Special Article

Milk A1 β-casein and health-related outcomes in humans: a systematic review

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Context: *Various epidemiological studies suggest a positive association between exposure to cow's milk A1 β-casein protein and risk for noncommunicable chronic diseases. The consumption of A2 cow's milk is increasing, likely because A2 milk is postulated to have positive effects on digestive health.* ***Objective:*** *A systematic review was conducted to investigate associations between A1 β-casein and health-related outcomes in humans.* ***Data Sources:*** *Five electronic databases, 3 clinical trial registries, and the internet were searched systematically.* ***Study Selection:*** *Using predefined inclusion criteria, 2 authors independently selected studies investigating the effect of A1 β-casein or β-casomorphin-7 intake/exposure on any health-related outcome in humans. Discrepancies were resolved by consensus.* ***Data Extraction:*** *Two authors independently extracted data and assessed risk of bias. The certainty of evidence per outcome was evaluated using the GRADE approach. Discrepancies were resolved by consensus.* ***Results:*** *Fifteen randomized controlled trials, 2 case-control studies, and 8 ecological studies were included. Most randomized controlled studies and case-control studies investigating a potential effect on various outcomes were based on intermediate markers and found no significant difference between the 2 milk types. In contrast, most ecological studies reported that population-level A1 β-casein exposure is associated with adverse health outcomes. The certainty of the evidence for the included outcomes, as assessed by the GRADE approach, was rated as moderate for digestive symptoms and as low to very low for all other outcomes.* ***Conclusions:*** *Human-based evidence from clinical trials and epidemiological studies published prior to October 2017 provides moderate certainty for adverse digestive health effects of A1 β-casein compared with A2 β-casein but low or very low certainty for other health effects. These conclusions may change in the future, given the emergent nature of this topic and the ongoing research in this area.* ***Systematic Review Registration:*** *PROSPERO registration number CRD42016043795.*

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INTRODUCTION

Cow's milk is an important component of the human diet worldwide, providing energy and nutrients that support proper bone mass formation and contribute to adequate growth in children. Additionally, milk is important for neuropsychological development.[1,2] However, concerns about potential adverse outcomes associated with regular bovine milk consumption (eg, an increased risk of noncommunicable diseases such as cardiovascular disease, cancer, or diabetes) have been raised repeatedly over the last few decades.[3–6]

Milk consists of protein (mainly casein and whey), fats (a spectrum of saturated, monounsaturated, and polyunsaturated fatty acids), carbohydrates (mainly lactose and oligosaccharides), and water. Approximately one-third of the protein fraction consists of β-caseins. β-Caseins are insoluble milk proteins that are present in various genetically determined forms in milk, including the A1 and A2 variants. The relative concentrations of A1 and A2 β-casein proteins in milk vary between different species of cattle. While most African and Asian cattle produce only A2 β-casein, cattle from Europe, the United States, Australia, and New Zealand produce both A1 and A2 β-casein.[7,8] In general, milk containing a higher concentration of A1 β-casein than A2 β-casein is known as A1 milk, while A2 milk contains predominantly A2 β-casein.

β-Caseins A1 and A2 are distinguished by only a single amino acid. During digestion, the A1 variant releases a bioactive opioid peptide, β-casomorphin-7, while the A2 variant does not. β-Casomorphin-7 is known to influence the nervous, endocrine, and immune systems by activating μ-opioid receptors that are expressed throughout the gastrointestinal tract and body, leading to different effects that include analgesia, sedation, slightly reduced blood pressure, nausea, decreased respiration, decreased bowel motility, and others. Thus, β-casomorphin-7 is thought to be responsible for potential adverse outcomes associated with A1 β-casein milk (A1 milk), such as increased risk of diabetes. In contrast, the consumption of milk containing exclusively the A2 β-casein variant (A2 milk) has been promoted as being associated with positive health effects, including reduced gastrointestinal symptoms, when compared with the consumption of milk containing both A1 and A2 ß-caseins (regular milk).[9,10] However, study design variations, inconsistent findings, and different ways of assessing A1 and A2 β-casein intake have thus far precluded definitive conclusions about the effect of these proteins on health outcomes.

A systematic review was conducted to assess whether A1 β-casein consumption is associated with an increased risk of noncommunicable diseases and other adverse health outcomes. The main objectives of this systematic review were as follows: (1) to identify all primary studies evaluating health-related outcomes of A1 β-casein consumption/exposure in humans; (2) to quantitatively summarize results by conducting meta-analyses for all health-related outcomes, provided at least 2 comparable studies (eg, in terms of study type) were available; and (3) to narratively summarize those results for which meta-analysis was not possible.

METHODS

All steps of the systematic review process were conducted according to the methods described in the *Cochrane Handbook for Systematic Reviews of Interventions*.[11] The systematic review was registered prospectively in PROSPERO, an international prospective register for systematic reviews (ID no. CRD42016043795). The methodology and the results are reported according to the PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) guidelines for systematic reviews (see Appendix S1 in the Supporting Information online).[12]

Eligibility criteria

The research question was defined according to the PICOS approach (Participants, Interventions, Comparison, Outcomes, Study design) (Table 1[13]). Studies investigating the effect of A1 β-casein or β-casomorphin-7 intake/exposure on any health-related outcome in humans were included. Any comparison group was considered, and the following types of comparisons were defined: comparison I, A1 β-casein vs A2 β-casein (any dose and any duration of consumption/exposure); comparison II, higher intake of or exposure to A1 β-casein vs lower intake of or exposure to A1 β-casein (ie, comparing different dosages or durations of A1 β-casein); and comparison III, A1 β-casein vs no A1 β-casein (ie, no milk intake/exposure).

In addition, to be included, studies had to report on any health-related outcome. Intermediate or surrogate markers were considered as proxies for relevant outcomes where available (eg, plasma insulin concentration, concentration of blood lipids, etc). No restrictions on study design or language were applied. Laboratory and animal studies were excluded.

Systematic literature search

To identify all published studies investigating the effect of A1 β-casein on health-related outcomes, the following electronic databases were searched from inception until April 2016 (an updated search from April 2016 to

Table 1 **PICOS criteria for inclusion and exclusion of studies**[a]

Criterion	Description
Participants	Humans of both sexes, without any restriction
Intervention/exposure	A1 β-casein or its derivate, β-casomorphin-7
Comparison	Any comparison group was eligible, including: (I) A2 β-casein intake/exposure (II) Different dosages of A1 β-casein intake/exposure (III) No A1 β-casein intake/exposure (ie, no milk intake/exposure)
Outcome	Any health-related outcome, including intermediate markers (eg, plasma cholesterol concentrations)
Study design	No restriction. Any study type was eligible, including controlled studies, studies without a control group, and ecological studies

[a]According to Sackett et al (1996).[13]

October 2017 was performed in October 2017): Ovid MEDLINE, Ovid MEDLINE In-Process & Other Non-Indexed Citations, Ovid MEDLINE Daily Update, the Cochrane Library, Web of Science, BIOSIS, and Ovid Embase. The search strategy used in Ovid MEDLINE is shown in Appendix S2 in the Supporting Information online. This search strategy was adapted for the other databases as required. Additionally, reference lists of eligible articles were screened for further relevant references and ongoing studies. The following clinical trials registries were searched for unpublished and ongoing trials: ClinicalTrials.gov, the World Health Organization (WHO) International Clinical Trials Registry Platform, and the Australian New Zealand Clinical Trial Registry. The internet was also searched using Google, Google Scholar, OpenGrey, and the World Intellectual Property Organization (WIPO) Patent Cooperation Treaty register of patents to identify further relevant studies not published in electronically available journals. For the internet search, the terms "A1 milk" and "A2 milk" were used. The Google search retrieved more than 8000 records, of which the first 100 were screened. Google Scholar retrieved 120 records, all of which were included in the screening process. The websites of relevant organizations, including a2milkcompany.com, βcasein.net, βcasein.org, and keithwoodford.wordpress.com, were also screened for studies.

Study selection

Two authors (D.K. and S.L.) independently selected studies on the basis of predefined inclusion criteria using the Covidence online software.[14] First, titles and abstracts of studies retrieved through the searches were screened to exclude obvious irrelevant references. Second, full-text publications of potentially relevant studies were obtained and checked for final inclusion. Any disagreement was resolved through consensus or by discussion with a third author (J.J.M.).

Data extraction and risk-of-bias assessment

Again, 2 authors (D.K. and S.L.) carried out data extraction and risk-of-bias assessment independently, and any disagreement was resolved through consensus or discussion with a third author (J.J.M.).

The following data were extracted for each included study: study characteristics (including bibliographic details, objective[s], details of funding, study design, and number of participants in groups), characteristics of the included population (including age, sex, and health status), characteristics of both the intervention/exposure and the control intervention (including type, dosage, and mode of administration), and outcome data (including definition, direction of the effect, and time of measurement).

The tools used for the risk-of-bias assessment varied, depending on the study design. The Cochrane risk-of-bias tool was used to assess risk of bias in randomized controlled trials (RCT), including specific potential risk of bias due to crossover design.[15] For case-control studies, the Cochrane risk of bias in non-randomized studies – of interventions (ROBINS-I) tool was used,[16] and for ecological studies, the National Institute for Health and Care Excellence (NICE) quality appraisal checklist for quantitative studies reporting correlations and associations was used.[17]

Data synthesis and statistical analysis

First, to provide an overview of the research landscape, health outcomes (ie, each study result) were displayed visually by intake of or exposure to A1 and A2 β-casein, using a bubble plot in which study results were categorized by study design and type of outcome (eg, type 1 diabetes, gastrointestinal conditions, etc).[18]

When studies were comparable in terms of the intervention, outcome, and study design, estimates from different studies were pooled using a random-effects meta-analysis. For continuous outcomes, the mean difference (MD) with 95%CI was tested, and heterogeneity was assessed using the I^2, χ^2, and τ^2 tests. A combination of I^2 value greater than 50% and $P < 0.05$ was considered an indicator of heterogeneity. For studies that used a crossover design and reported results for both intervention periods together, meta-analysis was performed as if the studies were parallel group trials. This

approach gives rise to a unit-of-analysis error because each study participant is exposed to more than 1 intervention.[19] For outcomes that did not allow a quantitative pooled summary estimate, the results are described narratively.

Subgroup and/or sensitivity analyses were planned (eg, stratified by characteristics of the study population, stratified by published/unpublished studies, etc) but could not be conducted because of the limited number of included studies.

Assessment of the certainty of evidence

The certainty of evidence for each outcome was assessed using the GRADE (Grading of Recommendations, Assessment, Development and Evaluation) approach.[20] The GRADE assessment takes into account, among other factors, study design, risk of bias (ie, study limitations), inconsistency of results across available studies (ie, differences in estimates of effect across studies that assessed the same comparison), indirectness (ie, differences in the patient population, intervention/exposure, or outcome measures), and imprecision of the results (ie, 95%CIs that are wide and/or include or are close to null effect around the point estimate). For observational studies, upgrading the certainty of the evidence for large magnitude of effect, dose response, and effect of all plausible residual confounding factors was considered, provided no important threats to validity were present. The certainty of the evidence was categorized for each outcome as high, moderate, low, or very low. The GRADE assessment was also carried out by 2 authors (D.K. and P.K.) independently, and again, any disagreement was resolved through consensus or discussion with a third author (J.J.M.).

A GRADE assessment was conducted only on relevant or other important outcomes. The importance of outcomes was determined pragmatically, ie, outcomes that were the primary outcome in any of the studies, or that were considered as secondary outcomes in 2 or more studies, were GRADEd. Secondary outcomes that were considered in only a single study, such as specific blood parameters, were not formally GRADEd (see Appendix S3 in the Supporting Information online).

RESULTS

Systematic literature search

A total of 9092 potentially relevant records were identified through the systematic literature search of electronic databases. An additional 73 records were identified through other sources. The updated search identified 514 new records. After removing duplicates, 6529 unique records were assessed for eligibility. From these, 6188 records were excluded after title and abstract screening, and another 316 were excluded after full-text screening. Finally, 25 records fulfilled the inclusion criteria (Figure 1). Of the 25 included references, 2 reported results on the same study, and 1 reported the results of 2 different studies. Therefore, 25 studies were included in this systematic review. Most studies were identified in scientific journals, 3 were identified in patent registers,[21,22] and 8 were identified in clinical trial registries.[23–30]

Included studies

Included studies varied in study design: 15 of 25 studies (60%) were RCTs with either parallel[21,26,29,31] or crossover designs,[9,10,23–25,27,28,30,32–35] 2 of 25 studies (8%) were case-control studies,[36,37] and the remaining 8 of 25 studies (32%) used an ecological design.[21,22,38–43] All RCTs, 1 case-control study, and 1 ecological study analyzed health outcomes after intake of or exposure to A1 β-casein in comparison with intake of or exposure to A2 β-casein (comparison I). One case-control study and the remaining ecological studies investigated different A1 β-casein dosages (comparison II); 1 of these ecological studies also compared A1 with A2 β-casein for 1 specific outcome (comparison I). Moreover, 1 of the identified RCTs compared the intake of regular milk (considered to be A1 milk) with no A1 β-casein intake/exposure (ie, placebo)[31] (comparison III).

Figure 2 displays the reported outcomes across included studies, grouped according to the study design and the type of control intervention. Each dot in the figure represents 1 outcome listed in Tables 4 to 8. In cases in which an outcome was reported stratified, eg, by age, the results were summarized into 1 dot.

