidant effects, important in the prevention of cancer and other civilization diseases.

The bioactive peptides originating in certain dairy products (cheese, sour milk) during manufacturing are natural components of foods. The thermal processing, especially at high temperatures, *e.g.*, the sterilization temperatures, can cause fundamental changes in the structure of casein and bioactive peptides. For example, dephosphorylation of casein by heat produces not just structural changes in it, but reduces also the content of phosphopeptides, thus minimizing or completely eliminating its capacity to transfer minerals. As a result of changes caused by high temperature the digestion enzymes react with structurally changed proteins as if they were completely different and unnatural substrates. Extreme thermal and alkaline processing of milk creates inter- and intramolecular covalent bonds, which are resistant to the action of hydrolytic enzymes. This, in turn, can promote racemic conversion of L-amino acids to D-amino acids, which leads to indigestible peptide bonds.

8 HUMAN MILK AND ITS IMMUNOPROTECTIVE ACTIVITIES

Human (breast) milk is a complex physiological liquid that plays a key role in the gastrointestinal tract of a newborn child in terms of its postnatal adaptation, including the formation of its digestive maturity, establishment of a beneficial symbiotic microflora, and development of gut-associated lymphoid tissue – GALT. The system of acquired immunity of a newborn child is immature and naïve. Also several parts of its natural immunity are not yet fully functional. Therefore the natural immune mechanisms of breast milk are the most important supplement to the normal function of the mucosal barrier in the developing intestine. Their essential components include bioactive proteins, peptides, lipids and saccharides acting as activating and regulating signals (Field 2005). The number and efficiency of these compounds is much higher than was originally expected. The biological activity of many of them (*e.g.*, peptides or FAs) is not demonstrable in native milk; they manifest themselves only after fermentative modification.

The human milk contains four main bioactive subsystems (Table I): cells and molecules of the immune system, hormones and growth factors, lipids and saccharides (Donovan 2006). The majority of them exert a protective effect against intestinal pathogens, they stimulate the establishment of mucosal immunity, the induction of oral immunological tolerance and probiotic microflora in the developing intestine (Newburg 2005). In most cases the efficiency of individual factors is amplified through their synergy.

The best known human milk protective molecules are immunoglobulins, especially secretory IgA (sIgA), which accounts for ≈90 % of their total quantity. The majority of other molecules are involved in the mechanisms of natural immunity. Their important characteristic is the ability to stimulate the growth of probiotic intestinal microflora. The intestinal microflora of children that are breast-fed and those that are fed with a formula differ significantly. The microflora composition of infants fed with a formula is similar to the microflora of adults, while the intestine of infants fed with breast milk contains a high percentage of probiotic milk bacteria, especially *Bifidobacterium bifidum*. This is due to the presence of a nondegradable glycan in human milk. The glycans are complex molecules containing an oligosaccharide skeleton. The nonreduction ends of their molecules contain motifs similar to human intestinal receptors for some pathogens. Therefore, glycans can inhibit the binding of pathogens to these receptors and thus an onset of infectious diarrhea (Morrow *et al.* 2005).

In addition to glycans human milk contains other components of the natural immune system, which are usually divided into two groups: biologically polyfunctional (fats, lactoferrin, α-lactalbumin) and immunomodulatory (cytokines, soluble cytokine receptors, nucleic acids, antioxidants).

Among fats the most important are free fatty acids (FFAs) and monoacylglycerols, which are released from the milk triacylglycerols in the stomach. Compared to adult individuals, in breast-fed infants this release takes place faster. As a result of that the stomach content of the breast-fed infants contains greater quantities of FFAs and monoacylglycerols that have antiviral, antibacterial and antiprotozoal activities. Thus in the case of breast-fed infants their stomach can act as a barrier against the consumed pathogens. The stomach of infants fed with a formula does not have such capacity. FFAs, such as oleic or linoleic acid, increase also the antimicrobial activity of milk peptides (Isaacs 2005).

Lactoferrin either directly or indirectly through lactoferricin, which is produced through its proteolytic degradation, exerts antioxidant, antimicrobial and immunomodulatory effects.

α-Lactalbumin is a part of an enzyme complex that synthesizes lactose and it is at the same time the essential protein of human milk. In the infant's stomach this protein is modified to a conformation which exerts an important antitumor activity. This conformation is stabilized by free oleic acid. In this way an active complex is formed. It was assigned the acronym HAMLET – human α-lactalbumin made lethal to tumor cells (Gustafsson *et al.* 2005). HAMLET binds to normal as well as tumor cells. However, it is transported only to the nucleus of tumor cells, where it inhibits links between histones and DNA, resulting in apoptosis. The animal models have shown that this mechanism inhibits the growth of many malignant tumors.

In breast milk there are several mediators of natural immunity that not just reduce the risk of cancer, but also infections, allergies and autoimmunity (Armogida *et al.* 2004). These include some cytokines, cytokine receptors, defensins, cathelicidins, receptors similar to toll – TLRs (toll-like receptors), including their agonists and antagonists, anti-inflammatory agents and nucleotides controlling the inflammatory reaction (Newburg and Walker 2007).

In human colostrum and milk >30 different cytokines were found, including IL-8, IL-12, epidermal growth factor – EGF, angiogenin, transforming growth factor β-2 – TGFβ-2, monocyte chemotactic protein 1 – MCP-1. The concentrations of individual cytokines differ in milk samples from different mothers, and they vary also depending on the time that elapsed since the delivery (Kverka *et al.* 2007).

The composition of breast milk during lactation is subject to changes that depend on the developmental need of an infant. The concentrations of growth factors and protective immunoproteins (including sIgA, lactoferrin and lysozyme) in the colostrum are high. At the next lactation stage the concentrations of growth factors and antimicrobial proteins drop and the concentration of casein increases, which serves also as a source of amino acids (Donovan 2006).

