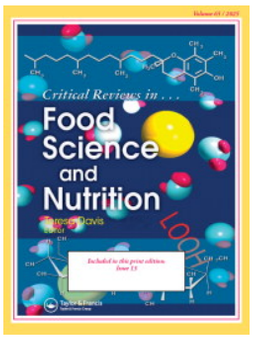

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REVIEW ARTICLE

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Cottage cheese, a relatively underexplored cultured dairy product with potential health benefits?

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ABSTRACT

Cottage cheese (CC) is a member of the "fresh cheese" family of cheeses and is widely consumed due to its culinary versatility and some perceived health benefits. However, the evidence of direct health effects of CC is not well established. This review describes the production and nutritional characteristics of CC, before exploring the evidence of health effects from human intervention, *in vitro*, and *in vivo* models. Despite widespread consumption and advocated health benefits, there is a dearth of evidence pertaining to the health effects of CC from high-quality human randomized controlled trials. To date, a limited number of human intervention models with CC have explored nutrient bioavailability, metabolic health, and appetite regulation, in small, niche study populations. Findings with *in vitro* and *in vivo* models suggest that CC may be an efficacious vehicle for bioactive compounds. In conclusion, CC is a cultured dairy product that could impose a myriad of benefits across health outcomes including cardiometabolic, gastrointestinal, body composition, appetite regulation, and nutrient status. However, there is a need for high-quality human randomized controlled trials to develop a substantiated evidence base relating to the full potential of CC in human health.

KEYWORDS

Cottage cheese; cheese; dairy; protein; health

Introduction

Cottage cheese (CC) is a member of the "fresh cheese" family of cheeses, i.e., cheeses so named because of being ready for consumption without the need for a period of ripening that can range from weeks to years depending on variety. A critical step in the manufacture of all cheese varieties involves coagulation of the milk. In the manufacture of fresh cheeses, this is achieved through acidification, acidification with a small amount of rennet, or a combination of acid and heat. Fresh cheeses are considered the oldest of cheese types as their manufacturing technology is relatively simple and may have evolved naturally arising from the "souring" (lactic acid fermentation) of stored milk (Kindstedt 2012). The origin of CC is not documented, but as its name implies, it likely emerged as a cheese made from surplus milk produced on very small land holdings traditionally associated with a cottage. However, large scale commercial production of CC began in the USA around 1916 (Reidy and Hedrick 1971).

CC is a popular cheese variety, consumed across the globe because of its culinary versatility. This may be at least partially due to its mild flavor (Bodyfelt and Potter 2009), enabling CC to be integrated into a number of dishes, including both savory and sweet. CC is consumed widely in many markets including North America, Europe, Australia, and New Zealand. It is estimated that CC accounts for ~5% of global cheese production (Lucey 2022) with a global market value of US$91 billion in 2022, with an expected compound annual growth rate of ~5.8% in the period 2023–2030 (GII 2023).

The popularity of CC is also driven by advocation amongst the fitness and health-conscious community due to perceived health benefits relating to facilitating muscle mass augmentation and weight loss. However, the scientific basis for some of these health benefits is not always clear. The aim of the present review is to describe CC production, its nutritional composition, and the evidence that exists concerning direct health effects from *in vitro*, *in vivo*, and human intervention models. We will then bring the review to a conclusion by discussing the current evidence base and suggesting directions for future research.

Cottage cheese production

CC is manufactured from pasteurized skim milk or reconstituted low-heat skim milk powder (Lucey 2022; Farkye 2004).

