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Review Article

Bovine Milk Proteins and Human Health: Unveiling Interplay, Surmounting Challenges and Future Prospects

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ABSTRACT
This review explores our current understanding of bovine milk proteins, examining their structural architecture and their impact on human health, particularly in enhancing milk quality. Recent advancements have illuminated the similarities between the bovine lactation genome and the regulators of human cell growth and physiology, revealing a complex interplay of cellular signalling pathways. These insights have paved the way for the development of milk protein-based functional foods tailored to specific health needs, with promising applications in precision medicine. This article not only unravels the complexities of milk proteins in transforming nutrition and health but also serves as a foundation for future innovations aimed at improving human well-being.

Keywords: Human health, Milk proteins, Nutrition, Precision medicine, Sustainability.

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INTRODUCTION

The first source of a complete meal for almost every mammalian offspring, one that meets all its nutritional needs, is its mother's milk. Mother's milk is easily digestible, provides essential nutrients, from macronutrients such as carbohydrates, proteins and lipids, to micronutrients such as vitamins and minerals. These essentials are tailored to support a young's growth and development. In addition to these nutrients, milk also contains antibodies that bolster the infant's immune system, providing protection against infections. Breastfed young are often found to exhibit superior physical and cognitive health similar to adults. Nature has fine-tuned mother's milk to meet the developmental and immunological requirements of an offspring, playing a crucial role in establishing lifelong health. This elixir, as a natural resource with its specific nutritional profile, has remained evolutionarily preserved across species, reflecting its critical role in survival and well-being.[1,2]

Bovine milk, produced by cows (*Bos taurus*), has become a staple in human diets, largely due to the cultural practices that led to domestication of cattle, as well as due to the consequential availability of this complete meal as a reliable and sustainable source of nutrition. While cow's milk remains the most widely consumed form of milk globally, other mammalian milk sources, such as buffalo, camel, goat, sheep and yak, are also popular, contributing significantly to its dietary diversity.[3] Owing to its nutritional significance and widespread availability, there are decades of extensive research on the unparalleled benefits of this superfood.[4]

This complex biological fluid, characterized by its white, viscous consistency maintains an appropriate ratio of carbohydrates, fats, proteins, vitamins and minerals, rendering it as a complete and an indispensable dietary source. Lactose, a disaccharide composed of glucose and galactose, is the main carbohydrate present in milk. Other oligosaccharides like lacto-N-tetraose and 3'-sialyllactose, serve as prebiotics. Though these are present in smaller quantities, they display a significant health implication due to their crucial role in gut microbiota modulation. These prebiotics promote the growth of beneficial gut bacteria such as *Bifidobacterium* species and have been shown to support the immune system. The concentration of oligosaccharides in human milk is higher than in bovine milk, which has led to their use in infant formulas designed to mimic the prebiotic effects of breast milk.[5]

Bovine milk fat is a complex mixture of triglycerides, phospholipids and free fatty acids, serving as an essential component of human nutrition. Saturated fatty acids like palmitic acid, stearic acid; monounsaturated fatty acids like oleic acid; polyunsaturated fatty acids like linoleic acid and alpha-linolenic acid are present in milk,

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making it more nutritionally rich. Also, it provides a rich source of energy and fat-soluble vitamins like A, D, E and K. Bovine milk is a rich source of Vitamins, like A, D, B2 and B12 with small amounts of B5 and B9. Calcium is the most abundant mineral in bovine milk, crucial for bone and teeth health, muscle function, blood clotting and nerve transmission. Phosphorus is equally rich and bioavailable in milk. The calcium-to-phosphorus ratio in milk is ideal for skeletal health, a find in bone mineralization and growth. It contains moderate amounts of magnesium which supports bone health, mental cognition and may enhance the effectiveness of calcium in bone mineralization. It is also an excellent source of potassium, which is an essential electrolyte that helps maintain proper fluid balance, muscle function and nerve transmission.[6]

Milk proteins are generally classified into two distinct categories: acid-insoluble caseins and acid-soluble whey proteins.[7] Both casein and whey, along with their derived peptides, exhibit a wide range of biological and physiological properties. These include antibacterial and antiviral activity, as well as potential antihypertensive, immune-modulating and even opioid-like effects.[8,9] As advancements in nutritional science progress, there is a growing need to revisit and deepen our understanding of milk proteins and their complex interactions with human health.

Bovine milk contains a variety of proteins, which are primarily divided into caseins (80%) and whey proteins (20%) (Figure 1). Caseins are compactly organized in a structure known as micelle which are responsible for the white appearance of milk, contributing to its structural complexity.[10] It is a heterogeneous phospho conjugated protein comprising of four major fractions: αS1, αS2, β and κ caseins.[11] Whey proteins, which are water-soluble, remain in the liquid fraction of milk after casein removal, during processes such as cheese-making. These proteins are rich in essential amino acids and are highly digestible, contributing significantly to the nutritional value of milk. The whey fraction includes a diverse array of proteins with distinct biological functions, including Alpha-Lactalbumin (α-LA), Beta-Lactoglobulin (β-LG), Serum Albumin, Immunoglobulins (IgG, IgA, IgM), Lactoferrin (LF), Lactoperoxidase (LP), Bovine Serum Albumin (BSA), lysozyme, Glycomacropeptide (GMP), peptidoglycans and protease-peptones.[12] Each of these proteins plays a unique role in the bioactive milieu of milk, contributing to its immune-boosting, antimicrobial and antioxidant properties.

In addition to the whey proteins, milk contains other important bioactive peptides, such as Caseinomacropeptide (CMP),[13] a peptide fragment derived from κ-casein during milk processing. Furthermore, Milk Fat Globule Membrane (MFGM) proteins, which encapsulate milk fat globules, are integral to the digestion and absorption of fat. Key MFGM proteins include butyrophilin, xanthine oxidase and fatty acid-binding proteins.[14] Together, these proteins enhance the nutritional and functional attributes of milk, offering a broad spectrum of health benefits beyond basic nourishment.

This paper aims to explore the multifaceted role of bovine milk proteins in human health, examining its nutritional composition, health benefits and the ongoing scientific advancements that continue to shape its significance in modern diets.

ALPHA CASEIN (AS1-CASEIN): MOLECULAR DIVERSITY

Alpha-S1-casein (αS1-casein) is a major protein component in bovine milk, constituting a significant portion of the casein fraction. As a phosphoprotein, αS1-casein plays a crucial role in the structure and function of casein micelles and is involved in nutrient delivery and digestion. It exhibits a remarkable diversity manifested through its various molecular variants, denoted as A, B, C, D, E, F, G, H and I. This diversity arises from genetic polymorphisms within the αS1-casein gene, resulting in distinct amino acid sequences and structural characteristics among different variants.[15] These genetic polymorphisms can influence the abundance of specific variants in milk,[16] thereby affecting its nutritional value and other properties. This knowledge not only sheds light on the mechanisms governing milk protein behaviour, but also holds implications for human health and nutrition, thereby underscoring the importance of elucidating the molecular intricacies of αS1-casein in shaping our understanding of milk's biological effects.

