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# Estimating β-casomorphin-7 exposure from milk and dairy product consumption: a comprehensive assessment for the European population

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**ABSTRACT**

β-Casomorphin-7 (BCM-7), a heptapeptide derived from β-casein hydrolysis, has gained attention for its potential role in gastrointestinal discomfort and certain diseases, yet its effects remain controversial. This study aimed to provide the first reliable estimation of BCM-7 exposure for low, medium and high consumers of milk and dairy products in the European population. We developed a database on BCM-7 released after simulated digestion, combining literature review and direct analysis of milk and selected dairy products. Using European food consumption data, we estimated BCM-7 daily exposure for adults (132–2541 µg), adolescents (163–2594 µg) and children (200–2357 µg). Milk emerged as the most significant source of BCM-7 exposure across all population groups. Statistical analysis revealed differences in exposure levels between adults and children at medium consumption levels. This work provides the first comprehensive estimation of dietary BCM-7 exposure, laying the groundwork for future research on its potential health effects.

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β-Casomorphin-7; BCM-7; bioactive peptides; dairy consumption; exposure; *in vitro* digestion

## Introduction

Milk is a cornerstone of global nutrition, providing essential nutrients like high-quality proteins and calcium (Smith et al. 2021). Beyond its basic nutritional value, milk contains bioactive compounds such as immunoglobulins, oligosaccharides and peptides that may offer health benefits (Lin et al. 2021). Of particular interest are bioactive peptides, short amino acid sequences typically 3–20 residues long, which can be released from milk proteins through various mechanisms including gastrointestinal (GI) digestion and food processing (e.g. fermentation) (Kekkonen and Peuhkuri 2009; Capriotti et al. 2016; Punia et al. 2020).

Among these bioactive peptides, β-casomorphins, especially β-casomorphin-7 (BCM-7), have garnered significant attention (Thiruvengadam et al. 2021). BCM-7 is derived from β-casein, which comprises about 30% of cow's milk protein and exists in multiple genetic variants (Giribaldi et al. 2022). The two most common variants, A1 and A2, differ by a single amino acid at position 67. A1 β-casein contains histidine at this position, which makes it more susceptible to enzymatic cleavage that releases BCM-7. In contrast, A2 β-casein contains proline at position 67, rendering it more resistant to this cleavage (European Food Safety Authority (EFSA) 2009). The A1 variant is common in Holstein-Friesian, Ayrshire and Red cattle, while the A2 variant is more prevalent in Guernsey and Jersey breeds (Kamiński et al. 2007).

BCM-7 might negatively impact GI function and microbial composition (Jianqin et al. 2016; Aslam et al. 2020), and is considered a potential risk factor for various health outcomes in humans (McLachlan 2001; Tailford et al. 2003; Chia et al. 2017). Some studies suggest it may contribute to certain non-communicable diseases (for a recent review, see Bolat et al. 2024). However, the scientific community remains divided on this issue, and in 2009, the European Food Safety Authority (EFSA) concluded that a causal relationship between BCM-7 consumption and health issues could not be established (European Food Safety Authority (EFSA) 2009).

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Despite this, research on the topic continues to grow as does the debate about the potential health implications of consuming A2 or A1 milk (Thiruvengadam et al. 2021; Kaplan et al. 2022), with several studies producing conflicting results (Brooke-Taylor et al. 2017; Küllenberg de Gaudry et al. 2019; Sheng et al. 2019; Summer et al. 2020; Kay et al. 2021; Cieślińska et al. 2022; Fernández-Rico et al. 2022; Jiménez-Montenegro et al. 2022; de Vasconcelos et al. 2023; Gonzales-Malca et al. 2023). Evidence suggests that BCM-7 formation can occur in both A1 and A2 milk under physiologically relevant conditions, such as GI digestion (Cattaneo et al. 2023; Reiche et al. 2024), and can also be influenced by industrial processes like heat treatment (Lambers et al. 2021). Furthermore, a recent animal study found no significant differences in the health effects of A1A2 and A2A2 milk types over a 90-day period (Semwal et al. 2023), and neither BCM-7 from A1 and A2 milk digestion nor synthetic BCM-7 showed an impact on human immune cells (Gard et al. 2024).

