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Review

The Impact of A1- and A2 β-Casein on Health Outcomes: A Comprehensive Review of Evidence from Human Studies

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Featured Application

This scoping review can be used to provide evidence-based dietary recommendations for children, middle-aged adults, and athletes regarding A2 or conventional dairy intake and their expected health outcomes.

Abstract

The digestion of A1 β-casein present in conventional milk releases β-casomorphin-7 (βCM-7), a bioactive peptide with potential implications for gastrointestinal and neurological health. A scoping review was performed to respond to the following research question: What are the health effects of consuming milk containing the A1 β-casein variant compared to the exclusive consumption of the A2 variant in humans? The evidence collected in this review of human studies with different populations (i.e., children, middle-aged adults, athletes) suggests that the consumption of milk containing A1 β-casein may negatively influence gut health by altering microbial composition, reducing intestinal motility, and increasing colonic fermentation, leading to elevated gas production and altered short-chain fatty acid (SCFA) profiles. The release of βCM-7 upon digestion can also compromise intestinal-barrier integrity, which may exacerbate symptoms of lactose intolerance, irritable bowel syndrome (IBS), or other allergy-related sensitivities. Its ability to cross the blood–brain barrier raises concerns about potential neurological effects. In contrast, milk containing exclusively A2 β-casein is associated with improved gastrointestinal outcomes, including the enhanced abundance of beneficial bacteria such as *Bifidobacterium* spp. and reduced inflammatory markers.

Keywords: β-casein; A2 milk; health; immune function; human studies; children; middle-aged adults; athletes; scoping review

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1. Introduction

Cow's milk is a staple in many diets around the world, but the potential health effects of milk proteins remain a matter of debate, especially those related to the consumption of β-casein A1 and A2 variants. There are thirteen genetic variants of β-casein, but A1 and A2 are the most common [1]. Caseins comprise 80% of the protein in cow's milk, and β-casein represents about 22% of the total casein [2]. The A1 and A2 variants have attracted attention in the health field because of their potential implications in various diseases [3–5].

The only difference between A1 and A2 β-caseins is a single amino acid at position 67 of the protein chain: A1 has histidine, whereas A2 has proline [4]. This single substitution has significant consequences for digestion and physiological effects. The presence of histidine in A1 makes it susceptible to enzymatic cleavage during digestion, releasing a peptide called β-casomorphin-7 (βCM-7). The proline in A2 prevents "sterically hinders" cleavage, so βCM-7 is released in much smaller amounts, probably minimal or barely noticeable ones [4,6].

While food processing does not change one variant into another, certain processing methods can influence the release of these peptides into the processed food or during digestion in the intestine. For example, the curdling and aging processes in cheesemaking can alter the structure of casein. Cross-linking and other modifications cause individual casein molecules to no longer be in equilibrium with the micelle. The transport of intact molecules through the gut may therefore be prevented or hindered [7]. However, in Zinßius et al.'s study, βCM-7 concentrations were higher in the A1A1 cheeses after pressing and during ripening [8]. Standard processes applied to milk, such as heat treatment and homogenization, although they can affect the overall structure of milk proteins, such as β-lactoglobulin denaturation and complex formation, do not appear to influence βCM-7 release [9].

In 2009, the European Food Safety Authority (EFSA) concluded that there is no direct link between βCM-7 and non-communicable diseases like cardiovascular conditions, autism, or insulin-dependent diabetes [10]. But βCM-7 is an opioid peptide with moderate agonist activity at the μ4 receptors [11,12]. This means that it can have morphine-like effects in the body. It has been suggested that βCM-7 may be associated with several health problems, which has led to a growing interest in the consumption of A2 dairy products, which are marketed as a healthier alternative [13]. One hypothesis suggests that βCM-7's presence in the gut triggers the activation of opioid receptors, potentially disrupting the balance of intestinal microbiota. This imbalance may compromise the integrity of the intestinal barrier and interfere with the bile acid metabolism, leading to additional effects. Recent research has increasingly focused on the gut–brain axis as a key pathway mediating the effects of dietary peptides such as βCM-7 [14].

Animal studies have been instrumental in understanding the health effects of A1 and A2 β-caseins, allowing a controlled analysis of their effects on various biological systems [15]. The main findings of these studies are that β-casein A1 has been associated with delayed gastrointestinal transit in rodents, whereas A2 appears to facilitate faster digestion. This effect may be due to the release of βCM-7 during A1 digestion, which can affect intestinal motility [3]. A1 has also been associated with an increase in the activity of myeloperoxidase, a marker of inflammation, in the rat intestine. This finding suggests that A1 may contribute to intestinal inflammation, a factor implicated in several digestive diseases [16]. In mice, feeding A1 was associated with an increase in leukocyte infiltration and the production of interleukin-4 and immunoglobulin E, indicators of an inflammatory response. These results suggest that A1 may promote a Th2 inflammatory response associated with allergy and asthma. In contrast, A2 appears to have a protective effect in this context [3,16,17]. Studies in rabbits have shown that A1 can promote the formation of atheroma plaques in the arteries, a key factor in the development of cardiovascular disease. It has been suggested that this effect may be mediated by βCM-7′s ability to increase blood cholesterol levels. In contrast, A2 has been shown to have a small protective effect against the formation of these plaques [15,18]. A multi-center study in rats and mice with type 1 diabetes found no significant difference in disease incidence between animals fed A1 and A2 [19]. This finding challenges the hypothesis that βCM-7 released during A1 digestion may contribute to the development of type 1 diabetes [20]. A1 has been found to induce

lung inflammation in mice, whereas A2 does not [21]. This finding suggests that A1 may be involved in the development of respiratory diseases.

These studies offer valuable insights, yet the applicability of findings from animal models to humans remains limited. Physiological and metabolic differences between species can significantly influence the response to A1 and A2 β-caseins. While animal research has provided a critical foundation for understanding the potential impacts of these milk proteins, focusing on human studies is essential to directly evaluate how genetic variations in β-casein affect health outcomes such as digestion, inflammation, and the risk of chronic diseases. It is known that not all individuals are affected by the peptide. A small subset of people seems to be more susceptible to the exposure and effects of βCM-7, which may trigger symptoms in various ways, either individually or in conjunction with other health conditions [22].

This review emphasizes the gut–neural axis effects of βCM-7 as a central research thread. By integrating evidence from gastrointestinal, immunological, and neurological studies, the manuscript aims to provide a cohesive perspective on how βCM-7 derived from A1 β-casein may influence health through interconnected digestive and neural pathways. Our aims were to critically analyze and synthesize human studies on the health effects of A1 and A2 β-casein proteins, with a particular emphasis on three key populations: children, who represent the most vulnerable group due to their developmental stage and sensitivity to dietary factors; athletes and individuals with high protein intake, for whom milk protein composition can significantly affect not only muscle recovery and performance but also digestive comfort; and middle aged adults, particularly regarding cardiovascular risks and inflammation-related conditions, as well as digestive disturbances associated with β-casein A1.