Randomized controlled trials. Table 2 [9,10,21,23–35] presents the main characteristics of the 15 RCTs (7 published, 8 ongoing or completed but not yet published). In brief, 7 RCTs were conducted in Australia or New Zealand,[9,21,23,26,32–34] 4 were conducted in China,[10,24,29,30,35] 1 was completed in the United Kingdom,[31] and 3 are ongoing in England.[25,27,28] In 10 RCTs, the study population consisted of healthy adults,[9,10,23–26,28,31,32,34] and in 5 RCTs, children and adolescents were included.[21,27,29,30,33]

The published RCTs made the following comparisons (comparison I): Using a crossover design, Chin-Dusting et al[32] administered milkshakes containing A1 or A2 β-casein (10 g/d for 12 weeks) to 15 adults at high risk of cardiovascular disease. Using a parallel design, Crawford et al[21] investigated the difference between 1-time milk intake of either A1 or A2 β-casein (500 ml

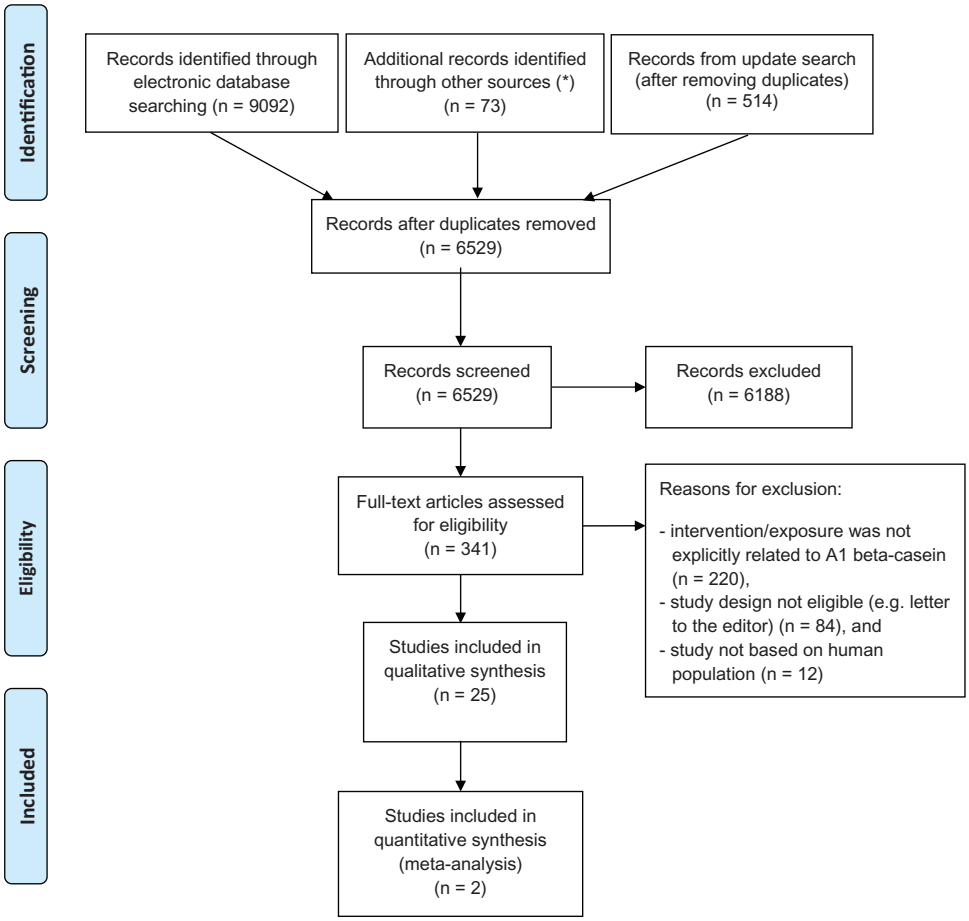


Figure 1 **Flow diagram of the literature search process.** (*) Other sources include results from the Internet search, as described in the methods section.

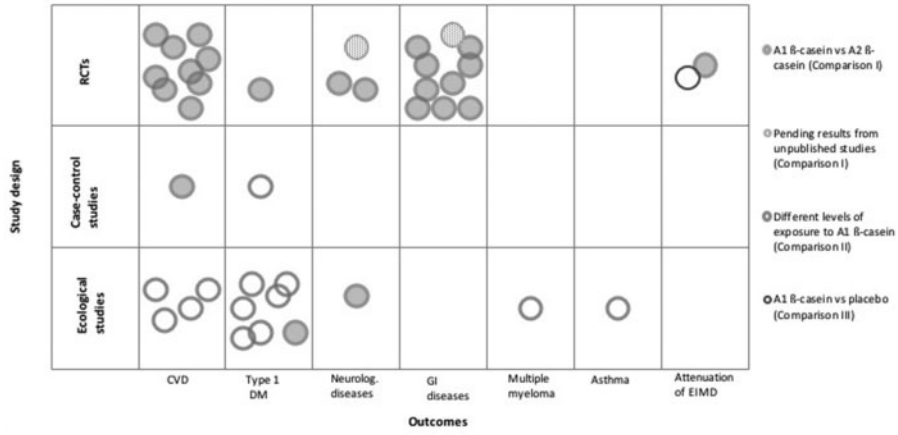


Figure 2 **A1 and/or A2 β-casein and health outcomes**. *Abbreviations*: CVD, cardiovascular disease; DM, diabetes mellitus; EIMD, exercise-induced muscle damage; GI, gastrointestinal; neurolog., neurological; RCTs, randomized controlled trials.

per 70 kg body weight, unclear sample size) in children and adolescents with autism. Crowley et al[33] used a crossover design to investigate intake of milk containing either A1 or A2 β-casein (400 ml/d for 2 weeks) in 26 children with chronic functional constipation. Ho et al[9] used a crossover design to compare A1 vs A2 milk (providing 750 ml of β-casein daily for 2 weeks) intake in 41 healthy adults. Jianqin et al[10] and Deth et al[35]

Table 2 **Characteristics of the included randomized controlled trials**

Study	Country	Study population	Reported outcomes	Study funding
Chin-Dusting et al (2006)[32]	Australia	Asymptomatic adult participants at high risk of developing CVD[a]. Sample size: n = 15 (crossover design)	Intermediate markers for CVD: plasma TC, LDL-C, HDL-C, plasma TGs, and BP Intermediate marker for diabetes: plasma insulin concentration	The a2 Milk Company Limited (formerly A2 Corporation Limited)
Crawford et al (2002)[21]	New Zealand	Children and adolescents aged ≤ 18 y of age, with diagnosed autism. Sample size: unknown (parallel design)	Intermediate marker for neurological disease: BCMs in urine	Not described
Crowley et al (2013)[33]	Australia	Children aged 1–12 y, diagnosed with CFC[b]. Sample size: n = 26 (crossover design)	GI symptoms, including constipation and bowel motions	Primary healthcare research and development program, as well as the University of Newcastle, Department of Rural Health
Ho et al (2014)[9]	Australia	Healthy adults without milk allergies or diagnosed lactose intolerance; no pregnancy/lactation; no cardiovascular events in the past 6 mo; no opioid consumption; no antibiotic treatment in the past 8 wk; no immunosuppressive or anti-inflammatory medications. Sample size: n = 41 (crossover design)	GI symptoms, including stool frequency and consistency, digestive discomfort, subjective measures of milk intolerance	The a2 Milk Company Limited (formerly A2 Corporation Limited)
Jianqin et al (2016)[10]; Deth et al[35]	China	Healthy adults (Chinese Han), who usually have a high rate of perceived lactose intolerance or report lactose malabsorption. Sample size: n = 45 (crossover design)	GI symptoms, including serum biomarkers, subjective measures of milk intolerance, stool frequency and consistency, GI transit time, stomach and bowel inflammation, fecal biomarkers Intermediate marker for neurological disease: computer-based reaction tests (speed of data processing) GSH concentration and BCM-7 concentration	The a2 Milk Company Limited (formerly A2 Corporation Limited)
Kirk et al (2017)[31]	United Kingdom	Healthy males who regularly compete in team-sports (Gaelic football, soccer, or rugby). Sample size: n = 21 (parallel design)	EIMD: VAS (muscle soreness), CMJ, MVIC, and 20-m sprint test	None
Venn et al (2006)[34]	New Zealand	Healthy adults with regular dairy product intake and total plasma cholesterol of 5–8 mmol/L, not taking lipid-lowering medications, and without any chronic disease. Sample size: n = 55 (crossover design)	Intermediate markers for CVD: plasma TC, LDL-C, HDL-C, and plasma TGs Plasma fatty acids (LA, ALA, AA, DHA)	Research grant from the University of Otago, New Zealand
Studies identified in trial registries				
Cameron-Smith (2016)[23]	New Zealand	Healthy female adults aged 20–30 y, with impaired digestion. Planned sample size: n = 40 (crossover design). Status: study completed in May 2017	Planned outcomes: Markers for GI symptoms: VAS score, GI symptoms diary, bowel movements, intestinal motility, inflammatory gene expression of TNF-α, MCP-1, IL-1β Plasma concentration of lipids, glucose, insulin, CRP, TNF-α, whole blood counts, urinary creatinine, breath metabolites	The a2 Milk Company Limited (formerly A2 Corporation Limited)

(continued)

Table 2 **Continued**

Study	Country	Study population	Reported outcomes	Study funding
Clarke (2016)[24]	China	Healthy adults aged 20–50 y, with self-reported milk intolerance. Planned sample size: n = 600 (crossover design). Status: study completed in March 2016	Planned outcomes: Markers for GI symptoms: subjective measures of milk intolerance, urinary galactose concentrations	The a2 Milk Company Limited (formerly A2 Corporation Limited)
Dickinson (2017)[25]	United Kingdom	Adults aged 18–45 y, diagnosed with asthma, who have regular endurance training at least 3 times weekly. Planned sample size: n = 24 (crossover design). Status: recruiting participants until January 2019	Planned outcomes: Markers of asthma: EVH challenge with lung function test, urine levels of prostaglandin (9α, 11β) and Clara cell protein (CC16), exhaled nitric oxide levels Markers of exercise performance: 3-km trial performance, gas exchange, perceptual responses, heart rate during exercise, blood lactate concentration Markers for GI symptoms: stool consistency Markers of inflammation, immune function, and intestinal integrity/intestinal epithelial cell damage, including WBC count, phagocyte oxidative burst, plasma CRP, cytokines and immunoglobulins, GSH, and lipid peroxidation markers, plasma BCM-7 concentration	The a2 Milk Company Limited (formerly A2 Corporation Limited)
Pal (2017)[26]	Australia	Overweight or obese adults (BMI 25–40 kg/m^2), aged 18–70 y. Planned sample size: n = 140 (parallel design). Status: not yet recruiting, but expected to end by December 2018	Planned outcomes: Markers for cardiometabolic risk: antibodies to oxidized LDL, serum cholesterol and triglyceride, arterial stiffness, blood pressure Markers for GI symptoms: self-reported tolerance to interventions, fecal calprotectin, gut microbiome, gut permeability Various serum concentrations, including dipeptidyl peptidase IV, glucose, insulin, leptin, inflammatory markers (including various ILs and CRP) Plasma BCM-7 concentration	The a2 Milk Company Limited (formerly A2 Corporation Limited)
Lodge (2016)[27]	United Kingdom	Children aged 5–10 y, diagnosed with ASD and features of ADHD. Planned sample size: n = 40 (crossover design). Status: participants recruited until December 2017	Planned outcomes: Autism rating scales Intermediate markers, including amino acids in urine, stool consistency	Northumbria University, ESPA research, and The a2 Milk Company Limited
Sandrine (2017)[28]	United Kingdom	Healthy adults aged 18–56 y. Planned sample size: n = 50 (crossover design). Status: participants recruited until March 2018	Planned outcomes: Markers for GI symptoms: GI inflammation (fecal calprotectin), VAS score, stool consistency, self-reported GI transit time, gut microbiota ecosystem Height, weight, BP, urinary and plasma metabolic profiles, systemic inflammation (CRP), lactose intolerance, and psychological behavior	University of Reading, United Kingdom

(continued)

Table 2 **Continued**

Study	Country	Study population	Reported outcomes	Study funding
Zhang (2017)[29]	China	Healthy infants aged 40–60 d, willing to formula feed and consuming at least 600 ml daily. Planned sample size: n = 33 (parallel design). Status: study completed in February 2016	Planned outcomes: Markers for GI symptoms: stool frequency, consistency, and color Fecal concentrations of myeloperoxidase, short-chain fatty acids, and microflora Body weight, height, head circumference, chest circumference, cry frequency and duration, sleep duration, milk regurgitation frequency	The a2 Milk Company Limited (formerly A2 Corporation Limited)
Zhang (2017)[30]	China	Healthy preschool children aged 5–6 y, with no regular milk consumption and with mild to moderate milk intolerance. Planned sample size: n = 80 (crossover design). Status: study completed in January 2017	Planned outcomes: Markers for GI symptoms: VAS score, stool frequency and consistency Serum concentrations of CRP, hemoglobin, IL-4, IgG, IgE, IgG1, IgG2a, BCM-7, GSH, calcium, iron, zinc Fecal short chain fatty acids and myeloperoxidase Subtle cognitive impairment test	The a2 Milk Company Limited (formerly A2 Corporation Limited)

Abbreviations: AA, arachidonic acid; ADHD, attention-deficit/hyperactivity disorder; ALA, α-linolenic acid; ASD, autism spectrum disorder; BCM, β-casomorphin; BMI, body mass index; BP, blood pressure; CFC, chronic functional constipation; CMJ, countermovement jump; CRP, C-reactive protein; CVD, cardiovascular disease; DHA, docosahexaenoic acid; EIMD, exercise-induced muscle damage; ESPA, Education and Services for People with Autism; EVH, eucapnic voluntary hyperpnea; GI, gastrointestinal; GSH, glutathione; HDL-C, high-density lipoprotein cholesterol; Ig, immunoglobulin; IL, interleukin; LA, linoleic acid; LDL-C, low-density lipoprotein cholesterol; MCP, monocyte chemotactic protein; MVIC, maximal voluntary isometric contraction; TC, total cholesterol; TG, triglyceride; TNF-α, tumor necrosis factor-α; VAS, visual analogue scale; WBC, white blood cell.