Given the ongoing scientific debate and the potential for consumer confusion, a comprehensive exposure assessment is essential to understand the potential effects of BCM-7, interpret health-related studies and guide future research directions. Indeed, BCM-7 has limited ability to cross the intestinal barrier and enter the bloodstream under normal physiological conditions due to its size (a heptapeptide) and enzymatic degradation by dipeptidyl peptidase IV (DPP-IV) (Asledottir et al. 2019; de Vasconcelos et al. 2023). However, in certain conditions, such as DPP-IV deficiency or increased intestinal permeability associated with some health conditions, BCM-7 absorption may be enhanced (de Vasconcelos et al. 2023). Therefore, the main research question concerns the extent of consumption of milk and dairy products that could result in concentrations of BCM-7 in the bloodstream causing any effect.

In this light, our study had two objectives:

i. Evaluating BCM-7 occurrence in milk (particularly A1 β-casein-containing milk) and various dairy products (including yogurt/fermented milk, cheese and milk powder) after simulated digestion, using both literature data and our laboratory findings.
ii. Estimating BCM-7 exposure levels for European populations (adults, adolescents and children), categorised into low, medium and high dairy consumers.

Our study is the first to provide a comprehensive population-level exposure assessment for BCM-7 across different age groups in Europe, filling a critical gap in the current literature. By providing this comprehensive analysis, we aim to provide valuable data in this complex field. Our aim is not to settle the A1 versus A2 milk debate, but rather to provide reliable, evidence-based information. This information can guide future research, support regulatory decision making and improve the scientific basis for public health considerations. In addition, this exposure data could inform future public health guidelines and food industry practices related to dairy consumption and processing.

## Methods

A schematic overview of the study design is presented in Figure 1. Our research employed a comprehensive, multi-faceted approach to estimate BCM-7 exposure in European populations. This approach integrated a systematic literature review, experimental data collection and analysis of milk and dairy consumption patterns.

### *Establishment of a dataset on BCM-7 released from milk and dairy products*

We conducted a systematic review of scientific literature to create a database on the quantity of BCM-7 released from selected foods: milk (with a particular focus on milk containing A1 β-casein), yogurt/fermented milk, cheese and milk-based powder. Milk-based powder was included due to its presence in widely consumed foods such as confectionery, bakery and meat products (Sharma et al. 2012). This

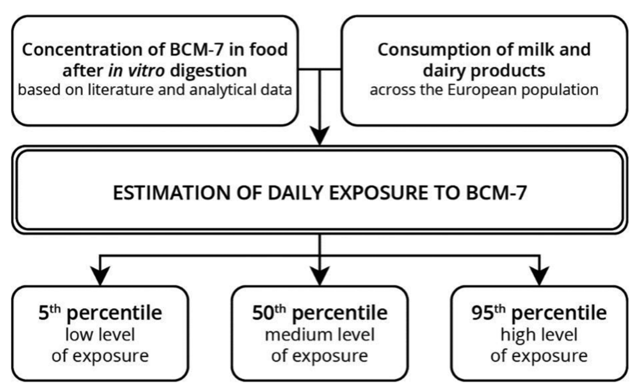


**Figure 1.** Schematic overview of the multi-step approach used to estimate the exposure to BCM-7 in milk and selected dairy products. The study involved a systematic literature review, experimental data collection, and the use of food consumption data from the European Food Safety Authority (EFSA) to calculate the estimated daily exposure to BCM-7 for different European population groups.

literature-based database was then supplemented with experimental data obtained from simulated digestion of selected foods (milk and milk powder).

### Literature review to estimate the occurrence and release of BCM-7 from milk and dairy products after simulated digestion

We performed a systematic literature search on 16 February 2023, using PubMed (http://www.ncbi.nlm.nih.gov/pubmed) and Scopus (http://www.scopus.com) databases. The search syntaxes used were:

PubMed: (beta-casomorphin 7[Title/Abstract] OR beta-casomorphin-7[Title/Abstract] OR β-casomorphin 7[Title/Abstract] OR β-casomorphin-7[Title/Abstract] OR BCM-7[Title/Abstract] OR BCM-7[Title/Abstract]) AND ((milk[Title/Abstract] OR cheese[Title/Abstract] OR yog*[Title/Abstract] OR dair*)[Title/Abstract])

Scopus: (TITLE-ABS-KEY(beta-casomorphin 7 OR beta-casomorphin-7 OR β-casomorphin 7 OR β-casomorphin-7 OR BCM-7 OR bcm-7) AND TITLE-ABS-KEY(milk OR cheese OR yog* OR dair*))