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Use of pasteurization is important to ensure the safety of the product, which is consumed fresh. It is important to note that the more intense heat treatments often applied to other fresh cheeses is to be avoided as it leads to high levels of whey denaturation, which can lead to soft body defects (Lucey 2022). The total solids (TS) content of the milk is often adjusted to the range 10–13% through the addition of nonfat skim milk powder, milk ultrafiltrate retentate, and milk protein concentrates (Lucey 2022; Patel, Sonia, and Agarwal 2014). However, the cost-benefit of this must be assessed by the manufacturer as the additional cheese yield advantage obtained by increasing TS is offset by the ingredient costs as TS increases. Further, TS >10.5% increases the buffering capacity of the milk, increasing the time taken for the milk pH to reduce to 4.6, the isoelectric point of caseins at which point coagulation occurs and thus, slows down the manufacturing process (Farkye 2004). The length of time taken for the milk gel to solidify prior to cutting the curd can vary from 5 to 16 h and thus, the "set time" is often used to distinguish different types of CC, i.e., short- medium- and long- set times. The set time is influenced by the acidification process and incubation temperature. Traditionally, CC was manufactured using non-gas producing *Lactococcus lactis subsp. lactis* and *L. lactis subsp. cremoris* strains, but direct acidification can also be used (Farkye 2004). The starter culture addition level and incubation temperatures range from 1 to 5% and 21 to 33 °C, respectively, depending on the target set times, i.e., short-, medium-, long. Low CO_2 producing citrate positive strains of *L. lactis subsp. lactis biovar. diacetylactis* or *Leuconostoc* strains are sometimes added to produce diacetyl, an important flavor compound in CC. However, as the CO_2 produced as part of this process has the potential to result in curd defects and, as much of the diacetyl produced is lost in the whey, diacetyl is now routinely added to the cheese during the creaming process at the end of manufacture (Farkye 2004). Exopolysaccharide producing starter cultures are also used in CC manufacture as a means of increasing cheese yield (van Ven Tempel and Carlson 2014). Use of direct acidification is achieved by initially acidifying the milk to pH ~5.3 at a low temperature (2–12 °C) by direct addition of an acid followed by heating the milk to 32 °C, and addition of the acidogen, glucono-δ-lactone (GDL), which facilitates the acidification of the curd to pH 4.75–4.6 (depending on addition of rennet). The benefit of direct acidification is that it facilitates greater control over the process as it is not dependent on the activity of starter cultures. Hydrolysis of GDL results in the formation of gluconic acid within the milk matrix and the amount of GDL required to achieve a particular pH can be accurately determined, thus adding even greater control to the process. In addition, gluconic acid has a tangy taste suitable for CC but is less sour than other acids. Rennet is typically added, at a low level during the commercial production of CC. Addition of rennet helps to form a stronger gel and facilitates cutting of the curd at a high pH (4.75), which reduces the overall manufacturing time. Once the desired pH and curd strength is achieved, the gel is cut into particles of specific size and the curd/whey mixture is then allowed to rest for a period of time. Cutting of the curd is an important step in the manufacture of CC as it dictates the final structure of the cheese, which involves the curd particles. Following the rest period, the curd/whey mix is gently stirred, and the temperature is slowly increased in a process referred to as "cooking." The rate of temperature increases, and the final temperature depends on the variety of CC and the starter cultures used, i.e., the heat treatment of the curd during the cooking process inactivates the starter culture and thus prevents excessive acid production, but it usually takes 1–3 h with a final cook temperature in the range 47–56 °C. After cooking, the whey is drained, and the curds are washed and cooled by adding water to achieve a final temperature of 2–5 °C. The cooling achieved during the washing process helps to prevent further acid development and, thus, further syneresis of the curd. It also removes some of the residual lactose from the curd, which is important from a nutritional perspective. The final step in CC manufacture involves mixing the curd particles with a "dressing" that can contain pasteurized cream, salt, and sometimes flavorings including diacetyl and herbs, spices and condiments depending on the variety/brand of CC. The dressing contributes to the final flavor, texture and fat content of the cheese, increases the cheese to pH ~5.1 and ensures the composition of the final product meets the regulatory standards (Lucey 2022; Farkye 2004).

Nutritional composition of cottage cheese

CC (per 100 g) has a high-water content (~79 g) and moderate protein content (present as casein and whey (11–13.8 g) relative to other cheeses (Holland, Unwin, and Buss 1989; Weaver 2021; Thorning et al. 2017). However, the fact that portion sizes for CC tend to be larger than for other cheeses means that it can be a good source of protein. Furthermore, as is the case for cheese in general, the fermentation process results in the progressive breakdown of casein to peptides and free amino acids meaning that considerable 'pre-digestion' has occurred even before consumption takes place (McSweeney 2004), facilitating more rapid digestion and absorption in the gut. Importantly, the protein content of CC is delivered in a form that is very low fat (2.3–3.9 g) and cholesterol contents (13 mg) relative to other cheeses and dairy products. This is widely regarded as the most appealing aspect of CC for consumers. While low- and nonfat forms of CC are produced, the levels of fat present in standard CC are low, so there is not a lot to be gained by choosing these alternatives, especially as levels of protein can be compromised.

Carbohydrate levels, though modest, are relatively higher (2.1–4.3 g) than many other cheeses. Most of this is present as lactose and, thus, CC is less suitable for lactose-intolerant consumers than other cheese varieties. Despite relatively higher carbohydrate content, total energy values are low at 98 kcal or 413 kJ, a trait that is attributable to the low-fat content (Holland, Unwin, and Buss 1989; Weaver 2021; Thorning et al. 2017).

The low-fat content of CC also impacts the levels of fat-soluble vitamins and pro-vitamins (vitamin precursors) present, with concentrations being lower than for many

other cheese varieties. Examples of fat-soluble vitamins present at relatively lower levels include (per 100 g) retinol (vitamin A; 44 µg), carotene (provitamin A; 10 µg), vitamin D (0.3 µg) and vitamin E (0.08 mg). The abundance of water-soluble vitamins relative to other cheeses (per 100 g) varies. Levels of riboflavin (B2; 0.26 mg), vitamin B6 (0.08 mg) and vitamin B12 (0.7 µg) are low, while those for thiamin (vitamin B1; 0.03 mg), niacin (vitamin B3; 0.13 mg), folate (vitamin B9; 27 µg), biotin (vitamin B7; 3 µg) are comparable to other cheese varieties, while pantothenate (vitamin B5; 0.4 mg) levels are relatively high (Holland, Unwin, and Buss 1989; Weaver 2021; Thorning et al. 2017).

In general, the mineral content (mg/100g) of CC is low relative to other cheeses. This is true for Na (380), K (89), Ca (73–123), Mg (Holland, Unwin, and Buss 1989), P (107–160), Fe (0.1) and Zn (0.6) (Holland, Unwin, and Buss 1989; Weaver 2021; Thorning et al. 2017). As previously highlighted, CC has a relatively larger serving size, and due to this, it is sometimes regarded as a potentially high source of sodium (Marsh, Klippstein, and Kaplan 1980), with some efforts having been made to produce low salt varieties to mitigate this. The low content of some of these minerals is a trait typical of the acid-coagulated fresh cheeses more generally relative to rennet-coagulated ripened cheeses (Renner 1993). It should be noted that both major and trace mineral components of CC are influenced by manufacturing practices. Indeed, larger curd size, added calcium chloride and fewer washes are all associated with higher iron and zinc (Wong, LaCroix, and Vestal 1977).