[a]Participants at high risk of developing CVD were defined as those who had at least 2 of the following risk factors: current smoker, BMI > 25 mg/m^2, total serum cholesterol > 6 mmol/l, systolic blood pressure > 145 mmHg, family history with at least 1 parent showing onset of CVD under the age of 60 y.

[b]CFC defined as fewer than 8 bowel motions in 14 d, unresolved by medication or diet.

used a crossover design to investigate A1 milk (A1:A2 ratio of 40:60) compared with milk containing A2 only (both study arms received 500 ml of milk daily for 14 days) in 45 healthy adults. Kirk et al[31] used a parallel design to compare the effect of 500 ml (1-time intake) of regular milk (containing both A1 and A2 β-casein) with 500 ml of milk containing solely A2 β-casein or placebo (50 g of maltodextrin mixed with water, comparison III) in 21 healthy men who regularly competed in team sports. Venn et al[34] evaluated, in a crossover design, consumption of milk and cheese containing both A1 and A2 β-casein compared with consumption of milk and cheese containing A2 β-casein only (participants in each study arm were provided with 500 ml of milk and 28 g of cheese daily for 4.5 weeks) in 55 healthy adults.

Table 2 shows the characteristics of the unpublished RCTs (identified in clinical trial registries). All studies compared the effect of A1 milk and A2 milk on various outcomes (including gastrointestinal symptoms and intermediate markers of asthma and autism) (comparison I).

Case-control studies. Table 3 [21,22,36–43] presents the main characteristics of the 2 case-control studies included. In brief, 1 of the studies investigated, retrospectively, whether A1 and A2 milk intake is associated with low-density lipoprotein cholesterol (LDL-C) concentrations in 82 healthy adults in Albania (comparison I).[36] The retrospective exposure to A1 and A2 β-casein was analyzed in relation to the plasma LDL-C concentrations of cases (healthy adults with high LDL-C plasma concentrations) and controls (healthy adults with low LDL-C plasma concentrations). The second study examined, in 220 children aged 3 to 19 years, whether incidence of type 1 diabetes is associated with former nursing habits (length of lactation) and/or milk exposure, respectively[37] (comparison II). The exposure to cow's milk (considering the A1 β-casein content and the duration of lactation as the timing of first exposure to cow's milk) was analyzed in patients with type 1 diabetes and in healthy controls.

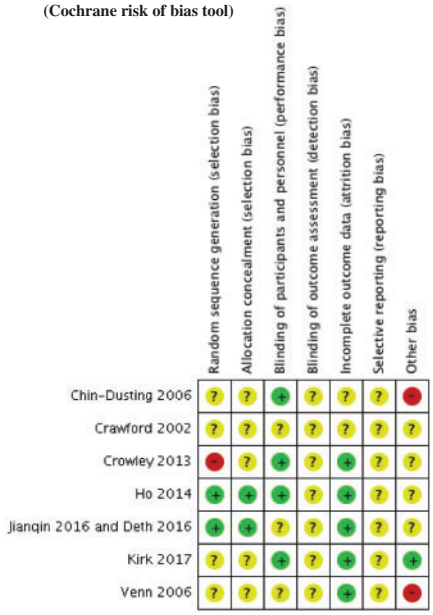
Ecological studies. Table 3 presents the main characteristics of the ecological studies. In brief, the 8 studies investigated whether A1 β-casein exposure is a risk factor for the development of selected diseases in various countries worldwide. In most studies, the regular A1 β-casein exposure was calculated from the estimated per capita milk intake, taking into account the cattle breeds present in the respective regions. The regular

Table 3 **Characteristics of the included case-control and ecological studies**

Study	Country	Study population	Reported outcomes	Study funding
Case-control studies				
Laknori et al (2010)[36]	Albania	Not described; probably healthy adults. Sample size: n = 82	Intermediate marker for CVD: LDL-C	Not described
Thorsdottir et al (2000)[37]	Iceland	Children and adolescents aged 3–19 y with diagnosed T1DM, and age- and sex-matched healthy controls. Sample size: n = 220 (55 cases, 165 controls)	Incidence of T1DM	Not described
Ecological studies				
Birgisdottir et al (2006)[38]	Iceland and Scandinavia (Norway, Denmark, Sweden, Finland)	Children and adolescents up to age 14 y. Data for children aged 2 y and for children aged 11–14 y were analyzed separately	Incidence of T1DM	Grants from the Icelandic Research Council and the Foundation for Research, Science and Technology, New Zealand
Casu et al (2001)[39]	Italy	Children and adolescents aged 0–14 y	Incidence of T1DM	Not described
Crawford et al (2002)[21]	Australia, Canada, Denmark, Finland, Germany, Iceland, New Zealand, Norway, Sweden, USA	Not described	Mortality due to neurological disease	Not described
Elliott et al (1999)[40]	Australia, Canada, Denmark, Finland, Germany, Iceland, New Zealand, Norway, Sweden, USA	Children and adolescents aged 0–14 y	Incidence of T1DM	National Child Health Research Foundation and the New Zealand Dairy Board
Elliott & Laugesen (2001)[22]	Various countries (not specified)	Not described	Mortality due to CVD Incidence of asthma Incidence of multiple myeloma	Not described
Laugesen & Elliott (2003)[41]	Various countries (n = 22)	Adults Children and adolescents aged 0–14 y	Mortality due to CVD Incidence of T1DM	The a2 Milk Company Limited (formerly A2 Corporation Limited)
McLachlan (2001)[42]	Various countries (n = 21)	Male adults aged 30–69 y, and older adults (men and women aged > 65 y)	Mortality due to CVD	The a2 Milk Company Limited (formerly A2 Corporation Limited)
	Germany	Male adults from different states in Germany (Schleswig-Holstein, Niedersachsen, Nordrhein-Westfalen, Saarland, Hessen, Rheinland-Pfalz, Baden-Württemberg, Bayern)	Mortality due to CVD	
	Various countries (not specified)	Children and adolescents aged 0–14 y	Incidence of T1DM	
Wasmuth et al (1999)[43]	Germany	Children	Incidence of T1DM	Not described

Abbreviations: CVD, cardiovascular disease; LDL-C, low-density lipoprotein cholesterol; T1DM, type 1 diabetes mellitus.

A Risk of bias for randomized controlled trials (Cochrane risk of bias tool)



B Risk of bias for case-control studies (Cochrane ROBINS-I tool)

Risk of Bias	Case-control studies	
	Laknori et al. (2010)[35]	Thorsdottir et al. (2000)[36]
1. Bias due to confounding	Serious	Serious
2. Bias in selection of participants into study	Serious	Serious
3. Bias in classification of intervention/exposure	Moderate	Moderate
4. Bias due to deviations from intended intervention/exposure	No information	No information
5. Bias due to missing data	No information	No information
6. Bias in measurement of outcomes	Moderate	Moderate
7. Bias in selection of the reported results	Low	Low
8. Overall risk of bias	Serious	Serious

C Risk of bias for ecological studies (quality appraisal checklist-quantitative studies reporting correlations and associations)

Ecological studies	1. Domain[a] Population	2. Domain[a] Definition of the exposure	3. Domain Endpoints	4. Domain[a] Analysis	5. Summary Internal validity	5. Summary External validity
Birgisdottir et al (2006)[37]	+ / ++ / ++	++ / NR / NR	++	- / NR/ ++	+	++
Casu et al (2001)[38]	+ / ++ / +	+ / NR / NR	++	- / NR/ ++	+	++
Crawford et al (2002)[20]	+ / ++ / +	NR / NR / NR	++	- / NR/ ++	+	++
Elliott et al (1999)[39]	+ / ++ / ++	NR / NR / NR	++	- / NR/ +	+	++
Elliott et al (2001)[21]	- / - / NR	NR / NR / NR	++	- / NR/ ++	+	+
Laugesen et al (2003)[40]	+ / ++ / +	NR / NR / NR	++	- / NR/ ++	+	++
McLachlan et al (2001)[41]	+ / ++ / +	NR / NR / NR	++	- / NR/ +	+	++
Wasmuth et al (1999)[42]	+ / + / +	NR / NR / NR	++	- / NR/ +	+	+

[a]Domain 1, 2, and 4 included various sub-categories tob e evaluated, thus several answers were possible. The assessment (++) means low risk of bias, (+) means unclear risk of bias, (-) means high risk of bias

Figure 3 **Risk-of-bias assessment for all included studies.** (**A**): Risk of bias for randomized controlled trials (Cochrane risk of bias tool); (**B**) Risk of bias for case-control studies (ROBINS-I tool); (**C**) Risk of bias for ecological studies (quality appraisal checklist—quantitative studies reporting correlations and associations). *Abbreviations*: ROBINS-I, Risk Of Bias In Non-randomized Studies – of Interventions; NR, not reported.

A1 β-casein exposure per capita in each country was correlated with various health conditions, including incidence and/or prevalence data obtained from registries for the outcome/disease of interest (eg, type 1 diabetes or cardiovascular disease). Hence, different dosages of A1 β-casein exposures could be analyzed. Seven of these studies evaluated exposure to different dosages of A1 β-casein (comparison II), and 1 ecological study compared exposure to A1 β-casein with exposure to A2 β casein (comparison I).

Funding of studies. Twelve of the 25 included studies reported the a2 Milk Company Limited (formerly A2 Corporation) as sponsor (10 RCTs and 2 ecological studies).[9,10,23–26,29,30,32,35,41,42] Of the remaining 13 studies, 5 were supported by different independent funding agencies, including various health research programs[28,33,34,38,40]; 1 had no financial support[31]; and 7 provided no information on funding.[21,22,36,37,39,43] Of these, 2 studies were published as journal articles,[36,37] 2 as abstracts of conference proceedings,[39,43] and 3 as patent register entries.[21,22]

Risk of bias in included studies

Figure 3[9,10,20,21,31–35,37–42] shows the results of the risk-of-bias assessment for all included studies. Most RCTs did not provide sufficient information for an assessment of low or high risk of bias, resulting in unclear risk of bias for most domains. Two RCTs were judged to have a high risk of bias owing to potential carryover effects because neither crossover trial included a washout period between the interventions.[32,34]

The 2 case-control studies were categorized to have serious risk of bias,[36,37] mainly owing to bias in the selection of participants and because they did not control for confounding variables such as usual milk intake apart from the intervention.

The results of risk-of-bias assessment of the ecological studies were similar across the studies. Overall, internal validity was judged to be unclear because of missing information, and generalizability (ie, external validity) was considered high for 6 of the 8 studies.[21,38–42]

Health-related outcomes

A1 β-casein and diabetes. Results for type 1 diabetes are summarized in Table 4.[32,37–43] One crossover RCT (comparison I) showed a decrease in the mean plasma insulin concentration in both groups, without a significant difference between groups (from 11.8 to 8.8 mU/L in the A1 group and from 11.8 to 9 mU/L in the A2 group after 12 weeks of intervention).[32] The incidence of type 1 diabetes was not associated with A1 β-casein consumption, either in 1 case-control study or in 2 ecological studies [all comparison II: Thorsdottir et al[37] and Wasmuth et al,[43] both described only narratively, and Casu et al[39]: $r(p) = 0.4$ (0.6)]. In contrast, 4

ecological studies found significant correlations between A1 β-casein exposure and the incidence of type 1 diabetes [($r(p) = 0.9$ ($P = 0.037$); $r(p) = 0.774$ ($P < 0.01$); $r(p) = 0.92$ ($P < 0.001$); $r(p) = 0.75$ (P value not described)].[38,40–43] Of note, 1 of these studies found a significant positive correlation only in 2-year-old children, whereas no correlation was found in children aged 11 to 14 years (described only narratively).[38] One ecological study also investigated the correlation between A2 β-casein exposure and the incidence of type 1 diabetes and found a weak significant positive correlation [comparison I: $r(p) = 0.47$ (0.05)].[41]

A1 β-casein and cardiovascular disease. All results for cardiovascular disease are summarized in Table 5[22,32,34,36,41,42,44]. Two crossover RCTs showed no significant difference between A1 and A2 milk for the following outcomes (estimated effects expressed as MD in mmol/L [95%CI]): plasma total cholesterol, −0.18 [−0.45 to 0.08]; plasma high-density lipoprotein cholesterol, −0.00 [−0.09 to 0.08]; plasma LDL-C, −0.08 [−0.21 to 0.04]; and plasma triglycerides, −0.17 [−0.46 to 0.11]) after 4.5 and 6 weeks of intervention (Figure 4).[32,34] Chin-Dusting et al[32] also investigated whether there was an effect on systolic and diastolic blood pressure; however, no differences were detected after 12 weeks of intervention (systolic blood pressure in A1 group, 131 ± 4 mmHg; systolic blood pressure in A2 group, 131 ± 5 mmHg; diastolic blood pressure in A1 group, 77 ± 2 mmHg; diastolic blood pressure in A2 group, 75 ± 2 mmHg). A case-control study showed significantly higher plasma LDL-C concentrations in participants with regular intake of A1 milk than in participants with regular intake of A2 milk (A1 milk, 129.29 ± 16.67 mg/dL; A2 milk, 120.15 ± 8.47 mg/dL).[36]

Mortality due to cardiovascular disease was correlated with increasing A1 milk consumption in 3 ecological studies [Elliott and Laugesen,[22] data from patent register: $r(p) = 0.72$ ($P < 0.001$) in males and 0.64 ($P < 0.01$)] in females; Laugesen and Elliott,[41] $r(p) = 0.76$–0.82 ($P < 0.001$); and McLachlan,[42]:$r(p) = 0.71$ (P value not described)].