Publications were selected based on specific inclusion/exclusion criteria. We excluded data on BCM-7 released from declared A2-milk-based products, as the literature suggests that A2-milk is less effective in releasing BCM-7 (Lambers et al. 2021). Furthermore, the European dairy market is predominantly composed of milk from Holstein-Friesian cows (Jeong et al. 2024), which contain significant amounts of A1 β-casein, making this variant particularly relevant for a population-level exposure assessment in Europe. Additionally, we excluded publications reporting the presence of BCM-7 in milk and dairy products not subjected to *in vitro* simulated digestion to avoid underestimating BCM-7 exposure from these products. After removing duplicates, we screened records based on title and abstract. Subsequently, we performed a full-text analysis to collect information on the type of product, reported BCM-7 content, and unit of measurement for each study.

### BCM-7 release from milk and milk powder after in vitro simulated digestion

Commercial whole milk samples ($n$ = 10) were defatted via centrifugation (4 °C, 4500 rpm for 10 min), and the supernatant was used for analysis. Skimmed milk powder samples ($n$ = 16) were resuspended in water and subjected to ultrasound treatment for 20 min prior to *in vitro* digestion. The simulated digestion process, designed to closely mimic the physiological environment of the human GI tract, was as follows:

i. *Gastric phase*: 0.5 g of pepsin from porcine gastric mucosa (≥250 units/mg solid) was added to 25 mL of defatted milk or resuspended milk powder. The pH was adjusted to 2.2–2.4 with 1 N HCl, and the mixture was incubated at 37 °C for 90 min.
ii. *Intestinal phase*: The pH was adjusted to 7.5 with 1 N NaOH, and the final volume was measured. A 25 mL aliquot was transferred to a centrifuge tube, and 0.5 g of pancreatin from porcine pancreas (8 × USP specifications) was added. This mixture was incubated at 37 °C for 150 min.

Following the intestinal phase, enzyme deactivation was achieved by heating the solution at 90 °C for 10 min. The mixture was then centrifuged at 4 °C (4500 rpm for 10 min). For LC–MS/MS analysis, samples were prepared by diluting 0.050 mL of the supernatant with 0.950 mL of LC–MS/MS mobile phase and filtering through a 0.45 µm filter.

The LC–MS/MS system consisted of an LC 1.4 Surveyor pump (Thermo Fisher Scientific, San Jose, CA), a PAL 1.3.1 sampling system (CTC Analytics AG, Zwingen, Switzerland), and a Quantum Discovery Max triple quadrupole mass spectrometer, controlled by Excalibur 1.4 software (Thermo Fisher Scientific, San Jose, CA). Chromatographic separation was achieved using an X Select HSS T3 column (2.5 µm particle size, 100 × 2.1 mm i.d., Waters Corporation, Milford, MA) with a gradient elution of $H_2O$–$CH_3CN$ (both acidified with 0.2% formic acid). The linear gradient was from 25% to 65% $CH_3CN$ within 4 min, isocratic for 4 min, followed by column conditioning for 6 min. The flow rate was 0.2 mL/min. Ionisation was performed in positive mode (ESI interface). The fragment ions monitored were: 229, 286, 383 and 530 $m/z$ (M + 791 $m/z$). The limit of detection (LOD) and limit of quantification (LOQ) were 0.1 and 0.25 mg/kg, respectively. A representative chromatogram of BCM-7 in a sample is provided in Supplementary Figure 1.

The complete dataset of BCM-7 content in milk and dairy products after *in vitro* simulated digestion was built and consolidated from both literature data and our experimental results.

### Consumption of milk and dairy products in the European population

We accessed the Comprehensive European Food Consumption Database (FoodEx 2) (European Food

Safety Authority (EFSA) 2015), developed by the EFSA, on 1 May 2023. This database provided overall consumption data for milk, yogurt/fermented milk, cheese and milk powder across Europe. Data were extracted at hierarchical level 5 (L5) for milk, yogurt/fermented milk and cheese to obtain more detailed information specifically on cow's milk and yogurt made from cow's milk. For milk powder, data were extracted at hierarchical level 4 (L4), as there were fewer surveys available at L5 for this product category. This approach allowed us to maintain a balance between specificity and data availability across all dairy product types.