The highlights of the nutritional profile of CC surround the provision of bioavailable protein with limited contributions from fat, making it an attractive source of dairy protein. This trait is the basis for most perceived health benefits attributed to CC.

Evidence of health effects from *in vitro* models

Overall, the current landscape of *in vitro* evidence predominantly arises from manipulating CC to ameliorate characteristics such as antioxidant capacity and cholesterol content, rather than evaluating the direct influence of unadulterated CC in its pure form. Nonetheless, we briefly summarize the main findings of studies which have utilized *in vitro* bioassays to provide indications of the purported health benefits of CC.

One such study reported the production of a new CC supplemented with bovine colostrum and probiotics (Abdeen, Hamed, and Ismail 2024). The authors added 1%, 2% and 3% of the ABT probiotic culture mix (consisting of *Lactobacillus acidophilus*, *Streptococcus thermophilus* and *Bifidobacterium* spp.) and bovine colostrum powder (BCP) to CC produced via cream fermentation. They subsequently evaluated the antimicrobial and antioxidant activity as well as determining the fatty acid profile of the fortified CC. While organoleptic properties such as chewiness and springiness were attenuated for the fortified cheeses, an increased cohesiveness, hardness, and gumminess was observed in CC supplemented with probiotic culture and BCP. Medium-chain fatty acids were the most abundant fatty acids detected in functional probiotic CC, with moderate levels of short-chain and long-chain fatty acids, and relatively low levels of monounsaturated fatty acids. Incorporation of BCP to CC samples enhanced their antioxidant activities significantly and conferred antimicrobial activity against pathogenic bacteria, coliforms, mold, and yeast, whilst concurrently promoting the growth of beneficial *bifidiobacteria* and Lactic Acid Bacteria (LAB). Thus, it was determined that the addition of 2% BCP displayed the highest nutritional value and associated health benefits (Abdeen, Hamed, and Ismail 2024).

Ribeiro and colleagues reported the incorporation of rosemary aqueous extracts to CC and downstream evaluation of antioxidant activities (Ribeiro et al. 2016). It was shown that higher concentrations of phenolic compounds were present in CC supplemented with rosemary extract. However, antioxidant bioactivity associated with these compounds was attenuated in the enriched CC samples after seven days of storage, highlighting that antioxidant bioactivity diminishes over time when adding rosemary extracts in free form. To mitigate this and prolong the antioxidant bioactivity, microencapsulation was implemented utilizing an atomization and coagulation technique. The authors reported that the samples added with the microencapsulated extracts maintained their antioxidant properties more efficiently throughout storage, thus increasing the bioavailability upon ingestion. Overall, the study found that the nutritional value of CC was not affected by the introduction of the extract in either free or microencapsulated forms, but microencapsulation was superior in maintaining antioxidant capacity of rosemary extracts, demonstrating CC to be an effective vehicle for bioactive compounds (Ribeiro et al. 2016). A separate study by Josipovic et al. evaluated the microbiological safety profile of CC containing spices with a view to enhancing the biological value and prolonging the shelf life of these cheeses, whilst concurrently ensuring that the cheese exhibited acceptable sensory and organoleptic properties (Josipović et al. 2015). The authors evaluated the antioxidant and antimicrobial activity of up to 30 types of cheese, which were supplemented with spices such as garlic, pepper, parsley, rosemary, and dill. The phenolic compound concentrations were also measured. Sensory profiles were found to be superior for cheeses supplemented with fresh sweet red pepper, while antioxidant and antimicrobial properties were identified to be most potent with cheeses which were supplemented with dry rosemary on account of the high concentrations of rosmarinic and caffeic acid, in addition to the presence of phenolic diterpenes and flavones. Encouragingly, the plant extracts elicited a decrease in the numbers of the foodborne pathogens *Listeria monocytogenes*, *Escherichia coli*, *Salmonella typhimurium* and *Staphylococcus aureus*. Thus, the addition of these extracts to CC has the potential not only to prolong the shelf life of CC, but also to facilitate functional food properties in the form of antimicrobial activity, with potential of attenuating illnesses caused by foodborne pathogens (Josipović et al. 2015). This is therefore a characteristic that would carry practical applications in food processing and be of interest to manufacturers when desiring to produce safe products for consumers.

As well as the addition of compounds, *in vitro* work has also sought to remove constituents of CC, one study described

the use of β-cyclodextrin treatment to develop low-cholesterol CC and downstream assessment of the textural and color profiles of such CC (Kolarič, Kántorová, and Šimko 2022). By adding β-cyclodextrin to CC, 97.9% of the total cholesterol was removed, with this reduction envisaged to confer benefits via cardiovascular health. While it is established that dietary cholesterol ought not be viewed with as much concern as once thought with respect to cardiovascular disease risk, intakes exceeding the average display a relationship with increased blood lipoproteins subfractions, and therefore, intakes are advised to be managed (Carson et al. 2020).

