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Dairy bioactive proteins and peptides: a narrative review

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Milk proteins are known for their high nutritional quality, based on their essential amino acid composition, and they exhibit a wide range of bioactivities, including satiety, antimicrobial, mineral-binding, and anti-lipidemic properties. Because of their unique water solubility, milk proteins are readily separated into casein and whey fractions, which can be further fractionated into many individual proteins, including alpha-S1- and alpha-S2-caseins, beta-casein, and kappa-casein, and the whey proteins alpha-lactalbumin, lactoferrin, beta-lactoglobulin, and glycomacropeptide. Many of these proteins have unique bioactivities. Further, over the past 30 years, peptides that are encrypted in the primary amino acid sequences of proteins and released along with amino acids during digestion are increasingly recognized as biologically active protein metabolites that may have beneficial effects on human health. This review examines the current state of the science on the contribution of dairy proteins and their unique peptides and amino acids to human health.

INTRODUCTION

Milk and dairy products are widely recognized as sources of dietary calcium, vitamin D, and protein and are recommended as an essential part of a healthy diet.[1] Milk is naturally a whole food, and its nutrients are among its many biologically important components in a complex structural matrix.[2] Considerable progress has been made in understanding of the biological functions of the individual components of milk and their health benefits. Milk has long been recognized as important for growth and development, and a growing body of evidence from systematic reviews and meta-analyses of well-controlled cohort studies indicates beneficial or neutral associations between the consumption of milk and dairy products and the risks of hypertension,[3–5] cardiovascular disease,[3,6,7] and type 2 diabetes[3,8–10] for adults.

Milk proteins, which are known for their high nutritional quality, based on their essential amino acid composition, also exhibit a wide range of bioactivities, including satiety, antimicrobial, mineral-binding, and anti-lipidemic properties. Further, over the past 30 years, peptides that are encrypted in the primary amino acid sequences of proteins and released along with amino acids during digestion are increasingly recognized as biologically active protein metabolites that may have beneficial effects on human health.[11]

Dairy products account for 16% of the average daily protein intake by adults in the U.S. (∼13 g/d).[12,13] If dairy consumption in the U.S. increased from the current 1.7 servings to 3 servings per day as recommended by the U.S. Dietary Guidelines for Americans,[1] the daily contribution of dairy foods to total protein intake could be to as high as 22%–31%.[14] Food pattern

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modeling studies conducted by the U.S. Dietary Guidelines Advisory Committee demonstrated that 3 daily servings of low-fat or fat-free dairy foods in healthy dietary patterns are needed to achieve recommended nutrient intakes, especially for calcium, vitamin D, and potassium, which are considered at-risk nutrients in the U.S. diet.[14]

The proteins in milk and dairy products are present within a complex matrix that encompasses diverse attributes of milk and dairy products. These attributes include structural elements (eg, casein micelles, milkfat globules), different forms (eg, solid, gel, liquid), textural features, and how dairy's nutrients and other biologically important components are compartmentalized.[15] The architecture of the dairy matrix, together with its components, positively affects digestive processes, absorption of nutrients and other compounds, and physiological functions.

This review examines the current state of the science on the contribution of dairy proteins and their unique peptides and amino acids to human health.

MILK AND DAIRY PRODUCTS

Milk is a unique food matrix that consists of 87% water, 4% lipids, and 9% water-soluble compounds (protein, lactose, and various minerals and vitamins)[16] and that allows for fractionation and isolation. Early examples of taking advantage of milk characteristics include separation of cream for making butter, and precipitation of the casein curd for cheese. Fractionation and processing methods have evolved considerably.[16–18] For example, concentrated forms of milk proteins are widely available today for use as ingredients and dietary supplements.

Milk, cheese, and yogurt are the most widely consumed dairy foods worldwide. Milk is available as whole milk, lower-fat milks, and non-fat milk, in both liquid and powder forms. The many varieties of cheese are made from raw or pasteurized milk and broadly characterized as natural or processed cheeses. In cheese production, heat treatment, bacterial fermentation, culture times, pH, the sequence of processing steps, use of salting or brining, and block formation processes vary depending on the type of cheese. Bacterial starter cultures (ie, lactic acid bacteria) produce lactic acid, which lowers the pH and aids with coagulation. Lactic acid, together with the addition of the enzyme rennet, and heat treatment cause casein to coagulate, forming the cheese curd and the aqueous whey fraction.

Yogurt is a fermented milk product that is produced using bacterial cultures. Varieties of yogurt include plain, Greek-style, and sweetened flavored yogurt. In yogurt production, the starter cultures, typically *Lactobacillus bulgaricus* and *Streptococcus thermophilus*, ferment lactose to produce lactic acid, which lowers the pH and aids in formation of the soft protein gel that is characteristic of yogurt. Fermented milks, such as cultured milk and sour milk, also are produced using one or more lactobacilli strains. *Lactobacillus* species not only form lactic acid, but also hydrolyze milk proteins, releasing free amino acids (which are needed for their growth) and peptides.[19] The pattern of amino acids and peptides produced varies considerably depending on the species and strain of *Lactobacillus* bacteria and the culture conditions.