Human milk has significant antioxidant activities, which include enzymes and scavengers of oxygen radicals. The enzymes include superoxide dismutase (it catalyzes the dismutation of superoxide anion into oxygen and hydrogen peroxide), catalase (it catalyzes the decomposition of hydrogen peroxide), and glutathion peroxidase (it decomposes hydrogen peroxide and organic peroxides). The function of oxygen radical scavengers is exhibited by cysteine and vitamins C and E (Tsopmo and Friel 2007). Antioxidant activity of milk is especially important in prematurely born infants, who in order to maintain the normal arterial oxygen pressure require its higher concentrations in inhaled air. This, however, has a side effect – higher generation of reactive oxygen radicals. As a protection against their damaging effect this has to be combined with antioxidant defense (Friel *et al.* 2002).

9 CONCLUSIONS

In addition to macro- and micro-nutrients milk contains many biologically active agents that started to be studied in the late 1980s. The primary structure of milk proteins contains encrypted peptides with multiple biological activities. These peptides become physiologically active through the digestion of the milk proteins by the host's enzymes, or by enzymes of lactic acid bacteria during production of cheeses and fermented milks. These peptides directly influence numerous biological processes evoking gastrointestinal, immunological, neurological, hormonal and nutritional responses. Bioactive milk components exert many effects favorable for human health, and they significantly contribute to the prevention of several diseases, including hypertension, coronary vascular diseases, obesity, osteoporosis, cancer – especially of large intestine, and diabetes of type II, as well as of some transmissible diseases. Several applications of bioactive milk's components have already evolved. For example, phosphopeptides derived from casein are currently used as both dietary and pharmaceutical supplements (Reynolds 1999; Aimutis 2004). Several milk-derived growth factors are nowadays increasingly used in pharmaceuticals products, such as in the treatment of skin disorders and gastrointestinal diseases (Pouliot and Gauthier 2006). Health promoting effects in humans were observed in leg ulcers and psoriasis (Smithers 2004; Paulin *et al.* 2005), in gut health (Fell *et al.* 2000) and in tissue bone regeneration – osteoporosis (Toba *et al.* 2001). Although in terms of prevention of chronic nontransmissible diseases the effects of some milk components are impressive, health nutrition experts assume that their complex action in sufficient abundance can increase the resistance of the organism against risk-bearing environmental factors.

Epidemiological studies confirm that higher consumption of fats, fresh meat, high intake of total joules combined with low intake of fruits, vegetables, fiber and milk products increases the risk of colorectal cancer. Unbalanced food and a complex of habits that are called incorrect lifestyle significantly contribute to the epidemiological expansion of nontransmissible civilization diseases. A certain role in this has also been played by myths that consumption of milk can cause different health problems. The people, who worship the myths on unhealthiness of milk, which as they claim is the cause of obesity, acne, asthma and especially overproduction of mucous substances, promote the concept that cow milk is only good for vealers. Dissemination of such myths goes hand in hand with significant reduction of milk consumption. It should be stressed that so far there has not been a single competent study confirming the afore-mentioned myths (Pinnock *et al.* 1990; Pinnock and Arney 1993; Wijga *et al*. 2003; Wuthrich *et al.* 2005). In contrast, milk and other milk products that are the main source of calcium and other essential nutrients help to reduce the risk of majority of chronic diseases and so contribute to a healthier lifestyle (Van der Meer *et al*. 1998). The functional proteins, bioactive peptides, essential FAs, calcium, vitamin D and other milk components exert different positive effects on the immune and cardiovascular systems, as well as gastrointestinal tract and intestinal health. Based on the studies of the health-promoting activities of lipids, the possibility of using such lipids as active ingredients in prophylactic and therapeutical dosage is considered (Haug *et al.* 2007; Thormar and Hilmarsson 2007). Full-fat milk has been shown to increase the mean gastric emptying time compared to half-skimmed milk (Haug *et al*. 2007). Clinical trials indicate that the consumption of recommended levels of dairy products, as part of a healthy diet, can contribute to lower blood pressure, and can reduce the risk of low bone mass. Specific peptides associated with casein and whey proteins can significantly lower blood pressure (Huth *et al.* 2006). Body fat and body-mass loss occurs when adequate calcium is provided by an equivalent amount of calcium supplied from dairy foods. High-protein diets and, in particular those that contain whey proteins, including medium-chain FAs, may reduce hunger and food intake, thereby reducing fat deposition and improving insulin sensitivity. Dietary regulation of food intake by dairy products and their component has the potential to contribute to the prevention and management of the obesity pandemic (Pfeuffer and Schrezenmeir 2006; Dunshea *et al.* 2007; Luhovyy *et al.* 2007). Milk and dairy foods also contribute substantial amounts of other essential nutrients to the diet including: calcium, phosphorus, magnesium, zinc, potassium, vitamin A, vitamin B_{12}, riboflavin and others.

REFERENCES

Agerholm-Larsen L., Raben A., Haulrik N., Hansen A.S., Manders M., Astrup A.: Effects of 8 weeks intake of probiotic milk products on risk factors for cardiovascular diseases. *Eur.J.Clin.Nutr*. **54**, 288–297 (2000).

Aimutis W.R.: Bioactive properties of milk proteins with particular focus on anticariogenesis. *J.Nutr.* **134**, 989S–995S (2004).

Alférez M.J.M., Barrionuevo M., López Aliagua I., Sanz Sampelayo M.R., Lisbona F., Campos M.S.: The digestive utilization of goat and cow milk fat in malabsorption syndrome. *J.Dairy Sci.* **68**, 451–461 (2001).

Armogida S.A., Yannaras N.M., Melton A.L., Srivastava M.D.: Identification and quantification of innate immune system mediators in human breast milk. *Allergy Asthma Proc.* **25**, 297–304 (2004).

Babayan V.K.: Medium chain length fatty acids esters and their medical and nutritional applications. *J.Am.Oil Chem.Soc.* **59**, 49A–52A (1981).