Despite this diversity, αS1-casein typically possesses a molecular weight of approximately 23 kDa and spans 199 amino acids, with a notable abundance of acidic amino acids compared to basic ones. Structurally, αS1-casein is delineated by three hydrophobic regions located between residues 1 to 44, 90 to 113 and 132 to 199, interspersed with two hydrophilic domains spanning residues 45 to 89 and 114 to 131. These structural features contribute to the functional versatility of αS1-casein, allowing it to interact with various molecules and participate in diverse physiological processes. Of significance are the eight phosphoseryl residues present within αS1-casein, rendering it highly susceptible to calcium modulation, which plays a crucial role in its conformational changes and functionality[17] that influence its solubility, stability and interaction with other milk components.

Moreover, αS1-casein exhibits a propensity for self-association, forming chains whose extent is influenced by factors such as ionic strength and pH conditions, adding another layer of complexity to its behaviour.[18] This self-association phenomenon contributes to the formation of larger protein aggregates, such as casein micelles, which are essential for the maintenance of milk's colloidal stability and texture.[19] Recent studies have further highlighted the impact of genetic variations on the structural and functional properties of αS1-casein, emphasizing its role as a key determinant of milk composition and quality.

ALPHA CASEIN: (AS2 CASEIN) VARIETAL COMPLEXITY

Within the realm of alpha caseins, αS2-casein is one of the four main caseins in bovine milk, contributing significantly to its nutritional and functional properties. It emerges as a molecular tapestry with four distinct genetic variants (a, b, c, d), each contributing to the intricate diversity of this milk protein. Comprising 207 amino acid residues and boasting a molecular weight of 25 kDa, αS2 casein showcases unique structural attributes that define its functional properties.[20] The amino acid sequence of αS2-casein unfolds with negatively charged residues predominantly located at the N-terminal, creating an electrostatic balance with positively charged amino acid residues concentrated at the C-terminal. This juxtaposition of charges imparts a distinctive character to αS2-casein, rendering it highly hydrophilic among its casein counterparts.[21] Notably, this hydrophilicity, coupled with an acute sensitivity to calcium, positions αS2 casein as a dynamic player in the intricate dance of molecular interactions within milk.[7,10] As a major protein fraction, αS2-casein plays a vital role in the formation of casein micelles, which are crucial for the stability and nutrient absorption of milk. Recent studies have shown that αS2-casein may also have important implications for human health, particularly concerning the gut microbiome. While research on αS2-casein's direct influence on the microbiome is still emerging, its impact on digestion, immune function and overall gut health warrants further exploration. Detailed elucidation of the structural and functional properties of αS2 casein holds promise for enhancing our comprehension of its role in milk composition, processing and product development. Unraveling the varied complexities in αS2 casein, will not only contribute to the advancement of dairy science but also pave way for novel strategies in dairy product optimization and personalized nutrition approaches.

BETA CASEIN (Β-CASEIN): VARIANTS AND STRUCTURAL DYNAMICS

Distinguished by its extensive variant repertoire, β-casein also emerges as a multifaceted player within the group of milk proteins. As the second most abundant casein, it has nearly 12 genetic variants (A1, A2, A3, B, C, D, E, F, G, H1, H2, I). This protein has a molecular weight of 24 kDa and comprises of 209 amino acids.[22] It is a proline-rich protein, with 35 proline residues displaying a distinctive structural profile. The amphiphilic nature of β-casein is nullified by a highly charged N-terminal region and a hydrophobic C-terminal domain. They are seen to play a crucial role in the structure of casein micelles, ensuring the stability and efficient delivery of nutrients. In contrast to αS caseins, β-casein exhibits a reduced sensitivity to calcium precipitation, marking a divergence in its calcium-dependent behaviour. The ability of beta casein to self-associate and form polymers is intricately governed by factors such as ionic strength, temperature and its concentration within a solution.[23]

Furthermore, recent studies suggests that phosphorylation and glycosylation, the two post-translational modifications, further alter the structural dynamics and functional properties of β-casein. Drawing insights from the works of renowned researchers[10] who have delved into the intricacies of β-casein, we can uncover a nuanced understanding of its structural dynamics. The β-casein monomer, characterized by a less extended structure and high hydration, adds another layer to its functional complexity.[24] Through comprehensive analysis and integration of diverse research findings, this section seeks to enrich our comprehension of the molecular intricacies that contribute to beta casein's unique properties and functional significance within the intricate matrix of milk proteins. As a protein, beta-casein has a diverse range of biological functions beyond its nutritional contribution. Increasing evidence points to its influence on human health, particularly regarding the gut microbiome.

KAPPA CASEIN (Κ-CASEIN): DIVERSITY AND FUNCTIONAL DYNAMICS

In the intricate tapestry of milk proteins, κ-casein stands as a molecular marvel, characterized by an expansive spectrum of nearly 14 genetic variants (A, A1, B, B2, C, D, E, F1, F2, G1, G2, H, I, J). This 19 kDa protein, consisting of 169 amino acids, holds a distinct position as the most resilient casein against calcium precipitation. Its remarkable 20 proline residues contribute to its structural uniqueness, imparting specific attributes to its functional role.[25] One of the defining features of κ-casein lies in its capacity to stabilize other caseins, showcasing a pivotal role in maintaining the structural integrity of the milk matrix. The catalytic activity of chymosin and pepsin, concentrated between amino acids 105 and 106 of κ-casein, leads to the release of a fragment of 1-105 length, aptly named para-casein. At neutral pH, this peptide exhibits high hydrophobicity and a positive charge, adding a layer of complexity to its biochemical interactions.

Further delving into the structure of κ-casein, the second fragment spanning amino acids 105-169 is identified as the Casein Macro Peptide (CMP), showcasing a distinctly hydrophilic nature.[26] Recent research has highlighted the functional versatility of κ-casein beyond its role in protein stabilization, including its potential as a carrier for bioactive compounds and also its involvement in the formation of dairy gels and emulsions.[27] Although much attention has been given to the broader casein family (alpha-and beta-caseins), κ-casein has unique properties that differentiate it from other casein proteins. Recent studies have begun to explore the influence of κ-casein on human health, particularly its correlation with the human gut microbiome.