Homogenization and pasteurization of milk for consumption or further processing employ well-established standards.[16] Homogenization reduces the size of milkfat globules and evenly distributes the milkfat in the liquid dairy matrix. Pasteurization destroys pathogens, reduces spoilage microorganisms, and inactivates endogenous enzymes that may decrease the quality of the milk. The most common heat treatment is high-temperature short-time (HTST, 72°C, minimum of 15 s).[18,20] Ultra-high-temperature pasteurization (UHT; 135–150°C, minimum of 2 s) is more common in some countries[21] and has the benefits of allowing longer shelf life and storage at room temperature when packaged aseptically.[22] Dairy processing standards and country-specific regulations across the globe provide for the safe consumption of dairy foods, consistency in composition and labeling, and quality assurance for global trade.

Physicochemical changes in dairy proteins can occur during the processing of milk and dairy products.[23] Depending on the exact processing conditions, the modifications to whey and/or casein may include denaturation, aggregation, glycation, oxidation, cross-linking, racemization, and dephosphorylation. A systematic review of descriptive research, in vitro digestibility models, animal studies, and a single human clinical trial[21] found no overall effect of heat treatments used in processing liquid milk on dairy protein digestibility or bioavailability.[21,23] In vitro and animal studies similarly demonstrated no effects of dairy protein denaturation on overall protein digestibility, but glycation was shown to decrease dairy protein digestibility and amino acid availability.[23] Research on other processing effects is limited mainly to descriptive studies. Human clinical research examining dairy processing effects on health-related outcomes, however, is scarce.[21,24–26] Although there is a lack of human trials evaluating the human health implications of dairy processing effects on casein and whey proteins, decades of research on the nutritional quality and human health benefits of dairy proteins have been conducted with milk and dairy products produced using processes consistent with industry and regulatory standards.

DAIRY PROTEINS

The major proteins in milk are casein and whey. The minor proteins (which are mainly components of the outer layer of milkfat globule membranes and include membrane globular proteins, glycoproteins, and lipoproteins) account for less than 2% of total milk proteins. Casein proteins form aggregates within the milk matrix that give milk its white color. The biological activities of caseins derive from their amino acid content and their mineral-carrying activity, which helps in the passive absorption of minerals.

Whey is the water fraction resulting from cheesemaking and contains dozens of high-quality proteins (with respect to essential amino acid content, bioavailability, and bioactivities).[27]

Of the 9 essential amino acids, whey proteins are notably rich in lysine, methionine, leucine, and tryptophan, which are generally the most limiting amino acids in other foods, and 1 or more of these 4 amino acids are always limiting in plant proteins.[28] Protein quality scores (ie, Digestible Indispensable Amino Acid Scores; DIAASs) for each of these amino acids range from 2.3 to 3.3 for whey proteins, while the DIAASs range from 0.9 to 1.0 for soy protein isolate and are less than 0.8 for wheat proteins.[29] The exceptional protein quality makes milk proteins, and specifically whey proteins, ideal for use as complementary proteins and for creating balanced meals.

Whey proteins

Whey protein gained attention for its protein quality in the late 1990s with the confluence of multiple research areas that recognized the importance of skeletal muscle in maintaining both functional mobility and cardiometabolic health. Aging, weight management, type 2 diabetes, bed rest, acute injury, and chemotherapy are all conditions in which body composition, muscle mass, and metabolic health are determinates of long-term outcomes.[27,30–32] The key finding that linked whey protein to skeletal muscle health was the discovery of the role of mechanistic target of rapamycin (mTOR) in regulating post-meal anabolic responses in skeletal muscle. mTOR is an intracellular protein complex that integrates multiple nutrients, energy, and hormonal signals to determine whether the cell environment can support an anabolic response of protein synthesis.[33] Regulation of mTOR differs across tissues and with age. In skeletal muscle, mTOR integrates 4 signals: (1) hormones (insulin and IGF-1), (2) energy (ATP status via AMPK), (3) physical activity (REDD1), and (4) amino acid concentrations (specifically, the branched-chain amino acid leucine).[34] When these components are correctly balanced, mTOR triggers the initiation phase of protein synthesis in skeletal muscle. In children and young adults, growth hormones and energy dominate the mTOR signal response, while in adults the critical signal is the intracellular leucine concentration.

The importance of the mTOR signal and the high concentration of leucine in whey protein can only be appreciated in the context of daily protein turnover. Every day, adults need to make 250–to 300 g of new proteins to repair and replace existing proteins. This process occurs with continuous cycling between the synthesis and breakdown of proteins, and while skeletal muscle accounts for approximately 50% of total body protein, it receives only about 25% of the newly formed proteins.[35] The body prioritizes de novo protein synthesis to essential organs, including the liver, heart, and GI tract. This distribution of new proteins is important for understanding age-related loss of skeletal muscle (ie, sarcopenia), and it becomes critically important during acute catabolic conditions such as weight loss for treatment of obesity, during bed rest for illness or surgery, and after intense exercise.[36,37]

During post-absorptive periods, such as during overnight fasting, the mTOR signal in skeletal muscle is turned off, reducing protein synthesis, and shifting muscle to net protein breakdown. Amino acids released from muscle proteins become available for protein synthesis and energy use in essential organs such as the liver, spleen, and GI tract. This cycling of protein turnover allows muscle to serve as a type of amino acid reservoir for essential organs during periods of fasting, starvation, or stress. This signal remains catabolic until a meal provides adequate leucine to stimulate mTOR. Extensive research has shown this triggering threshold for mTOR to require a minimum of 2.5 g of leucine.