Beaulieu J., Dupont C., Lemieux P.: Whey proteins and peptides: beneficial effects on immune health. *Therapy* **3**, 69–78 (2006).

Bellamy W.R., Yamauchi K., Wakabayashi H., Takase M., Shimamura S., Tomita M.: Antifungal properties of lactoferricin, a peptide derived from the N-terminal region of bovine lactoferrin. *Lett.Appl.Microbol.* **18**, 230–233 (1994).

Bergendi Ľ., Beneš L., Ďuračková Z., Ferenčík M.: Chemistry, physiology and pathology of free radicals. *Life Sci*. **65**, 1865–1874 (1999).

Bertolami M.C., Faludi A.A., Batlouni M.: Evolution of the effects of a new fermented milk product (Gaio) on primary hypercholesterolemia. *Eur.J.Clin.Nutr.* **53**, 97–101 (1999).

Biancone L., Monteleone I., Blanco G.D., Vavassori P., Pallone F.: Resident bacterial flora and immune system. *Digest.Liver Dis*. **34**, 537–543 (2002).

Böttcher M.F., Jenmalm M.C., Björkstén B.: Cytokine, chemokine and secretory IgA level in human milk in relation to atopic disease and IgA production in infants. *Pediatr.Allergy Immunol.* **14**, 35–41 (2003).

Bounous G., Gold P.: The biological activity of undenatured dietary whey proteins: role of glutathione. *Clin.Invest.Med.* **14**, 296–309 (1991).

Brandsch M., Brust P., Neubert K., Ermisch A.: β-Casomorphins – chemical signals of intestinal transport systems, pp. 207–219 in V. Brantl, H. Teschemacher (Eds): *β-Casomorphins and Related Peptides: Recent Developments.* VCH, Weinheim (Germany) 1994.

Brody E.P.: Biological activities of bovine glycomacropeptide. *Brit.J.Nutr.* **84** (Suppl. 1), S39–S46 (2000).

Brown E.M.: Interaction of β-lactoglobulin and α-lactalbumin with lipids: a review. *J.Dairy Sci.* **67**, 713–722 (1984).

Calligaris S., Manzocco L., Anese M., Nicoli M.C.: Effect of heat-treatment on the antioxidant and peroxidant activity of milk. *Internat.Dairy J.* **14**, 421–427 (2004).

Cho Y., Batt C.A., Sawyer L.: Probing the retinol-binding site of bovine β-lactoglobulin. *J.Biol.Chem.* **269**, 1102–1107 (1994).

Clancy R.: Immunobiotics and the probiotic evolution. *FEMS Immunol.Med.Microb*. **38**, 9–12 (2003).

Clare D.A., Swaisgood H.E.: Bioactive milk peptides: a prospectus. *J.Dairy Sci*. **83**, 1187–1195 (2000).

De Lorgelil M., Renaud S., Mamelle N., Salen P., Martin J.L., Monjaud I., Guidollet J., Touboul P., Delaye J.: Mediterranean α-linoleic acid rich in secondary prevention of coronary heart disease. *Lancet* **343**, 1454–1459 (1994).

De Witt J.N., van Hooydonk A.C.M.: Structure, function and applications of lactoperoxidase in natural antimicrobial systems. *Netherlands Milk Dairy J.* **50**, 227–244 (1996).

Dhiman T.R., Nam S.-H.N., Ure A.L.: Factors affecting conjugated linoleic acid content in milk and meat. *Crit.Rev.Food Sci.Nutr.* **45**, 463–482 (2005).

Donovan S.M.: Role of human milk components in gastrointestinal development: current knowledge and future needs. *J.Pediatr*. **149** (Suppl. 1), S49–S61 (2006).

Dunshea F.R., Ostrowska E., Ferrari J.M., Gill H.S.: Dairy proteins and the regulation of satiety and obesity. *Austral.J.Exper. Agric.* **47**, 1051–1058 (2007).

Elfstrand L., Mansson H.L., Paulsson M., Hyberg L., Akisson B.: Immunoglobulins, growth factors and growth hormone in bovine colostrum and effects of processing. *Internat.Dairy J.* **12**, 879–887 (2002).

Farnworth E.R.: Kefir – a complex probiotic. *Food Sci.Technol.Bull.* **2**, 1–17 (2005).

Fell J.M., Paintin M., Arnaud-Battandier F., Beattie R.M., Hollis A., Kitching P., Donnet Hughes A., MacDonald T.T., Walker Smith J.A: Mucosal healing and a fall in mucosal pro-inflammatory cytokine mRNA induced by a specific oral polymeric diet in pediatric Crohn's disease. *Aliment.Pharmacol.Ther.* **14**, 281–289 (2000).

Ferenčík M., Ebringer L.: Probiotics, allergy and asthma. (In Slovak) *Alergie* **5**, 224–230 (2003)

Field C.J.: The immunological components of human milk and their effect on immune development in infants. *J.Nutr.* **135**, 2–4 (2005).

Filipčík P., Cente M., Ferenčík M., Hulín I., Novák M.: The role of oxidative stress in the pathogenesis of Alzheimer's disease. *Bratisl.Med.J.* **107**, 384–394 (2006).

Fitzgerald R.K., Murray B.A.: Bioactive peptides and lactic farmentations. *Internat.J.Dairy Technol.* **59**, 118–125 (2006).

Forssén K.M., Jägerstadt M.I., Wigertz K., Witthoft C.N.: Folates and dairy products: a critical update. *J.Am.Coll.Nutr.* **19**, 100S–110S (2000).

Fox P.F.: Indigenous enzymes in milk, pp. 447–467 in P.F. Fox, P.L.H. Sweeney (Eds): *Advanced Dairy Chemistry, Vol. 1, Proteins*. Kluwer Academic–Plenum Publishers, New York 2003.

Friel J.K., Martin S.M., Langdon M., Herzberg G.R., Buettner G.R.: Milk from mothers of both premature and full-term infants provides better antioxidant protection than does infant formula. *Pediatr.Res.* **52**, 612–618 (2002).