We focused on chronic consumption data (expressed in g/day) for three population groups: adults, adolescents and children (excluding toddlers and infants aged 0–3 years due to their unique dietary patterns). For each country and population group, we used the most recent dietary surveys available.

To simulate varying levels of dairy consumption, we extracted the 5th, 50th and 95th percentiles of consumption for each analysed food type, representing "low", "medium" and "high" consumption, respectively.

### *Estimation of exposure to BCM-7 for the European population*

We estimated daily exposure to BCM-7 at the 5th, 50th and 95th percentiles for adults, adolescents and children using the following formula:

$$\text{Daily exposure to BCM}-7 = \sum\left(\text{BCM}-7\ \text{Concentration}_{\text{food}} \times \text{Daily consumption}_{\text{food}}\right)$$

where "BCM-7 Concentration$_{\text{food}}$" is the medium occurrence in the digested food, based on our BCM-7 content dataset, and "Daily consumption$_{\text{food}}$" is the daily consumption of the food for each percentile, as reported in the FoodEx 2 database.

Exposure values for all food categories (milk, fermented milk/yogurt, cheese and milk powder) were summed in low, medium and high consumers for each population subgroup, providing a comprehensive view of BCM-7 exposure across varying dietary habits. This approach accounted for all relevant dairy sources in the diet. Additionally, we assessed the percentage contribution of each dairy product to the daily BCM-7 exposure for medium consumers (50th percentile) across European population groups to identify the major contributors to potential bioactive effects of BCM-7.

### *Statistical analysis*

We assessed the distribution of data using the Shapiro–Wilk test. For non-normally distributed data, we expressed results as median with interquartile range (IQR). To assess differences in total daily BCM-7 exposure among the three population groups (adults, adolescents and children), we performed a Kruskal–Wallis test (the non-parametric equivalent of one-way ANOVA) followed by Dunn's multiple comparisons test for post hoc analysis where the Kruskal–Wallis test indicated significant differences. Statistical significance was set at $p < 0.05$. All statistical analyses were conducted using GraphPad Prism version 10.3.0 for Windows (GraphPad Software, La Jolla, CA).

## Results

### *Estimation of BCM-7 released from milk and dairy products after in vitro simulated digestion*

The literature review process is illustrated in Figure 2. Initially, 261 publications were identified through database searches. After removing duplicates, 177 studies underwent screening based on title and abstract, resulting in 19 potentially relevant publications. These 19 studies were subjected to full-text review, leading to the exclusion of 10 records. Ultimately, nine publications met the eligibility criteria and were included in the dataset for estimating BCM-7 release after *in vitro* digestion of milk containing A1 β-casein and selected dairy products.

The release of BCM-7 after *in vitro* simulated digestion for each category of dairy products (milk, yogurt/fermented milk, cheese and milk powder) was calculated based on data from the literature review (Jarmołowska et al. 1999; Sienkiewicz-Szłapka et al. 2009; De Noni and Cattaneo 2010; Cieślińska et al. 2012; Asledottir et al. 2017, 2018; Lambers et al. 2021; Nguyen et al. 2021; Cattaneo et al. 2023) and experimental results. A complete list of data considered in this analysis is provided in Supplementary Table 1. Within each food category, particularly milk and cheese, a high variability in BCM-7 content was observed. This was possibly related to several factors: (i) different analytical methods used across studies, with varying sensitivity and specificity for BCM-7 detection; (ii) diverse digestion protocols employed by different research groups, which can significantly impact peptide release, as also noted by Cattaneo et al. (2023); and (iii) variations in food matrices and processing conditions, which affect protein structure and subsequent peptide release during digestion. Of