2. Methodology

Search Strategy and Inclusion Criteria

An exploratory review was carried out to gather and evaluate the breadth of existing evidence related to the topic, aiming to map out the current state of the research. The methodology was guided by the PRISMA Extension for Scoping Reviews (PRISMA-ScR) guidelines [23]. The process involved clearly stating a research question, searching for and identifying relevant studies, carefully selecting appropriate sources, organizing and extracting key data, and ultimately summarizing and presenting the findings as outlined below [24].

The core question that guided this review was

- What are the health implications for humans of consuming milk with the A1 β-casein protein variant compared to consuming milk that contains only the A2 β-casein variant?

Secondary questions:

- How do βCM-7 and the microbiota interact in gut health and disease regulation?
- How does individual susceptibility to βCM-7 exposure influence the observed effects of A1 β-casein consumption?
- How does the consumption of A1 β-casein versus A2 influence digestive health in different populations?
- Is there an association between A1 β-casein consumption and an increased risk of inflammation or cardiovascular disease?
- How does A1 β-casein affect muscle performance and recovery in athletes?
- How does A1 β-casein influence immune function?
- What is the impact of A1 β-casein on neurological and cognitive health?

In March 2025, an extensive search for the relevant literature was conducted using databases such as PubMed, Scopus, and the Web of Science Core Collection. There were no restrictions placed on the publication dates. Both the keywords and standardized terms related to five primary concepts were applied to the titles, abstracts, and keywords of the articles identified:

- Types of β-casein (A1 β-casein, A2 β-casein, β-casein variants, milk proteins);
- βCM-7 (βCM-7, BCM-7, β-casomorphin-7, β-casomorphin, opioid peptides);
- Human studies (clinical trials, intervention studies, observational studies, cohort studies, case-control studies);
- Microbiota perturbations and βCM-7 (gut–brain axis, intestinal barrier, gut microbiome, gut microbiota);
- Human health outcomes (chronic diseases, gastrointestinal health, cardiovascular health, diabetes, mental health, autism, autoimmune diseases, muscle performance and recovery);
- Population (children, middle-aged adults, athletes).

Original research papers investigating associations between the effects of A1 β-casein or βCM-7 compared to A2 β-casein on health-related outcomes were included. Only full-length articles and patents were selected.

Various types of studies involving human participants were considered, including randomized controlled trials, prospective and cohort studies, as well as cross-sectional observational and epidemiological research. Review articles were not included in the analysis. Information was gathered from the complete texts of the selected articles, which were then thoroughly examined. Each study was first evaluated on its own, and then the results were compared and synthesized.

The selection of studies and the extraction of relevant data were carried out independently by two reviewers. The findings were presented both in tabular format and through descriptive summaries.

3. Results

3.1. Synthesis

A total of 157 records were initially identified from the database searches, after applying the inclusion and exclusion criteria and removing duplicate entries. Screening of the titles and abstracts resulted in 50 studies being chosen for full-text evaluation. A total of 30 publications fulfilled the eligibility requirements and were incorporated into this scoping review. The entire selection process and its results are illustrated in the PRISMA flow diagram (Figure 1).

The details of the data abstraction, including an overview of the study design, population size, sample characteristics, methodology, primary parameters, observed outcomes, and key findings for all of the studies included in the review, are summarized in Table 1.

The studies reviewed here were conducted in various countries, including Australia [25–28], New Zealand [29–31], China [32–37], South Korea [38], USA [39–43], United Kingdom [44], Indonesia [45], and Russia [46,47].

From the 30 studies included, 3 were epidemiological studies [27,48,49], 24 were interventional clinical trials [25–41,43–46,50–53], and 3 were not dietary intervention studies [22,46,47].

In terms of the population studied, 12 studies involved children and/or adolescent population [22,25,27,34,35,37,38,45–47,53,54]. The rest of the trials involved adults.

Human studies investigating the potential effects of βCM-7 are limited and challenging to conduct. Most dietary-intervention studies have focused on evaluating the association between milk consumption and gastrointestinal symptoms (or related serum or urinary

markers). The link between A1/A2 milk consumption and the nervous and immune systems is primarily restricted to epidemiological association studies.

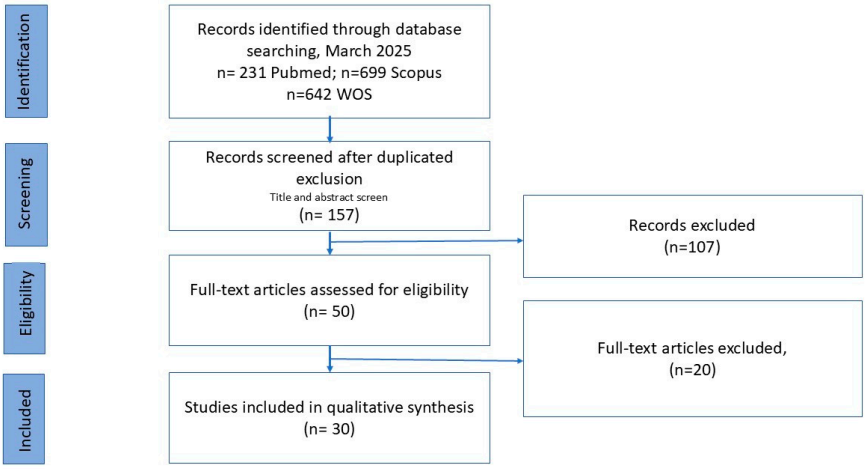

Figure 1. PRISMA flow diagram [23].

Table 1. Summary of clinical studies on the effects of milk β-casein variants in human health outcomes (children, middle-aged adults, and athletes).