Finally, in a foundational study on *in vitro* digestibility, Bodwell et al. reported the outcomes of comparing the digestibility of *in vitro* digestions with that of *in vivo* digestions of CC (Bodwell, Satterlee, and Hackler 1980). The reason for inclusion of this study in the present section covering *in vitro* models rather than the following *in vivo* model section is due to the emphasis on evaluating the performance of the *in vitro* digestions in this work. The authors evaluated the digestibility of preparations of CC, as well as five other sources of protein (spray dried whole egg, canned tuna, peanut flour, soy isolate, and wheat gluten), in 4–5 men and rats, with a view to comparing the digestibility data to three *in vitro* enzymatic digestion protocols. The correlation coefficient between true digestibility in rats and humans was found to be 0.46 for all protein sources, including CC. High correlations with r values higher than 0.90 were found when either the three animal proteins independently (including CC) were correlated with *in vitro* digestion data and human/rat digestibility data. The overall conclusion from the study was that due to the vast differences in correlation coefficients when plant proteins were correlated with *in vitro* digestion data, and when animal protein was compared to *in vitro* digestion data, that different equations would be required for gaining meaningful insights into the digestibility profiles of different protein sources, using any such correlation analyses.

To summarize, it is difficult to extrapolate significant conclusions from *in vitro* models concerning the health effects CC. However, the evidence suggests that CC provides a food matrix that can be manipulated to incorporate putative bioactive compounds with a view to enhancing characteristics of relevance to manufacturers and consumers.

Evidence of health effects from *in vivo* animal models

There have been two earlier *in vivo* studies incorporating animal models concerning CC. However, like the above *in vitro* work, these investigations were exploring ameliorating CC characteristics, rather than understanding direct health effects. Specifically, work from Kaup, Greger, and Lee (1991) followed by Reykdal and Lee (1993) investigated the capacity to apply calcium fortification to CC in an effort to increase calcium content, which as highlighted above, is generally lower than other rennet cheeses. In summary of the work, CC represents a candidate for effective calcium fortification, with increasing concentrations of calcium applied to the CC matrix enabling greater bioavailability in rats, the authors also reported that addition of guar gum is effective at attenuating bitter sensory properties which arise from calcium fortification. While this evidence does not provide an understanding of the direct health effects of CC per se, it does, as above, further demonstrate the capacity of CC to be a vehicle for additional bioactive compounds and nutrient biofortification.

Evidence of health effects from human intervention studies

At the time of the present review, there is a relative dearth of high-quality evidence from human intervention models exploring the direct health effects of CC consumption (Table 1). The current literature includes investigations in nutrient breakdown and availability, metabolic health, and impact on energy expenditure and appetite related variables. This evidence will be summarized below.

Nutrient bioavailability

Three studies have elucidated the bioavailability of nutrients following CC consumption, specifically, these investigations explored postprandial protein and calcium metabolism.

In a dated study in 1958, Watts et al. (1959) employed a crossover study design in eight healthy young men to determine the availability of essential amino acids (AA), as well as cysteine and tyrosine, following five diets which were: (i) a low nitrogen basal diet, consisting of mainly fruit and vegetables, containing 0.9 g nitrogen which was supplemented with sucrose to achieve a total daily energy intake of 43 kcal/kg; (ii) the basal diet supplemented with egg to make up to 1 g/kg protein intake (500–659 g of whole egg (approximately 12 eggs) depending on body weight); (iii) milk diet, matched for protein to the egg diet (approximately 2 quarts of milk); (iv) a milk and egg diet (approximately 1 quart of milk with 6 whole eggs); (v) a CC diet (450–600 g CC). The diets were followed for 10 days before moving to the next diet. The egg diet was included as a reference protein to compare the bioavailability of dairy proteins alone and when combined with egg. Bioavailability was assessed by urinary creatinine and nitrogen balance, as well as fecal AA. The authors reported marginal differences in AA bioavailability between diets, with the CC diet lower than the egg reference diet, but greater than the milk diet. While this study provides some evidence for AA bioavailability for a selection of protein sources, it dates from over 60 years ago and methodologies that have since been modernized significantly. Furthermore, there is no evidence of a randomization protocol, and no statistical tests were performed to determine effect size or significance for the difference between diets.

Khan, Gannon, and Nuttall (1992) performed a small study in a population of five healthy male adults to determine the fate of deaminated AA following protein ingestion. The authors hypothesized the majority of carbon skeletons present following AA deamination would be converted to glucose in the liver. Using a crossover design, tritiated glucose dilution tracers were used to determine the effect of a protein meal on the glucose appearance rate in plasma

Table 1. Human intervention studies included in the present review investigating the health effects of cottage cheese consumption.