Galdeano C.M., Perdigon G.: The probiotic bacterium *Lactobacillus casei* induces activation of the gut mucosal immune system through innate immunity. *Clin.Vacc.Immunol.* **13**, 219–226 (2006).

Garcia Unciti M.: Therapeutic utility of the medium-chain triglycerides. Ketogenic diets in infantile epilepsy. (In Italian) *Nutr.Clin.* **16**, 7–35 (1996).

Garofalo R.P., Goldman A.S.: Cytokines, chemokines, and colony-stimulating factors in human milk: the 1997 update. *Neonatology* **74**, 134–142 (1998).

German J.B.: Butyric acid – a role in cancer prevention. *Nutr.Bull.* **24**, 293–299 (1999).

German J.B., Dillard C.J.: Saturated fats: what dietary intake? *Am.J.Clin.Nutr.* **80**, 550–559 (2004).

German J.B., Dillard C.J.: Composition, structure and absorption of milk lipids: a source of energy, fat-soluble nutrients and bioactive molecules. *Crit.Rev.Food Sci.Nutr.* **46**, 57–92 (2006).

Gibson G.R., Probert H.M., Van Los J., Rastall R.A., Roberfroid M.B.: Dietary modulation of the human colonic microbiota: updating the concept of prebiotics. *Nutr.Res.Rev.* **17**, 259–275 (2004).

Gill H.S., Guarner F.: Probiotics and human health: a clinical perspective. *Postgrad.Med.J.* **80**, 516–526 (2004).

Gilliland S.E., Nelson C.R., Maxwell C.: Assimilation of cholesterol by *Lactobacillus acidophilus*. *Appl.Environ.Microbiol.* **49**, 377–381 (1985).

Grappin R., Beuvier E.: Possible implications of milk pasteurization on the manufacture and sensory quality of ripened cheese. *Internat.Dairy J.* **7**, 751–761 (1997).

Grundy S.M.: Influence of stearic acid on cholesterol metabolism relative to other long-chain fatty acids. *Am.J.Clin.Nutr.* **60**, 986S–990S (1994).

Guarner F., Schaafsma G.J.: Probiotics. *Internat.J.Food Microbiol.* **39**, 237–238 (1998).

Gustafsson L., Biers C., Hallgren O., Mossberg A.K., Pettersson J., Fischer W., Aronsson A., Svanborg C.: HAMLET kills tumor cells by apoptosis: structure, cellular mechanisms, and therapy. *J.Nutr.* **135**, 1299–1303 (2005).

Hachelaf W., Boukrelda M., Coquin P., Desjeux J.F., Boudraa G., Touhami M.: Digestibility of goat milk fat in children with a digestive origin malnutrition. (In French) *Lait* **73**, 593–599 (1993).

Halliwell B., Gutteridge M.C.: *Free Radicals in Biology and Medicine*, 2nd ed. Clarendon Press, Oxford (UK) 1989.

Harrison R.: Structure and function of xanthin oxidoreductase: where we are now? *Free Rad.Biol.Med.* **33**, 774–797 (2002).

Hartmann R., Meisel H.: Food-derived peptides with biological activity: from research to food applications. *Curr.Opin.Biotechnol.* **18**, 163–169 (2007).

Haug A., Høstmark A.T., Harstad O.M.: Bovine milk in human nutrition – a review. *Lipids Health Dis.* **6**, 25 (2007).

Hayashida K., Kaneko T., Takeuchi T., Shimizu H., Ando K., Harada E.: Oral administration of lactoferrin inhibits inflammation and nociception in rat adjuvant-induced arthritis. *J.Vet.Med.Sci.* **66**, 149–154 (2004).

Hernández-Ledesma B., Amigo L., Recio I., Bartolomé B.: ACE-inhibitory and radical-scavenging activity of peptides derived from β-lactoglobulin *f* (19-25). Interactions with ascorbic acid. *J.Agric.Food Chem.* **55**, 3392–3397 (2007).

Huth P.J., DiRienzo D.B., Miller G.D.: Major specific advances with dairy foods in nutrition and health. *J.Dairy Sci.* **89**, 1207–1221 (2006).

Iigo M., Kuhara T., Ushida Y., Sekine K., Moore M.A., Tsuda H.: Inhibitory effects of bovine lactoferrin on colon carcinoma 26 lung metastasis in mice. *Clin.Exp.Metastasis* **17**, 35–40 (1999).

Isaacs C.E.: Human milk inactivates pathogen individually, additively, and synergistically. *J.Nutr.* **135**, 1286–1288 (2005).

Isawa M., Kaito M., Ikoma J.: Lactoferrin inhibits hepatitis C virus in chronic hepatitis C patients with high viral loads and HVC genotype 1b. *Am.J.Gastroent.* **97**, 766–767 (2002).

Isolauri E., Sutas Y., Kankaapää P., Arvilommi H., Salminen S.: Probiotics: effect on immunity. *Am.J.Clin.Nutr.* **73** (Suppl.) 444S–450S (2001).

Jaziri M., Migliore-Samour D., Casablanca-Pigred M.R., Keddat K., Morgat J.L., Jolles P.: Specific binding sites on human phagocytic blood cells for Gly-Leu-Phe and Val-Glu-Pro-Ile-Pro-Tyr, immunostimulatory peptides from human milk proteins. *Biochim.Biophys.Acta* **1160**, 251–261 (1992).

Jenssen H.: Anti-herpes simplex virus activity of lactoferrin/lactoferricin – an example of antiviral activity of antimicrobial protein/peptide. *Cell.Mol.Life Sci.* **24**, 3302–3313 (2005).

Kaito M., Iwasa M., Fujita N., Kobayashi Y., Kojima Y., Ikoma J., Imoto I., Adachi Y., Hamano H., Yamauchi K.: Effect of lactoferrin inpatients with chronic hepatitis C: combination therapy with interferon and ribavirin. *J.Gastroent.Hepatol.* **22**, 1984–1997 (2007).