A1 β-casein and neurological disease. All results for neurological disease are summarized in Table 6.[10,21] Urinary concentration of β-casomorphins in children was increased after A1 milk consumption vs A2 milk consumption in autistic children, while age-matched normal children had almost no β-casomorphin in their urine (described only narratively in an RCT, data from patent register).[21] Results on the speed and effectiveness of information processing in 45 healthy adults (comparison I), found slower response times and higher error rates after 2 weeks of A1 milk intake compared with A2 milk intake (described only narratively).[10] One ecological study found a positive significant correlation between A1 β-casein exposure and mortality due to neurological disorders (comparison I), whereas a negative, nonsignificant correlation was described for A2 β-casein exposure [A1 β-casein, $r(p) = 0.795$ (0.006); A2 β-casein, $r(p) = -0.219$ (0.544); data from patent register].[21]

A1 β-casein and gastrointestinal conditions. All results for gastrointestinal conditions are summarized in Table 7[9,10,33]. One RCT showed no significant difference between A1 and A2 milk in resolution of constipation or the number of bowel motions (A1 milk, 10.05 ± 5.75 bowel motions; A2 milk, 10.56 ± 5.24 bowel motions; assessed after 2 weeks of intervention).[33] Stool consistency (assessed with the Bristol stool scale) was measured in 2 RCTs after a 2-week intake of both milk types (Ho et al,[9] 3.87 ± 0.02 after A1 milk and 3.56 ± 0.02 after A2 milk; and Jianqin et al,[10] 4.42 ± 0.74 in participants consuming A1 milk in their first trial period, 4.35 ± 1.11 in participants consuming A1 milk in their second trial period, 4.05 ± 0.25 in participants consuming A2 milk in their first trial period, and 4.08 ± 0.61 in participants consuming A2 milk in their second trial period). Additionally, in participants with self-reported milk intolerance, Ho et al[9] reported higher rates of flatus, bloating, abdominal pain, and voiding difficulty after a 2-week intake of A1 milk compared with a 2-week intake of A2 milk (described only narratively).[9] Results for stool frequency were contradictory in the 2 RCTs: Ho et al[9] found no difference, while Jianqin et al[10] reported higher stool frequency rates after a 2-week intake of A1 milk (11.05 ± 4.21 number/week in participants taking A1 milk in their first trial period, 10.43 ± 3.46 motions/week in participants taking A1 milk in their second trial period, 7.91 ± 1.15 number/week in participants taking A2 milk in their first trial period, and 7.87 ± 1.91 number/week in participants taking A2 milk in their second trial period). Furthermore, Jianqin et al[10] showed increased gastrointestinal transit time after a 2-week intake of A1 milk in comparison with A2 milk (whole gastrointestinal transit time: 39.95 ± 8.45 hours after A1 milk first, 40.14 ± 6.81 hours after A1 milk second, 33.41 ± 5.68 hours after A2 milk first, and 34.36 ± 6.9 hours after A2 milk second).

A1 β-casein and the attenuation of exercise-induced muscle damage. Results for the attenuation of exercise-induced muscle damage are summarized in Table 8[31]. The RCT of Kirk et al[31] found a significantly quicker recovery when assessing the countermovement jump and the time after a 20-meter sprint in participants who

Table 4 **Studies investigating A1 β-casein and diabetes**

Randomized controlled trials

Study	Intervention	Control intervention	Sample size	Outcome	Time of outcome measurement[a]	Results		
						Intervention A1, mean ± SD	Control A2, mean ± SD	MD
Chin-Dusting et al (2006)[32]	Comparison I[b]: Dairy shakes containing A1 β-casein (25 g A1 β-casein powder daily, mixed with water or juice). Dosage: 10 g A1 β-casein daily for 12 wk	Dairy shakes containing A2 β-casein (25 g A2 β-casein powder daily, mixed with water or juice). Dosage: 10 g A2 β-casein for 12 wk	15 (crossover design)	Plasma insulin concentration	Baseline After 6 wk After 12 wk	11.8 ± 0.93 mU/L 10.1±0.59 mU/L 8.8 ± 0.36 mU/L	11.8 ± 0.93 mU/L 7.8 ± 0.31 mU/L 9 ± 0.36 mU/L	0 2.3 mU/L 0.2 mU/L

Case-control studies

Study	Exposure	Controls	Sample size (no.)	Outcome	Results	
					Exposure A1	Exposure A2 (control)
Thorsdottir et al (2000)[37]	Comparison II[b]: Exposure to cow's milk (considering the A1 β-casein content and the duration of lactation as the timing of first exposure to cow's milk)	Not described	220 (55 cases, 165 controls)	Incidence of T1DM	Only narrative results reported: "No significant difference on the incidence of DM1 between cases and controls. Breastfeeding habits were similar in both groups."	

Ecological studies

Study	Exposure	Calculated dosage	Sample size (no.)	Outcome	Results	
					Exposure A1, *r*(p)[c]	Exposure A2, *r*(p)
Birgisdottir et al (2006)[38]	Comparison II[b]: Exposure to A1 and A1 + B β-casein [d]	For children aged 2 y: mean exposure to A1 β-casein (grams/day): Ireland, 1.7; Norway, 2,0; Denmark, 2.7; Sweden, 2.1; Finland, 2.8	NA	Incidence of T1DM	0.9 (0.037)	NA[e]
		For boys aged 11–14 y: mean exposure to A1 β-casein: Ireland, 3.7; Norway, 4.9; Denmark, 3.7; Sweden, 4.8; Finland, 4.7		Incidence of T1DM	No correlation	NA[e]
		Mean exposure to A1 β-casein: Ireland, 2.8; Norway, 3.6; Denmark, 3.0; Sweden, 3.7; Finland, 3.5		Incidence of T1DM (girls aged 11–14 y)	No correlation	NA[e]

(continued)

Table 4 **Continued**

Ecological studies

Study	Exposure	Calculated dosage	Sample size (no.)	Outcome	Results	
					Exposure A1, *r*(p)[c]	Exposure A2, *r*(p)
Casu et al (2001)[39]	Comparison II[b]: Exposure to A1 + B β-casein	Not described	NA	Incidence of T1DM	0.4 (0.6)	NA[e]
Elliott et al (1999)[40]	Comparison II[b]: Exposure to A1 and A1 + B β-casein	Nonweighted mean exposure to A1 β-casein (grams/day): Canada, 2.589; Australia, 1.99; Denmark, 3.075; Finland, 3.862; Germany, 1.93; Iceland, 2.425; New Zealand, 1.749; Norway, 3.135; Sweden, 3.895; San Diego (USA), 2.154	NA	Incidence of T1DM	0.774 (< 0.01)	NA[e]
		Weighted mean exposure to A1 β-casein: Canada, 2.686; Australia, 1.929; Denmark, 2.735; Finland, 3.87; Germany, 1.93; Iceland, 2.453; New Zealand, 1.734; Norway, 3.401; Sweden, 3.714; San Diego (USA), 2.201		Incidence of T1DM	0.726 (< 0.05)	NA[e]
Laugesen & Elliott (2003)[41]	Comparison I[b]: Exposure to A1 and A2 β-casein	Mean exposure to A1 β-casein (grams/day): Finland, 2.93; Sweden, 2.92; Norway, 1.94; Japan, 0.73; Venezuela, 0.50; France, 0.01; Italy, 1.11; Switzerland, 1.27; Israel, 0.90; Australia, 2.12; Iceland, 1.65; Denmark, 1.29; Germany, 1.18; Canada, 1.79; Austria, 0.94; Hungary, 1.25; USA, 1.63; New Zealand, 2.0; UK, 2.14	NA	Incidence of T1DM	0.92 (< 0.001)	0.47 (< 0.05)
McLachlan (2001)[42]	Comparison II[b]: Exposure to A1 and A1 + B β-casein [d]	Not described	NA	Incidence of T1DM	0.75[f]	NA[e]
Wasmuth et al (1999)[43]	Comparison II[b]: Exposure to A1 β-casein	Not described	NA	Incidence of T1DM	Only narrative results reported: "No significant correlation was found between the exposure of A1 beta-casein and the incidence of DM1. A correlation to A2 beta-casein was not analyzed."	

Abbreviations: MD, mean difference; mU/L, milliunit per liter; NA, not applicable; T1DM, type 1 diabetes mellitus.

[a]For all randomzied controlled trials, the results include information on the time points when outcomes were measured. No time points are included for case-control studies or ecological studies, as no explicit time point can be defined because of the nature of the study design.

[b]Refers to the comparison investigated in the study. Comparison I: A1 β-casein intake/exposure compared with A2 β-casein intake/exposure. Comparison II: different dosages of A1 β-casein.

[c]Ecological studies calculated either Pearson's correlation coefficient (when values had a normal distribution) or Spearman's correlation coefficient (when values were not normally distributed). The nature of this study design means that the number of participants cannot be defined.

[d]Some studies included B β-casein in their analysis because it has properties similar to those of A1 β-casein. However, the concentration of B β-casein in milk is normally very low compared with concentrations of the other β-casein variants.

[f]No information about the significance level (*P* value) was reported. *r*(p): correlation coefficient and statistical significance (*P* value). Interpretation of *r*: 0.1–0.5 = weak correlation, 0.51–0.8 = medium correlation, 0.81–1.0 = strong correlation.

[e]Only the correlation with A1 β-casein, and not the correlation with A2 β-casein, was analyzed.

Table 5 **Studies investigating A1 β-casein and cardiovascular disease**

Randomized controlled trials								
Study	Intervention	Control intervention	Sample size	Outcome	Time of outcome measurement[a]	Results		
						Intervention A1, mean ± SD	Control A2, mean ± SD	MD
Chin-Dusting et al (2006)[32]	Comparison I[b]: Dairy shakes containing A1 β-casein (25 g A1 β-casein powder daily, mixed with water or juice). Dosage: 10 g A1 β-casein daily for 12 wk	Dairy shakes containing A2 β-casein (25 g A2 β-casein powder daily, mixed with water or juice). Dosage: 10 g A2 β-casein daily for 12 wk	15 (crossover design)	TC	Baseline	6.3 ± 0.08 mmol/L	6.3 ± 0.08 mmol/L	0
					After 6 wk	5.9 ± 0.08 mmol/L	6.2 ± 0.08 mmol/L	0.3 mmol/L
					After 12 wk	5.6 ± 0.05 mmol/L	5.7 ± 0.08 mmol/L	0.1 mmol/L
				LDL-C	Baseline	3.7 ± 0.05 mmol/L	3.7 ± 0.05 mmol/L	0
					After 6 wk	3.6 ± 0.05 mmol/L	3.7 ± 0.05 mmol/L	0.1 mmol/L
					After 12 wk	3.3 ± 0.05 mmol/L	3.4 ± 0.05 mmol/L	0.1 mmol/L
				HDL-C	Baseline	1.8 ± 0.05 mmol/L	1.8 ± 0.05 mmol/L	0
					After 6 wk	1.8 ± 0.03 mmol/L	1.8 ± 0.05 mmol/L	0
					After 12 wk	1.6 ± 0.03 mmol/L	1.7 ± 0.05 mmol/L	0.1 mmol/L
				TGs	Baseline	1.4 ± 0.03 mmol/L	1.4 ± 0.05 mmol/L	0
					After 6 wk	1.2 ± 0.03 mmol/L	1.5 ± 0.03 mmol/L	0.3 mmol/L
					After 12 wk	1.4 ± 0.05 mmol/L	1.3 ± 0.05 mmol/L	0.1 mmol/L
				SBP[c]	Baseline	127 ± 4.0 mmHg	127 ± 4.0 mmHg	0
					After 6 wk	131 ± 4.0 mmHg	127 ± 4.0 mmHg	4 mmHg
					After 12 wk	131 ± 4.0 mmHg	131 ± 5.0 mmHg	0
				DBP[c]	Baseline	77 ± 3.0 mmHg	77 ± 3.0 mmHg	0
					After 6 wk	76 ± 2.0 mmHg	73 ± 2.0 mmHg	3 mmHg
					After 12 wk	77 ± 2.0 mmHg	75 ± 2.0 mmHg	2 mmHg
Venn et al (2006)[34]	Comparison I[b]: Milk and cheese (containing both A1 and A2 β-casein). 500 ml milk and 28 g cheese daily for 4.5 wk. Dosage: A1 β-casein 2.5 g/d and A2 β-casein 3.6 g/d	Milk and cheese containing A2 β-casein. 500 ml milk and 28 g cheese daily for 4.5 wk. Dosage: 0.4 g A1 β-casein and 5.8 g A2 β-casein daily	55 (crossover design)	TC	Baseline	5.92 ± 0.92 mmol/L	5.92 ± 0.92 mmol/L	0
					After 4.5 wk	5.60 ± 0.77 mmol/L	5.63 ± 0.81 mmol/L	0.03 mmol/L
				LDL-C	Baseline	3.97 ± 0.66 mmol/L	3.97 ± 0.66 mmol/L	0
					After 4.5 wk	3.73 ± 0.70 mmol/L	3.75 ± 0.75 mmol/L	0.02 mmol/L
				HDL-C	Baseline	1.28 ± 0.36 mmol/L	1.28 ± 0.36 mmol/L	0
					After 4.5 wk	1.26 ± 0.34 mmol/L	1.27 ± 0.37 mmol/L	0.1 mmol/L
				TGs	Baseline	1.48 ± 0.72 mmol/L	1.48 ± 0.72 mmol/L	0
					After 4.5 wk	1.33 ± 0.51 mmol/L	1.34 ± 0.63 mmol/L	0.1 mmol/L