Reference	Study Design	Population	Methodology	Outcome	Results	Main Finding
(McLachlan, 2001) [48]	Epidemiological	WHO MONICA PROJECT. Ecological data (1985–1990), 17 countries	Correlation between β-casein A1 and IHD mortality rates	IHD mortality rates	Strong correlation (r^2 = 0.86)	A1 β-casein may be a dietary risk factor in IHD
(Boland MJ et al., 2002) [27]	Correlational epidemiological	Twenty "healthcare-affluent" countries	Estimated A1/A2 intake and mental disorder mortality	Mental disorder	Positive correlation with A1 intake	A1 β-casein may be linked to mental disorder mortality
(Crawford et al., 2002) [27]	Epidemiological	67 autistic children	Milk with histidine or proline β-casein; urine BCM levels	βCM-7 in urine	Higher βCM-7 (urine from autistic children) in histidine group	Histidine variant may contribute to neurological disorders in susceptible individuals
(Laugesen & Elliott, 2003) [49]	Epidemiological	Data from: 20 countries IHD mortality; 51 countries DM-1	Correlation between per capita A1 β-casein and incidence IHD and DM-1	IHD and DM-1 rates	Strong correlations	Further studies needed to confirm A1's role in CHD and DM-1
(Chin-Dusting et al., 2006) [26]	RCT	15 high-CVD-risk adults	12-week A1/A2 intake; vascular function markers	CVD risk biomarkers	No significant differences	No cardiovascular disadvantage with A1
(Venn et al., 2006) [31]	Crossover trial	62 adults	4.5-week A1/A2 vs. A2 dairy consumption	Plasma lipid profile	No significant differences	A1/A2 do not impact cholesterol
(Kost et al., 2009) [46]	Longitudinal observational	90 infants	Plasma βCM-7, psychomotor evaluation	βCM-7 levels and development	Elevated βCM-7 in formula-fed with delays	A1 milk may affect development; breastfeeding beneficial
(Crowley et al., 2013) [25]	RCT crossover	52 children	Soy vs. cow's milk, then A1 vs. A2	Constipation resolution	Soy milk resolved CFC; no diff. A1/A2	Cow's milk (not casein type) linked to CFC
(Ho et al., 2014) [28]	RCT crossover	41 adults	2 weeks A1 then A2 milk	Bowel habits, pain	Higher stool consistency and pain with A1	A1 linked to GI symptoms
(Sokolov et al., 2014) [47]	Case-control	20 children aged 4–8 years: 10 healthy controls, 10 ASD	βCM-7 in urine, autism severity	βCM-7 vs. CARS	Higher βCM-7 in autistic children	βCM-7 may play role in ASD
(Jianqin et al., 2015) [33]	RCT crossover	45 adults	A1/A2 vs. A2 milk	GI symptoms, inflammation, cognition	A1 increased symptoms and inflammation	A2 milk improved GI and cognitive outcomes
(Deth et al., 2015) [39]	RCT crossover	45 adults	A1/A2 vs. A2 milk	Glutathione, βCM-7	A2 milk ↑GSH, ↓βCM-7	A2 may boost antioxidant capacity
(He et al., 2017) [32]	Multicenter RCT	600 adults	A1/A2 vs. A2 milk	GI symptoms	A2 milk reduced GI symptoms	A2 milk better for lactose intolerance

Table 1. *Cont.*

Reference	Study Design	Population	Methodology	Outcome	Results	Main Finding
(Kirk et al., 2017) [44]	RCT placebo-controlled	21 athletes	Post-exercise milk intake	Muscle recovery	A2 and regular milk > placebo	A2 helps in recovery for A1-intolerant
(Clarke AJ & Yelland GY, 2017) [53]	RCT crossover	40 adults + 30 preschool children	A1/A2 vs. A2 milk	Cognitive tests (DSST)	A2 improved processing speed	A2 enhances cognition and comfort
(Jarmołowska et al., 2019) [22]	Case-control	137 children	βCM-7, DPPIV assays	Serum/urine levels	↑βCM-7 and DPPIV in ASD	Dairy peptides may relate to ASD
(Sheng et al., 2019) [34]	RCT crossover	75 preschoolers	5 days A1/A2 vs. A2 milk	GI symptoms, cognition	↓Symptoms, inflammation with A2	A2 improves comfort and cognition
(Milan et al., 2020) [29]	RCT	40 women	Milk challenge by type	Digestive symptoms	A2 ↓symptoms in lactose-intolerant	A2 may benefit LI but not NLDI
(Ramakrishnan et al., 2020) [43]	RCT crossover	33 adults	Single meal A1/A2 vs. A2	GI symptoms, H2 breath	Less pain with A2	A2 reduces symptoms in LI/maldigestion
(Ramakrishnan et al., 2020) [40]	RCT	21 adults	A1A2/Jersey/A2/Lactose free milk	GI symptoms, H2 breath	Less H2 with A2 and Jersey	A2 reduces symptoms in LI/maldigestion
(Lijun C et al., 2021) [52]	Cohort	60 adults	A1/A2 vs. A2 milk	GI symptoms, microbiota	A2 ↑Bifidobacterium	A2 improves gut health
(Prodhan et al., 2022) [30]	RCT crossover	40 women	3 milk types, plasma AAs	Protein digestion	No differences overall	Digestion not affected by intolerance
(Ramakrishnan et al., 2023) [41,50]	RCT crossover	10 LI adults	MRI gastric emptying	Gastric emptying time	A1 milk emptied faster	A1 leads to more symptoms
(Meng et al., 2023) [37]	RCT	387 toddlers	GUM A2 vs. conventional	GI symptoms, constipation	Less constipation in A2	A2 well tolerated in toddlers
(Choi et al., 2024) [38]	RCT crossover	Adults (n/s)	A2 vs. A1/A2 milk	GI symptoms, calprotectin	Less discomfort with A2	A2 milk better tolerated
(Ramakrishnan et al., 2024) [50]	RCT crossover	16 LI adults	2 weeks A2 vs. A1/A2	Symptoms, biomarkers	↓Bloating with A2	A2 improves symptoms over time
(Saiprasad et al., 2024) [51]	RCT crossover	16 LI adults	16 LI adults	GI symptoms, H2 breath, inflammatory markers and GSH levels	↓acute fecal urgencyH2 breath	A1/A2 increases digestive discomfort in LI
(Sheng et al., 2024) [36]	RCT	180 infants	A2 formula vs. standard	Growth, stool, bone	Better outcomes with A2	A2 formula improves infant health
(Novika et al., 2025) [45]	Quasi-experimental	30 children	3-month A2 milk	Growth, inflammation	↑Growth, ↓TNF-α and cortisol	A2 supports growth in stunted kids
(Yu et al., 2025) [35]	Open-label RCT	200 toddlers	A1PF vs. conventional formula	ARI, diarrhea, tolerance	↓ARI duration, better GI tolerance	A1-free formula may reduce illness duration

Note: AA (Amino Acids), A1PF (A1 Protein-Free Formula), ASD (Autism Spectrum Disorder), βCM-7 (β-casomorphin-7), CARS (Childhood Autism Rating Scale), CHD (Coronary Heart Disease), CFC (Chronic Functional Constipation), DPPIV (Dipeptidyl Peptidase-4), GI (Gastrointestinal), GSH (Glutathione), GUM A2 (Growing-Up Milk with A2 β-casein), H2 (Hydrogen gas, used in breath testing), IHD (Ischemic Heart Disease), LI (Lactose Intolerant), MRI (Magnetic Resonance Imaging), MONICA (MONItoring trends and determinants in CArdiovascular disease), NLDI (Non-Lactose Dairy Intolerant), RCT (Randomized Controlled Trial), TNF-α (Tumor Necrosis Factor Alpha), and WHO (World Health Organization). ↑ (increase), ↓ (decrease).