Leucine accounts for 11% to 12% of whey proteins, approximately 8% to 9% of animal proteins (eggs, meats, and fish) and approximately 6% to 8% of plant proteins (soy, pea, wheat, oat, and quinoa).[28] Hence, a meal with 20–25 g of whey protein can provide the leucine threshold of 2.5 g, while meats require approximately 30 g of protein and plants require 35–40 g of protein to provide adequate leucine to trigger the post-meal anabolic response in skeletal muscle.[37] Whey proteins have gained extensive use for anabolic recovery, generating maximum response with the least total protein and least total calories, which are important considerations for weight management, conditions of limited food intake such as illness or aging, or for post-exercise recovery, including muscle-building use for athletes.[38]

Alpha-lactalbumin

Whey protein has also become an important source of individual proteins with unique bioactivities. The most studied of the individual proteins in the whey fraction are alpha-lactalbumin (α-Lac), lactoferrin, and glycomacropeptide.[27] Alpha-lactalbumin accounts for approximately 22% of the total protein and 36% of the whey protein in human milk. It is a water-soluble protein containing 129 amino acids and is notable for its comparatively high content of tryptophan, lysine, cysteine, and the branched-chain amino acids. Beyond its high protein quality supporting growth and development, α-Lac exhibits diverse bioactivities involved in sleep, mood, gastrointestinal function, mineral absorption, and immunity. There is no consensus about the precise mechanism(s) for these observed bioactivities, but most research points to the unique amino acid composition and specifically the tryptophan and cysteine concentrations in α-Lac.[28]

Alpha-lactalbumin contains nearly 4 times the level of tryptophan compared with that of egg, beef, soy, or wheat proteins.[28] Tryptophan is the direct precursor of serotonin, the neurotransmitter produced in the brain, kidney, lung, and the gut epithelial cells. In the brain, serotonin has been shown to enhance sleep, improve mood, and regulate food intake. Tryptophan supplements have been shown to increase serotonin production, enhancing sleep, including sleep latency (time to fall asleep), sleep depth, and sleep duration. Although research using α-Lac as a source of tryptophan is less consistent, a 20-g α-Lac supplement providing 4.8 g of tryptophan has been shown to enhance sleep quality.[39]

Alpha-lactalbumin also has a unique ratio of cysteine/methionine of nearly 5:1 (compared with ratios of approximately 1:2 in egg, beef, soy, or wheat proteins), plus the α-Lac structure contains 4 unique disulfide bridges, contributing to its tertiary configuration. Cysteine is the amino acid precursor of glutathione and taurine, and the disulfide bridges appear to contribute to prebiotic-like characteristics of α-Lac.[28]

Alpha-lactalbumin, along with other whey proteins, including lactoferrin and immunoglobulins, exert diverse physiological roles on gastrointestinal function, including cell development, motility, and antimicrobial activity.[28] The activity of α-Lac may be associated with tryptophan and serotonin production, cysteine as a direct precursor for production of the antioxidant glutathione, or with peptides such as pentapeptides linked by a disulfide bridge or glycyl-leucyl-phenylalanine produced by trypsin digestion in the small intestine.[40] These peptides have been shown to have antibacterial activity in animal and isolated cell systems[40,41]; however, the physiological significance of these peptides in the human gastrointestinal tract remains largely unknown.

Because of its unique amino acid profile and its high concentration in breast milk, α-Lac has been extensively studied for use in infant formulas. Most dairy-based infant formulas are supplemented with whey proteins and often with α-Lac–enriched preparations. Alpha-lactalbumin has received relatively little attention for use in adult nutrition.[28]

Lactoferrin

Lactoferrin (Lf) is an iron-binding protein expressed in virtually all secretory fluids, including tears, saliva, nasal secretions, vaginal fluids, semen, bile, and gastrointestinal fluids, and it is most highly concentrated in milk. Lactoferrin is the second-most predominate protein in human milk. Lactoferrin is a glycoprotein containing approximately 690 amino acids, and it is highly conserved across all mammalian species. It contains 2 mineral-binding sites that strongly bind iron but can also bind copper, zinc, and manganese. Initial studies of breast milk reported that Lf facilitates intestinal iron absorption in the newborn. Lactoferrin supplements have been shown to improve the iron status of female distance runners and during pregnancy; however, comparisons of infant formulas with or without Lf demonstrate similar iron absorption.[42–44]

Beyond being an important food for essential amino acids and iron delivery, Lf exhibits a wide range of bioactivities related to host defenses, including antibacterial and antiviral functions, enhanced intestinal cell development, and immunity.[43] The mechanisms for these bioactivities have been extensively investigated; however, conclusive modes of action remain speculative.