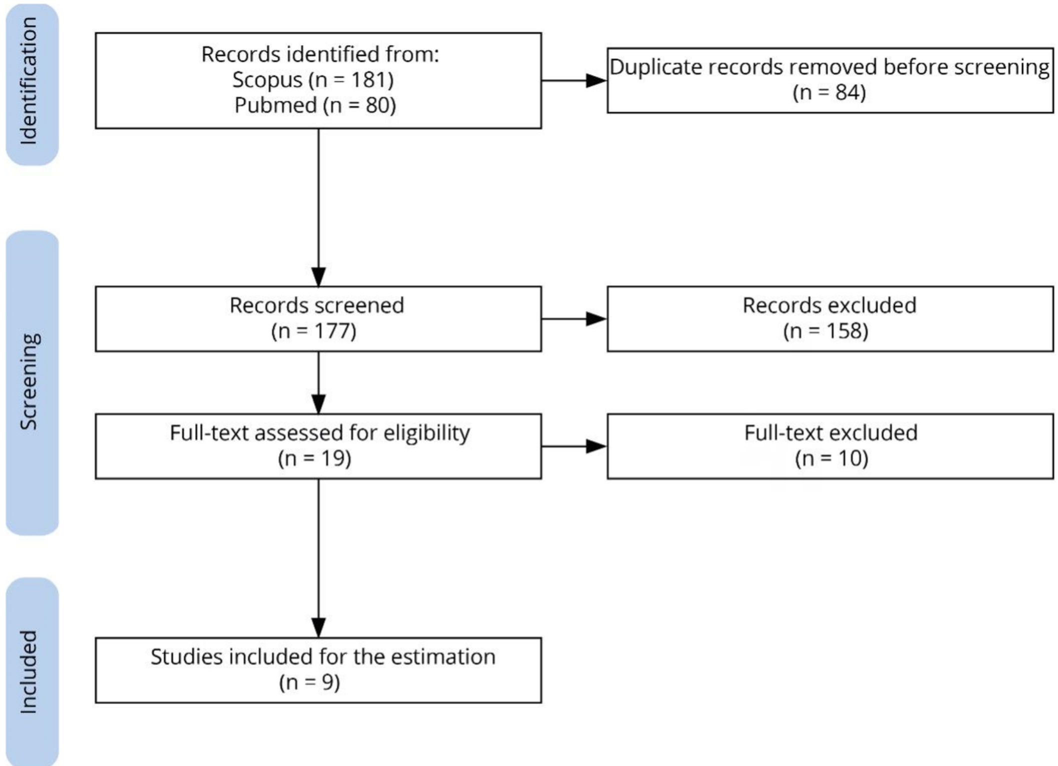


**Figure 2.** Flowchart of the study selection process for the systematic literature review on BCM-7 released from milk and dairy products after *in vitro* simulated digestion.

**Table 1.** Daily consumption of milk and dairy products considered in this study based on the FoodEx 2 database (European Food Safety Authority (EFSA) 2015).

| Type of food | Low consumption (g/day) 5th percentile | Medium consumption (g/day) 50th percentile | High consumption (g/day) 95th percentile |
|---|---|---|---|
| *Milk* | | | |
| Adults | 10.0 (7.2–17.1) | 134.3 (84.2–191.9) | 486.2 (300.0–616.9) |
| Adolescents | 13.7 (9.1–39.0) | 200.0 (125.0–272.1) | 511.3 (400.0–629.0) |
| Children | 24.0 (14.1–64.4) | 212.7 (177.4–304.5) | 553.2 (446.2–667.9) |
| *Yogurt and fermented milk* | | | |
| Adults | 29.7 (14.0–50.0) | 83.3 (62.5–100.0) | 249.9 (200.0–275.0) |
| Adolescents | 25.1 (14.5–50.1) | 76.3 (61.9–100.0) | 208.9 (175.3–250.1) |
| Children | 25.0 (13.2–45.0) | 66.7 (58.3–77.4) | 178.2 (143.0–225.0) |
| *Cheese* | | | |
| Adults | 4.2 (2.5–6.8) | 12.8 (8.5–19.4) | 41.5 (28.0–57.7) |
| Adolescents | 4.5 (2.4–7.9) | 13.3 (8.0–16.0) | 29.5 (16.4–49.7) |
| Children | 4.4 (1.3–7.0) | 8.6 (4.8–13.1) | 23.8 (14.3–32.1) |
| *Milk powder* | | | |
| Adults | 0.6 (0.3–2.2) | 2.1 (1.2–4.8) | 7.5 (5.0–14.8) |
| Adolescents | 0.5 (0.2–3.7) | 1.3 (0.7–4.4) | 2.8 (1.7–6.4) |
| Children | 0.2 (0.1–1.0) | 0.8 (0.5–1.5) | 1.9 (1.1–6.7) |

Data are reported as median and interquartile range (IQR; Q1–Q3) (g/day) for adults, adolescents and children for the 5th, 50th and 95th percentiles, representing the "low", "medium" and "high" consumption, respectively.

note, in milk our experimental data were consistent with the literature data (average BCM-7 content: 0.46 mg/100 g and 0.32 mg/100 g, respectively). The average BCM-7 release varied across dairy categories, with milk powder showing the highest release at 5.02 mg/100 g, likely due to its concentrated form, followed by cheese (1.45 mg/100 g), milk (0.35 mg/100 g) and yogurt/fermented milk (0.08 mg/100 g).