3.2. How Do βCM-7 and the Microbiota Interact in Gut Health and Disease Regulation?

The gastrointestinal mucosa represents the interface between the internal and the external environment. It is composed of multiple layers with specific functions whose integrity is essential for the body's health and defense [55]. The gut microbiome, considered a "hidden organ", plays a fundamental role in metabolism and immunity, highlighting its importance in health and disease management. Diet is a determining factor in gut-microbiota regulation, providing nutrients that influence its population. The microbiota, in turn, impacts nutrient absorption, metabolism, and host physiology [56].

βCM-7 acts as an agonist of μ-opioid receptors, which are predominantly found on nerve cells within the enteric nervous system that innervates the intestinal lining [57]. The activation of these receptors plays a vital role in numerous gastrointestinal functions, including maintaining the integrity of the intestinal barrier, regulating secretions, and

controlling motility [58]. Milk that contains both A1 and A2 β-casein may influence the gut microbiota differently than milk with only the A2 variant. Through its action, βCM-7 could indirectly impact the composition of the microbiota, thereby affecting gut motility, mucus secretion, and potentially contributing to inflammation [38].

Some research suggests that consuming only A2 β-casein may support better gastrointestinal function, possibly by promoting beneficial microbial populations. In contrast, A1 β-casein may be linked to slower digestion in the small intestine and increased fermentation in the colon, leading to changes in gas production and modifications in the microbial ecosystem [37]. For example, one study found that milk containing A2 β-casein can significantly boost the levels of *Bifidobacterium*, a bacterial genus known for its positive effects on gut health [52]. Additionally, other studies have indicated that the intake of milk with A1 β-casein is associated with alterations in the production of short-chain fatty acids—such as butyric, acetic, and propionic acids—which are important for colon health [33,40,43].

Regarding gut-barrier function, animal studies have shown that the consumption of A1 milk can trigger an inflammatory response characterized by increased myeloperoxidase (MPO) activity and increased neutrophil infiltration into the colon [17]. Furthermore, βCM-7 activates the TLR-2 and TLR-4 receptors, enhancing the immune response and potentially compromising the intestinal barrier function by increasing its permeability. Intestinal inflammation, which has been associated in some studies with the consumption of A1 β-casein, can disrupt the composition of the colonic microbiota and promote the growth of pathogens [28]. It is important to note that research on the specific effects of A1 and A2 β-casein proteins on the human microbiota is ongoing, and results may vary between studies due to factors such as the population studied, the duration of the intervention, and the methodology used [35].

3.3. How Does Individual Susceptibility to βCM-7 Exposure Influence the Observed Effects of A1 β-Casein Consumption?

βCM-7 may influence intestinal health, inflammation, and neurological function. An individual's response to its consumption depends on factors such as enzyme activity, gut microbiota, intestinal permeability, opioid-receptor expression, and genetic predisposition. Also, the amount of βCM-7 released during A1 β-casein digestion varies depending on each individual's digestive enzymes and intestinal conditions.

In order for proteins to be absorbed in the intestinal tract, they first need to be broken down into amino acids or short-chain peptides, such as dipeptides and tripeptides. The digestion of casein starts in the stomach, where enzymes act to hydrolyze peptides of different lengths. This process continues in the duodenum and concludes in the jejunum, where further breakdown and absorption of smaller peptides takes place [59].

Dairy protein digestion showed no significant differences between people with or without lactose intolerances, based on blood amino-acid levels after consuming different types of milk. Only a slight variation in lysine was observed in the tolerant group, suggesting that intolerances do not significantly affect protein digestion [30].

βCM-7 degradation is largely dependent on dipeptidyl peptidase IV (DPP-4), an enzyme present in various cells, including enterocytes and neurons. DPP-4 is highly expressed in the membrane of small intestinal enterocytes and is secreted into the intestinal lumen via exosomes. The availability and activity of DPP-4 determine the rate of βCM-7 degradation in the small intestine [60,61]. However, elevated DPP-4 activity in the small intestinal membrane can exacerbate the inflammatory process and can compromise the integrity of the intestinal barrier. Furthermore, single nucleotide polymorphisms (SNPs) in the DPP-4 gene have been associated with an increased risk of diseases such as heart failure, type 2 diabetes, and dyslipidemia, suggesting that these genetic variations may influence βCM-7 metabolism [60,61]. DPP-4 activity is highly variable, and individuals with lower

activity may have higher circulating levels of βCM-7 after consuming A1 β-casein. Studies such as Crawford et al. found that urine from autistic children consuming histidine β-casein milk showed higher BCM peptide levels compared to proline β-casein milk. Age-matched controls had no detectable BCM peptides [27]. Jarmolowska et al. found elevated serum levels of βCM-7 in children with autism spectrum disorder (ASD) compared to healthy children, suggesting a possible role for βCM-7 metabolism in this group [22].

Intestinal permeability also varies between individuals, meaning that some may absorb more βCM-7 than others, intensifying its effects. As we saw in the previous section, animal studies have shown that the consumption of A1 milk triggers an inflammatory response, enhancing the immune response and possibly altering the intestinal barrier function. This inflammatory response can lead to increased intestinal permeability, compromising the barrier [17]. The inflammatory response to βCM-7 exposure can vary considerably between individuals due to genetic differences, gut microbiota, and overall health status. The study by Jianqin et al. included participants with self-reported milk intolerance and found that the consumption of milk containing both A1 and A2 β-casein was associated with higher concentrations of inflammation-related biomarkers compared to milk containing only A2 β-casein [33]. Ho et al. observed in their pilot study that some individuals showed higher levels of fecal calprotectin, a marker of gut inflammation, with consumption of A1 milk [28].

Research conducted by Deth et al. [39] on healthy adults showed that drinking milk containing only the A2 β-casein variant led to a more significant rise in the plasma levels of glutathione (GSH), a key antioxidant, compared to consuming milk with both A1 and A2 β-casein. The authors propose that the peptide βCM-7 may interfere with cysteine absorption—an amino acid essential for glutathione synthesis. Reduced glutathione availability could be associated with elevated oxidative stress and inflammation. Similarly, He et al., [32] in a study involving Chinese individuals who reported lactose intolerance, hypothesized that the inflammation observed with A1 β-casein intake might explain the more severe gastrointestinal symptoms seen when compared to A2 milk consumption. Previous studies have also reported increases in inflammatory markers or related mechanisms following exposure to A1 β-casein, including, in addition to the involvement of βCM-7 in glutathione synthesis [39], increased fecal calprotectin [28] and the effects of βCM-7 on oxidative stress [62] by inhibiting cysteine uptake with redox and epigenetic consequences. In this latest study, it is reported that gluten-derived exorphin and βCM-7 share a mechanistic pathway to induce oxidative stress in cultured human intestinal epithelial cells and neuronal cells [62].