Kaizu H., Sasaki M., Nakajima H.: Effect of antioxidative lactic acid bacteria on rats fed a diet deficient in vitamin E. *J.Dairy Sci*. **46**, 2493–2499 (1993).

Kang S.H., Kim J.U., Imm J.Y., Oh S., Kim S.H.: The effects of dairy processes and storage on insulin-like growth factor-1 (IGF-1) content in milk and in model fortified dairy product. *J.Dairy Sci.* **89**, 402–409 (2006).

Kepler C.R., Tove S.B.: Biohydrogenation of unsaturated fatty acids. *J.Biol.Chem.* **242**, 5682–5686 (1967).

Khanal R.C., Olson K.C.: Factors affecting conjugated linoleic acid (CLA) content in milk, meat, and egg: a review. *Pakistan J.Nutr.* **3**, 82–98 (2004).

Kiesling G., Schneider J., Jahreis G.: Long-term consumption of fermented dairy products over 6-months increases HDL cholesterol. *Eur.J.Clin.Nutr*. **56**, 843–849 (2002).

Kitts D.D., Yuan Y.V.: Caseinophosphopeptides and calcium bioavailability. *Trends Food Sci.Technol*. **3**, 31–35 (1992).

Koppová I., Lukáš F., Kopečný J.: Effect of fatty acids on growth of conjugated-linoleic-acids-producing bacteria in rumen. *Folia Microbiol.* **51**, 291–293 (2006).

Korhonen H., Pihlanto A.: Bioactive peptides: production and functionality. *Internat.Dairy J.* **16**, 945–960 (2006).

Kullisaar T., Songisepp E., Mikelsaar M., Zilmer K., Vihalemm T., Zilmer M.: Antioxidative probiotic fermented goats' milk decreases oxidative stress-mediated atherogenicity in human subjects. *Brit.J.Nutr*. **90**, 449–456 (2003).

Kverka M., Buriánová J., Lodinová-Žádníková R., Kocourková I., Cinová J. Tučková L., Tlaskalová-Hogenová H.: Cytokine profiling in human colostrum and milk by protein array. *Clin.Chem.* **53**, 955–962 (2007).

Lahov E., Regelson W.: Antibacterial and immunostimulating casein-derived substances from milk: casecidin, isracidin peptides. *Food Chem.Toxicol*. **34**, 131–145 (1996).

Lerebours E., N'Djitoyap Ndam C., Lavoine A., Hellot M., Antoine J.M., Collin R.: Yoghurt and fermented-then-pasteurized milk: effects of short-term and long-term ingestion on lactose absorption and mucosal lactase activity in lactase-deficient subjects. *Am.J.Clin.Nutr*. **49**, 823–827 (1989).

Ling M.Y., Yen C.L.: Antioxidative ability of lactic acid bacteria. *J.Agric.Food Chem*. **47**, 1460–1466 (1999).

Liu Q., Raina A., Smith M., Sayre L., Perry G.: Hydroxynonenal, toxic carbonyls, and Alzheimer disease. *Molec.Aspects Med*. **24**, 305–313 (2003).

Ljungh A., Lan J., Yanagisawa N.: Isolation, selection and characteristics of *Lactobacillus paracasei* subsp. *paracasei* F16. *Microb. Health Dis*. **3** (Suppl.), 4–6 (2002).

Luhovyy B.L., Akhavan T., Anderson G.H.: Whey proteins in the regulation of food intake and satiety. *J.Am.Coll.Nutr.* **26**, 704S–712S (2007).

MacFarlane S., MacFarlane G.T., Cummings J.H.: Review article: prebiotics in the gastrointestinal tract. *Aliment.Pharmacol. Therap.* **24**, 701–714 (2006).

Machnicki M., Zimecki M., Zagulski T.: Lactoferrin regulates the release of tumor necrosis factor-α and interleukin-6 *in vivo*. *Internat.J.Exp.Pathol.* **74**, 433–439 (1993).

MacRae J., O'Reilly L., Morgan P.: Desirable characteristics of animal products from a human health perspectives. *Livestock Prod.Sci.* **94**, 95–103 (2005).

Maga E.A., Anderson G.B., Culler J.S., Smith W., Murray J.D.: Antimicrobial properties of human lysozyme transgenic mouse milk. *J.Food Protect*. **62**, 51–56 (1998).

Malkoski M., Daspher S.G., O`Brien-Simpson N.M., Talbo G.H., Macris M., Cross K.J., Reynolds E.C.: Kappacin, a novel antibacterial peptide from bovine milk. *Antimicrob.Agents Chemother.* **45**, 2309–2315 (2001).

Markus C.R., Olivier B., de Haan E.H.: Whey protein rich in α-lactalbumin increases the ration of plasma tryptophan to the sum of the other large neutral amino acids and improves cognitive performance in stress-vulnerable subjects. *Am.J.Clin.Nutr.* **75**, 1051–1056 (2002).

Matar C., Valdez J.C., Medina M., Rachid M., Perdigon G.: Immunomodulating effects of milks fermented by *Lactobacillus helveticus* and its non-proteolytic variant. *J.Dairy Res*. **68**, 601–609 (2001).

McCann K.B., Shiell B.J., Michalski W.P., Lee A., Wan J., Roginski H., Coventry M.J.: Isolation and characterization of a novel antibacterial peptide from bovine α_{S1}-casein. *Internat.Dairy J.* **16**, 316–323 (2006).

Mehra R., Marnila P., Korhonen M.: Milk immunoglobulins for health promotion. *Internat.Dairy J*. **16**, 1262–1272 (2006).

Meisel H.: Biochemical properties of regulatory peptides derived from milk proteins. *Biopolymers* **43**, 118–128 (1997).

Meisel H.: Bioactive peptides from milk proteins: a perspective for consumers and producers. *Austral.J.Dairy Technol.* **56**, 83–92 (2001).