WHEY PROTEINS: COMPOSITION AND STRUCTURAL ELEGANCE

Whey proteins are a complex and varied mixture of substances that are obtained from the liquid residue that remains after casein precipitates. These include proteins, peptides, lactose, lipids, mineral salts (such calcium, potassium and sodium), vitamins and water.[28] Whey proteins add 5 to 7 g of protein per litre (or around 20% of total milk proteins) to milk's nutritional composition. They are characterized by their densely folded, globular structure, rendering them sensitive to heat. The distinctive absence of proline, coupled with a high helical predominance, facilitates the formation of their compact, globular configuration. Unlike caseins, they showcase a uniform distribution of hydrophilic and hydrophobic amino acids all over the polypeptide chains, underscoring their structural elegance.

Bovine Serum Albumin (BSA), Immunoglobulins (Igs), α-Lactalbumin (α-LA) and β-Lactoglobulin (β-LG) are the main whey proteins present in milk. Notably, β-LG and α-LA have been extensively studied, contributing to a deeper understanding of the functional roles played by whey proteins in the biological milieu.[29] As a member of the lipocalin protein family, β-LG facilitates the transportation and absorption of hydrophobic substances like retinol and fatty acids by acting as a carrier for them. On the other hand, α-LA is involved in lactose synthesis, acting as a regulatory protein in lactose biosynthesis. Additionally, α-LA also exhibits antimicrobial properties, contributing to the innate immune defence provided by whey proteins. Furthermore, BSA serves as a carrier for various endogenous and exogenous compounds, including fatty acids, hormones and drugs, facilitating their transport within the circulatory system.[28]

ALPHA-AND BETA-LACTOGLOBULIN: UNRAVELLING VARIETAL DIVERSITY AND STRUCTURAL CONFIGURATION

Within the family of whey proteins, Beta-Lactoglobulin (β-LG) emerges as a captivating entity, boasting several genetic variants (A, B, C, D, E, F, G, H, I, J, W). This globular protein, with a molecular weight of 36.6 kDa, exhibits a unique structural elegance that contributes to its diverse functional roles. β-LG is mostly present as a dimer at typical room temperature and pH range of 5-7. It has two identical subunits, each of which has a molecular weight of 18.3 kDa.[30] The genetic variants of β-LG add a layer of complexity to its molecular diversity, influencing its physiological and biochemical properties. This structural intricacy contributes to the versatile roles played by β-LG in various biological processes. The two main groups of beta-globulin proteins, immunoglobulins and serum albumin, have unique functions that contribute to both immune modulation and metabolic health.

α-lactalbumin stands out by its compact, globular structure and genetic variability, featuring three distinct variants (A, B, C). It accounts for approximately 25% of the protein content in the whey fraction. This small, water soluble, acidic protein exerts its influence as a Ca^{2+} -binding entity, boasting a molecular weight of 14.2 kDa. Characterized by its high hydrophilicity, α-lactalbumin defies precipitation from milk at its isoelectric point, showcasing its resilience within the complex matrix of whey proteins. As a member of the whey protein family, α-lactalbumin is particularly sensitive to thermal treatments, with notable modifications in its structure observed as denaturation, which initiates at temperatures exceeding 65ºC.[31] The three genetic variants further contribute to the nuanced functional roles and interactions of α-lactalbumin within the biological milieu. Jovanović's insights into the thermal dynamics of α-lactalbumin offer valuable perspectives on the protein's behaviour under heat stress, shedding light on the factors influencing its structural integrity.

Furthermore, recent research has highlighted the diverse functional roles played by α-lactalbumin beyond its calcium-binding properties. For instance, α-lactalbumin has been implicated in immune modulation, antimicrobial activity and even as a potential therapeutic agent for certain medical conditions.[32] Additionally, its role in lactose synthesis, in conjunction with β-1,4-galactosyltransferase, forms the lactose synthase enzyme complex, essential for lactation and milk production.[33] Beyond its well-known function in lactation, α-LA possesses various bioactive properties that have significant implications for human health, particularly in relation to the gut microbiome, immune function and metabolic health.[34] By unravelling the molecular intricacies of this whey protein, we can gain a deeper understanding of its role in the dynamic interplay of milk proteins and its significance in various biological processes.

BOVINE SERUM ALBUMIN: STRUCTURAL INTRICACIES IN BLOOD AND MILK

Bovine Serum Albumin (BSA) is a unique whey protein that is not produced by the mammary gland. It is found in both milk and blood. Comprising 582 amino acids, this substantial protein possesses a molecular weight of 66 kDa. Unlike other whey proteins, BSA passively diffuses from the bloodstream into milk, constituting a trace protein that contributes to a mere 1% of the total milk protein content. It demonstrates remarkable stability even under high pressures of 800 MPa, showcasing its resilience in various physiological environments. Its structural complexity is highlighted by multiple domains, encompassing three structural domains and nine subdomains. The intricate architecture of BSA contributes to its multifaceted functions within the dynamic contexts of blood and milk.[35]

Functionally, BSA plays a pivotal role in transporting fatty acids across membranes, underscoring its significance in lipid metabolism and transportation for cellular energy production.

This process may support overall metabolic health, particularly in individuals with metabolic disorders such as obesity and type 2 diabetes. Moreover, the regulation of blood pressure and fluid balance by BSA can contribute to cardiovascular health. It is also seen that it is essential in the transfer of numerous endogenous and exogenous substances, such as hormones, vitamins and medications, within the circulatory system by acting as a carrier protein for them, which suggests that it may play a role in supporting muscle metabolism and tissue repair. BSA also has antioxidant qualities that help shield cells from oxidative stress by scavenging free radicals.[36]

IMMUNOMODULATION AND ALLERGIC RESPONSES OF MILK PROTEINS

Delving deeper into the immunomodulatory potential of milk proteins, casein hydrolysates emerge as reservoirs of immunoactivity peptides. These peptides, released during hydrolysis, exert regulatory functions on the intestinal immune system, further highlighting the multifaceted roles played by milk proteins in immune modulation. Also, lactoferrin, an abundant whey protein, exhibits potent immunomodulatory properties, including anti-inflammatory effects and regulation of immune cell proliferation and differentiation.

β-casein, a highly conserved casein fraction across mammals, exhibits variability in amino acid composition among different species. β-casein demonstrates immunomodulatory effects. Notably, actinase-digested peptides from β-casein induce macrophage migration and activation, suggesting a crucial role in promoting innate immune responses in the host.[37] Studies suggest that β-casein-derived peptides can influence immune function by enhancing the Gut-Associated Lymphoid Tissue (GALT) response and modulating both innate and adaptive immunity.[38] These peptides may potentially be utilized for therapeutic applications in immune-related disorders or to enhance the immune system's ability to respond to infections. Among these peptides, BCCY-1, derived from human β-casein, showcases immunoregulatory functions. This peptide not only induces chemokine activity but also activates the NF-KB and MAPK signaling pathways, unveiling its intricate role in shaping immune responses.[39] K-casein, the lone glycoprotein within the casein fractions, adds another layer of intricacy to the immunomodulatory spectrum. Its contribution to immune regulation signifies the complexity of milk proteins in influencing the host's immune landscape.[40] Interestingly, the immune-modulatory properties of αS1-casein are being studied for their potential therapeutic benefits. Recent research indicates that αS1-casein-derived peptides and κ-casein-derived peptides may help regulate the immune system by enhancing the GALT response, potentially improving mucosal immunity and modulating systemic inflammation. Some studies suggest that peptides of αS2-casein may have a role in regulating immune responses by enhancing mucosal immunity and balancing Th1/Th2 responses, potentially offering therapeutic benefits for inflammatory conditions.[38] However, further research is needed to fully understand these immune-modulatory effects in the context of αS2-casein consumption.