Early research demonstrated Lf to have bacteriostatic activity against intestinal pathogens, including *Escherichia coli, Helicobacter pylori,* and *Vibrio cholerae*. Lactoferrin adheres to the bacterial cell wall, and its strong iron-binding capacity allows it to compete with the bacteria for iron, ultimately depriving the bacteria of an essential nutrient. Iron-binding sites on Lf are typically only approximately 30% saturated in milk, allowing Lf to retain significant capacity to bind additional iron.[45]

Subsequent studies of Lf reveal that the bactericidal function may also be associated with direct interaction of Lf with the bacterial cell membrane. Lactoferrin binds directly to the cell surface of bacteria, damaging the external cell membrane through interaction with lipopolysaccharides, causing increased permeability and cell damage from lysozyme activity.[45]

Lactoferrin also binds to enterocytes on the gut mucosa, stimulating normal cell development and reducing inflammation. In animal studies, Lf has been shown to inhibit the production of inflammatory cytokines TNF-α, IL-1b, and IL-6.[43] In total, Lf has been shown to enhance iron status in infants and at-risk groups and to have potential therapeutic effects in relation to bacterial infection and inflammation.

Beta-lactoglobulin

Beta-lactoglobulin (β-Lg) is the predominate whey protein in milk from ruminant animals. It is absent, or present possibly in minor quantities, in human milk.[46] In bovine milk, β-Lg accounts for approximately 10% of total milk protein and approximately 58% of the whey proteins. Beta-lactoglobulin is a relatively small peptide chain containing 162 amino acids. It exists in bovine milk as a 36 kDa molecular weight dimer. Beta-lactoglobulin exhibits high solubility and clarity over a broad pH range (ie, pH 3 to 7) and has excellent gelling and foaming properties, making it useful for food applications.[47] However, as a protein absent in human milk, there are concerns about β-Lg allergies.[48]

Research suggests a diverse array of potential roles of β-Lg in milk, including enhancing transport and uptake of hydrophobic ligands (ie, retinol and long-chain fatty acids), enzyme regulation, and development of passive immunity for the neonate.[47] However, the most obvious role is serving as a rich source of essential amino acids.[49] Because of its solubility, rapid digestibility, and high biological value, β-Lg is a major contributor to the characteristics that make whey proteins attractive for protein supplements, protein-fortified beverages, and sports drinks.

Functional roles of β-Lg in ruminant offspring and applications in human nutrition have been investigated but remain speculative.[47,50] Many of the bioactivities attributed to β-Lg are derived from peptides produced by enzyme hydrolysis and largely studied in isolated cell systems.[51] These bioactivities include inhibition of angiotensin-converting enzyme (ACE inhibitor), antimicrobial activity, and inhibition of pathogen adhesion.[47]

Various peptides derived from proteolytic digestion of β-Lg have been shown to inhibit ACE. Intact β-Lg has minimal ACE inhibitory activity; however, peptides derived from digestion with pepsin, trypsin, or chymotrypsin have high activity. Again, most of these studies demonstrate ACE inhibition using in vivo or in vitro systems. Perhaps the most promising ACE-inhibiting peptide is the tetrapeptide termed "β-lactosin B" (alanine-leucine-proline-methionine; derived from the *f*142–145 sequence of the β-Lg chain), which has been shown to have antihypertensive activity when administered orally to hypertensive rats.[52]

While no definitive role for β-Lg has been identified, the most compelling evidence supports its role in molecular transport. Beta-lactoglobulin can bind a variety of lipids, including retinol, long-chain fatty acids, and vitamin D. It has been proposed that β-Lg lipid binding may facilitate lipid transport in milk and intestinal uptake of retinol and fatty acids in the neonate.[50]

Glycomacropeptide

Glycomacropeptide (GMP) is a phosphorylated and glycosylated bioactive peptide chain of 64 amino acids, derived from casein in bovine milk. GMP is released from kappa casein (κ-casein) during cheese-making by the enzymatic action of chymosin (rennet), and the water-soluble peptide is removed with the whey. Glycomacropeptide may occur naturally in the human gastrointestinal tract via pepsin-mediated proteolysis of milk.[53] Glycomacropeptide accounts for approximately 20% of the total amino acid nitrogen in rennet whey and is normally present in whey protein isolates and concentrates. Glycomacropeptide is an unusual peptide, being devoid of the aromatic amino acids (phenylalanine, tyrosine, and tryptophan), which made it virtually invisible to early protein detection methods. Glycomacropeptide is also devoid of cysteine and contains only a single methionine.[54,55]

In the early 1990s, methods were developed for isolation and purification of GMP. In its purified form, GMP is routinely used to create medical foods for treatment of the inborn error of phenylketonuria for individuals lacking the enzyme phenylalanine hydroxylase to metabolize phenylalanine to tyrosine.[56] Without the enzyme, phenylalanine accumulates, producing high blood levels, resulting in brain damage and severe intellectual disability. Glycomacropeptide is used as a protein source for the creation of food products low in phenylalanine; however, GMP is also low in other amino acids, including arginine, cysteine, histidine, tyrosine, and tryptophan, which must be supplemented into the phenylketonuria diet.