Author, Country	Year	Study design	*N*	Study population	CC product	Comparator	Outcomes	Results
von Post-Skagegård et al. 2006, Sweden	2006	RCCT	17 (female, 17)	Healthy 30–65 years BMI 22–32 kg/m^2	1% fat 295 g	1. 1. Cod fillet (225 g) 1. Soy protein isolate (43 g)Meals consisted of: 1. energy, 2300 kJ/500 kcal; protein, 44 g; carbohydrate, 56–57 g; fat, 16–17 g.	AUC blood glucose; serum insulin; serum TAG; serum NEFAs; plasma C-peptide.	Larger AUC serum insulin following CC. Higher insulin/C-peptide and insulin/glucose ratios after CC.
Gannon et al. 1988, United States	1988	RCCT	17 (male, 17)	Type II diabetic without treatment. 40–75 years	Providing 25g protein w/ 50g glucose.	Following sources providing 25g protein w/ 50 g glucose: (1) Lean beef; (2) Turkey; (3) Gelatin; (4) Egg white; (5) Fish; (6) Soy.	Relative AUC plasma insulin ; relative AUC plasma glucose. Absolute AUC plasma glucagon, AAN, and urea nitrogen.	Relative AUC insulin greatest following ingestion of meal with CC.
Marsset-Baglieri et al. 2015, France	2015	RCCT	30 (female, 16; male, 14)	18–40 years BMI 18–25 kg/m^2	Providing: energy, 1346 kJ; protein, 26 g; carbohydrate, 8 g; fat, 21 g.	Egg (as omelet) matched for energy and macronutrient composition.	Plasma urea, glucose, cholesterol, tAG, AAs, insulin, glucagon, GIP, GLP-1, peptide YY, ghrelin. Ad libitum lunch intake; subjective satiety via elapsed time between test meal and lunch request and VAS.	No difference in satiety metrics, blood glucose, TAG, and cholesterol. Slower plasma AA and urea response after eggs vs CC. GIP and insulin increased significantly after CC. Glucogan and GLP-1 delayed with egg.
Leyh et al. 2018, United States	2018	RCCT	10 (female, 10)	Healthy Active (moderate to vigorous activity >4 d/week ≥ 12 months)	Providing: protein, 30 g; carbohydrate, 10 g; fat, 0 g.	(1) Casein protein supplement energy and protein matched; (2) Placebo (0 kJ)	REE and appetite	No difference between intervention products.
Howe 1990, United States	1990	UT*	8 (female, 8)	Postmenopausal 51–65 years	Liquid meals consisting of: 2. 15 g protein 3. 45 g protein	Liquid meals with same protein content of: 1. Beef 2. Soy isolate 3. Placebo (0 g protein)	Serum calcium; serum phosphorous, serum creatinine, urine creatinine; serum insulin; serum PTH; serum Ct; urine GFR.	Significant difference in calcium metabolism metrics following protein containing meals vs placebo. No significant difference between protein source.
Khan et al. 1992, United States	1992	NRCT	(male, 8)	Healthy	Providing 50g protein	Water	8 h glucose appearance rate via 3H-glucose infusion; urea production from deaminated protein.	Urea production and glucose appearance only account for at most 43% protein metabolized or 23% total amount of protein ingested.
Nuttall and Gannon 1990, United States	1990	RCCT	7 (male, 7)	Healthy	Providing 50g protein.	Egg white providing 50g protein.	8 hr iAUC serum insulin; C-peptide; glucagon; α-amino-nitrogen and urea nitrogen.	All metrics 50% lower after egg white which was associated with 50% smaller conversion of protein to urea. 70% of CC vs 47% egg white accounted for by urea formation.
Gannon et al. 1998, United States	1998	RCCT	7 (male, 7)	42–75 years Untreated diabetes	Providing 25 g protein.	1. 25 g fructose 2. 25 g CC + 25 g fructose 3. 50 g glucose (control)	Plasma glucose; serum insulin; serum TAG; plasma glucagon; plasma urea nitrogen; plasma AAN; plasma C-peptide; serum NEFAs.	Insulin response 2.5-fold greater following protein vs fructose, and the response to co-ingestion was additive and quantitatively similar to response of 50 g glucose.
Watts et al. 1959, United States	1955	UT	8 (male, 8)	34–38 years Healthy	450–600 g (quantity of cottage cheese to reach 1 g protein/kg/day).	Isocaloric diets: 1. Low protein diet. 2. Diets providing 1 g protein/kg/day: 3. Whole egg (500-659 g/12 eggs); 4. Milk (2 qts); 5. Milk (1 qt) + egg (6 eggs)	Urinary creatinine and nitrogen balance; stool AA; AA availability.	AA availability in CC hgiher than milk diet, but lower than milk + egg (except isoleucine, leucine, and lysine).

CC: cottage cheese; RCCT: randomized crossover controlled trial; NRCT: non-randomized controlled trial; UT: uncontrolled trial; REE: resting energy expenditure; TAG: triglycerides; AUC: area under the curve; iAUC: area under the incremental curve; NEFAs: non-esterified fatty acids; AA: amino acids; AAN: **α**-amino nitrogen; PTH: parathyroid hormone; Ct: calcitonin; GFR: glomerular filtration rate; GIP: glucose-dependent insulinotropic peptide; GLP-1: total glucagon-1 like peptide; VAS: visual analogue scales.

*Unclear if study was performed as randomized protocol.

following consumption of CC providing 50 g protein. The 8-h glucose appearance rate was evaluated with the use of 3H-glucose infusion as well as quantification of protein deaminated and converted into urea. The study reported an inability to report on the fate of all deaminated AA in CC, with urea production accounting for 29.3 g (58.8%) of protein ingested. Glucose appearance in plasma was deemed to account for 9.7 g protein. The authors reported that based on the gluconeogenic potential of CC (which would comprise 42.3 g glucose from 50 g protein), this could only account for 23% of the total amount of protein ingested. It is difficult to extrapolate definitive findings from this study as CC was not compared to any other food or protein source, as well as including a small homogenous sample size.