The immunoglobulins in bovine milk beta-globulin (IgG, IgA, IgM) are vital for immune defense. IgG is seen to play a central role in protecting the body against infections by binding to pathogens such as bacteria, viruses and fungi. In the context of milk, these immunoglobulins are particularly beneficial in infants, where they provide passive immunity by protecting mucosal surfaces (e.g., in the gut or respiratory tract) from infections. Research has shown that the ingestion of bovine milk immunoglobulins can support the immune system in both infants and adults, enhancing pathogen resistance and potentially reducing the incidence of infections.[41]

Alpha-Lactalbumin (α-LA) in bovine milk also plays a critical role in supporting the immune system. While its primary function is in the synthesis of lactose, recent studies have highlighted its ability to influence immune responses. As a bioactive protein, α-LA is rich in essential amino acids and can exert immunomodulatory effects. It has been shown to enhance the activity of immune cells such as macrophages and neutrophils, which are involved in defending the body against infections. Additionally, α-LA has been reported to have antimicrobial properties, which may help protect against infections by inhibiting the growth of certain bacteria and viruses, contributing to overall immune defense.[34]

It has been conclusively shown by several *in vitro* and *in vivo* investigations that milk whey proteins positively affect immunological responses. Mice given whey protein concentrate for 12 weeks had considerably stronger mucosal antibody responses to ovalbumin and cholera toxin than those fed a regular diet.[42] An extensive study also exists on the effects of whey protein on T cell populations. Whey protein concentrate fed mice (for 4 weeks) displayed larger numbers of helper cells and a higher helper/suppressor ratio than those fed an isocaloric casein diet. Additionally, mice fed with alpha-whey isolates demonstrated higher levels of total white blood cells, CD4+ and CD8+ lymphocyte counts and gamma-Interferon (IFN-γ) production by spleen cells compared to mice fed with casein and soy protein fractions.[43] In an alternate study with the administration of bovine lactoferrin in a dose dependent manner, the mice exhibited significant improvement in their delayed-type hypersensitivity towards a broad range of antigens.[44] Prescription of whey protein in cancer patients has been shown to normalise the quantity of blood leukocytes. Additionally, whey protein supplementation has been observed to improve plasma glutathione levels and Natural Killer (NK) cell activity in individuals with chronic hepatitis B.[45]

Immunoglobulins (Ig), intricate and diverse glycoproteins endowed with potent antibody activity, serve as the vigilant defenders of immune responses. In bovine milk, four out of the five Ig classes are present, namely IgG, IgA, IgE and IgM, each contributing to the multifaceted immune landscape. Among these, IgG emerges as the most abundant, constituting 2% of the total milk protein and 10% of the whey protein. This prevalence emphasizes its pivotal role in the immune defence mechanisms within milk.[46] The fundamental structure of Ig monomers involves two light chains (20 kDa) intricately connected with two heavy chains, the latter varying in size (50-70 kDa) depending on the specific Ig class. This intricate structure contributes to the diverse functionalities of immunoglobulins in orchestrating immune responses within the biological milieu. Moreover, recent research has shed light on the unique properties of IgA, which plays a crucial role in mucosal immunity, protecting against pathogens at mucosal surfaces of the gastrointestinal and respiratory tracts.

Additionally, IgE has been implicated in allergic responses, triggering immune reactions against harmless substances. However, the effectiveness of immunoglobulins is not without vulnerability, as they prove to be heat-sensitive proteins. The delicate balance of their structural integrity is influenced by thermal treatments, shedding light on the importance of considering temperature effects in preserving their functionality.[46] Beta-casein (β-casein) is also a known allergen, particularly in individuals with Cow's Milk Allergy (CMA), a condition most common in young children. Symptoms of CMA may include gastrointestinal disturbances, skin rashes and even severe anaphylactic reactions. In some individuals, αS1-casein is a potent allergen, particularly in children, leading to symptoms such as hypersensitive reactions and skin rashes. Like other casein proteins, αS2-casein and kappa-casein are known to be allergens in sensitive individuals, particularly in infants and young children.

In essence, the immunomodulatory prowess of specific milk protein fractions opens avenues for understanding their role in sculpting immune responses. As we unravel the molecular intricacies and functional outcomes of these proteins, a comprehensive picture emerges of milk proteins not merely as nutritional entities, but as dynamic regulators of the immune system, contributing to the intricate dance of immune responses within the human body. This understanding holds promise for the development of novel therapeutic interventions targeting immune-related disorders and enhancing overall health and well-being.

METABOLOMIC IMPACTS OF BOVINE MILK PROTEINS ON HUMAN PHYSIOLOGY

Metabolomics, with its study of a complete profile of low-molecular-weight metabolites generated in a biological system, provides an invaluable perspective on the complex interactions between milk proteins and metabolism. In metabolomic analyses, biological samples-typically feces, urine, or blood-are scrutinized using advanced techniques such as LC-MS (Liquid Chromatography-Mass Spectrometry), GC-MS (Gas Chromatography-Mass Spectrometry), or NMR (Nuclear Magnetic Resonance).[47] This analytical approach enables the identification and quantification of metabolites, providing insights into metabolic pathways influenced by dietary components. Research has illuminated the intricate interplay between diet, microbiota and insulin-related pathways. Both di*et al*one and diet combined with specific microbiota compositions can influence insulin resistance and the expression of insulin signaling genes.[48,49]

The intricate interplay between milk proteins and human physiology spans a spectrum of dynamic interactions that surpass basic nutritional functions. Milk proteins, including casein and whey, play a pivotal role by not only providing essential amino acids vital for tissue maintenance but also generating bioactive peptides during digestion. These peptides offer a myriad of potential health benefits, ranging from antioxidant properties to immunomodulatory effects.