βCM-7 is classified as an exorphin because it is an exogenous opioid peptide with the same classification as morphine [39]. Between 30 min and 6 h after β-casein ingestion, βCM-7 is liberated into the intestinal lumen acting as an agonist of μ-opioid receptors in enteric neurons. Its affinity for these receptors is due to its structural similarity to endogenous opioid peptides, such as enkephalins and endorphins. characterized for the presence of tyrosine (Tyr) at its N-terminal end [34]. These receptors and their endogenous ligands are implicated in immune-mediated gastrointestinal pathophysiology [58]. Some studies have found that milk with A1 β-casein is associated with increased gastrointestinal symptoms in individuals with milk intolerance [32,33].

Beyond the enteric nervous system, μ-opioid receptors are also present in the central nervous system (CNS), including the cortical and subcortical regions, the brainstem, and the hypothalamus, which regulate homeostasis. βCM-7 can cross the blood–brain barrier and interact with μ-opioid receptors in the CNS, potentially inducing effects such as sedation, mild analgesia, and respiratory depression [63]. All of this highlights the complexity of the interaction between βCM-7, μ-opioid receptors, and overall health.

The opioid system regulates pain, the endocrine response, emotion, memory, and the gastrointestinal function.

Sensitivity to βCM-7 depends on the expression of μ-opioid receptors in the gastrointestinal tract and nervous system [39]. Elevated levels of βCM-7 have been observed in individuals with autism, suggesting a decreased ability to metabolize this peptide [22,39,46]. Some individuals may be more susceptible to its effects, especially those with lactose intolerance [29,32,33,40,43], irritable bowel syndrome [29], or autism spectrum disorders [22,46,47].

In conclusion, individual response to βCM-7 depends on multiple factors, including enzyme activity, intestinal permeability, opioid receptor expression, and genetic predisposition. This complex interaction between βCM-7, μ-opioid receptors, and overall health underscores the importance of considering individual variations when studying the effects of A1 β-casein on human health.

3.4. How Does the Consumption of A1 β-Casein Versus A2 Influence Digestive Health in Different Populations?

The consumption of A1 β-casein versus A2 β-casein appears to influence digestive health differently in various populations, primarily due to the production of the βCM-7 peptide during A1 β-casein digestion, whereas A2 β-casein produces minimal amounts of βCM-7.

Several studies conducted with children of different ages suggest that milk containing only A2 β-casein may be more beneficial in terms of gastrointestinal discomfort compared to A1/A2 (regular) milk. Thus, in a study with infants, an A2 β-casein-based formula without A1 was compared with a conventional infant formula (IF). Better gastrointestinal tolerance was observed in the A2 IF group, with stool frequency and consistency more like those of exclusively breastfed infants [36].

Two trials carried out with toddlers (1–3 years) evaluated conventional growth formulas with comparable nutritional compositions versus those free of A1 β-casein. Formulas made exclusively with A2 β-casein were better tolerated and linked to lower constipation scores as reported by parents, when compared to standard formulas [37]. In healthy toddlers experiencing mild digestive issues, the use of A2-based formula led to noticeable improvements in gastrointestinal comfort and related symptoms within just one week. In another study, while the incidence of acute respiratory infections and diarrhea remained similar between groups, children who consumed formulas free of A1 β-casein experienced shorter episodes of respiratory infections and better outcomes related to diarrhea. Additionally, they showed reductions in bloating, flatulence, and regurgitation [35].

In Crowley's study were included children (1 to 12 years) diagnosed by a pediatrician with chronic functional constipation (CFCs). The authors concluded that there is an association between CFCs and cow's milk consumption, but no effect was found for casein type on the resolution of constipation. They suggest that some other component in cow's milk, common to both A1 and A2 milk, might be causing the problem in these susceptible children [25]. In 5- to 6-year-old children with milk intolerance, consumption of milk containing A2 β-casein was shown to be associated with a reduction in parent-reported gastrointestinal symptoms compared to conventional milk [34].

Some studies investigated the effects of A2 milk on the digestive health of adults. The reviewed trials found that in individuals who experienced gastrointestinal discomfort after milk consumption, switching to A2 milk caused less abdominal pain, fecal urgency, and rumbling compared to A1/A2 milk [32,33,38,40–43]. Milk containing A2 β-casein could significantly reduce the proportion of abdominal bloating and bowel movement difficulty, increase bowel movement frequency, and change stool characteristics compared to regular milk (containing A1 and A2).

Some studies have compared the effect of A2 milk, conventional milk, and lactose-free conventional milk on individuals with different types of lactose intolerance. According to the study by Ramakrishnan et al. using MRI for people with lactose maldigestion, gastric emptying was significantly faster after consuming conventional milk (containing both A1 and A2 β-casein) compared to milk containing only A2 β-casein. Abdominal pain symptoms tended to be fewer with A2 milk, although the difference did not reach statistical significance in this small sample, leading the authors to suggest that this difference in gastric transit time could mediate the symptoms of lactose intolerance [41]. A2 milk could offer some advantages such as decreasing the sensation of bloating and fullness and fecal urgency in both milk-tolerant and non-lactose-intolerant subjects [40,43,50,51,64]. Furthermore, these results appear to become more evident over time [50]. Gastrointestinal symptoms caused by the consumption of conventional milk are accompanied by higher concentrations of inflammatory biomarkers such as fecal calprotectin and lower total fecal short-chain fatty acids [28], alterations in cysteine availability and glutathione synthesis [39], and the induction of oxidative stress [39,62].

Kirk et al. suggest that A2 milk could be an alternative for athletes who are intolerant to A1 protein, implying that some athletes may experience digestive problems with regular milk due to the presence of A1 β-casein [44].

In summary, the evidence suggests that β-casein A1 may contribute to a higher incidence of adverse gastrointestinal symptoms and inflammation compared to A2 β-casein in children and adults, especially those with milk or lactose intolerance. Milk containing only A2 β-casein is often associated with better gastrointestinal tolerance. However, further research in different populations and settings is needed to fully confirm and understand these effects.

3.5. Is There an Association Between A1 β-Casein Consumption and an Increased Risk of Cardiovascular Disease?

Human trials evaluating the relationship between A1 β-casein consumption and cardiovascular disease have yielded mixed results.

On the one hand, Chin-Dusting et al. [26] analyze diverse biochemical and diagnostic markers associated with cardiovascular disease. To assess cardio protection, various parameters have been studied, including endothelial function (related to nitric oxide bioavailability, an early marker of atherosclerosis), arterial function (vascular biomechanics), resting blood pressure, plasma lipids, and biochemical markers of inflammation. Endothelial function is a key predictor of future cardiovascular events, while increased arterial stiffness has been associated with decreased survival in hypertensive patients [65]. Plasma markers associated with cardiovascular disease (CVD) include hyperinsulinemia, hyperhomocysteinemia, and C-reactive protein (CRP). The study concluded that no cardiovascular disadvantages were found associated with the consumption of A1 casein compared to A2 casein.