(continued)

Table 5 **Continued**

Case-control studies

Study	Exposure	Control intervention	Sample size	Outcome	Results		
					Intervention A1, mean ± SD	Control A2, mean ± SD	MD
Laknori et al (2010)[36]	Comparison I[b]: Exposure to A1 or A2 β-casein	Not described	82	LDL-C	129.29 ± 16.67 mg/dL	120.15 ± 8.47 mg/dL	9.14 mg/dL

Ecological studies

Study	Exposure	Calculated dosage	Sample size	Outcome	Results	
					Exposure A1, *r*(p)[d]	Exposure A2, *r*(p)
Elliott & Laugesen (2001)[22]	Comparison II[b]: Exposure to A1 β-casein	Not described	NA	Mortality due to CVD (males)	0.72 (< 0.001)	NA[e]
				Mortality due to CVD (females)	0.64 (< 0.01)	NA[e]
Laugesen & Elliott (2003)[41]	Comparison II[b]: Exposure to A1 β-casein (in milk and cream)	Mean exposure to A1 β-casein (grams/day): Japan, 0.73; France, 0.93; Guernsey, 0.03; Italy, 1.19; Switzerland, 1.20; Jersey, 0.29; Israel, 0.91; Australia, 2.0; Iceland, 1.82; Sweden, 2.82; Denmark, 1.65; Germany, 1.06; Canada, 1.90; Norway, 1.99; Austria, 0.92; USA, 1.60; Finland, 3.11; New Zealand, 2.42; UK, 2.31; Ireland, 1.63	NA	Mortality due to CVD	0.76–0.82 (< 0.001)	NA[e]
	Comparison II[b]: Exposure to A1 β-casein (in milk, cream, and cheese)	Mean exposure to A1 β-casein (grams/day): Finland, 2.93; Sweden, 2.92; Norway, 1.94; Japan, 0.72; Venezuela, 0.50; France, 1.01; Italy, 1.11; Switzerland, 1.27; Israel, 0.90; Australia, 2.12; Iceland, 1.65; Denmark, 1.29; Germany, 1.18; Canada, 1.79; Austria, 0.94; Hungary, 1.25; USA, 1.63; New Zealand, 2.0; UK, 2.14	NA	Mortality due to CVD	0.66–0.79 (< 0.01)	NA[e]

(continued)

Table 5 **Continued**

Ecological studies

Study	Exposure	Calculated dosage	Sample size	Outcome	Results	
					Exposure A1, *r*(p)[d]	Exposure A2, *r*(p)
McLachlan (2001)[42]	Comparison II[b]: Exposure to A1 β-casein	Mean exposure to A1 β-casein (grams/day): Australia, 3.15; Austria, 1.58; Canada, 2.4; Denmark, 3.68; Finland, 4.07; France, 2.3; Germany, 2.4; Iceland, 2.67; Ireland, 3.72; Israel, 2.22; Japan, 0.91; New Zealand, 3.76; Norway, 3.77; Sweden, 3.94; Switzerland, 1.76; UK, 3.57; USA, 2.7; England and Wales, 3.53; Scotland, 3.63; Northern Ireland, 4.08	NA	Mortality due to CVD (males, 30–69 y)	0.71[f]	NA[e]
				Mortality due to CVD (males, > 65 y)	0.84[f]	NA[e]
				Mortality due to CVD (females, > 65 y)	0.73[f]	NA[e]
		Mean exposure to A1 β-casein (grams/day): Schleswig-Holstein, 2.92; Lower Saxony, 2.80, North Rhine-Westphalia, 2.93; Saarland, 2.77; Hesse, 2.27; Rhineland-Palatinate, 2.81; Baden-Württemberg, 1.70; Bavaria, 1.47		Mortality due to CVD (males)	0.66[f]	NA[e]

Abbreviations: CVD, cardiovascular disease; DBP, diastolic blood pressure; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; MD, mean difference; NA, not applicable; SBP, systolic blood pressure; TG, triglyceride.

[a]For all randomized controlled trials, the results include information on the time points when outcomes were measured. No time points are included for case-control studies or ecological studies, as no explicit time point can be defined because of the nature of the study design.

[b]Refers to the comparison that was investigated in the study. Comparison I: A1 β-casein intake/exposure in comparison with A2 β-casein intake/exposure. Comparison II: different exposure/intake dosages of A1 β-casein.

[c]According to the American Heart Association, normal blood pressure is below 120/80 mmHg.[44]

[d]Ecological studies calculated either Pearson's correlation coefficient (when values had a normal distribution) or Spearman's correlation coefficient (when values were not normally distributed). Because of the study design used for ecological studies, no number of participants can be defined.

[e]Study did not analyze the correlation with A2 β-casein, only with A1 β-casein.

[f]Study gave no information about the significance level (*P* value).

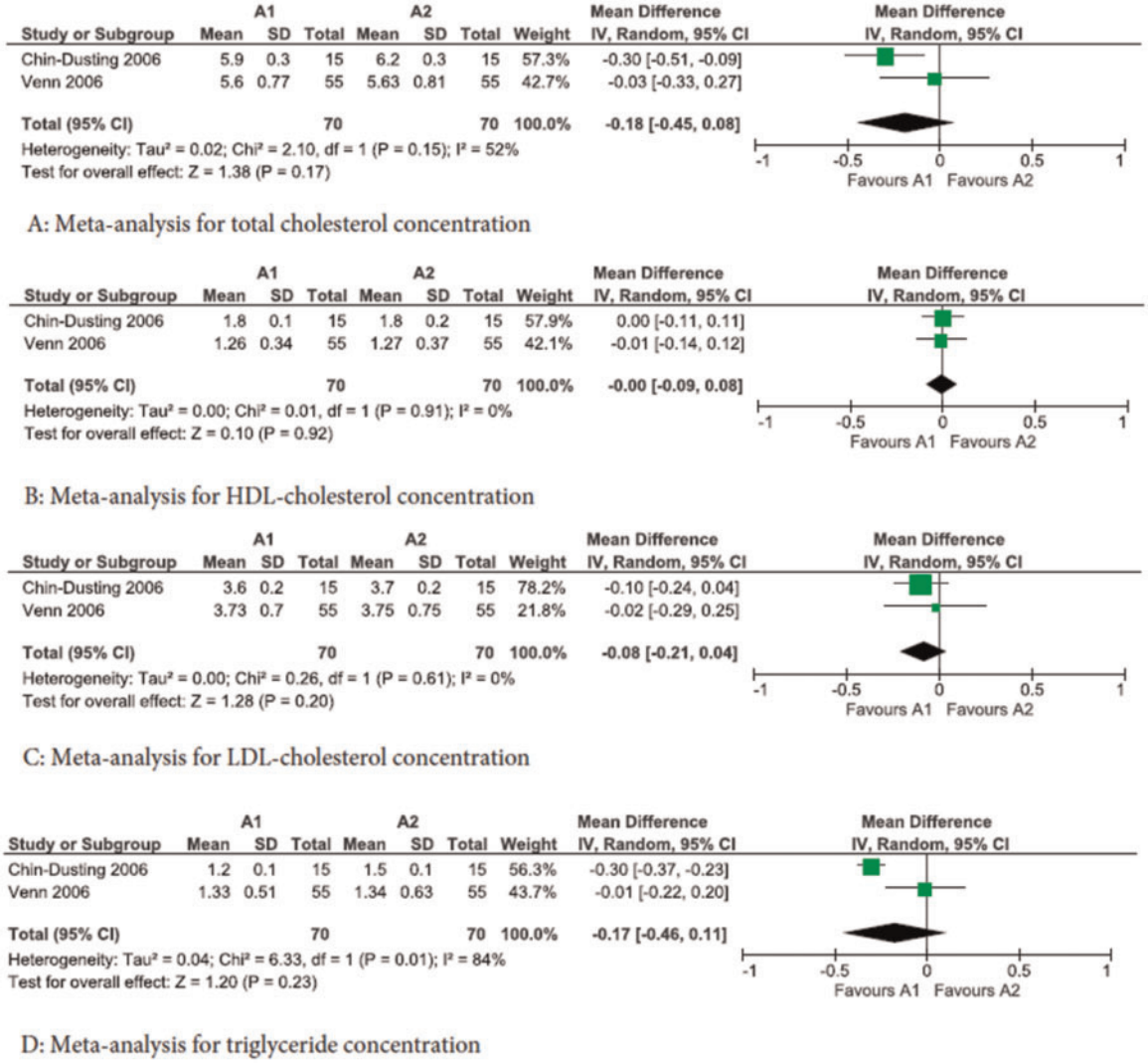


Figure 4 **Meta-analyses of the comparative effects of A1 and A2 milk on cholesterol concentrations in 2 randomized controlled trials.** Outcomes were measured at different times in each study. To allow comparison between the 2 studies, results from the nearest measuring time points were considered: Chin-Dusting et al (2006)[32] (outcome measured after 6 weeks of intervention) and Venn et al (2006)[34] (outcome measured after 4.5 weeks of intervention). (**A**) Meta-analysis for total cholesterol concentration; (**B**) Meta-analysis for HDL-C concentration; (**C**) Meta-analysis for LDL-C concentration; (**D**) Meta-analysis for triglyceride concentration. *Abbreviations*: HDL, high-density lipoprotein; LDL, low-density lipoprotein.

consumed either A1 or A2 milk compared with placebo. Results suggest that A2 milk could be equally effective as A1 milk in attenuating exercise-induced muscle damage, presenting an alternative to athletes intolerant to regular milk.

A1 β-casein and further health-related outcomes. All further results for other health-related outcomes are summarized in Table 9[10,22,34,35] (results from 2 RCTs and 1 ecological study). Briefly, 1 of the RCTs found significantly higher levels of interleukin 4, immunoglobulin (Ig) G, IgE, IgG1, acetic acid, butanic acid, total small-chain fatty acids, and β-casomorphin-7 after A1 milk intake in comparison with A2 milk intake; no difference in C-reactive protein or hemoglobin; and significantly higher concentrations of plasma glutathione after A2 milk intake in comparison with A1 milk intake (comparison I).[10,35] A further crossover RCT found significantly lower plasma concentrations of α-linolenic acid after A2 milk intake in comparison with A1 milk intake, but no difference in concentrations of other plasma fatty acids (lactic acid, arachidonic acid, and docosahexaenoic acid).[34]

One ecological study found a significant positive correlation between A1 β-casein exposure and multiple myeloma in males [$r(p) = 0.62$ ($P < 0.01$)] and in

Table 6 **Studies investigating A1 β-casein and neurological disease**

Randomized controlled trials

Study	Intervention	Control intervention	Sample size	Outcome	Time of outcome measurement[a]	Results	
						Intervention A1	Control A2
Crawford et al (2002)[21]	Comparison I[b]: Milk containing A1 β-casein. One-time intake of 500 ml per 70 kg of body weight	Milk containing A2 β-casein. One-time intake of 500 ml per 70 kg of body weight	Not described	BCM concentration in urine[c]	Before and after intervention	Narrative results only: "Autistic children had a 10-fold higher beta-casomorphine concentration in urine after the single intake of A1 milk when compared to children who drank A2 milk."[d]	
Jianqin et al (2016)[10]	Comparison I[b]: Milk containing A1 and A2 β-casein at a 40:60 ratio (500 ml/d for 14 d)	Milk containing A2 β-casein (500 ml/d for 14 d)	45 (crossover design)	Information processing (speed and effectiveness)	After 2 wk	Narrative results only: ". . .the intake with milk containing A1 and A2 beta-casein participants showed significant slower information processing time and higher error rates when compared to participants under the control intervention (milk containing only A2 beta-casein)."	

Ecological studies

Study	Intervention/exposure	Calculated dosage	Sample size	Outcome	Results	
					Exposure A1, *r*(p)[e]	Exposure A2, *r*(p)
Crawford et al (2002)[21]	Comparison I[b]: Exposure to A1, A1 + B[f], and A2 β-casein	Not described	NA	Mortality due to neurological disease (including Iceland)[c]	0.795 (0.006)	−0.219 (0.544)
				Mortality due to neurological disease in males (including Iceland)[c]	0.731 (0.016)	−0.379 (0.280)
				Mortality due to neurological disease in females (including Iceland)[c]	0.801 (0.005)	−0.137 (0.706)
				Mortality due to neurological disease (excluding Iceland)[c]	0.855 (0.003)	0.584 (0.099)
				Mortality due to neurological disease in males (excluding Iceland)[c]	0.863 (0.003)	0.584 (0.099)
				Mortality due to neurological disease in females (excluding Iceland)[c]	0.832 (0.005)	0.570 (0.109)

Abbreviations: BCM, β-casomorphin; NA, not applicable.

[a]For all randomized controlled trials, the results include information on the time points when outcomes were measured. No time points are included for case-control studies or ecological studies, as no explicit time point can be defined because of the nature of the study design.

[b]Refers to the comparison investigated in the study. Comparison I: A1 β-casein intake/exposure in comparison with A2 β-casein intake/exposure.

[c]Icelandic milk is known to have lower concentrations of A1 and B β-casein fractions and higher concentrations of A2 β-casein fractions when compared with milk from other Nordic countries. Therefore, this study analyzed correlations with and without the Icelandic population to find differences.

[d]Study gave no further results.

[e]Ecological studies calculated either Pearson's correlation coefficient (when values had a normal distribution) or Spearman's correlation coefficient (when values were not normally distributed). Because of the study design used for ecological studies, no number of participants can be defined.