β-casein's unique properties, such as its slow digestion and its ability to form gels in the stomach, offer various health benefits. Upon digestion, it releases several bioactive peptides that have been shown to possess physiological effects. Notably, beta-casein-derived peptides, such as casomorphins, have opioid-like effects that may influence gastrointestinal motility, satiety and digestion. For instance, casomorphins are known to interact with opioid receptors in the gut, potentially aiding in the regulation of gastrointestinal transit time and promoting gut health. Other peptides released from β-casein, such as antihypertensive peptides, have shown promise in reducing blood pressure, further extending the potential benefits of this protein for cardiovascular health.[50] Notably, the consumption of A1 β-casein has been linked to an increased incidence of Type-1 Diabetes (T1D) in genetically susceptible mice, with effects evident in subsequent generations.[51] This finding underscores the potential impact of milk protein variants on metabolic health outcomes and the importance of understanding their physiological effects. Metabolomics approaches, employing NMR and LC-MS techniques, have further delineated the distinct metabolic outcomes associated with different variants of β-casein. A2 β-casein intake, for instance, resulted in the release of metabolites such as lactose, proline and methionine, while A1 β-casein variant led to the release of citric acid, cAMP, choline and glycine.[52] Examining the impact of β-casein variants on toddlers, a study found that growing-up milk, containing A2 β-casein, improved digestive comfort and alleviated gastrointestinal symptoms in healthy toddlers with minor GI distress. Intriguingly, ingestion of A1 β-casein, rather than lactose, was associated with milk-related GI symptoms in certain individuals.[53] These findings suggest that the composition of milk proteins, particularly β-casein variants, can influence gastrointestinal health outcomes, highlighting the importance of personalized dietary interventions. Beyond diabetes-related effects, hyperlipidemia, a global metabolic syndrome characterized by elevated blood lipids such as triglycerides, low-density lipoprotein and cholesterol, has also been a focus of study. Reports suggest that oral treatment with casein-derived peptides has the potential to ameliorate hyperlipidemia.[54]

αS1-casein is also known for its role in promoting growth and development due to its high content of essential amino acids, calcium-binding properties and slow digestion profile. These attributes contribute to its bioavailability and ability to support various physiological processes, including muscle growth, bone health and immune function. However, its impact on human health is more complex than simply being a source of nutrients. The digestion of αS1-casein in the gastrointestinal tract results in the release of bioactive peptides, which may have diverse physiological effects. Some studies suggest that peptides derived from αS1-casein and αS2-casein, such as casokinins, exhibit anti-hypertensive, antioxidant and immune-modulating activities.[50] However, the slow digestion of αS1-casein, particularly in individuals with lactose intolerance or those with milk protein allergies, may cause gastrointestinal discomfort or adverse reactions.

κ-casein helps to form a gel-like structure in the stomach during digestion, slowing down gastric emptying and promoting satiety. This slow digestion allows for the gradual release of amino acids and other nutrients, which enhances nutrient absorption and improves the bioavailability of minerals such as calcium. This prolonged nutrient release is particularly beneficial in the context of growth and development, especially in infants and children, where efficient nutrient absorption is critical. Like other

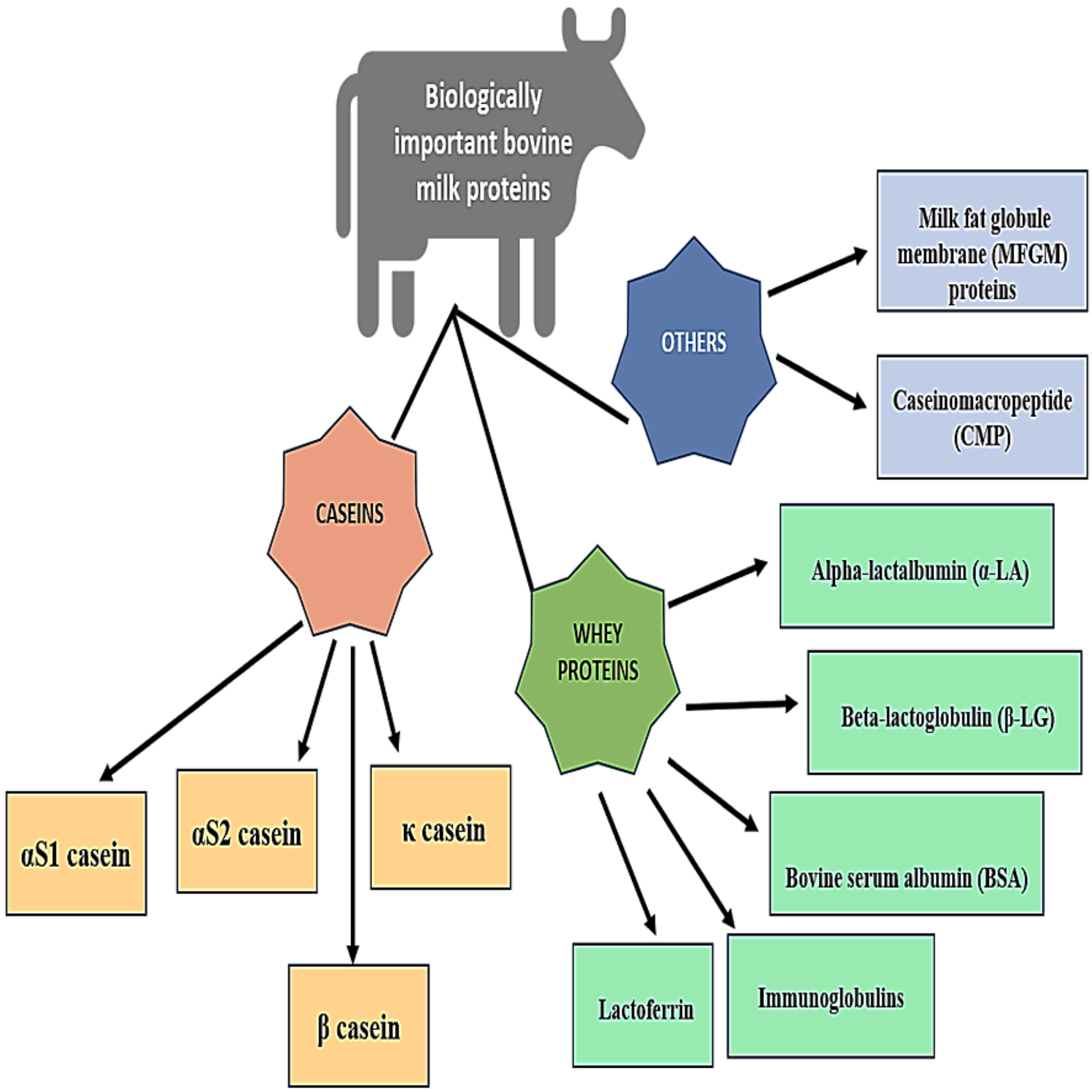

Figure 1: Types of Bovine Milk Proteins.

milk proteins, κ-casein is a source of bioactive peptides that are released during digestion. These peptides also have been found to possess a range of physiological effects, such as anti-hypertensive, anti-inflammatory and antioxidant properties. Some of the peptides released from κ-casein, include casokinins as seen earlier, have been shown to exhibit potential therapeutic effects, such as modulating blood pressure and reducing oxidative stress, which may have implications for cardiovascular health.[50]

Serum albumin, a protein in the β-globulin fraction, plays an essential role in transporting nutrients, such as fatty acids, hormones and vitamins, throughout the body. Serum albumin helps maintain protein homeostasis, regulate blood pressure and balance fluids in the body. By improving nutrient absorption and transport, bovine milk beta-globulin proteins may support metabolic health, including the regulation of blood glucose, lipid metabolism and overall energy balance. These properties suggest that bovine milk beta-globulin could potentially be beneficial in managing conditions related to metabolic dysfunction, such as obesity, type 2 diabetes and cardiovascular diseases. Additionally, by improving gut health and promoting a balanced microbiome.