However, this review found that several human trials suggest a possible relationship between A1 β-casein consumption and increased inflammation in adults through the study of biomarkers as IL-4, myeloperoxidase, monocyte chemoattractant protein-1, IgE, IgG, IgG1, IgG2a, Toll-like receptors 1 and 2, TNF-α, cortisol, LBP, CD14, fecal calprotectin, and hs-CRP. Additionally, glutathione has been analyzed as a marker of redox balance. Jianqin et al. observed that the consumption of conventional milk containing A1 and A2 β-casein increased the serum levels of IL-4 and other inflammatory markers [33]. Ho et al. measured fecal calprotectin, finding an increase in its levels after exposure to A1 β-casein [28]. Deth et al. addressed the redox homeostasis of thiols in the context of neurodegenerative diseases, highlighting the role of glutathione. The study showed that the consumption of milk containing only A2 β-casein was associated with a greater increase in plasma glutathione

concentrations compared to the consumption of milk containing both types of β-casein (A1 and A2) [39]. Lower levels in A1 β-casein are due to a limitation in the cellular uptake of cysteine, which is a limiting precursor for GSH synthesis. Oxidative stress and inflammation are relevant processes in the pathogenesis of cardiovascular disease, and glutathione plays a role in modulating these processes, so an indirect relationship can be inferred. A1 casein-induced glutathione reduction could contribute to increased oxidative stress and inflammation, which in turn could have implications for the development of cardiovascular disease. Glutathione plays a role in protecting nitric oxide (NO) bioavailability by reducing oxidative stress. Decreased NO bioavailability is a hallmark of early atherosclerosis and has predictive value for future cardiovascular events [26]. Glutathione, on the other hand, might protect against LDL oxidation. Ramakrishnan et al. investigated the relationship between A1 casein consumption (comparing A1/A2 milk with A2 milk) and various markers of inflammation. He used ELISA assays to measure IgG1, GSH, and IL-4, as well as hs-CRP, total immunoglobulin G (IgG), and CRP. The study found no significant differences in most of these markers (IgG1, hs-CRP, GSH) between the consumption of milk containing A1/A2 casein and milk containing only A2 casein. The exception was a difference in total IgG between the two groups after two weeks of consumption [50]. In contrast, the effect of removing A1 β-casein on various immune markers (specifically, immuno-globulin [Ig]G, IgG1, IgE, and interleukin-4) was reported by Yu et al. [35] Finally, Novika et al. [45] identified significant differences in TNF-α and cortisol levels when β-casein A1 was consumed.

Scientific evidence demonstrates a well-established relationship between elevated levels of circulating inflammatory and cardiovascular risk. Therefore, the increase in inflammatory markers related to the consumption of A1 β-casein could be reflected in an increased cardiovascular risk in individuals who consume this type of protein.

The possible relationship between β-casein A1 consumption and the increased risk of CVD has been explored in two epidemiological studies. McLachlan et al. hypothesized that β-casein A1 is a specific risk factor for ischemic heart disease (IHD), based on correlations between consumption of this protein and IHD mortality in different countries and regions [48]. Laugesen and Elliott found a positive correlation was found between the per capita supply of A1 β-casein and IHD mortality in 20 countries [49].

On the other hand, dietary trials have offered less conclusive results. Chin-Dusting et al. investigated whether dietary supplementation with A1 β-casein increased the risk of CVD compared to A2 β-casein. This 24-week study included 15 asymptomatic individuals (six men and nine women) at high risk of CVD, with a mean age of 52 years (several over 60). The results showed no significant differences in vascular function, blood pressure, plasma lipids, or biochemical markers between the two interventions [26]. Venn et al. conducted a randomized crossover trial in New Zealand adults to evaluate the impact of the A1 and A2 β-casein variants on cholesterol levels. After replacing all dairy products in the diet with low-fat milk and cheese containing varying proportions of A1 and A2 β-casein for two 4.5-week periods, no significant differences were found in plasma cholesterol levels [31].

In summary, although some epidemiological studies have reported a positive correlation between the consumption of β-casein A1 and an increased risk of cardiovascular diseases, these findings are based on population-level analyses that do not allow for establishing a direct causal relationship. In contrast, intervention trials conducted in adults at elevated cardiovascular risk have not shown significant differences between the consumption of β-casein A1 and A2 in terms of cardiovascular outcomes. These discrepancies could be explained by confounding factors present in observational studies, such as variations in dietary patterns, genetic background, lifestyle factors, or differences in the characteristics of the studied populations. The previous factors, often not adequately controlled for in

epidemiological research, may influence the observed associations. Therefore, the lack of effect in intervention trials suggests that the associations reported in population studies may not be causal, highlighting the need for further investigation into the underlying mechanisms through more rigorous and well-designed studies.

3.6. How Does A1 β-Casein Affect Muscle Performance and Recovery in Athletes and Individuals with High Protein Intakes?

Athletes require efficient recovery after intense exercise to maintain performance and prevent overtraining or injury. If A1 β-casein interferes with digestion, amino acid absorption, or induces gastrointestinal inflammatory responses, it could potentially impede optimal recovery, even with high protein intake [44]. Athletes, especially those who consume large amounts of milk or other dairy products to achieve their protein goals, may be more susceptible to experiencing gastrointestinal discomfort induced by A1 β-casein and βCM-7 release. These discomforts can include bloating, altered gastrointestinal transit, and inflammation. A compromised gastrointestinal system can impair the absorption of essential nutrients and therefore performance and recovery. Furthermore, βCM-7 production can reduce levels of glutathione, a crucial antioxidant. Athletes subject their bodies to increased oxidative stress due to intense exercise, so maintaining optimal antioxidant levels is important for recovery and overall health.

Regarding how A1 β-casein affects muscle performance and recovery in athletes and individuals with high protein intakes, we can infer some relevant points. So, Ho et al. suggested that A1 β-casein could be linked to increased intestinal inflammation in some individuals, which could have implications for the absorption of nutrients crucial for muscle recovery [28]. Jianqin et al. reported that consuming milk with A1 β-casein was linked to heightened gastrointestinal inflammation, more severe post-dairy digestive discomfort, slower gut transit time, and reduced cognitive performance in terms of speed and accuracy, compared to milk containing only A2 β-casein [33]. Although the study focused on individuals who self-identified as intolerant to milk rather than on athletes with high protein needs, it raises the possibility that inflammation and digestive issues may hinder an athlete's nutrient intake and recovery capacity after training or competition.

Deth et al. hypothesized that βCM-7 might influence levels of glutathione, an important antioxidant. Limited glutathione availability could impair recovery by influencing oxidative stress associated with intense exercise [39]. The study by Kirk et al. investigated the effect of A2 milk versus regular milk containing both A1 and A2 β-casein on recovery from exercise-induced muscle damage (EIMD) in athletes. While the study did not isolate the effect of A1 β-casein alone, it found that both A2 milk and regular milk were effective in limiting declines in dynamic muscle function at 48 h post-exercise compared to a placebo. This suggests that the presence of A1 β-casein in regular milk did not impede the muscle recovery benefits in these athletes. However, the study mentions that, in some individuals, A1 β-casein could be associated with negative gastrointestinal effects that could potentially affect nutrition, hydration, and therefore muscle recovery. The authors suggest that A2 milk could be an ergogenic aid after exercise-induced muscle damage and could offer an alternative to athlete's intolerant to A1 protein [44].