Howe (1990) conducted a crossover study in eight postmenopausal women (age 51–65 years) to characterize the postprandial response in calcium metabolism to meals varying in protein quantity and source. After fasting overnight, the women consumed a liquid meal containing either 15 g or 45 g of protein from CC, beef, or soy isolate, as well as a basal meal which provided 0 g protein. Blood and urine samples were collected for measuring metrics pertinent to postprandial calcium metabolism. With reference to findings of interest for CC, the study observed that calcium reabsorption was significantly reduced following the meals containing 45 g protein from CC and soy isolate. In addition, with relevance to the studies to follow concerning cardiometabolic health, the authors reported differential postprandial insulinemia between the meals, with CC instigating the greatest response out of the protein sources. There are factors to consider when extrapolating these results, including the small study population comprising a specific subgroup of the population which would impact both power and generalizability of the findings. In addition, it is unclear if randomization was implemented in the study design which also introduces caution to the results.

Metabolic health

Four studies have investigated the influence of CC on outcomes pertaining to metabolic health. von Post-Skagegård, Vessby, and Karlström (2006) evaluated the difference in metabolic effects between different dietary proteins within a composite meal consisting of the same macronutrient profile (energy 2300 kJ, protein 44 g, carbohydrate 56–57 g, fat 16–17 g) using a randomized crossover controlled trial (RCCT) in a study population of seventeen healthy female participants (mean age, 46 years). Participants consumed three test meals varying in protein sources consisting of CC (representing milk protein arm), cod fillet (cod protein arm) or soy protein isolate (soy protein arm), with a 1-week washout period between each test meal. Blood samples were collected for the measurement of blood glucose, serum insulin, serum triglycerides, serum non-esterified fatty acids (NEFAs), and plasma C-peptide in the fasting state and at 20, 40, 60, 90, 120, 180, and 240 min after commencing the test meals. The study reported that the CC meal caused the largest increase in serum insulin calculated up to 240 min post meal, and a higher insulin/C-peptide and insulin/glucose ratio at 120 min post meal compared to cod fillet and soy protein isolate.

Gannon et al. (1988) investigated the effect of consuming 25 g protein on its own or in conjunction with 50 g glucose on serum insulin levels in 17 untreated type 2 diabetic participants. Regarding "untreated" status, the authors reported that "none of the subjects were treated with either hypoglycaemic agents or insulin before the study." The investigators tested several protein sources including CC, lean beef, turkey, gelatin, egg white, fish, and soy. On separate days pertaining to each arm, participants consumed randomized test meals following an overnight fast, with blood collected at 30, 60, 120, 180, 240, 300 min post meal for the analysis of response in plasma insulin, glucose, glucagon, amino acid nitrogen (ANN) and urea nitrogen. The study reported that all protein sources stimulated a greater insulin response when consumed in conjunction with glucose, with CC causing the largest increase in plasma insulin response compared to the remaining protein sources.

Nuttall and Gannon performed a follow-on study in seven healthy males where they compared the response to 50 g protein provided via egg white or CC (Nuttall and Gannon 1990). The reasoning was that in the previous work discussed above, egg white stimulated the smallest insulin stimulatory effect and CC the greatest, while the authors hypothesized egg white would have the greater insulinemic effect due to egg white being considered a readily digestible and high-quality protein. Following an afternoon fast, participants consumed the test foods on separate days pertaining to the separate arms, following which blood was collected before and 0.5, 1, 2, 3, 4, 5, 6, 7 and 8 h post meal for the analysis of plasma glucose, serum insulin, glucagon, AAN, C-peptide, and NEFAs. The study found the metabolic response to CC and egg white differed, with egg white stimulating a marginal increase, whereas CC a minor reduction in plasma glucose, while both caused an increase in serum insulin, C-peptide, and glucagon concentrations, with a twofold greater response after CC consumption. The increase in AAN concentration, which is an index of the total amino acid concentration, was also almost twofold greater after CC.

In a study 10 years later, Gannon et al. tested the metabolic effects of 25 g fructose, and 25 g protein and fructose combined, in comparison to a 50 g glucose control, in a study population of seven untreated (reporting "untreated" status as above) type 2 diabetic participants (Gannon et al. 1998). After an overnight fast, participants consumed test meals as a single breakfast meal on different days in a randomized order. The meals consisted of 25 g fructose (fructose dissolved in water), 25 g protein (as 147 g CC), or 25 g fructose and 25 g protein. Fifty grams glucose was provided on two separate occasions as a control and on another separate occasion, participants consumed only water to serve as a baseline measure. Blood was collected before and 0.5, 1, 2, 3, 4, and 5 h after the beginning of the meal for analysis of plasma glucose, serum insulin, glucagon, C-peptide, urea nitrogen, triglyceride, NEFAs, AAN. The authors reported that combining fructose and protein resulted in a 2.5-fold greater response in serum insulin compared to fructose alone and was similar to the 50 g glucose used as a control.

Considering the above studies, the cardiometabolic effects of CC are difficult to interpret due to small sample sizes without indication of sample size calculations, variations in health status, different study regimens and the lack of comparator foods against CC. However, to extrapolate the limited findings, the studies suggest that CC may elicit a greater insulinemic response compared to other protein sources which could be due to a more rapid digestibility and bioavailability. It may also suggest that for at-risk populations, CC may not be beneficial when seeking to manage acute blood glucose and insulin levels in the postprandial state.