Meisel H.: Biochemical properties of peptides encrypted in bovine milk proteins. *Curr.Med.Chem.* **12**, 1905–1919 (2005).

Meisel H., Bockelmann W.: Bioactive peptides encrypted in milk proteins: proteolytic activation and tropho-functional properties. *Anthonie van Leeuwenhoek* **76**, 207–216 (1999).

Mensink R.P., Katan M.B.: Effect of dietary fatty acids on serum lipids and lipoproteins: a meta-analysis of 27 trials. *Artherioscl. Thromb.* **12**, 911–919 (1992).

Mensink R.P., Zock P.L., Kester A.D., Atan M.B.: Effects of dietary fatty acids and carbohydrates on the ration of serum total to HDL cholesterol and on serum lipids and apolipoproteins: a meta-analysis of 60 controlled trials. *Am.J.Clin.Nutr.* **77**, 1146–1155 (2003).

Meydani S.N., Ha W.-K.: Immunologic effects of yoghurt. *Am.J.Clin.Nutr*. **71**, 861–872 (2000).

Mezzaroba L.F.H., Carvalho J.E., Ponezi A.N., Antonio M.A., Monteiro K.M., Possenti A., Sgarbieri V.C.: Antiulcerative pro-perties of bovine α-lactalbumin. *Internat.Dairy J.* **16**, 1005–1112 (2006).

Mikeš Z., Ferenčík M., Jahnová E., Ebringer L., Čižnár I.: Hypocholesterolemic and immunostimulatory efects of orally applied *Enterococcus faecium* M-74 in man. *Folia Microbiol*. **40**, 639–646 (1995).

Mikeš Z., Ebringer L., Boča M., Dušinský R., Jahnová E.: Some risk factors for cardiovascular diseases in using traditional Slovak sheep cheese: results of a pilot study. (In Slovak) *Geriatria* **1**, 29–36 (2005).

Mistry N., Drobni P., Nasland J., Sunkari V.G., Jenssen H., Evander M.: The antipapillomavirus activity of human and bovine lactoferricin. *Antivir.Res.* **75**, 258–265 (2007).

Miyauchi H., Hashimoto S., Nakajima M., Shinoda I., Fukuwatari Y., Hayasawa H.: Bovine lactoferrin stimulates the phagocytic activity of human neutrophils: identification of its active domain. *Cell.Immunol.* **187**, 34–37 (1998).

Mizushima S., Ohshige K., Watanabe J., Kimura M., Kadowaki T., Nakamura Y., Tochikubo O., Ueshima H.: Randomized controlled trial of sour milk and blood pressure in borderline hypertensive men. *Am.J.Hypertens.* **17**, 701–706 (2004).

Montagne P.M., Tregoat V.S., Cuilliere M.L., Bene M.C., Taure G.C.: Measurement of nine human milk proteins by nephelometric immunoasssays: application to the determination of nature milk protein profile. *Clin.Biochem.* **33**, 181–186 (2000).

Morgan F., Bodin J.-P., Gaborit P.: Link between goat milk lipolysis and sensorial quality of lactic goat cheeses made from raw or pasteurized milk. *Lait* **81**, 743–746 (2001).

Morrow A.L., Ruiz-Palacios G.M., Jiang X., Newburg D.S.: Human-milk glycans inhibit pathogen binding protect breast-feeding infants against infectious diarrhea. *J.Nutr.* **135**, 1304–1307 (2005).

Nagaoka S., Futamura Y., Miwa K., Awano T., Yamauchi K., Kanamaru Y., Tadashi K., Kuwata T.: Identification of novel hypocholesterolemic peptides derived from bovine milk β-lactoglobulin. *Biochem.Biophys.Res.Commun.* **281**, 11–17 (2001).

Newburg D.S.: Innate immunity and human milk. *J.Nutr.* **135**, 1308–1312 (2005).

Newburg D.S., Ruiz-Palacios G.M., Morrow A.L.: Human milk glycans protect infants against enteric pathogens. *Ann.Rev.Nutr.* **25**, 37–58 (2005).

Newburg D.S., Walker W.A.: Protection of the neonate by the innate immune system of developing gut and of human milk. *Pediat.Res.* **62**, 2–8 (2007).

Ochoa J.J., Farguharson A.J., Grant I., Moffat M.E., Heys S.D., Wahle K.W.: Conjugated linoleic acid (CLAs) decrease prostate cancer cell proliferation: different molecular mechanisms for *cis* 9,*trans* 11 and *trans* 10,*cis* 12 isomers. *Carcinogenesis* **25**, 1185–1191 (2004).

Odriozola-Serrano I., Bendicho-Porta S., Martin-Belloso O.: Comparative study on shelf life of whole milk processed by high-intensity pulsed electric field or heat treatment. *J.Dairy Sci.* **89**, 905–911 (2006).

Parodi P.W.: Cow's milk folate binding protein: its role in folate nutrition. *Austral.J.Dairy Technol.* **52**, 109–118 (1997).

Parodi P.W.: Conjugated linoleic acid and other anticarcinogenic agents of bovine milk fat. *J.Dairy Sci.* **82**, 1339–1349 (1999).

Parodi P.W.: Milk in human nutrition. *Austral.J.Dairy Technol.* **59**, 3–59 (2004).

Paulin Y., Pouliot Y., Lamiot E., Aattouri N., Gauthier S.F.: Safety and efficacy of a milk-derived extract in the treatment of plaque psoriasis: an open label study. *J.Cutan.Med.Surg.* **9**, 271–275 (2005).

Pecquet S., Bovetto L., Maynard F., Fritsche R.: Peptides obtained by tryptic hydrolysis of bovine β-lactoglobulin induce specific oral tolerance in mice. *J.Allergy Clin.Immunol.* **105**, 514–521 (2000).

Pellegrini A.: Antimicrobial peptides from food proteins. *Curr.Pharmaceut.Design* **9**, 1225–1238 (2003).