## *Consumption of milk and dairy products across the European population*

The consumption data for milk and dairy products among European adults, adolescents and children were extracted from the open-access FoodEx 2 system-based dataset (European Food Safety Authority (EFSA) 2015) and are presented in Table 1.

**Table 2.** Estimated daily exposure to BCM-7 (μg/day) for European adults, adolescents and children, calculated using the formula given in Section "Estimation of exposure to BCM-7 for the European population", for the different levels of consumption of milk and dairy products.

| | Low exposure to BCM-7 (μg/day) 5th percentile | Medium exposure to BCM-7 (μg/day) 50th percentile | High exposure to BCM-7 (μg/day) 95th percentile |
|---|---|---|---|
| *Adults* | | | |
| Milk | 34.6 (24.9–59.1) | 464.9 (291.5–664.3) | 1683.1 (1038.5–2135.3) |
| Yogurt and fermented milk | 22.2 (10.5–37.4) | 62.4 (46.8–74.8) | 187.0 (149.7–205.8) |
| Cheese | 61.0 (36.9–98.2) | 185.0 (123.6–281.1) | 602.2 (406.9–837.4) |
| Milk powder | 30.4 (15.3–108.8) | 107.7 (62.2–238.4) | 376.4 (249.7–742.6) |
| Total | 131.8 (69.8–226.1) | 790.8 (571.3–948.8) | 2540.7 (2065.5–3519.0) |
| *Adolescents* | | | |
| Milk | 47.5 (31.5–135.0) | 692.3 (432.7–941.9) | 1769.9 (1384.6–2177.3) |
| Yogurt and fermented milk | 18.8 (10.8–37.5) | 57.1 (46.3–74.8) | 156.3 (131.2–187.2) |
| Cheese | 64.8 (34.2–114.3) | 193.5 (116.5–232.8) | 428.1 (237.6–721.7) |
| Milk powder | 26.8 (11.9–184.2) | 66.9 (37.4–221.7) | 141.2 (85.3–323.6) |
| Total | 162.7 (82.6–256.1) | 903.4 (647.9–1128.0) | 2594.2 (1818.4–3281.8) |
| *Children* | | | |
| Milk | 83.2 (48.8–222.9) | 736.4 (614.2–1054.2) | 1915.0 (1544.7–2311.8) |
| Yogurt and fermented milk | 18.7 (9.9–33.7) | 49.9 (43.7–57.9) | 133.4 (107.0–168.4) |
| Cheese | 63.3 (18.2–101.8) | 124.5 (70.0–189.9) | 346.0 (208.1–466.3) |
| Milk powder | 11.8 (4.3–50.2) | 37.6 (23.4–75.3) | 93.2 (53.9–334.4) |
| Total | 199.6 (105.4–403.3) | 967.0 (849.6–1332.0) | 2356.5 (2052.1–2921.3) |
| *p* Value | 0.1095 | 0.0176 (adults vs. children, $p = 0.0148$) | 0.6933 |

Data are reported as median and interquartile range (IQR; Q1–Q3). Statistical differences among the three population groups were assessed using the Kruskal–Wallis test, which showed a significant difference only at the medium exposure level. Subsequent Dunn's multiple comparisons test revealed a significant difference specifically between adults and children, while other pairwise comparisons were not significant.

Milk consistently emerged as the most consumed dairy product across all age groups. Interestingly, there was a clear age-related trend in milk consumption, with children showing the highest intake, followed by adolescents, and then adults. This pattern likely reflects the emphasis on milk consumption for growing children and adolescents in many European dietary guidelines (Herforth et al. 2019). Yogurt and fermented milk products showed a different consumption pattern. Adults exhibited the highest consumption of these products, particularly in the medium and high exposure categories, compared to adolescents and children. This trend might be attributed to increased health consciousness and the marketing of probiotic products to adult consumers (Özer and Kirmaci 2010). Cheese consumption demonstrated less variation across age groups compared to milk and yogurt. However, a slight increase in consumption was observed from children to adults, possibly reflecting evolving taste preferences and dietary habits with age (Hjartåker et al. 2002). As expected, milk powder was the least consumed product across all age groups and exposure categories.