[f]Some studies included B β-casein in their analysis since it has similar properties to A1 β-casein. However, the concentration of B β-casein in milk is normally very low compared with concentrations of the other β-casein variants.

Table 7 **Studies investigating A1 β-casein and gastrointestinal symptoms**

Randomized controlled trials

Study	Intervention	Control intervention	Sample size	Outcome	Time of outcome measurement[a]	Results		
						Intervention A1, mean ± SD	Control A2, mean ± SD	MD
Crowley et al (2013)[33]	Comparison I[b]: Milk containing A1 β-casein (400 ml/d for 2 wk)	Milk containing A2 β-casein (400 ml/d for 2 wk)	26 (crossover design)	Resolution of constipation	After 2 wk	n = 14 participants (64%)	n = 16 participants (64%)	n = 2
				No. of bowel motions per 2 wk		10.05±5.75	10.56±5.24	0.51
Ho et al (2014)[9]	Comparison I[b]: Milk containing A1 β-casein (750 ml/d for 2 wk). Dosage: A1 β-casein approx. 7.5 g/d	Milk containing A2 β-casein (750 ml/d for 2 wk). Dosage: approx. 7.5 g A2 β-casein daily	41 (crossover design)	Stool consistency (BSS[c])	During the 2-wk intervention period (self-reported)	3.87±0.02	3.56±0.02[d]	0.31
				Stool consistency (BSS[c]) for individuals self-described as milk tolerant	During the 2-wk intervention period (self-reported)	3.82±0.02	3.47±0.02[d]	0.35
				Stool consistency (BSS[c]) for individuals self-described as milk intolerant	During the 2-wk intervention period (self-reported)	4.02±0.04	3.87±0.05	0.15
				Bowel frequency	During the 2-wk intervention period (self-reported)	Narrative results only: "No significant difference between the groups."		
				Digestive discomfort (bloating, abdominal pain, flatus, voiding difficulty)	During the 2-wk intervention period (self-reported)	Narrative results only: "Participants self-described as milk-intolerant had significant higher values of bloating and flatus after A1 beta-casein when compared to A2 beta-casein intake."		
				Fecal calprotectin	After 2 wk[e]	41.6 μg/g[f]	35.8 μg/g[f]	5.8 μg/g
Jianqin et al (2016)[10]	Comparison I[b]: Milk containing A1 and A2 β-casein at a 40:60 ratio (500 ml/d for 14 d)	Milk containing A2 β-casein (500 ml/d for 14 d)	45 (crossover design	SBTT (hours)	After the intervention[e]	(I) 3.62±1.46	(III) 4.02±1.45	0.4
						(II) 3.79±1.89	(IV) 3.90±1.85	0.11
				CTT (hours)	After 2 wk[e]	(I) 35.41±8.68	(III) 28.23±5.50[d]	7.18
						(II) 35.31±6.92	(IV) 29.62±7.41[d]	5.69
				WGTT (hours)	After 2 wk[e]	(I) 39.95±8.45	(III) 33.41±5.68[d]	6.54
						(II) 40.14±6.81	(IV) 34.36±6.90[d]	5.78
				Gastrointestinal inflammation	Between intervention periods	Narrative results only: "When participants switched from milk containing A1 and A2 beta-casein to milk containing only A2 beta-casein, 36.4% showed an improvement of small bowel inflammation and 22.7% an improvement of stomach inflammation. In contrast, when participants switched from milk containing only A2 beta-casein to milk with A1 and A2 beta-casein, 11.1% showed an improvement of small bowel and stomach inflammation."		

(continued)

Table 7 **Continued**

Randomized controlled trials

Study	Intervention	Control intervention	Sample size	Outcome	Time of outcome measurement[a]	Results		
						Intervention A1, mean ± SD	Control A2, mean ± SD	MD
				Stool frequency (no./wk)	Baseline[e]	(I) 7.86±1.98	(III) 7.95±2.3	0.09
						(II) 7.83±1.59	(IV) 7.57±1.95	0.26
					After 2 wk[e]	(I) 11.05±4.21	(III) 7.91±1.15	3.14
						(II) 10.43±3.46	(IV) 7.87±1.91	2.56
				Stool consistency (BSS[c])	Baseline[e]	(I) 4.05±0.65	(III) 4.08±0.46	0.03
						(II) 4.07±0.51	(IV) 4.09±0.67	0.02
					After 2 wk[e]	(I) 4.42±0.74	(III) 4.05±0.25	0.37
						(II) 4.35±1.11	(IV) 4.08±0.61	0.27
				Diarrhea (measured as adverse event)	During the 14-d intervention period (self-reported)	8 events in 5 participants	3 events in 3 participants	

Abbreviations: approx., approximately; BSS, Bristol stool scale; CTT, colon transit time; MD, mean difference; SBTT, small bowel transit time; SD, standard deviation; WGTT, whole gastrointestinal transit time.

[a]For all randomized controlled trials, the results include information on the time points when outcomes were measured. No time points are included for case-control studies or ecological studies, as no explicit time point can be defined because of the nature of the study design.

[b]Refers to the comparison investigated in the study. Comparison I: A1 β-casein intake/exposure in comparison with A2 β-casein intake/exposure.

[c]Scoring defined as follows: 1 = severe constipation; 2 = mild constipation; 3 and 4 = normal; 5 = lacking fiber; 6 = mild diarrhea; 7 = severe diarrhea.

[d]Results were significantly different between intervention and control ($P < 0.05$).

[e]Crossover trials reported results for each period of intervention separately, as follows: (I) participants who received A1 intervention in their first trial period, (II) participants who received A1 intervention in their second trial period, (III) participants who received A2 intervention in their first trial period, and (IV) participants who received A2 intervention in their second trial period.

[f]Results reported without SDs.

Table 8 **Studies investigating A1 β-casein and the attenuation of exercised-induced muscle damage (EIMD)**

Randomized controlled trials

Study	Intervention	Control intervention	Sample size	Outcome	Time of outcome measurement	Results	
						Intervention A1, mean ± SD	Control A2, mean ± SD
Kirk et al (2017)[31]	Comparisons I and III[a] Regular milk (containing A1 and A2 β-casein) (1-time intake of 500 ml)	Comparison I: Milk containing A2 β-casein, or Comparison III: placebo (50 g maltodextrin mixed with water) (1-time intake of 500 ml)	21 (parallel design)	20-m sprint time	After 24 h, 48 h, and 72 h	Sprint time recovered quicker in A2 and regular milk groups (3.3 ± 0.1 m and 3.3 ± 0.3 m) compared with placebo group (3.6 ± 0.3m) after 48 h of EIMD. No significant difference observed after 24 h or 72 h. Therefore, regular milk intake or A2 milk intake reduced decrements in 20-m sprint time (5.1% and 5.2%, respectively) compared with placebo	
				Height of CMJ	After 24 h, 48 h, and 72 h	After 48 h, recovery of CMJ height was quicker in A2 group (33.4 ± 6.6 cm) and regular milk group (33.1 ± 7.1 cm) than in placebo group (29.2 ± 3.6 cm). Therefore, regular milk intake or A2 milk intake reduced decrements in CMJ height (7.2% and 6.3% respectively), compared with placebo	
				Maximal voluntary isometric contraction	After 24 h, 48 h, and 72 h	No different effects between intervention and control groups	
				Muscle soreness	After 24 h, 48 h, and 72 h	No different effects between intervention and control groups	

Abbreviation: CMJ, countermovement jump.

[a]Refers to the comparison investigated in the study. Comparison I: A1 β-casein intake/exposure in comparison with A2 β-casein intake/exposure. Comparison III: A1 β-casein in comparison with no A1 β-casein intake/exposure.

Table 9 **Studies investigating A1 β-casein and other health-related outcomes**

Randomized controlled trials

Study	Intervention	Control intervention	Sample size	Outcome	Time of outcome measurement[a]	Results		
						Intervention A1, mean ± SD	Control A2, mean ± SD	MD
Jianqin et al (2016)[10]	Comparison I[b] Milk containing A1 and A2 β-casein at a 40:60 ratio (500 ml/d for 14 d)	Milk containing A2 β-casein (500 ml/d for 14 d)	45 (crossover design)	Serum CRP	Baseline[c]	(I) 1.0±0.7 mg/L	(III) 0.97±0.58 mg/L	0.03 mg/L
						(II) 1.01±0.98 mg/L	(IV) 1.03±1.03 mg/L	0.02 mg/L
					After 14 d[c]	(I) 1.17±0.64 mg/L	(III) 1.10±0.58 mg/L	0.07 mg/L
						(II) 1.18±1.04 mg/L	(IV) 1.02±1.11 mg/L	0.16 mg/L
				Serum Hb	Baseline[c]	(I) 141.7±17.5 g/L	(III) 136.7±23.2 g/L	5.0 g/L
						(II) 137.5±25.2 g/L	(IV) 142.8±20.1 g/L	5.3 g/L
					After 14 d[c]	(I) 145.1±17 g/L	(III) 143.9±16.4 g/L	1.2 g/L
						(II) 142±18.1 g/L	(IV) 145.5±17.7 g/L	3.5 g/L
				Serum IL-4	Baseline[c]	(I) 11.8±4.2 ng/L	(III) 11.1±3.4 ng/L[d]	0.7 ng/L
						(II) 11.8±3.4 ng/L	(IV) 11.9±4.3 ng/L[d]	0.01 ng/L
					After 14 d[c]	(I) 14.1±5.2 ng/L	(III) 11.0±3.2 ng/L[d]	3.1 ng/L
						(II) 14.1±4.6 ng/L	(IV) 12.0±3.7 ng/L[d]	2.1 ng/L
				Serum IgG	Baseline[c]	(I) 10.3±2.1 g/L	(III) 10.2±1.7 g/L[d]	0.1 g/L
						(II) 10.8±1.8 g/L	(IV) 10.6±2.1 g/L[d]	0.2 g/L
					After 14 d[c]	(I) 11.6±2.3 g/L	(III) 10.6±1.4 g/L[d]	1 g/L
						(II) 12.2±1.7 g/L	(IV) 11.1±1.9 g/L[d]	1.1 g/L
				Serum IgE	Baseline[c]	(I) 61.3±29 IU/mL	III) 63.3±30.1 IU/mL[d]	2.0 IU/mL
						(II) 56.7±31.3 IU/mL	(IV) 58.6±31.2 IU/mL[d]	1.9 IU/mL
					After 14 d[c]	(I) 69.8±38 IU/mL	(III) 66.2±28.9 IU/mL[d]	3.6 IU/mL
						(II) 64.4±34.2 IU/mL	(IV) 60.7±33.3 IU/mL[d]	3.7 IU/mL
				Serum IgG1	Baseline[c]	(I) 29.4±31.3 μg/mL	(III) 31.0±33.1 μg/mL[d]	1.6 μg/mL
						(II) 32.9±27.2 μg/mL	(IV) 33.0±28.3 μg/mL[d]	0.1 μg/mL
					After 14 d[c]	(I) 37.4±39.1 μg/mL	(III) 30.3±32.9 μg/mL[d]	7.1 μg/mL
						(II) 37.4±31.4 μg/mL	(IV) 28.5±28.5 μg/mL[d]	8.9 μg/mL
				Fecal acetic acid	Baseline[c]	(I) 0.42±0.15%	(III) 0.4±0.14%[d]	0.02%
						(II) 0.39±0.17%	(IV) 0.39±0.19%[d]	0
					After 14 d[c]	(I) 0.42±0.15%	(III) 0.46±0.11%[d]	0.04%
						(II) 0.36±0.11%	(IV) 0.46±0.19%[d]	0.1%
				Fecal butanoic acid	Baseline[c]	(I) 0.17±0.07%	(III) 0.16±0.07%[d]	0.01%
						(II) 0.17±0.08%	(IV) 0.17±0.09%[d]	0
					After 14 d[c]	(I) 0.16±0.07%	(III) 0.20±0.08%[d]	0.04%
						(II) 0.16±0.05%	(IV) 0.23±0.09%[d]	0.07%
				Fecal total SCFAs	Baseline[c]	(I) 0.76±0.24%	(III) 0.72±0.24%[d]	0.04%
						(II) 0.74±0.28%	(IV) 0.73±0.33%[d]	0.01%
					After 14 d[c]	(I) 0.76±0.24%	(III) 0.83±0.19%[d]	0.07%
						(II) 0.69±0.18%	(IV) 0.88±0.33%[d]	0.19%

(continued)

Table 9 **Continued**

Randomized controlled trials

Study	Intervention	Control intervention	Sample size	Outcome	Time of outcome measurement[a]	Results		
						Intervention A1, mean ± SD	Control A2, mean ± SD	MD
Jianqin et al (2016)[10]; Deth et al (2016)[35]				Plasma BCM-7[c]	Baseline[c,e]	(I) 0.63 ng/mL (II) 0.65 ng/mL	(III) 0.71 ng/mL[d] (IV) 0.56 ng/mL[d]	0.08 ng/mL 0.09 ng/mL
					After 14 d[c,e]	(I) 0.976 ng/mL (II) 0.87 ng/mL	(III) 0.71 ng/mL[d] (IV) 0.73 ng/mL[d]	0.27 ng/mL 0.14 ng/mL
				Plasma gluthatione	Mean change from baseline	1.99±0.5 nmol/mL	4.01±0.61 nmol/mL[d]	2.02 nmol/mL
Venn et al (2006)[34]	Comparison I[b] Milk and cheese (containing both A1 and A2 β-casein). 500 ml milk and 28 g cheese daily for 4.5 wk. Dosage: A1 β-casein 2.5 g/d and A2 β-casein 3.6 g/d	Milk and cheese containing A2 β-casein. Daily 500 ml milk and 28 g cheese for 4.5 wk. Dosage: A1 β-casein 0.4 g/d and A2 β-casein 5.8 g/d	55 (crossover design)	Plasma lipid LA	Baseline	13.9±4.67 mol%	13.9±4.67 mol%	0
					After 4.5 wk	13.8±3.9 mol%	13.8±3.87 mol%	0
				Plasma lipid ALA	Baseline	1.26±0.43 mol%	1.26±0.43 mol%	0
					After 4.5 wk	1.3±0.39 mol%	1.2±0.41 mol%[d]	0.1 mol%
				Plasma lipid AA	Baseline	0.97±0.34 mol%	0.97±0.34 mol%	0
					After 4.5 wk	1.02±0.31 mol%	1.09±0.38 mol%	0.07 mol%
				Plasma lipid DHA	Baseline	0.61±0.53 mol%	0.61±0.53 mol%	0
					After 4.5 wk	0.73±0.86 mol%	0.61±0.42 mol%	1.2 mol%

Ecological studies

Study	Intervention/ exposure	Calculated dosage	Sample size	Outcome	Results	
					Exposure A1, *r*(p)[f]	Exposure A2, *r*(p)
Elliott & Laugesen (2001)[22]	Comparison II[b] Exposure to A1 β-casein	Not described	NA	Incidence of multiple myeloma in males	0.62 (< 0.01)	NA[g]
				Incidence of multiple myeloma in females	0.71 (< 0.001)	NA[g]
				Prevalence of asthma	No correlation	NA[g]

Abbreviations: AA, arachidonic acid; ALA, α-linolenic acid; BCM-7, β-casomorphin-7; CRP, C-reactive protein; DHA, docosahexaenoic acid; EIMD, exercise-induced muscle damage; Hb, hemoglobin; Ig, immunoglobulin; IL, interleukin; IU/mL, international unit per milliliter; LA, linoleic acid; MD, mean difference; NA, not applicable; SCFA, small-chain fatty acid.