In summary, while the Kirk's study in athletes did not show a difference in muscle recovery between regular (A1) and A2 milk, it does suggest that A2 milk may be beneficial for those with A1 protein intolerance. Evidence from other studies indicates that A1 β-casein may contribute to gastrointestinal inflammation and other digestive symptoms in some people, which could potentially indirectly affect recovery and performance in athletes, especially those sensitive to A1 β-casein. A2 milk may be beneficial for muscle recovery in athletes, offering an alternative for those with possible A1 β-casein intolerance, the importance of this factor is magnified in athletes with high protein intakes due to

their increased exposure to A1 β-casein and the potential negative impact on digestion, gastrointestinal function, inflammation, and oxidative stress, all crucial factors for athletic performance and recovery.

However, conclusions regarding the benefits of A2 milk for muscle recovery in athletes should be drawn with caution, as the current evidence is limited and does not conclusively demonstrate a direct negative effect of A1 β-casein on recovery. Further controlled and well-designed studies are needed to clarify whether there are significant differences between A1 and A2 milk in this context and to better understand their potential role in athletic performance and post-exercise recovery.

3.7. How Does A1 β-Casein Influence Immune Function?

Casein, which makes up about 80% of the protein content in cow's milk [25,35], has a complex interaction with the immune system. Research suggests that both casein itself and the peptides produced during its digestion may affect immune function in various ways. Casein is thought to generally support immune function by enhancing the immune system. Casein-derived peptides have been shown to strengthen immune responses, induce the death of malignant cells, and play a role in developing the mucosal immune system. Additionally, casein has been found to prevent colon cancer induced by azoxymethane in rats. There is also evidence pointing to the involvement of α-casein in cancer suppression [66].

However, the digestion of A1 β-casein can release βCM-7, a bioactive peptide that can affect immune function and potentially contribute to the onset of allergies. βCM-7 has an affinity for opioid receptors and can influence inflammation and immune responses [22,39]. Animal studies suggest that A1-like β-casein variants (A1/A1 and A1/A2) may trigger inflammatory reactions in the gastrointestinal tract by activating the Th2 pathway [13], as demonstrated by increased inflammatory markers and greater leukocyte infiltration. These effects are believed to be driven by both βCM-7 and BCM-5 [14]. Such inflammatory responses have also been linked to intolerance reactions like asthma and eczema [67].

SCFAs, known for their anti-inflammatory effects and their role in supporting colon cell function [68], have been found at lower levels in individuals who drink milk containing both A1 and A2 β-casein compared to those who consume milk with only the A2 variant. The exclusive consumption of A2 β-casein supports the production of microbial SCFAs, helping to prevent colonic health issues linked to insufficient SCFA production [33].

βCM-7 has a high affinity for μ-opioid receptors, present not only in the nervous system but also in immune cells. By binding to these receptors, βCM-7 can modulate the activity of these immune cells. In vitro studies have shown that βCM-7 can alter lymphocyte proliferation and the release of inflammatory markers. The consumption of bovine milk containing βCM-7 can induce an inflammatory response in the gut by activating the Th2 pathway, which is central to the development of allergic responses, including IgE production [33]. Jarmolowska et al. suggest that it may contribute to the etiology of food allergies [22]. In fact, a subcutaneous injection of βCM-7 can cause local pseudoallergic reactions, with wheal formation and erythema, mast cell degranulation, and histamine secretion even in healthy children, suggesting the allergenic potential of casein-derived peptides [69].

The study by Ramakrishnan et al. investigated the effects of the long-term (two-week) consumption of milk containing A1 and A2 β-casein (A1/A2) compared to milk containing only A2 β-casein in individuals with lactose maldigestion. A key finding was that while reductions in some gastrointestinal symptoms were observed with A2 milk over time, most serum immunological and antioxidant markers did not show significant differences between the two types of milk. The specific markers evaluated were IgG1,

hs-CRP, and GSH. The exception was total IgG, which did show a difference between groups [50]. The lack of significant differences in these markers could suggest that, in the studied population of lactose intolerants over a two-week period, the type of β-casein did not induce noticeable systemic changes in these aspects of immunity. However, it could suggest that the type of β-casein could be affecting other IgG subclasses, resulting in a difference in total IgG without significantly affecting IgG1. The observed difference in total IgG requires further investigation to understand its significance. It is crucial to remember that this research focused on individuals with lactose indigestion. Their immune response to milk components could differ from that of individuals without this condition. Gastrointestinal symptoms associated with lactose indigestion could be more prominent than subtle alterations in immune markers related to the type of β-casein over a two-week period [50,51].

In summary, casein and its bioactive peptides, especially βCM-7 derived from A1 β-casein, may influence the immune system by promoting inflammatory responses, modulating immune-cell activity, and potentially contributing to the development of allergies. However, the immunostimulatory actions of casein and its peptides have also been suggested.

3.8. What Is the Impact of A1 β-Casein on Neurological and Cognitive Health?

The impact of the role of the βCM-7 peptide in neurological and cognitive health has been the subject of various investigations, with findings suggesting possible associations with neurological and cognitive health.

Particularly in relation to certain neurodevelopmental disorders, an association between elevated βCM-7 immunoreactivity and delayed psychomotor development in infants has been reported [33,46,47]. The study carried out by Kost et al. found that the basal level of bovine BCM immunoreactivity increased two-fold in the blood plasma of infants with developmental delay [46]. In 2014, a possible causal relationship between circulating BCM levels and delayed psychomotor development in infants was proposed [47].

The involvement of milk-derived opioid peptides in neurodevelopmental conditions like autism spectrum disorder (ASD) has been gaining more attention. One of the main theories in this area is the "opioid excess theory" which suggests that excessive exposure to peptides like β-casomorphin-7 (βCM-7) which can be released during the digestion of A1 β-casein may disrupt normal brain development by interacting with neurotransmitter systems—especially opioid and serotonin receptors [22,27,33,46,47]. These peptides are thought to cross the intestinal barrier and possibly accumulate in the brain, especially in individuals with increased blood–brain barrier permeability or impaired peptide degradation. Elevated levels of dipeptidyl peptidase-4 (DPPIV), an enzyme involved in breaking down these peptides, have been found in children with ASD [22]. Furthermore, higher serum levels of βCM-7 have been found in autistic children compared to controls, supporting the hypothesis of increased systemic exposure in this population potentially contributing to symptoms associated with ASD [33]. Some researchers argue that the presence of hyperpeptidemia and compromised peptide clearance might lead to the accumulation of βCM-7 in the brain, influencing dopaminergic, serotonergic, and cholinergic neurotransmission [19,44].