In addition to the nutritional value of GMP for the management of phenylketonuria, it exhibits several other bioactive functions that may have therapeutic potential, including antibacterial, prebiotic, and enhanced immunity functions. The specific mechanisms promoting these bioactive roles remain speculative, but in vitro and in vivo evidence suggests the activity may at least in part be associated with post-translation glycosylation of the κ-casein protein. Glycomacropeptide has been found to contain the oligosaccharides N-acetylgalactosamine (GalNAc) and N-acetylneuraminyl (sialic acid) attached to threonine units in the peptide chain.[54,55] In early studies, researchers recognized that the cholera toxin caused cell damage by binding to similar oligosaccharide links on intestinal cells, causing the cholera damage. Glycomacropeptide has been shown to inhibit the binding of the cholera toxin to the oligosaccharide chains on healthy enterocytes. Subsequent research has shown GMP to be effective in preventing binding of other pathogenic bacteria, including *E. coli* in the intestine and the cariogenic bacteria *S. mutans* in the oral cavity.[54]

In addition, GMP may have a role as a prebiotic in promoting the growth of healthy intestinal microbiota, in particular *Bifidobacterium*, which may prevent or blunt the growth of pathogenic bacteria.[55,57,58] Mechanisms for prebiotic effects of GMP remain speculative, but GMP has been shown to increase the production of short-chain fatty acids in the colon and to modulate immune responses. However, most of the immune response research has been done with direct administration of GMP to isolated cell systems, and the efficacy of oral administration remains unknown. Taken together, the antibacterial and prebiotic findings suggest bioactive potential for GMP that may be important for normal gut development or in pathological conditions like inflammatory bowel disease; however; definitive human trials have not been done.[58]

Casein

Casein (Latin word *caseus* for cheese) represents a group of phosphoproteins and accounts for approximately 80% of the protein in bovine milk. The primary proteins are α_{S1}- and α_{S2}-caseins, β-casein, and κ-casein, representing approximately 38%, 10%, 36%, and 13% of the casein fraction, respectively. Together, the casein proteins provide a complete balance of essential amino acids, but they are also characterized by an unusually high content of proline (~16% of amino acids) and are nearly devoid of cysteine. Because of the proline and cysteine contents, casein lacks the disulfide bridges and alpha-helix structures typical of most proteins. Further, the phosphoproteins have a high affinity for binding calcium and are hydrophobic, making them insoluble in water. In native milk, caseins exist in a micellular structure to maintain water solubility; however, in acidic environments ($pH < 4.6$), casein proteins curdle, forming a curd, providing the characteristics that led to its use in cheese-making.[59]

Likewise, the low solubility in acidic environments causes casein to curdle in the low pH of the stomach, slowing gastric emptying and prolonging digestion and absorption. Because of the low pH qualities, casein is often characterized as a slow-digesting protein, which tends to enhance satiety and prolong the post-prandial period of amino acid absorption and appearance in the blood circulation.[60,61] The digestive properties of proteins have been extensively studied for use in protein powders to support protein synthesis in skeletal muscle, and the optimal protein choices depend on the application.[38,62,63] For example, whey proteins are routinely used as protein supplements, because they have a high leucine content (~12% w/w) and are rapidly digested, producing the rapid rise in plasma and intracellular leucine concentrations essential for the mTOR trigger for muscle protein synthesis.[38] Whey is ideal for providing a maximum response with the least amount of protein and calories, plus it is water-soluble and has a clean flavor profile. Whey is widely used by athletes for post-exercise recovery and with elderly or bed-rest patients when food intake may be restricted. Casein has a more moderate leucine content (~9%), is slow digesting, and has low solubility, resulting in a lower and more prolonged leucine absorption profile. Hence, a meal contain 25 g of whey protein will trigger the mTOR response, while 40 g of casein may be required to reach the same intracellular leucine concentration to trigger mTOR.[64] Casein can be used to enhance and prolong post-meal anabolic responses in muscles when the total amount of protein is not restricted. Likewise, casein has been used for nighttime supplements for athletes, to produce a prolonged anabolic period during sleep; the natural combination of whey plus casein in milk has been shown to be effective for post-exercise recovery in athletes.[65]

DAIRY-DERIVED BIOACTIVE PEPTIDES

A newer area of protein research is the study of the bioactive peptides that are encrypted in the native structure of food proteins, including casein and whey. Food-derived peptides are released from food proteins by proteolysis that occurs naturally during the digestive process, through fermentation of foods, or by enzymatic hydrolysis in laboratory settings.[66] Dairy protein–derived peptides and those from other food proteins, are commonly referred to as bioactive peptides, ie, "protein fragments that have a positive influence on physiological and metabolic functions or condition of the body and may have ultimate beneficial effects on human health".[11] Bioactive peptides typically contain 2 to 20 amino acids. Present understanding of bioactive peptides comes mainly from computer modeling and preclinical research; therefore, the role of food-based peptides in human physiology and health is uncertain. The term "bioactive peptides" as used in the present review is not intended to indicate that health benefits have been convincingly demonstrated in humans, but rather for consistency with its use in the scientific literature based on evidence derived largely from isolated cell systems and animal studies.