Current research endeavours confirm the profound impact of milk proteins on cellular signalling pathways and metabolic processes. Moreover, age-dependent effects bring to light the diverse physiological impacts of milk proteins across various life stages, from infancy through old age. An evolving comprehension of how milk proteins shape human physiology, holds promise for targeted nutritional interventions and the development of functional foods aimed at optimizing human health. This intricate exploration into the metabolomic effects of milk proteins sheds light on their diverse roles in shaping metabolic responses and highlights the potential for targeted nutritional interventions aimed at improving metabolic health outcomes. Through continued research in this field, we can further elucidate the mechanisms underlying the metabolic effects of milk proteins and develop innovative strategies for optimizing dietary interventions to promote overall health and well-being.

INTERPLAY BETWEEN BOVINE MILK PROTEINS AND THE GUT MICROBIOME

The gut microbiome is a complex ecosystem of microorganisms that plays a pivotal role in health, influencing digestion, metabolism and immune function. The intricate relationship between milk proteins and the gut microbiome unfolds a tapestry of influences on human health, spanning from early development to aging and various physiological states. Throughout evolution, a mutualistic relationship between humans and their gut microbial ecosystem has provided information about additional metabolic and genetic traits.[55] This ecosystem, residing in the gut, plays a pivotal role in influencing host behavior and brain development. Notably, the choice of milk proteins can impact the gut microbiome, consequently affecting emotional behavior. A study reports that the intake of A1 β-casein variant milk in early postnatal life may negatively influence microbiome development, linking it to emotional behavior.[56] The modulatory effects of milk proteins extend to conditions like Type 2 diabetes, where GHP casein (glycomacropeptide hydrolysate) has shown to alter gut microbiota composition. Specific bacterial strains were seen to increase with GHP supplementation, potentially mitigating diabetic effects.[57]

Moreover, cow milk consumption, as observed in postmenopausal women, not only enhances beneficial bacterial taxa but also impacts functional modules, including biotin synthesis.[58] Ageing mice fed with A2 milk exhibit improved gut villi morphology and increased short-chain fatty acids, influencing CD4+ T cell differentiation and enhancing gut health.[59] In hypertensive rats, probiotic fermented milk containing *Lactiplantibacillus plantarum* strains demonstrates the ability to regulate the intestinal microecology, lower blood pressure and attenuate renal injury.[60] Lactoferrin, being a milk protein, emerges as a key player in cognitive function in male obese mice with Western diet-induced cognitive impairment, acting through the microbiome-gut-brain axis.[61] Additionally, lactoferrin has preventive effects on chronic alcoholic liver injury.[62]

Additionally, Milk Fat Globule Membrane (MFGM) supplementation proves beneficial in neurodevelopmental delay, especially in offspring of pre-pregnancy obese individuals and those consuming a high-fat diet during pregnancy and lactation.[63] Stress-sensitive rats, when fed with a diet enriched with *Lacticaseibacillus rhamnosus* HN001 and MFGM, demonstrate synergistic physiological changes affecting neural pathways involved in anxiety, memory depression and fear.[64] Distinct bacterial responses are noted with the consumption of cow milk versus plant-based drinks, with an increase in *Bifidobacteriacea* bacteria observed in cow Milk Consumers.[65] Protein Hydrolysate (WPH) supplementation, in addition to being a rich source of essential amino acids, positively modulates the infant gut microbiome, stimulating probiotic bacteria like *Lactobacillus acidophilus* and altering the Firmicutes/Bacteroidetes ratio.[66]

The intake of casein, including αS1-casein, αS2-casein, β-casein and κ-casein, play a crucial role in gut microbiota diversity, composition and modulation due to the stimulation of beneficial bacteria such as *Lactobacillus* and *Bifidobacterium* species. These are partly because of the prebiotic functionalities of the bioactive peptides released from the food during digestion, which increase the growth of beneficial microbes while inhibiting pathogenic bacteria, hence promoting intestinal health and immune responses.[67] Casein-derived peptides increase the gut barrier function since they regulate the tight junction proteins, reduce intestinal permeability and decrease states of IBS, IBD and leaky gut syndrome. SCFAs (Short-Chain Fatty Acids) such as butyrate, produced as a result of the fermentation of casein, preserve the gut barrier integrity as well as modulate immune responses and lead to healthy metabolic outcomes of glucose metabolism and lipid profiles.

α-LA, BSA and β-LG have important roles in modulating the gut microbiota and their health. α-LA, on its part, also selectively promotes good bacteria strains with reduced pathogenic strains through its bioactive peptides that promote the growth of SCFA and protects the integrity of the gut barrier. It also binds to GALT (Gut-Associated Lymphoid Tissue) that aids the maintenance of the immune response with healthy intestinal health. Likewise, BSA provides a set of essential amino acids and peptides that promote good microbial flora growth, suppress inflammation and improve gut barrier function. These effects of α-LA and BSA lead to low systemic inflammation, increased diversity in microbes and balanced modulation in immunity resulting from increased or reduced risks of diseases and syndromes like IBS (Irritable Bowel Syndrome), IBD (Inflammatory Bowel Disease) and metabolic disorders as shown with conditions like obesity and diabetes.[68]

The complex interplay between milk proteins and the gut microbiome extends beyond nutritional considerations, influencing a spectrum of physiological processes and health outcomes. This intricate relationship highlights the need for a holistic understanding of the impact of milk proteins on the human body, underscoring the importance of further research in elucidating these mechanisms for the development of targeted nutritional interventions aimed at optimizing health and well-being as well as maintaining overall metabolic homeostasis. The diverse health benefits of a few important milk proteins have been consolidated in (Table 1).

CELLULAR SIGNALING PATHWAYS OF BOVINE MILK PROTEINS WITHIN CELLS

Within the intricate interweave of cellular signaling pathways, milk proteins emerge as influential communicators, exerting pronounced effects on specific cellular processes. Mechanistic Target of Rapamycin Complex 1 (mTORC1) is the master

Table 1: Summary of health benefits of some important bovine milk proteins.