[a]For all randomized controlled trials, the results include information on the time points when outcomes were measured. No time points are included for case-control studies or ecological studies, as no explicit time point can be defined because of the nature of the study design.

[b]Refers to the comparison that was investigated in the study. Comparison I: A1 β-casein intake/exposure in comparison with A2 β-casein intake/exposure. Comparison II: different exposure/intake dosages of A1 β-casein.

[c]Crossover trials reported results for each period of intervention separately: (I) participants who received A1 intervention during their first trial period; (II) participants who received A1 intervention during their second trial period; (III) participants who received A2 intervention during their first trial period; and (IV) participants who received A2 intervention during their second trial period.

[d]Results were significantly different between intervention and control ($P < 0.05$).

[e]Results were reported without SDs.

[f]Ecological studies calculated either Pearsons's correlation coefficient (when values had a normal distribution) or Spearman's correlation coefficient (when values were not normally distributed). Because of the nature of the study design used for ecological studies, no number of participants can be defined.

[g]Study did not analyze the correlation with A2 β-casein, only with A1 β-casein.

females [$r(p) = 0.71$ ($P < 0.001$)] (data from patent register), but no correlation with asthma was identified.[22]

GRADE assessment

Table 10 shows the results of the GRADE assessment for at least 1 outcome of each outcome class, including incidence of type 1 diabetes, mortality due to cardiovascular or neurological disease, and gastrointestinal conditions (including some intermediate markers). When a study gave more than 1 result per outcome, ie, outcome data were grouped by age or by region, the results were summarized into 1 composite measure (ie, mean of all values was measured). The certainty of the evidence according to GRADE was judged as very low for most outcomes. This was mainly due to the fact that many outcomes were investigated in ecological studies, in which the certainty of the evidence is lower than in RCTs due to the inherent limitations of this study design. The certainty of evidence for risk of diabetes (assessed with plasma insulin concentration) was rated as low, and the certainty for outcomes of gastrointestinal conditions (including score on the Bristol stool scale, total gastrointestinal transit time, and resolution of constipation) was rated as moderate. Most outcomes reported in RCTs were downgraded for imprecision, because of the small number of participants and/or events, and for indirectness, because many studies investigated only intermediate markers of various diseases.

DISCUSSION

Principal findings

The present systematic review assessed the available evidence on the impact of A1 β-casein on health-related outcomes in humans. Currently, the results of the studies investigating A1 β-casein are inconclusive. Nevertheless, ongoing studies were identified in study registries, and new studies have been registered during the past few months. Therefore, the evidence will likely continue to change while new studies are being performed and their results published. For example, 1 of the ongoing studies identified (Clarke[24]), which investigated gastrointestinal effects after intake of conventional milk vs milk containing A2 β-casein in 600 Chinese adults with self-reported lactose intolerance, was recently published.[45] The results showed that gastrointestinal conditions (borborygmus, flatulence, bloating, abdominal pain, stool frequency, and stool consistency) were significantly less frequent in participants consuming milk with A2 β-casein.[45] Results from this study were not included in the current systematic review because the study was published after the literature search was updated. This highlights the emerging nature of this topic and the importance of updating this systematic review in the near future.

The included RCTs reported only on intermediate markers of various diseases and on gastrointestinal conditions after the intake of A1 or A2 milk. Those results were mostly inconclusive, mainly owing to a variety of results that could not be pooled and to the many ongoing trials with pending results. In addition, some studies reported significant differences between A1 and A2 β-casein intake when intermediate markers were assessed, but the differences were not clinically relevant, eg, differences in the Bristol stool scale, stool frequency, and exercise-induced muscle damage. Only the ecological studies assessed relevant outcomes and showed significant correlations between exposure to A1 β-casein and type 1 diabetes, cardiovascular disease, neurological disease, and multiple myeloma. Ecological studies usually analyze the correlation between a risk factor (eg, dietary exposure to A1 β-casein) and the presence of a disease at the population level. This means that the unit of analysis is not an individual, but a group of participants. Therefore, ecological studies must be interpreted with caution, since they only allow a correlation between A1 β-casein exposure and an outcome to be described on a population level; hence, no causal inferences can be established on the basis of such results. Overall, most interventional studies had a follow-up time of 12 weeks or less and included a small number of participants, leading to imprecise results. In addition, most outcomes assessed were intermediate markers rather than relevant outcomes. Another consideration when interpreting the results of this review is that the included studies measured only short-term effects of the interventions. According to GRADE, the certainty of the evidence was rated as moderate for outcomes related to digestive symptoms and as low to very low for all other outcomes assessed.

Furthermore, it is important to mention that industry funding was present in at least 12 included studies, and therefore the possibility that economic interests could have influenced the study results cannot be excluded. At least 5 published studies received financial support from the A2 Milk Company Limited (Auckland, New Zealand)[9,10,32,35,41,42] and, with the exception of 1 study,[32] the results of these studies favored the interests of the funder.

Strength and weaknesses of the review

Included studies were extremely heterogeneous in terms of study design, type of intervention, control group characteristics (doses and time of intake or exposure),

Table 10 **GRADE evidence profile table: results of GRADE assessment**

Quality assessment							No. of participants		Effect		Quality[a]
No. of studies	Study design	Risk of bias	Inconsistency	Indirectness	Imprecision	Other considerations	A1	A2	Relative (95%CI)	Absolute (95%CI)	
Incidence of T1DM											
1	Case-control	Serious[b]	Not serious	Serious[c]	Not serious	None	Study found no significant difference in the incidence of T1DM between cases and controls				⊕○○○ Very low
Incidence of T1DM											
6	Ecological	Serious[b]	Not serious	Not serious	Not serious	None	Studies found a significant positive correlation between A1 β-casein exposure and incidence of T1DM				⊕○○○ Very low
Risk of diabetes (assessed with plasma insulin concentration)											
1	RCT	Not serious	Not serious	Serious[d]	Serious[e]	None	15	15	–	MD 0.2 mU/L lower(1.13 lower to 0.73 higher)	⊕⊕○○ Low
Mortality due to cardiovascular disease											
3	Ecological	Serious[b]	Not serious	Not serious	Not serious	None	Studies found a significant positive correlation between A1 β-casein exposure and mortality due to cardiovascular disease				⊕○○○ Very low
Cardiovascular disease (assessed with plasma total cholesterol concentration)											
2	RCT	Not serious	Serious[d,f]	Serious[d]	Serious[e]	None	70	70	–	MD 0.18 mmol/L lower(0.45 lower to 0.08 higher)	⊕○○○ Very low
Cardiovascular disease (assessed with LDL-C concentration)											
1	Case-control	Serious[b]	Not serious	Serious[d]	Serious[e]	None	Study found higher LDL-C concentrations in cases after the intake of A1 milk (129.29 $\pm$ 16.67 mg/dL) when compared with controls who consumed A2 milk (120.15 $\pm$ 8.47 mg/dL)				⊕○○○ Very low
Mortality due to neurological disease											
1	Ecological	Serious[b]	Not serious	Not serious	Not serious	None	Study found a significant positive correlation between A1 β-casein exposure and mortality due to neurological disease				⊕○○○ Very low
Gastrointestinal symptoms: stool consistency (assessed with the Bristol stool scale)											
2	RCT	Not serious	Not serious	Not serious	Serious[e]	None	58	59	–	MD 0.31 higher(0.25 higher to 0.37 higher)	⊕⊕⊕○ Moderate
Gastrointestinal symptoms: WGTT											
1	RCT	Not serious	Not serious	Not serious	Serious[e]	None	22	18	–	MD 6.15 h higher(3.09 higher to 9.21 higher)	⊕⊕⊕○ Moderate

(continued)

Table 10 **Continued**

Quality assessment							No. of participants		Effect		Quality[a]
No. of studies	Study design	Risk of bias	Inconsistency	Indirectness	Imprecision	Other considerations	A1	A2	Relative (95%CI)	Absolute (95%CI)	
Gastrointestinal symptoms: resolution of constipation											
1	RCT	Not serious	Not serious	Not serious	Serious[e]	None	Resolution of constipation achieved in 14 participants in A1 milk group and in 16 participants in A2 milk group				⊕⊕⊕◯ Moderate
Attenuation of EIMD (assessed with visual analogue scale [muscle soreness], height of CMJ, maximal voluntary isometric contraction, and time of 20-m sprint)											
1	RCT	Not serious	Not serious	Serious[c]	Serious[e]	None	Study found quicker recovery of muscle damage (measured by sprint time and height of CMJ) in participants who received A1 or A2 milk compared with participants who received placebo				⊕⊕◯◯ Low

Abbreviations: CMJ, countermovement jump; EIMD, exercise-induced muscle damage; LDL-C, low-density lipoprotein cholesterol; MD, mean difference; mU/L, milliunit per liter; RCT, randomized controlled trial; T1DM, type 1 diabetes mellitus; WGTT, whole gastrointestinal transit time.

[a]Quality of the evidence according to GRADE is defined as follows: high, high confidence that the true effect lies close to that of the estimate of the effect; moderate, moderate confidence in the effect estimate, ie, the true effect is likely to be close to the estimate of the effect, but there is a possibility that it is substantially different; low, limited confidence in the effect estimate, ie, the true effect may be substantially different from the estimate of the effect; very low, very little confidence in the effect estimate, ie, the true effect is likely to be substantially different from the estimate of the effect.

[b]Downgraded due to serious risk of bias or study design (in ecological studies, outcomes are evaluated with aggregated population data instead of individual data).

[c]Downgraded due to indirectness (study population).

[d]Downgraded due to indirectness: outcomes are intermediate markers of the outcome of interest.

[e]Downgraded due to imprecision: small study population.

[f]Downgraded due to inconsistency: high statistical heterogeneity.

reported outcomes, and included population; therefore, such data cannot be pooled. Additionally, poor reporting of the included studies often prevented a thorough interpretation of the data. One of the challenges during this systematic review was the assessment of all the different outcomes analyzed in the studies. Most intermediate markers (eg, gastrointestinal conditions and some blood parameters, which normally show a short response time) were analyzed in RCTs, while relevant outcomes were investigated mostly in ecological studies. The results have been separated by study design (and therefore separated indirectly by type of outcome) to present results as clearly and transparently as possible. Considering that new studies comparing A1 and A2 β-casein intake are currently under way and that new results relevant to this systematic review will emerge in the coming months, further systematic reviews focusing on particular conditions (such as digestive symptoms) could be performed individually.

The literature search for the current review also identified a substantial number of studies that evaluated casein against substances such as soy protein, other proteins (eg, whey), or isoflavones. These studies provided no information about the type of casein analyzed and were therefore not included in the review.

Eight unpublished studies were identified in study registries such as ClinicalTrials.gov, all of which were RCTs that compared the effect of A1 milk vs A2 milk on various outcomes, including gastrointestinal conditions and intermediate markers of asthma and autism. Approximately half of these studies have been completed, and, as previously mentioned, the publication of further results in the near future is likely.

The systematic literature search for this review identified 3 studies in the patent register. These studies include data that has not undergone peer review. In addition, a large number of animal and experimental studies investigating A1 vs A2 β-casein interventions were identified. Evidence mapping of these studies has been performed, and publication of the results is planned.

Other reviews

There have been previous initiatives to evaluate the available evidence on the health implications of A1 and A2 milk intake. B. Swinburn[48] (on behalf of the New Zealand Food Safety Authority) and the European Food Safety Authority[8] conducted systematic reviews investigating different health effects of A1 and A2 β-casein in 2004 and 2009, respectively. More recently, Brooke-Taylor et al[47] conducted a systematic review of in vitro and in vivo studies (all species) to examine the gastrointestinal effects of A1 compared with A2 β-casein. They found that A2 β-casein had favorable gastrointestinal effects in rodents and humans compare with A1 β-casein.[47] Chia et al[48] also published a review highlighting the theoretical evidence of A1 β-casein and its β-casomorphin-7 derivative as a causal factor of type 1 diabetes.