Over the past 30 years, research on bioactive peptides has proliferated. Scientific interest in identifying food-derived bioactive peptides that can help reduce or prevent the risk of chronic diseases has been motivated largely by potential functional food, dietary supplement, and pharmaceutical applications. Peptides isolated from animal tissues (eg, pituitary gland, placenta) or chemically synthesized have a long history of use in medicine, dating back more than half a century.[67] As of 2014, more than 7000 naturally occurring peptides had been identified, and the pharmaceutical industry had more than 60 peptide drugs on the market.[68] The recognition that peptides naturally present in both plant and animal foods are released during digestion and fermentation processes stimulated the rapid evolution of research on food-derived peptides.

Although many food proteins are sources of bioactive peptides, dairy-derived bioactive peptides are among the most extensively studied. Dairy peptides have been shown to exhibit antihypertensive, antimicrobial, antithrombotic, immunomodulatory, opioid, antioxidative, antidiabetic, and mineral-binding functions, with some exhibiting bioactivity in more than a single physiological system.[11,69,70] Bioinformatics (ie, computer modeling methodology), also known as *in silico* analyses, is a tool used to identify food-derived peptides with biological functionality.[71,72] Large databases of amino acid sequences for peptides that are encrypted in food proteins are widely available and are open access (eg, BIOPEP database).[71,72] Computer software is used extensively to detect amino acid sequences with known biological activity and to simulate proteolytic release of specific peptides with proteases.[71,72] Structure–activity relationships of bioactive peptides can be examined using quantitative structure–activity relationship modeling, which is based on existing knowledge of peptide structures, amino acid sequences, hydrophobicity, and bioactivity. Computer-based screening of large numbers of peptide sequences utilizes molecular docking technology to examine interactions between peptides and specific enzyme active sites and/or receptors and to determine IC_{50} values (amount of peptide needed for half of the maximum inhibitory activity). Confirmatory studies using in vitro and animal studies often verify predicted results from computer-based screening.

In order to be effective in humans, however, peptides need to resist degradation in the digestive tract and, if their bioactivity is not targeted solely in the gastrointestinal tract, they need to be absorbed into the bloodstream and reach tissues in an active form. Although much knowledge has been attained through bioinformatics, research on the bioavailability and effectiveness of bioactive peptides in humans is limited. In light of the systematic reviews and meta-analyses that have continued to show that consumption of dairy foods is linked to beneficial or neutral associations with risk of hypertension,[3–5] cardiovascular disease,[3,6,7] and type 2 diabetes,[3,8,9] examination of the research on dairy protein–derived peptides in relation to these three conditions of public health concern is of interest.

Blood pressure and antihypertensive peptides

A principal mechanism in the regulation of blood pressure is the angiotensin-converting enzyme (ACE), which catalyzes the conversion of angiotensin I to angiotensin II, a hormone that stimulates vasoconstriction and increases blood pressure.[73] ACE inhibitors block this conversion and are commonly prescribed as a treatment option for patients with hypertension. Identification of dairy protein–derived peptides with antihypertensive properties more than 30 years ago stimulated both scientific and commercial interest. Antihypertensive effects of dairy protein–derived peptides in sour (fermented) milk were first demonstrated in the mid-1990s.[74–76] Since then, many dairy-derived peptides have been shown to have antihypertensive properties[11,77,78]; however, the two tripeptides, isoleucine-proline-proline (IPP) and valine-proline-proline (VPP), are by far the most extensively studied. IPP is encrypted in β-casein and κ-casein, and VPP in β-casein.[74]

Beyond ACE inhibition, IPP and VPP also may mediate their antihypertensive effects through other biological activities. For example, anti-inflammatory and antioxidant properties of IPP and VPP may contribute to vascular function benefits.[79–81] In addition to IPP and VPP, other bioactive peptides encrypted in dairy proteins have been shown to have ACE inhibitory properties.[11,77,82] For example, two α_{s1}-casein derived peptides with opioid properties were shown in spontaneously hypertensive rats to have blood pressure–lowering effects.[83]

In addition to extensive in vitro and laboratory animal studies, human clinical trials in Japan and Europe examined blood pressure responses to a variety of dairy foods and hydrolysates shown to contain IPP and VPP. Between 1996 and 2012, more than 30 human clinical trials evaluated blood pressure responses after consuming fermented milks, yogurt drinks, or casein hydrolysates containing known amounts of IPP and VPP. Most were randomized controlled trials with hypertensive or prehypertensive populations. Nine systematic reviews and/or meta-analyses,[84–92] a Cochrane review and meta-analysis,[93] and a European Food Safety Authority (EFSA) review[94] of these studies have been published.