Protein	Gut microbiome modulation	Gut barrier function	Immunity	Metabolic health	Additional benefits	References
αS1-casein	Promotes *Lactobacillus* and *Bifidobacterium through prebiotic peptides; inhibits pathogens.*	Enhances tight junction proteins, reducing intestinal permeability and preventing leaky gut.	Modulates GALT, supports mucosal immunity and reducing systemic inflammation.	Improves glucose regulation and reduces risk of obesity.	Supports overall metabolic health by improving SCFA production and nutrient absorption.	[67]
αS2-casein	Modulates microbiota diversity; promotes SCFA production, especially butyrate.	Improves gut barrier integrity; reduces intestinal inflammation and supports tight junction problems.	Enhances Th1/Th2 immune balance; reduces systemic inflammation.	Regulates glucose metabolism and lipid profiles, reducing risks of obesity and type 2 diabetes.	Contributes to satiety through gel-like consistency; promotes cardiovascular health via anti-hypersensitive and anti-inflammatory peptides.	
β-casein	Acts as a prebiotic for *Lactobacillus* and *Bifidobacterium;* inhibits harmful microbes/ pathogens.	Strengthens intestinal epithelial integrity by modulating tight junctions.	Enhances innate and adaptive immunity through GALT activation; supports anti-inflammatory responses.	Improves lipid profiles, insulin sensitivity and reduces risks of obesity and metabolic syndrome.	Releases casomorphins that regulate gut motility; produces SCFAs for anti-inflammatory effects.	
κ-casein	Selectively promotes *Bifidobacterium* and *Lactobacillus* species; increases SCFA production.	Strengthens gut barrier by reducing permeability and supporting epithelial integrity; mitigates IBS and IBD symptoms.	Modulates GALT for improved immune function; reduces systemic and localized inflammation.	Reduces risks of cardiovascular disease and metabolic disorders like diabetes.	Offers potential therapeutic applications in hypersensitive and oxidative stress management through bioactive peptides.	

Protein	Gut microbiome modulation	Gut barrier function	Immunity	Metabolic health	Additional benefits	References
β-LG	Act as a prebiotic; fosters gut microbiota diversity; promotes SCFA production, including butyrate.	Also supports gut barrier by reducing intestinal permeability; promotes GALT development.	Enhances immune responses by preventing pathogen adhesion and supporting systemic immunity.	Reduces chronic inflammation and metabolic risks, including obesity and diabetes.	Acts as an antimicrobial agent against pathogens; supports nutrient transport and energy metabolism.	[68]
α-LA	Promotes *Lactobacillus* and *Bifidobacterium* growth; inhibits harmful bacteria; increases microbial diversity.	Maintains tight junction proteins, preventing intestinal inflammation and leaky gut.	Modulates immune responses via GALT; reduces systemic and gut inflammation; supports immune balance.	Improves energy metabolism and glucose regulation; reduces obesity risk by modulating fate deposition.	Exhibits anticancer properties through apoptosis induction; acts as an antioxidant, reducing oxidative stress.	
BSA	Supports beneficial microbes through amino acids and peptides; fosters microbial balance.	Maintains gut permeability and strengthens the intestinal barrier; reduces leaky gut symptoms.	Regulates immune responses through GALT interaction; reduces chronic inflammation, particularly in autoimmune and IBD diseases.	Enhances nutrient absorption and metabolic control; may reduce risks of metabolic disorders like obesity and diabetes.	Acts as a carrier for hormones and vitamins; supports systemic health by reducing oxidative stress and improving protein homeostasis.	

regulator that controls some of the most crucial cellular processes. A significant amount of research has uncovered the signaling pathways regulated by mTORC1 and the involvement of these signaling cascades in human diseases, such as cancer, diabetes and aging.[69] Milk consumption stimulates mTORC1 through five pathways: (1) growth factors (GH, insulin and IGF-1), (2) amino acids (BCAAs), (3) milk fat-derived palmitic acid, (4) lactose (β-D-galactopyranosyl-(1→4)-D-glucose) and (5) epigenetic modifiers (MEX-derived miRs). Milk consumption triggers Milk-Induced Growth Factor signaling which enhances the levels of Growth Hormone (GH) by replenishing the amounts of Insulin Growth Factor 1(IGF1).[70] Additionally, milk consumption has been shown to increase trophoblast mTORC1 activity which downstream affects placental-fetal transfer of amino acids and glucose, resulting in fetal growth and birthweight.[71] Moreover, whey proteins, being another essential component of milk, have been shown to modulate cellular signaling pathways associated with muscle protein synthesis. Specifically, whey protein consumption has been linked to the activation of the mTOR pathway, a key regulator of muscle protein synthesis, thereby promoting muscle growth and repair.

Milk is an excellent source of Micro RNA (MiR), particularly MiR 148a, which is highly conserved across mammals. Nucleotide sequences from human and cow have been shown to be identical, enabling MiR-based cross-species interaction. DNA methyltransferases (DNMT), which is one of the prime targets for MiR148a; inactivation of DNMT leads to the expression of developmental genes such as IGF1 and mTORC1, thereby promoting growth.[72]

Notably, in the context of Triple Negative Breast Cancer (TNBC), the deadliest breast cancer subtype, the milk protein α-casein takes center stage in orchestrating cellular responses, thereby showcasing their integral role in both prevention and treatment strategies. Research on Breast Cancer Stem Cells (BCSC) reveals that α-casein exhibits a remarkable capacity to reduce BCSC in the triple-negative MDA MB-231 cell line. Beyond this, its influence extends to impeding the transformation of these cells into a Cancer-Associated Fibroblast (CAF) phenotype within TNBC, a phenomenon mediated by HIF-1 alpha.[73] The multifaceted role of α-casein is further underscored by its novel potential as a suppressor of tumor growth and metastasis. It achieves this by down-regulating gene transcripts associated with stemness while concurrently up-regulating gene transcripts linked to interferon/STAT1 signaling.[74] Emerging research has suggested that α-LA possesses anticancer properties, particularly through its ability to induce apoptosis (programmed cell death) in certain cancer cells. α-LA may also act as an antioxidant, protecting cells from oxidative stress, which is a contributing factor in the development of various diseases, including cancer and cardiovascular disease. These bioactive properties could provide significant therapeutic benefits, particularly in the prevention of chronic diseases. Expanding our exploration into cellular signaling pathways, casein undergoing hydrolysis by microbial proteases, can yield peptides with distinct bioactive properties. Four such peptides-YQLD, FSDIPNPIGSEN, FSDIPNPIGSE, YFYP-identified through LC-MS/MS, undergo chemical synthesis and characterization in an oxidatively damaged HepG2 cell model. These peptides, when activated, demonstrate the potential to serve as antioxidant agents by engaging the Keap1-Nrf2 signaling pathway. This dual functionality positions them not only as candidates for pharmaceutical preparations but also as valuable additions to functional foods.[75]

The dynamic interplay between milk proteins and cellular signaling pathways unfolds a narrative of profound impact on cellular behavior, particularly in the context of breast cancer, antioxidant responses and muscle protein synthesis. These findings pave the way for a deeper understanding of the therapeutic potential of milk proteins and their peptides in modulating cellular processes, offering new horizons for both medical interventions and functional nutrition. Further research in this area holds promise for the development of targeted therapies and nutritional strategies to promote overall development and well-being.