### *Estimated daily exposure to BCM-7 for European adults, adolescents and children*

The estimated daily exposure levels to BCM-7 for the three European subpopulations are presented in Table 2. Overall, the total estimated daily exposure to BCM-7 was similar across the three population groups, except at the medium exposure level (50th percentile), where children showed a significantly higher potential exposure than adults.

Analysis of the contribution of different food groups to BCM-7 exposure for medium consumers (Figure 3) revealed distinct patterns across age groups. Milk emerged as the predominant source of BCM-7 exposure across all age categories, with its contribution decreasing from children to adolescents to adults. This trend was in line with the observed milk consumption patterns and highlighted the significance of milk as a dietary source of BCM-7, particularly in younger populations. Cheese presented an interesting contrast, emerging as the second most significant source of BCM-7 exposure for adults and adolescents, but contributing less significantly for children. Yogurt and fermented milk products, along with milk powder, contributed the least to BCM-7 exposure across all age groups. However, their combined contribution was not negligible, highlighting the importance of considering all dairy sources when assessing BCM-7 exposure.

## Discussion

BCM-7 is one of the most important β-casomorphins (Daniloski et al. 2021), exogenous peptides released from β-casein during digestion that can function as opioid-like molecules (De Noni et al. 2015). BCM-7 is considered a proinflammatory exogenous opioid peptide (food protein) that binds to μ-opioid receptors (MORs) mainly in the GI tract and central nervous system (Giribaldi et al. 2022). Bovine BCMs have up

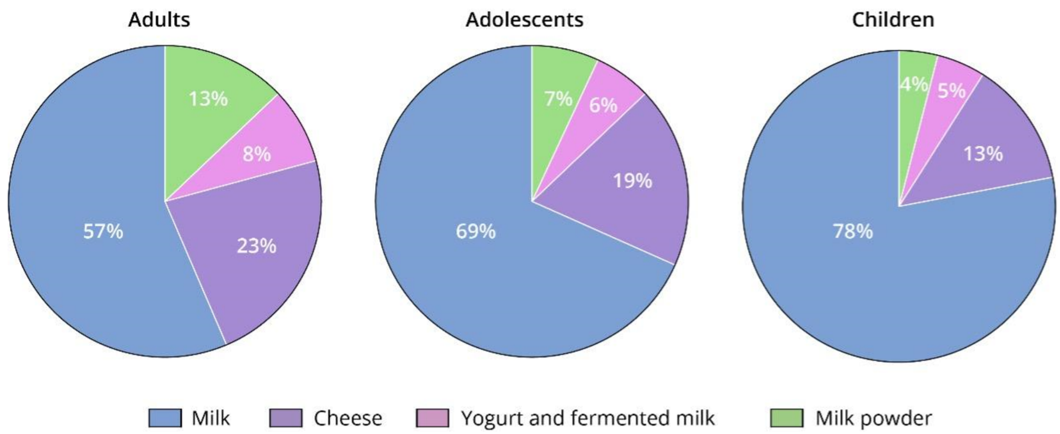


**Figure 3.** Contribution (%) of milk and dairy products to the estimated exposure to BCM-7 for medium consumers of milk and dairy products in three target groups of the European population: adults, adolescents and children. The pie charts illustrate the percent contribution of each food category (milk, yogurt/fermented milk, cheese and milk powder) to the total estimated BCM-7 exposure for each population group.

to 30 times more potency than human BCMs, so their possible effects after consuming milk and dairy products have attracted the attention of the scientific community.

In this study, the potential BCM-7 exposure related to milk and dairy product consumption in the European population was estimated for the first time. Our findings provide crucial insights into the variability of BCM-7 exposure across different age groups and highlight several important considerations for future research.

The limited number of studies suitable for estimating BCM-7 released after simulated digestion highlighted a significant gap in the current literature, and our approach of combining literature data with *in vitro* analysis allowed us to create a more robust dataset. The observed significant intra-category variability aligned with findings from Cattaneo et al., who reported substantial differences in BCM-7 release depending on digestion conditions and milk processing (Cattaneo et al. 2023), and emphasises the need for standardised methods in future studies.