All of the meta-analyses, with the exception of the Cochrane review, demonstrated significant reductions in both systolic and diastolic blood pressure. Mean reductions in systolic blood pressure were 1.3 to 5.6 mm Hg and in diastolic blood pressure were 0.6 to 2.4 mm Hg. The magnitude of these reductions, while moderate, is similar to that shown with diet-related reductions such as increased calcium, magnesium, and potassium intakes,[95] sodium restriction,[96] and consumption of the DASH (Dietary Approaches to Stop Hypertension) diet.[97] The Cochrane meta-analysis, which included 15 trials, found a modest main effect only for lower systolic blood pressure (–2.4 mm Hg). While it appears plausible that the biopeptides, IPP and VPP, may contribute, at least in part, to the benefits of consuming dairy foods and the reduced risk of hypertension as shown in the systematic reviews and meta-analyses,[3–5] significant research gaps exist. For example, little is known about the bioavailability of IPP and VPP in humans,[98] and their physiological targets and biological mechanisms of action in humans have not been characterized. Heterogeneity in study designs, especially the variability in the dairy products containing the IPP and VPP peptides and the rigorous selection criteria in the Cochrane review may explain the absence of diastolic blood pressure reductions in the Cochrane meta-analysis.[93] The EFSA evaluation of the clinical trials, together with review of the animal and in vitro research and other supporting materials, led the committee to conclude that IPP and VPP were sufficiently characterized but that a cause-and-effect relationship between the consumption of IPP and VPP and the claimed benefit of maintaining blood pressure in the general population was not supported.[94]

Cardiovascular disease and anti-atherogenic peptides

Cardiovascular disease is a group of disorders of the heart and blood vessels that can lead to heart failure or stroke, and it has often been linked to dietary cholesterol and saturated fats associated with animal-derived foods. However, systematic reviews and meta-analyses published in just the past 5 years add to a large body of research indicating that consumption of dairy foods is not linked to increased risk for cardiovascular disease,[3,6,7] coronary artery disease,[3,7,99] or stroke.[3,6,100]

Heart attacks and strokes commonly occur from a blockage that is caused by the build-up of atherosclerotic plaques and prevents blood flow to the heart and brain, respectively. Many bioactive peptides that are released from casein and whey proteins during digestion, by fermentation processes, and by enzymatic methods have been shown to mitigate cellular damage at different stages of atherogenic plaque formation.[81] These include dairy-derived peptides with antioxidative, antihypertensive, immunomodulatory, anti-inflammatory, and antithrombotic effects.[11,81,101,102] The available research assessing the potential benefits of these peptides, however, currently is limited to bioinformatics, in vitro studies, and laboratory animal research.

Vascular oxidative stress plays a central role in the initiation and progression of cardiovascular disease. Oxidative stress caused by excess production of reactive oxygen species (free radicals, superoxide, hydroxyl radicals, non-radical hydrogen peroxide) leads to oxidative changes in lipoproteins, activation of macrophages, and (over time) to the formation of atherogenic plaques.[103] Many casein- and whey-derived peptides have been shown, mainly through bioinformatics and in vitro studies, to exhibit antioxidant properties.[81,101] The antioxidant potential of individual peptides is affected by their amino acid content and the positions of amino acids in the peptide chain.[101] For example, antioxidant activities of dairy-derived peptides have been shown to be influenced by the presence of histidine with its peroxyradical trapping and chelating ability, and of hydrophobic amino acids that increase access of the peptide to hydrophobic targets.[81,101] Immunomodulatory activities involving complex immune system functions also have been identified for many dairy-derived peptides; however, specific mechanisms remain to be fully elucidated.[11,81] Inflammation when chronic and uncontrolled enhances the progression of atherosclerosis. Anti-inflammatory activities of dairy-derived peptides have been shown in vitro to be mediated, at least in part, through inhibition of the NF-κB pathway and a PPAR-γ–dependent mechanism.[81] Arterial thrombosis, which is the main cause of myocardial infarction and stroke, occurs from the rupture or erosion of atherogenic plaques, coupled with increased platelet aggregation and clot formation.[104] Peptides encrypted on κ-casein and Lf have been shown to inhibit platelet aggregation and related antithrombotic properties.[81,102]

Casein-derived peptides also have been linked to a cholesterol-lowering effect that has been attributed to a reduction in the solubility of cholesterol in bile salt mixed micelles, resulting in impaired cholesterol absorption.[11,105]

In the absence of human clinical research, however, the extent to which dairy peptides with antithrombotic, antioxidative, anti-inflammatory, immunomodulatory, and cholesterol-lowering properties may contribute to the observed neutral associations between consuming dairy foods and risk of cardiovascular disease and stroke remains theoretical.

Type 2 diabetes and antidiabetic peptides

Type 2 diabetes is a chronic metabolic disorder characterized by insulin resistance and elevated blood glucose.

Common comorbidities include obesity, hypertension, cardiovascular disease, and stroke. Systematic reviews and meta-analyses in recent years continue to add to evidence that consuming dairy foods has beneficial or neutral associations with the incidence of type 2 diabetes.[3,8–10]

Chronic elevation of blood glucose produces diverse cellular damage in many organs, including the pancreas, eyes, kidneys, heart, and peripheral blood vessels. Treatment strategies for type 2 diabetes include modification of diet and exercise, plus insulin and other medications to reduce postprandial glucose excursions. One category of medications in routine use is designed to blunt carbohydrate absorption by inhibiting the brush border digestive enzyme α-glucosidase.[106] Bioinformatics research has identified a large number of food protein sources of bioactive peptides, including those encrypted in casein and whey proteins, which exhibit α-glucosidase inhibitory potential.[107]