Pereira D., Gibson G.R.: Cholesterol assimilation by lactic acid bacteria and bifidobacteria. *Appl.Environ.Microbiol.* **68**, 4689–4693 (2002).

Pihlanto-Leppälä A., Koskinen P., Piilola K., Tupasela T., Korhonen H.: Angiotensin I-converting enzyme inhibitory properties of whey protein digests: concentration and characterization of active peptides. *J.Dairy Res.* **67**, 53–64 (2000).

Pinnock C.B., Arney W.K.: The milk-mucus believe: sensory analysis comparing cow's milk and a soy placebo. *Appetite* **20**, 61–67 (1993).

Pinnock C.B., Graham N.M., Mylvaganam A., Douglas R.M.: Relationship between intake and mucus production in adult volunteers challenged with rhinovirus-2. *Am.Rev.Resp.Dis.* **141**, 352–356 (1990).

Possemiers S., Van Camp J., Bolca S., Verstraete W.: Characterization of the bactericidal effect of dietary sphingosine and its activity under intestinal conditions. *Internat.J.Food Microbiol.* **105**, 59–70 (2005).

Pouliot Y., Gauthier S.F.: Milk growth factors as health products: some technological aspects. *Internat.Dairy J.* **16**, 1415–1420 (2006).

Prased S., Dhiman R.K., Duseja A., Chawla Y.K., Sharma A., Agarwal R.: Lactulose improves cognitive functions and health-related quality of life in patients with cirrhosis who have minimal hepatic encephalopathy. *Hepatology* **45**, 549–559 (2007).

Rachid M., Matar C., Duarte J., Perdigon G.: Effect of milk fermented with a *Lactobacillus helveticus* R389(+) proteolytic strain on the immune system and on the growth of 471 breast cancer cells in mice. *FEMS Immunol.Med.Microbiol.* **47**, 242–253 (2006).

Rainer L., Heiss C.J.: Conjugated linoleic acid: health implications and effects on body composition. *J.Am.Diet.Assoc.* **104**, 963–968 (2004).

Razafindrakoto O., Revelomanana N., Rasolofo A., Rakotoarimanana R.D., Bourque P., Coquin P., Briend A., Desjeux J.F.: May goat milk replace cow milk in undernourished children? (In French) *Lait* **73**, 601–611 (1993).

Reiter B., Perraudin J.P.: Lactoperoxidase, biological functions, pp. 144–180 in J. Everse, K.F. Everse, B. Brisham (Eds): *Peroxidases in Chemistry and Biology, Vol. II.* CRC Press, Boca Raton (USA) (1991).

Reynolds E.C.: Anticariogenic casein phosphopeptides. *Prot.Peptides Lett.* **6**, 253–303 (1999).

Riollet C., Rainard P., Poutrel B.: Cell subpopulation and cytokine expression in cow milk in response to chronic *Staphylococcus aureus* infection. *J.Dairy Sci.* **84**, 1077–1084 (2001).

Santosa S., Farnworth E., Jones P.J.: Probiotics and their potential health claims. *Nutr.Rev.* **64**, 265–274 (2006).

Sanz Sampelayo M.R., Chilliard Y., Schmidely P., Boza J.: Influence of type of diet on the fat constituents of goat and sheep milk. *Small Rumen Res.* **68**, 42–63 (2007).

Schanbacher F.L., Talhouk R.S., Murray F.A.: Biology and origin of bioactive peptides in milk. *Liv.Prod.Sci.* **50**, 105–123 (1997).

Schrezenmeir J., de Vrese M.: Probiotics, prebiotics, and synbiotics – approaching and definition. *Am.J.Clin.Nutr.* **73** (Suppl.), 361S–364S (2001).

Schuster G.S., Dirksen T.R., Ciarlonw A.E., Burnett G.W., Reynolds M.T., Lankford M.T.: Anticaries and antiplaque potential of free fatty acids *in vitro* and *in vivo*. *Pharmacol.Ther.Dent.* **5**, 25–33 (1980).

Seppo L., Jauhiainen T., Poussa T., Korpela R.: A fermented milk high in bioactive peptides has a blood pressure-lowering effect in hypertensive subjects. *Am.J.Clin.Nutr.* **77**, 326–330 (2003).

Seifu E., Buys E.M., Donkin E.F.: Significance of the lactoperoxidase system in the dairy industry and its potential applications: a review. *Trends Food Sci.Technol.* **16**, 137–154 (2005).

Silva S.V., Malcata F.X.: Caseins as source of bioactive peptides. *Internat.Dairy J.* **15**, 1–15 (2005).

Sipola M., Finckenberg P., Korpela R., Vapaatolo H., Nurminen M.-L.: Effect of long-term intake of milk products on blood pressure in hypertensive rats. *J.Dairy Res.* **69**, 103–111 (2002).

Smithers G.W.: Isolation of growth factors from whey and their application in food and biotechnology industries – a brief review, pp. 16–19 in *Bull. No. 389, Advances in Fractionation and Separation Processes for Novel Dairy Applications*. Internat. Dairy Federation, Brussels 2004.

Songisepp E., Kullisaar T., Hutt P., Elias P., Brilene T., Zilmer M., Mikelsaar M.: A new probiotic cheese with antioxidative and antimicrobial activity. *J.Dairy Sci.* **87**, 2013–2017 (2004).

Songisepp E., Kals J., Kullisaar T., Mändar R., Hutt P., Zilmer M., Mikelsaar M.: Evolution of the functional efficacy of an antioxidative probiotic in healthy volunteers. *Nutr.J.* **4**, 22–31 (2005).

Sprong R.C., Hulstein M.F., van der Meer R.: High intake of milk fat inhibits intestinal colonization of *Listeria* but not of *Salmonella* in rats. *J.Nutr.* **129**, 1382–1389 (1999).