The investigation of intake of A1 and A2 β-caseins in milk is an emerging topic in nutrition. This systematic review was conducted to include new evidence in humans across any health-related outcome. A number of new studies that were not included in the systematic reviews performed by Swinburn[46] and the European Food Safety Authority[8] were identified, and many ongoing studies that will provide data for future updates of this review were found. Similar to this systematic review, both reviews concluded that the evidence was not sufficient to reveal differences in health-related effects between the 2 milk types.[8,46]

CONCLUSION

In this systematic review, evidence from clinical trials and epidemiological studies conducted in humans and published prior to October 2017 provides moderate certainty that A2 β-casein compared with A1 β-casein provides benefits to digestive health, but low or very low certainty for other health benefits.

To more definitively evaluate further benefits and potential harms of A1 milk, including more-rare adverse health outcomes, high-quality RCTs that include more participants, use sufficiently long intervention and follow-up periods, and measure relevant outcomes are needed. Such studies are essential to provide reliable information on the health implications of A1 and A2 milk to both decision makers and the general public and to inform public health recommendations in the future.

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Supporting Information

The following Supporting Information is available through the online version of this article at the publisher's website:

Appendix S1 **PRISMA 2009 checklist**

Appendix S2 **Search strategy for Ovid MEDLINE**

Appendix S3 **List of outcomes included in systematic review and classification by whether these outcomes were GRADEd**

REFERENCES

1. Haug AH, Høstmark AT, Harstad, OM. Bovine milk in human nutrition—a review. *Lipids Health Dis.* 2007;6:25. doi:10.1186/1476-511X-6-25
2. Pereira PC. Milk nutritional composition and its role in human health. *Nutrition.* 2014;30:619–627.
3. Aune D, Navarro Rosenblatt DA, Chan DS, et al. Dairy products, calcium, and prostate cancer risk: a systematic review and meta-analysis of cohort studies. *Am J Clin Nutr.* 2015;101:87–117.
4. Patterson E, Larsson SC, Wolk A, et al. Association between dairy food consumption and risk of myocardial infarction in women differs by type of dairy food. *J Nutr.* 2013;143:74–79.
5. Hu D, Huang J, Wang Y, et al. Dairy foods and risk of stroke: a meta-analysis of prospective cohort studies. *Nutr Metab Cardiovasc Dis.* 2014;24:460–469.
6. Aune D, Norat T, Romundstad P, et al. Dairy products and the risk of type 2 diabetes: a systematic review and dose-response meta-analysis of cohort studies. *Am J Clin Nutr.* 2013;98:1066–1083.
7. Truswell AS. The A2 milk case: a critical review. *Eur J Clin Nutr.* 2005;59:623–631.
8. De Noni I, FitzGerald RJ, Korhonen HJT, et al; DATEX Working Group on β-Casomorphins. *Potential Health Impact of β-Casomorphins and Related Peptides: Report of the DATEX Working Group on β-Casomorphins.* Parma, Italy: European Food Safety Authority; 2009.
9. Ho S, Woodford K, Kukuljan S, et al. Comparative effects of A1 versus A2 beta-casein on gastrointestinal measures: a blinded randomised cross-over pilot study. *Eur J Clin Nutr.* 2014;68:994–1000.
10. Jianqin S, Leiming X, Lu X, et al. Effects of milk containing only A2 beta casein versus milk containing both A1 and A2 beta casein proteins on gastrointestinal physiology, symptoms of discomfort, and cognitive behavior of people with self-reported intolerance to traditional cows' milk [published correction appears in *Nutr J.* 2016;15:45]. *Nutr J.* 2016;15:35. doi:/10.1186/s12937-016-0147-z.
11. Higgins JPT, Green S, eds. *Cochrane Handbook for Systematic Reviews of Interventions.* Version 5.1.0. The Cochrane Collaboration; 2011. www.cochrane-handbook.org. Updated March 2011.
12. Moher D, Liberati A, Tetzlaff J, et al. Preferred reporting items for systematic reviews and meta-analyses: the PRISMA statement. *Ann Intern Med.* 2009;151:264–269.
13. Sackett DL, Rosenberg WM, Gray JA, et al. Evidence based medicine: what it is and what it isn't. *BMJ.* 1996;312:71–72.
14. *Covidence* [systematic review software]. Melbourne, Australia: Covidence; 2015.
15. Higgins JPT, Altman DG, Sterne JAC, et al, eds. Asessing risk of bias in included studies; chap 8. In: *Cochrane Handbook for Systematic Reviews of Interventions.* Version 5.1.0. The Cochrane Collaboration; 2011. www.cochrane-handbook.org. Updated March 2011.
16. Sterne JA, Hernán MA, Reeves BC, et al. ROBINS-I: a tool for assessing risk of bias in non-randomised studies of interventions. *BMJ.* 2016;355:i4919. doi:10.1136/bmj.i4919
17. National Institute for Health and Care Excellence. *Methods for the Development of NICE Public Health Guidance.* 3rd edn. London, England: National Institute for Health and Care Excellence; 2012. https://www.nice.org.uk/process/pmg4/chapter/appendix-g-quality-appraisal-checklist-quantitative-studies-reporting-correlations-and. Published September 2012. Accessed September 2016.
18. Miake-Lye IM, Hempel S, Shanman R, et al. What is an evidence map? A systematic review of published evidence maps and their definitions, methods, and products. *Syst Rev.* 2016;5:28. doi:10.1186/s13643-016-0204-x
19. Higgins JPT, Deeks JJ, Altman DG, et al, eds. Special topics in statistics; chap 16. In: *Cochrane Handbook for Systematic Reviews of Interventions.* Version 5.1.0. The Cochrane Collaboration; 2011. www.cochrane-handbook.org. Updated March 2011.
20. Langer G, Meerpohl JJ, Perleth M, et al. GRADE guidelines: 1. Introduction—GRADE evidence profiles and summary of findings tables [in German]. *Z Evid Fortbild Qual Gesundhwes.* 2012;106:357–368.
21. Crawford RA, Boland MJ, Norris CS, Hill JP, Fenwick RM, inventors; New Zealand Dairy Board, assignee. Milk containing β-casein with proline at position 67 does not aggravate neurological disorders. WIPO patent WO/2002/019832. March 14, 2002.
22. Elliott RB, Laugesen BM, inventors; New Zealand Milk Institute Limited, assignee. Prophylactic dietary supplement based on milk. WIPO patent WO/2001/000047A1. January 4, 2001.
23. Australian New Zealand Clinical Trials Registry. Sydney, New South Wales: NHMRC Clinical Trials Centre, University of Sydney; 2005. Identifier ACTRN12616001694404. A2 milk for gut comfort (principal investigator, Cameron-Smith D). Registered December 9, 2016. https://www.anzctr.org.au/Trial/Registration/TrialReview.aspx?id=371676. Accessed November 1, 2017.
24. ClinicalTrials.gov. Bethesda, MD: National Library of Medicine. Identifier NCT02878876. Incidence of lactose intolerance among self-reported lactose intolerant people (study director, Clarke AJ). Posted August 25, 2016. https://clinicaltrials.gov/ct2/show/NCT02878876. Accessed November 1, 2017.
25. Current Controlled Trials. London, England: BioMed Central. ISRCTN 29968077, Effects of A2 milk (compared to regular milk) on asthma symptoms, inflammation and exercise performance in athletes with asthma-related conditions (primary contact, Dickinson J). Updated August 22, 2017. http://www.isrctn.com/ISRCTN29968077?q=heart&filters=conditionCategory:Respiratory&sort=&offset=18&totalResults=177&page=1&pageSize=20&searchType=basic-search. Accessed November 1, 2017.
26. Australian New Zealand Clinical Trials Registry. Sydney, New South Wales: NHMRC Clinical Trials Centre, University of Sydney; 2005. Identifier ACTRN12617000403336. Effects of A1 versus A2 milk on cardiometabolic risk in overweight and obese Australians (principal investigator, Pal S). Registered March 17, 2017. https://www.anzctr.org.au/Trial/Registration/TrialReview.aspx?id=369412. Accessed November 1, 2017.
27. ClinicalTrials.gov. Bethesda, MD: National Library of Medicine. Identifier NCT02911194. A2 milk for autism and attention-deficit hyperactivity disorder (ADHD) (A2MT) (principal investigator, Lodge JK). Posted September 22, 2016. https://clinicaltrials.gov/ct2/show/NCT02911194. Accessed November 1, 2017.
28. ClinicalTrials.gov. Bethesda, MD: National Library of Medicine. Identifier NCT03060395. Effects of A2 milk on gastrointestinal function in non-lactose milk intolerance (principal investigator, Claus SP). Posted February 23, 2017. https://clinicaltrials.gov/ct2/show/record/NCT03060395. Accessed November 1, 2017.
29. ClinicalTrials.gov. Bethesda, MD: National Library of Medicine. Identifier NCT03081936. Comparative A1 vs A2 formula and breast feeding on alimentation and gastrointestinal digestion for the infant (study director, Zhang C). Posted March 17, 2017. https://clinicaltrials.gov/ct2/show/NCT03081936. Accessed November 1, 2017.
30. ClinicalTrials.gov. Bethesda, MD: National Library of Medicine. Identifier NCT03081845. A1 versus A2 milk on the gastrointestinal physiology, symptoms and cognitive behaviour for the preschool children (study director, Zhang L). Posted March 16, 2017. https://clinicaltrials.gov/ct2/show/NCT03081845. Accesssed November 1, 2017.
31. Kirk B, Mitchell J, Jackson M, et al. A2 milk enhances dynamic muscle function following repeated sprint exercise, a possible ergogenic aid for A1-protein intolerant athletes? *Nutrients.* 2017;9:94. doi:10.3390/nu9020094
32. Chin-Dusting J, Shennan J, Jones E, et al. Effect of dietary supplementation with β-casein A1 or A2 on markers of disease development in individuals at high risk of cardiovascular disease. *Br J Nutr.* 2006;95:136–144.
33. Crowley ET, Williams LT, Roberts TK, et al. Does milk cause constipation? A cross-over dietary trial. *Nutrients.* 2013;5:253–266.
34. Venn BJ, Skeaff CM, Brown R, et al. A comparison of the effects of A1 and A2 β-casein protein variants on blood cholesterol concentrations in New Zealand adults. *Atherosclerosis.* 2006;188:175–178.
35. Deth R, Clarke A, Ni J, Trivedi M. Clinical evaluation of glutathione concentrations after consumption of milk containing different subtypes of β-casein: results from a randomized, cross-over clinical trial. *Nutr J.* 2016;15:82. doi:10.1186/s12937-016-0201-x
36. Laknori O, Rexha T, Sulaj Leka F, et al. The ecological distribution of bovine races and its correlation with low density lipoprotein oxidation in Albanian populations. *Natura Montenegrina.* 2010;9:601–606.

37. Thorsdottir I, Birgisdottir BE, Johannsdottir IM, et al. Different β-casein fractions in Icelandic versus Scandinavian cow's milk may influence diabetogenicity of cow's milk in infancy and explain low incidence of insulin-dependent diabetes mellitus in Iceland. *Pediatrics*. 2000;106:719–724.
38. Birgisdottir BE, Hill JP, Thorsson AV, Thorsdottir I. Lower consumption of cow milk protein A1 β-casein at 2 years of age, rather than consumption among 11- to 14-year-old adolescents, may explain the lower incidence of type 1 diabetes in Iceland than in Scandinavia. *Ann Nutr Metab*. 2006;50:177–183.
39. Casu A, Fadda M, Bottazzo GF, et al. Type 1 diabetes and cow milk in Sardinia: casein variant consumption. *Diabetes* 2001;50(suppl 2):A209–A210.
40. Elliott RB, Harris DP, Hill JP, et al. Type I (insulin-dependent) diabetes mellitus and cow milk: casein variant consumption. *Diabetologia*. 1999;42:292–296.
41. Laugesen M, Elliott R. Ischaemic heart disease, type 1 diabetes, and cow milk A1 beta-casein. *N Z Med J*. 2003;116:U295.
42. McLachlan CNS. β-casein A1, ischaemic heart disease mortality, and other illnesses. *Med Hypotheses*. 2001;56:262–272.
43. Wasmuth HE, Rosenbauer J, Elliott RB, et al. β-Casein A1 consumption and incidence of type 1 diabetes in Germany [abstract]. *Diabetologia*. 1999;42(suppl 1):A88.
44. Understanding blood pressure readings. American Heart Association website. https://www.heart.org/en/health-topics/high-blood-pressure/understanding-blood-pressure-readings Updated November 30, 2017. Accessed May 14, 2018.
45. He M, Sun J, Jiang ZQ, et al. Effects of cow's milk beta-casein variants on symptoms of milk intolerance in Chinese adults: a multicentre, randomised controlled study. *Nutr J*. 2017;16. doi:10.1186/s12937-017-0275-0
46. Swinburn B. Beta-casein A1 und A2 in milk and human health: report to New Zealand Food Safety Authority. https://pdfs.semanticscholar.org/459b/20c652cb3a98f2945048aa254c7367e31046.pdf. Revised July 13, 2004. Accessed November 21, 2018.
47. Brooke-Taylor S, Dwyer K, Woodford K, et al. Systematic review of the gastrointestinal effects of A1 compared with A2 β-casein. *Adv Nutr*. 2017;8:739–748.
48. Chia JSJ, McRae JL, Kukuljan S, et al. A1 beta-casein milk protein and other environmental pre-disposing factors for type 1 diabetes. *Nutr Diabetes*. 2017;7:e274. doi:10.1038/nutd.2017.16