Energy intake and appetite

Two studies have explored the capacity of CC to impact energy intake and appetite variables in human participants. Leyh et al. (2018) implemented a randomized, cross-over design in a study population of ten healthy, active women. The study determined the impact of consuming CC (providing 30 g of protein, 10 g of carbohydrate and 0 g of fat) before sleep on both acute and next-morning resting energy expenditure (REE), resting energy rate (RER) and appetite, compared with an isoenergetic/isonitrogenous casein protein supplement and non-energetic placebo, with a view of evaluating the differences between a wholefood source and liquid source of protein. At 30–60 min prior to sleeping, participants consumed CC, a casein supplement, or placebo before measurement of REE. Upon waking, REE was repeated, and subjective appetite was recorded. There were no significant differences in acute REE, acute RER, morning REE or morning RER. In addition, the authors reported no difference in subjective measures of appetite between groups. The study findings suggests that in active women, pre-sleep consumption of CC does not alter REE or RER differently than either a liquid casein protein supplement or non-energetic placebo beverage.

Marsett-Baglieri et al. assessed the impact of CC on satiety in comparison to an egg omelet using a RCCT in a study population of thirty healthy participants (Marsset-Baglieri et al. 2015). The aim of the study was to decipher whether foods comprising similar nutrient compositions (i.e., containing proteins and lipids) but differing in food forms, may differentially modulate satiety metrics. On the test days, participants consumed a standardized meal, followed by CC or egg omelet 3 h later (providing 1342 kJ, 26 g protein, 21 g lipids, and 8 g lactose). The study outcomes included the elapsed time between the CC or omelet and lunch request, food intake at lunch, and satiety measured via visual analogue scales (VAS). Ten participants underwent a subgroup analysis where plasma metabolites and hormones were measured in blood samples. The study found that there were differences in the plasma insulin, gastric inhibitory polypeptide (GIP), urea and AA between foods, with a more rapid increase after CC. These marked differences were not reciprocated in satiety metrics, with no significant differences between foods in elapsed time to eat, food intake at lunch and VAS. The study findings suggest that while food form can impact nutrient kinetics and incretin secretion, CC does not modulate satiety differently from other protein sources when the energy and protein content are matched.

Considering the above studies, the findings suggests that CC does not impact energy expenditure, satiety or appetite differently compared to other protein sources when matched for energy, protein and other macronutrient content.

Discussion and future directions for CC research

The present review has summarized the production of CC and nutritional composition, suggesting how the nutritional characteristics could putatively elicit health benefits to consumers. We have also summarized the current evidence from human intervention, *in vitro*, and *in vivo* models as to the direct health effects of consuming CC. Overall, there is not a wealth of high-quality evidence from randomized controlled trials (RCTs); considered the gold standard in the hierarchy of evidence in assessing the effectiveness of interventions and causal relationships (Hariton and Locascio 2018). The outcomes that have been investigated to date include nutrient bioavailability, cardiometabolic health, energy expenditure and satiety metrics (Figure 1).

The human intervention models investigating metabolic health outcomes have primarily focussed on the insulin and glucose response to protein, and protein in conjunction with carbohydrate feeding, with an emphasis on clinical diabetic study populations (von Post-Skagegård, Vessby, and Karlström 2006; Gannon et al. 1988, 1998; Nuttall and Gannon 1990). The findings suggest that compared to other protein sources, CC may instigate a greater insulinemic response, which could be detrimental for diabetic populations who require large fluctuations in blood insulin and glucose levels to be kept to a minimum (Hanssen et al. 2020). However, away from a clinical diabetic perspective, protein initiated insulinemia can aid in enhancing anabolism and reducing catabolism in skeletal muscle tissue (Bennet et al. 1990; Biolo, Declan Fleming, and Wolfe 1995; Moller-Loswick et al. 1994). Ergo, a greater insulin response to CC compared to other protein sources could suggest that it may be more potent at initiating muscle protein synthesis pathways, which would be advantageous for those seeking to augment skeletal muscle mass. Nevertheless, the present landscape of evidence for metabolic outcomes following CC consumption comprises a small selection of studies in small study populations ($n = 7$–17) which reduces the power of the results. Therefore, more RCTs are required, recruiting larger sample sizes, to further characterize the metabolic effects of CC. In addition, when the desire is to elucidate the holistic impact on cardiometabolic health, more outcomes are required. Future RCTs could consider focussing on outcomes including blood lipids such as cholesterol and lipoprotein fractions (e.g., high-density lipoprotein (HDL) and low-density lipoprotein (LDL)), as well as blood pressure, which would help develop a full picture of the cardiometabolic impact of consuming CC.

The human studies which assessed bioavailability and nutrient metabolism also comprised small sample sizes, which make extrapolating the findings difficult. There are also issues arising from the studies including that the protein bioavailability study by Watts et al. was dated (1955) (Watts et al. 1959), with the methodology implemented reflective of this. Fecal AA are a poor reflection of bioavailability and