Sun C.Q., O'Connor C.J., Roberton A.M.: The antimicrobial properties of milk fat after partial hydrolysis by calf pregastric lipase. *Chem.Biol.Interact.* **140**, 185–198 (2002).

Svensson M., Hakansson A., Mossberg A.K., Linse C., Svanborg C.: Conversion of α-lactoglobulin to a protein inducing apoptosis. *Proc.Nat.Acad.Sci.USA* **97**, 4221–4226 (2000).

Tahri K., Grill J.P., Schneider F.: Bifidobacteria strains' behavior toward cholesterol coprecipitation with bile salts assimilation. *Curr.Microbiol.* **3**, 187–193 (1996).

Taylor M.J., Richardson T.: Antioxiodant activity of skim milk: effect of heat and resultant sulfhydryl groups. *J.Dairy Sci.* **63**, 1783–1795 (1980).

Teschemacher H., Brantl V.: Milk proteins derived atypical opioid peptides and related compounds with opioid antagonist activity pp. 3–17 in V. Brantl, T. Teschemacher (Eds): *β-Casomorphins and Related Peptides: Recent Developments.* VCH Publishers, Weinheim (Germany) 1994.

Thormar H., Isaacs E.E., Kim K.S., Brown H.R.: Interaction of visna virus and other enveloped viruses by free fatty acids and monoglycerides. *Ann.N.Y.Acad.Sci.* **724**, 465–471 (1994).

Thormar H., Hilmarsson H.: The role of microbicidal lipids in host defense against pathogens and their potential as therapeutic agents. *Chem.Phys.Lipids* **150**, 1–11 (2007).

Toba Y., Takada Y., Matsuoka Y., Morita Y., Motouri M., Hirai T., Suguri T., Aoe S., Kawakami H., Kumegawa M., Takeuchi A., Itabashi A.: Milk basic protein promotes bone formation and suppresses bone resorption in healthy adult men. *Biosci.Biotechnol.Biochem.* **65**, 1353–1357 (2001).

Trebichavský I., Šplíchal I.: Probiotics manipulate host cytokine response and induce antimicrobial peptides. *Folia Microbiol.* **51**, 507–510 (2006).

Tricon S., Burdge G.C., Kew S., Banerjee T., Russel J.J., Jones E.L., Grimble R.F., Williams C.M., Yaqoob P., Calder P.C.: Opposing effects of *cis*-9,*trans*-11 and *trans*-10,*cis*-12 conjugated linoleic acid on blood lipids in healthy humans. *Am.J. Clin.Nutr.* **80**, 614–620 (2004).

Tsopmo A., Friel J.K.: Human milk has anti-oxidant properties to protect premature infants. *Curr.Pediatr.Rev.* **3**, 45–51 (2007).

Ustundag D., Yilmaz E., Dogan Y., Akarsu S., Canatan H., Halifeoglu I., Cikim G., Aygun A.D.: Levels of cytokines (IL-1β, IL-2, IL-6, IL-8, TNF-α) and trace elements (Zn, Cu) in breast milk from mothers of preterm and term infants. *Mediat. Inflamm.* **6**, 331–336 (2005).

Van der Meer R., Bovee-Oudenhoven I.M.J., Sesink A.L.A., Kleibeuker J.H.: Milk products and intestinal health. *Internat.Dairy J.* **8**, 163–170 (1998).

Vegarud G.E., Langsrud T., Svenning C.: Mineral-binding milk proteins and peptides; occurrence, biochemical and technological characteristics. *Brit.J.Nutr.* **84**, S91–S98 (2000).

Vesper H., Schelma E., Nikolova-Karakashion M.N., Dillehay D.L., Lynch D.V., Mercill A.H.: Sphingolipids in food and the emerging importance of sphingolipids to nutrition. *J.Nutr.* **129**, 1239–1249 (1999).

Walker G., Cai F., Shen P., Reynolds C., Ward B., Fone C., Honda S., Koganei M., Oda M., Reynolds E.: Increased remineralization of tooth enamel by milk containing added casein phosphopeptide-amorphous calcium phosphate. *J.Dairy Res.* **73**, 74–78 (2006).

Wang Q., Allen J.C., Swaisgood H.E.: Binding of vitamin D and cholesterol to β-lactoglobulin. *J.Dairy Sci.* **80**, 1054–1059 (1997).

Wang W.P., Iigo M., Sato J., Sekine K., Adachi I., Tsuda H.: Activation of intestinal mucosal immunity in tumor-bearing mice by lactoferrin. *Japan.J.Cancer Res.* **91**, 1022–1027 (2000).

Weinberg E.D.: Antibiotic properties and applications of lactoferrin. *Curr.Pharmaceut.Design* **13**, 801–811 (2007).

Welsh J.K., May J.T.: Anti-infective properties of breast-milk. *J.Pediatr.* **94**, 1–9 (1979).

Wijga A.H., Smit H.A., Kerkhof M., de Jongste J.C., Gerritsen J., Neijens H.J., Boshuizen H.C., Brunekreef B.: Association of consumption of product containing milk fat with reduced asthma risk in pre-school children: the PIAMA birth cohort study. *Thorax* **58**, 567–572 (2003).

Wuthrich B., Schmid A., Walther B., Sieber R.: Milk consumption does not lead to mucus production or occurrence of asthma. *J.Am.Coll.Nutr.* **24**, 547S–555S (2005).

Yamauchi K., Wakabayashi H., Shin K., Takase M.: Bovine lactoferrin: benefits and mechanism of action against infections. *Biochem.Cell Biol.* **84**, 291–296 (2006).

Yoshida T., Owens G.K.: Molecular determinant of vascular smooth muscle diversity. *Circul.Res.* **96**, 280–291 (2005).

Yoshikawa M., Tani F., Chiba H.: Structure–activity relationship of opioid antagonist peptides derived from milk proteins, pp. 473–476 in T. Schiba (Ed.): *Peptide Chemistry*. Protein Research Foundation, Osaka (Japan) 1998.