However, it is important to emphasize that the evidence is still limited and inconsistent. While some findings point to a relationship between A1 β-casein, βCM-7, and neurodevelopmental disturbances such as autism or psychomotor delay, other studies using analytical methods such as mass spectrometry have failed to detect casomorphins in children with ASD [46,47]. These discrepancies underscore the need for further well-designed studies

to determine the significance of these peptides and clarify whether their presence has any causative role in ASD.

A1 β-casein consumption has also been linked to mental illness. In the epidemiological study by Boland et al., a correlation was found between β-casein A1 intake and mental-disorder mortality rates [27]. A study investigated the effects of milk containing only A2 β-casein versus milk containing both A1 and A2 β-casein on the cognitive behavior of people with self-reported cow's milk intolerance, using the Subtle Cognitive Impairment Test (SCIT). The results suggested that A1 β-casein and its peptide derivatives also affect information processing in the brain [33].

Although originally proposed in the context of autism spectrum disorders, the opioid excess theory outlines a plausible mechanism by which opioid peptides derived from milk, such as β-casomorphins, may influence central nervous system (CNS) function. According to this hypothesis, the increased permeability of the blood–brain barrier could facilitate the entry and accumulation of these peptides in the brain, where they may modulate not only the opioid neurotransmission system but also the serotonergic, dopaminergic, and cholinergic pathways [22,47]. β-CM-7, in particular, has been shown to act as an antagonist of serotonin 5-HT2 receptors. Additionally, the related peptide (D-Pro4)-β-CM-5 has been found to influence the synaptic transmission of acetylcholine in the hippocampus and dopamine in the striatum, possibly through interactions with serotonin and dopamine receptors [46].

Elevated concentrations of casomorphins may exert pathological effects on both opioid and serotonergic signaling. For instance, in vitro studies have demonstrated that β-CM-7 can displace the 5-HT2 receptor ligand ^{3}H-spiperone in rat frontal cortex membranes, as well as inhibiting serotonin-induced platelet aggregation in human blood samples. These findings support the idea that β-CM-7 may function as a 5-HT2 receptor antagonist. Furthermore, in vivo experiments have shown that β-CM-7 suppresses 5-hydroxytryptophan-induced hyperactivity of the serotonergic system in mice, an effect that was reversed by naloxone—suggesting an interaction between serotonergic and opioid systems [44].

Although most of the current literature explores these mechanisms in relation to autism, the influence of βCM-7 on the serotonergic system may also have broader implications for other neuropsychiatric conditions. These findings underscore the need for further investigation into the neuroactive properties of milk-derived peptides and their potential roles in modulating CNS function beyond developmental disorders.

As Hockey et al. [63] point out, significantly elevated concentrations of βCM-7 have been found in the blood plasma and urine of patients with schizophrenia, autism, and postpartum psychosis, reinforcing the potential similar involvement in other mental illnesses [47,70–72]. This study evaluated the comparative effects of dairy products containing only A2 β-casein versus conventional dairy products on symptoms of psychological distress in women with low mood. Research suggests that A1 β-casein is involved in pathways thought to be important for the etiology of mental disorders, including inflammation, oxidative stress, and gut microbiota [63].

The intestinal and systemic inflammatory response induced by βCM-7 [28,32,33], has been proposed as a pathway connecting diet with neurological and psychiatric disorders and depression [22,47,63,73].

The reduction in glutathione (GSH) associated with A1 β-casein consumption can increase the inflammatory, oxidative, and nitrosative stress load [39]. Oxidative stress is implicated in the pathogenesis of several mental disorders, including depression and schizophrenia [73]. Jarmołowska (2019) also suggests that reduced cysteine uptake and the resulting decrease in glutathione synthesis due to opioid peptides in milk could have consequences in neurological disorders [22].

The alteration of the gut microbiota due to the increased gastrointestinal transit time induced by βCM-7 could be relevant to mental disorders. The gut–brain axis is a bidirectional communication pathway, and alterations in microbiota composition and function have been linked to depression and other neuropsychiatric disorders [74–76]. Jianqin et al. observed lower levels of short-chain fatty acids (SCFAs) with A1 β-casein consumption, which could have implications for colon health and, through the gut–brain axis, potentially for mental health [33]. However, it is important to note that research in this field is complex and not always conclusive.

In summary, A1 β-casein and its digestion product, βCM-7, are implicated in the pathogenesis of neurological disorders such as autism and schizophrenia, as well as in psychomotor development. However, further research is needed to confirm these effects and elucidate the underlying mechanisms.

4. Conclusions

The interaction between βCM-7, a bioactive peptide released during the digestion of A1 β-casein, and the gut microbiota has significant implications for gut health and overall well-being. Studies suggest that milk containing A1 β-casein may negatively impact gut health by altering microbiota composition, reducing motility, and increasing fermentation in the colon. These effects are linked to the increased production of gases like hydrogen and changes in short-chain fatty-acid (SCFA) profiles. Conversely, milk containing only A2 β-casein has been associated with improved gastrointestinal outcomes, including an increased abundance of beneficial bacteria like *Bifidobacterium* spp. and reduced markers of inflammation.

Research indicates that βCM-7 can compromise intestinal barrier function by increasing permeability and triggering inflammatory responses. This may exacerbate gastrointestinal symptoms in individuals with lactose intolerance, irritable bowel syndrome (IBS), or other sensitivities. Furthermore, βCM-7′s ability to cross the blood–brain barrier and interact with μ-opioid receptors in the central nervous system highlights its potential neurological effects.

Individual responses to βCM-7 vary due to factors such as enzyme activity (e.g., DPP-4), genetic predisposition, gut microbiota composition, and intestinal permeability. For example, individuals with lower DPP-4 activity may experience higher circulating levels of βCM-7 after consuming A1 β-casein, intensifying its effects. Elevated levels of βCM-7 have been observed in populations with specific conditions, such as ASD, suggesting a potential role in these groups.

Clinical studies (30 of which 24 were interventional clinical trials, 3 epidemiological studies, and 3 non-interventional studies, including 12 studies with children and/or adolescents and multiple trials with adults) have consistently shown that milk containing only A2 β-casein is better tolerated than milk with both A1 and A2 β-caseins. Participants consuming A2 milk reported fewer gastrointestinal symptoms such as bloating, abdominal pain, and fecal urgency. Additionally, inflammatory biomarkers were lower in those consuming A2 milk compared to conventional milk.

In conclusion, the evidence underscores the distinct effects of A1 and A2 β-caseins on gut health. While A1 β-casein is associated with adverse gastrointestinal outcomes and inflammation, A2 β-casein offers a more favorable profile for individuals sensitive to dairy products. However, further research is needed to explore these effects across diverse populations and clinical settings to fully understand their implications for human health.

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