CHALLENGES AND FUTURE PROSPECTS

While milk proteins offer undeniable nutritional benefits, there are issues like allergies and ethical considerations to be carefully examined. The quest to surmount these challenges has led to notable advancements in biotechnology and sustainable agriculture, paving the way for innovative solutions to address these concerns. Particularly, alternative protein sources, such as plant-based and cell-cultured proteins, are gaining prominence as viable alternatives to mitigate the environmental impact associated with traditional milk production methods. Moreover, breakthroughs in allergen-free milk protein production techniques are revolutionizing the landscape of dairy consumption. These innovations not only cater to individuals with allergies but also contribute to a more inclusive and accessible dairy market. The transformation brought about by allergen-free production techniques signifies a positive shift towards enhancing the diversity and acceptance of dairy products within the broader consumer base. By leveraging cutting-edge technologies and scientific advancements, the dairy industry is poised to meet the evolving needs and preferences of consumers while addressing pressing concerns related to sustainability and allergen management.

Hence, the trajectory of milk proteins into the future hinges on the relentless pursuit of innovation, pushing boundaries and envisioning new horizons. This section of the review article embarks on an exploration of cutting-edge research and pioneering applications that are shaping the next frontier of milk proteins. Our journey into the future unfolds with a focus on groundbreaking research endeavours and their practical applications. From the inception of personalized nutrition recommendations rooted in individual milk protein profiles to the evolution of milk protein-based functional foods designed to address specific health needs, the potential avenues are boundless. We may delve into the synergies of science and nutrition, uncovering the transformative possibilities that milk proteins offer for customized dietary interventions. Emerging technologies, such as advanced proteomic analysis and bioinformatics, enable the precise characterization of milk protein variants, paving the way for precise dietary recommendations based on individual genetic makeup and metabolic profiles.

Furthermore, our exploration can extend to the realm of precision medicine, where the potential of milk proteins takes centre stage. Here, we may contemplate the prospect of tailored diets enriched with specific milk proteins as a proactive approach to prevent and manage various health conditions. By leveraging our understanding of how different milk proteins interact with the human body at a molecular level, we can design bespoke dietary interventions recommended for individual health goals and genetic predispositions. This visionary perspective underscores the pivotal role that milk proteins could play in the future landscape of precision medicine, offering nuanced and targeted dietary solutions for optimizing health and well-being. As we navigate through these frontiers, the potential applications of milk proteins in shaping a healthier and more personalized future come into sharper focus. Collaborations between interdisciplinary teams of scientists, nutritionists and healthcare professionals will be crucial in translating cutting-edge research findings into actionable strategies for improving human health. By harnessing the power of innovation and scientific discovery, we can unlock the full potential of milk proteins as versatile agents of health promotion and disease prevention in the years to come.

SUMMARY AND CONCLUSION

This comprehensive review has delved into the multifaceted world of milk proteins, shedding light on their pivotal role in human health and nutrition. Beginning with an exploration of their historical significance as essential contributors to human nutrition, providing vital amino acids and nutrients, the review has traversed through various dimensions to reassess our understanding of milk proteins in the context of human well-being. Throughout the investigation, recent advances in proteomics, metabolomics and molecular biology have been instrumental in uncovering the intricate mechanisms through which milk proteins influence various aspects of health. From their impact on the gut microbiome to their modulation of immune responses and cellular signalling pathways, milk proteins have been revealed as dynamic regulators of human physiology. Moreover, the review has addressed pressing challenges such as sustainability, allergies and ethical concerns surrounding milk protein production. Innovative solutions, including advancements in biotechnology and sustainable agriculture, have been explored, alongside promising developments in allergen-free milk protein production to promote inclusivity in dairy consumption. Looking ahead, the review envisions a future where milk proteins will play a central role in personalized nutrition and precision medicine. It serves as a call to action for stakeholders to recognize the invaluable contributions of milk proteins and harness their potential for the betterment of human well-being. By offering tailored dietary solutions and potentially serving as therapeutic agents in managing various health conditions, milk proteins hold transformative potential in revolutionizing nutrition and health for a more sustainable and healthier world.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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ABBREVIATIONS

α:LA: Alpha:Lactalbumin; **αS1:casein:** Alpha:S1:casein; **β:LG:** Beta:lactoglobulin; **BSA:** Bovine serum albumin; **BCSC:** Breast Cancer Stem Cells; **HIF:1** alpha:Hypoxia:inducible Factor 1:Alpha; **CAF:** Cancer: Associated Fibroblast; **CMP:** Caseinomacropeptide; **WPH:** Protein hydrolysate; **CMA:** Cow's milk allergy; **DNMT:** DNA Methyltransferases; **MEX:** derived; **miRs:** Milk Exosomal Micro RNAs; **(IFN:γ):** Gamma:interferon; **GC:MS:** Gas Chromatography:Mass Spectrometry; **GHP:** Glycomacropeptide hydrolysate; **GMP:** Glycomacropeptide; **BCAAs:** Branched:Chain Amino Acids; **GH:** Growth Hormone; **GALT:** Gut:associated Lymphoid Tissue; **IBD:** Inflammatory Bowel Disease; **IBS:** Irritable bowel syndrome; **Ig:** Immunoglobulins; **IGF:** Insulin Growth Factor; **STAT1:** Signal transducer and Activator of Transcription 1; **κ:CASEIN:** kappa Casein; **LF:** Lactoferrin; **LP:** Lactoperoxidase; **LC:MS:** Liquid Chromatography: Mass Spectrometry; **MAPK:** Mitogen:Activated protein kinase; **mTORC1:** Mechanistic Target of Rapamycin Complex 1; **MiR:** Micro RNA; **MFGM:** Milk fat globule membrane; **NK:** Natural killer cells; **NF: κ B:** Nuclear Factor Kappa B; **NMR:** Nuclear Magnetic Resonance; **SCFA:** Short:chain fatty acids; **Th1/Th2:** T helper cells; **TNBC:** Triple Negative Breast Cancer; **T1D:** Type:1 Diabetes.

ETHICAL STATEMENT

No laboratory studies or experiments were performed for this review article. All data, findings and references included in this work were sourced from previously published studies and proper attribution has been given to the original authors. The authors declare that there are no conflicts of interest and the manuscript adheres to ethical standards for academic publishing, including ensuring accuracy, transparency and integrity in the presentation of data and conclusions. No human or animal subjects were involved in this review.

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