Combining results from our dataset and consumption data, it was possible to estimate the daily BCM-7 exposure ranges for Europeans, providing a novel perspective on the potential dietary intake of this bioactive peptide. Exposure ranges were broadly similar across age groups, except in children at the medium consumption level. Given that children are in a critical developmental stage, this finding warrants further investigation for its potential implications for public health and dietary recommendations. Indeed, our results highlight the need for more targeted investigations of BCM-7's health effects, particularly in vulnerable populations such as children. The higher exposure levels observed in children, coupled with their potentially greater susceptibility to bioactive compounds due to ongoing developmental processes such as immature GI barrier function potentially affecting absorption (Landrigan and Goldman 2011), developing enzymatic systems for peptide metabolism (particularly DPP-IV), and differences in MOR expression and sensitivity (Giribaldi et al. 2022), suggest that this age group should be a priority in future research.

The emergence of milk as the primary contributor to BCM-7 exposure across all age groups, despite not having the highest BCM-7 content per unit, highlighted the critical interplay between food composition and consumption patterns in exposure assessment. This finding aligns with broader nutritional research emphasising the importance of considering dietary patterns rather than individual foods or nutrients in health outcomes (Hu 2002).

While our study provides valuable baseline data, it also highlights significant knowledge gaps. The lack of established safety thresholds represents a significant challenge in BCM-7 risk assessment. Unlike many food components and contaminants, BCM-7 does not have regulatory limits or tolerable daily intake values established by food safety authorities. This is due to several factors: the conflicting evidence regarding BCM-7's physiological effects as highlighted in the 2009 EFSA report (European Food Safety Authority (EFSA) 2009), the difficulties in establishing dose–response relationships given interindividual variability, and the presence of this peptide in widely consumed foods, making traditional toxicological approaches less applicable. Our exposure estimates provide a foundation for future *in vitro* and *in vivo* studies, allowing for the use of more physiologically relevant concentrations in experimental designs.

The limitations of our study, particularly the variability in BCM-7 content data, echo broader challenges in food peptide research. The need for harmonised digestion methods and standardised analytical techniques aligns with calls for improved methodology in bioactive peptide research (Sánchez-Rivera et al. 2014; Brodkorb et al. 2019). Additionally, the consumption data used in our study did not specify the A1/A2 protein content, as such detailed information about the β-casein phenotype in foods is not available. While we focused primarily on A1 β-casein due to its prevalence in the commercial milk market (Jeong et al. 2024), the growing interest in A2 milk necessitates further research comparing BCM-7 release and potential effects between A1 and A2 milk types under various conditions.

## Conclusions

Research studies on the effects of BCM-7 on human health are still limited. More studies are needed in the future perspective to fill the gaps in the literature. First, future research should prioritise developing standardised methods for BCM-7 identification and quantification, and bioactivity tests should be conducted using physiologically relevant concentrations. Mechanistic studies are also needed, as BCM-7 is currently studied focusing on its opioid mechanism, but some research highlights that it may indirectly trigger other protein mechanisms. Additionally, investigating potential health implications of BCM-7 exposure, particularly in vulnerable populations like children, is crucial. Longitudinal studies are needed to assess any long-term effects of BCM-7 exposure, and to establish safety thresholds for BCM-7.

Ultimately, this research, estimating the current level of exposure to this peptide, puts a piece into the complicated puzzle that links milk and dairy consumption to BCM-7, paving the way for evidence-based assessment of human health outcomes.

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## Author contributions

GA: methodology and writing – review and editing; AB: writing – review and editing; TB: formal analysis, writing – review and editing; FC: project administration, writing – review and editing; MDA: conceptualisation, methodology, data curation, formal analysis, original draft writing; FD: conceptualisation, methodology, original draft writing; GL: formal analysis, writing – review and editing; RM: conceptualisation; DR: conceptualisation; FR: writing – review and editing; MS: writing – review and editing.

## Disclosure statement

Federico Canzoneri and Roberto Menta are employed by Soremartec Italia Srl (Alba, Italy). At the time of conceptualisation, Davide Risso was employed by Soremartec Italia Srl, while his current employer is Tate & Lyle Italy SpA. The other authors declare no competing interests.

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