A second area of research relates to glucagon-like peptide-1 (GLP-1), an incretin hormone secreted by intestinal endocrine cells that controls gastric motility, slows gastric emptying, and stimulates the release of insulin from the pancreas.[108] Glucagon-like peptide-1 is inactivated primarily by dipeptidyl peptidase 4 (DPP-4), an enzyme that plays a key role in the regulation of blood glucose. Individuals with type 2 diabetes have decreased incretin responses, and consequently reduced insulin secretion and higher postprandial glucagon and blood glucose levels. Synthetic DPP-4 inhibitors, a successful class of drugs for treatment of patients with type 2 diabetes, improve glycemic control by impeding the degradation of GLP-1.[108] It has been proposed that DPP-4, which is expressed not only in the intestine but also in the pancreas, kidney, liver and by lymphocytes and monocytes, may function also as a local mediator of inflammation and insulin resistance in adipose and hepatic tissue.[108,109] Its potential broader role in glucose homeostasis, however, remains to be established.

Research on food-derived peptides that have DPP-4 inhibitory properties is still early, and current knowledge is mostly from bioinformatics and in vitro studies.[71,109] Animal studies are scant, and there is a clear absence of human studies. Dipeptidyl peptidase 4 inhibitory peptides are encrypted in many food proteins, including both casein and whey, and vary widely in amino acid sequences and chain lengths.[107,109] Casein and whey proteins, namely α_{s1}-casein, β-casein, κ-casein, β-Lg, α-Lac, and Lf, collectively, have been shown to contain more than 200 unique peptide fragments with DPP-4 inhibitory potential.[109] Dipeptidyl peptidase 4 inhibitory peptides also have been identified in cheese.[110] Bioinformatics research has identified casein as potentially the richest source of DPP-4 inhibitor peptides,[107] and the tripeptide isoleucine-proline-isoleucine appears to be one of the most potent DPP-4 inhibitory peptides (lowest IC_{50} of the peptides examined).[107,109] Dairy-derived peptides, including several with DPP-4 inhibitory potential, have been detected in the human intestinal tract after consumption of dairy products.[70] This body of research, collectively, gives an indication of the potential role of peptides with DPP-4 inhibitory properties in regulation of glucose homeostasis, but the effectiveness of dairy- and other food-derived DPP-4 inhibitory peptides in humans has not been examined. Their resistance to degradation by intraluminal or brush border peptidases, their bioavailability, and their specific cell targets and biological functions are topics for future research in both laboratory animals and humans.

A related avenue of research has examined indicators of glucose homeostasis after consumption of whey or casein hydrolysates; however, the peptide and amino acid patterns of the hydrolyzed proteins were not determined. These studies, which included patients with prediabetes,[111] type 2 diabetes,[112,113] or gestational diabetes,[114] or healthy adults[115–119] randomized to the protein hydrolysate or native form of whey or casein, found limited and inconsistent evidence of improved glucose control in the hydrolysate groups (eg, plasma levels of glucose-dependent insulinotropic polypeptide,[116] glucose,[118,120] and/or insulin[113,119–121]). Future clinical research is needed to validate these findings.

CONCLUSION

Milk is a complex food matrix serving as an essential food and protein source for all mammalian species, including humans. Milk proteins, which are known for their high nutritional quality, based on their essential amino acid composition, also exhibit a wide range of bioactivities. Currently, milk and dairy foods provide approximately 15% to 20% of daily protein intake in the United States. Because of their unique water solubility, milk proteins are readily separated into casein and whey fractions, which can be further fractionated into many individual proteins, including α_{S1}- and α_{S2}-caseins, β-casein, κ-casein, and the whey proteins. Many of these proteins have unique bioactivities that have been extensively studied.

Whey proteins have become popular as protein supplements for muscle health and development because of their exceptionally high concentration of the essential amino acid leucine (~12% w/w). Individual whey proteins such as α-Lac are available in high purity, and α-Lac is now widely incorporated into infant formulas to enhance protein quality. Likewise, GMP is the foundation of most medically defined diets for the management of the inborn error of metabolism, phenylketonuria.

Further, over the past 30 years, peptides that are encrypted in the primary amino acid sequences of proteins and released along with amino acids during digestion or during food processing such as fermentation or enzyme digestion are increasingly recognized as biologically active protein metabolites that may have beneficial effects on human health. The most studied of the peptides are the ACE inhibitors, which have been shown to reduce blood pressure to a similar degree to sodium restriction or the DASH (Dietary Approaches to Stop Hypertension) diet. New areas of research have focused on novel dairy peptides as aids in the management of type 2 diabetes. Peptides derived from casein have been shown in preclinical research to blunt post-meal increases in blood glucose by inhibiting the digestive enzyme α-glucosidase or by modulating the incretin hormone GLP-1. Use of these peptides represents a novel new approach to understanding the bioactivity of dairy proteins. Future studies that expand on the limited research in humans will further advance understanding of the physiological benefits of milk consumption. In total, milk remains a cornerstone of the human diet, and advanced food processing allows for isolation and unique applications of bioactive proteins and peptides from the milk matrix.

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