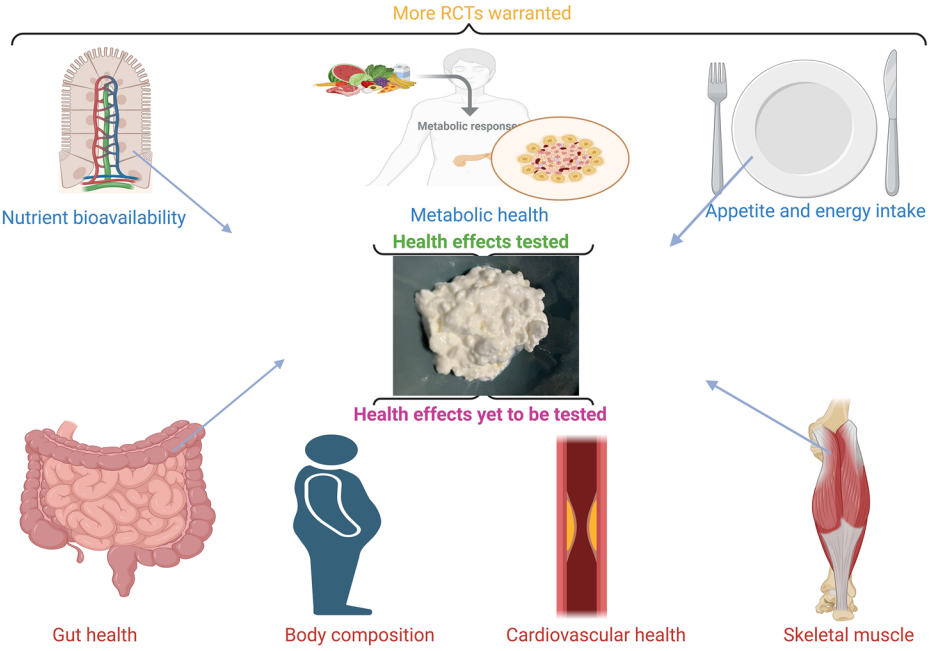

Figure 1. Summary of the health effects tested and health effects yet to be tested pertaining to cottage cheese. To date, investigations have been performed on the effects of cottage cheese on nutrient bioavailability, metabolic health and appetite and energy intake; although further randomized controlled trials are warranted due to investigations being performed in small, niche, study populations. There have been no investigations of the impact of cottage cheese on gut health, body composition metrics such as weight loss, cardiovascular health and skeletal muscle, investigations which are arguably warranted to garner a detailed picture of the impact of cottage cheese in health.

digestibility (Gaudichon and Calvez 2021), with measurement at the ileal level a more reflective marker due to AA absorption in the small intestine, whereas AA reaching the large intestine are fermented by the resident microbiota and transformed into other AA, or metabolites absorbed in the colon such as ammonia, indoles, phenols, hydrogen sulfide and branched chain fatty acids depending on nitrogenous nature of AAs (Moughan and Wolfe 2019). Further, there was a lack of randomization protocol to randomize participants to intervention arms and statistical analyses to disentangle the differences between the protein sources assessed. The protein bioavailability study by Khan, Gannon, and Nuttall (1992) only included CC as the protein source to evaluate protein bioavailability, thus making it difficult to interpret differences between CC and other protein sources. Howe (1990) investigated calcium bioavailability in a defined study population with relevance to a specified research question of calcium metabolism in an at-risk population (postmenopausal women). This makes the study difficult to generalize, but also the small study population also renders the results difficult to interpret toward the defined research question and arguably represents preliminary pilot data to build toward larger RCTs. Although a lower calcium content per 100 g compared to other cheeses, the higher serving sizes of CC would enable greater quantities of calcium to be consumed and suggest that CC could represent a source of calcium that may be efficacious in at-risk populations requiring high quality bioavailable sources such as postmenopausal women (The North American Menopause Society 2021; de Villiers and Goldstein 2021; Erdélyi et al. 2023). Therefore, future research should consider developing on this work to understand if CC should be recommended to improve calcium status.

Considering the limitations of the studies above, high quality studies comprising larger study populations are required to understand the nutrient bioavailability of CC.

One of the reasons for advocacy of CC is for facilitating weight loss. We identified two studies reporting appetite related outcomes, which would be of interest in the objective of weight loss. If foods and/or diets can aid satiety, this can help maintain dietary patterns which place individuals in a net negative energy balance to facilitate weight reduction via reduction of adipose mass (Aragon et al. 2017). The high casein content of CC could culminate in a slower digestibility profile, which may be able to lead to greater satiation between meals. This could facilitate the maintenance of energy restrictive dietary regimes for those seeking to lose adipose tissue and improve body composition (de Carvalho et al. 2020; Kohanmoo, Faghih, and Akhlaghi 2020; Moon and Koh 2020). The study by Leyh et al. found CC provided the same satiation as the equivalent protein in liquid form and a non-energetic beverage (Leyh et al. 2018), which would suggest no advantage for providing satiation, while the study by Marsett-Baglieri and colleagues (Marsset-Baglieri et al. 2015) suggests that CC could be as satiating as eggs, a protein food acknowledged for its satiating capacity. These two studies differed in sample size ($n = 10$ vs $n = 30$), participant characteristics (healthy active women vs mixed study population), as well as research questions (effect of pre sleep consumption on acute and next morning appetite vs effect of consumption on acute and same day appetite). It is therefore difficult to draw definitive conclusions and more RCTs are required to determine the impact of CC on satiation, both in the acute postprandial phase as well as over a longer duration post consumption (e.g., 8–24 h).

Another reason for the advocacy of CC in weight loss is due to the lower fat, and thus higher protein to fat content of CC compared to other cheeses, which would provide advantages toward achieving energy restrictive diets and facilitating weight loss, as well as providing a high-quality protein source with limited energy from other macronutrients for those aiming to achieve high protein intakes such as athletic and health-conscious populations.

Therefore, in addition to satiety metrics, the effects of chronic consumption on body composition ought to be considered in future RCTs to determine whether CC could be viewed as a facilitator of weight loss and provide evidence to support the advocacy of CC amongst consumers for this purported benefit.

Related to the topic of body composition is the lack of evidence for CC with respect to facilitating skeletal muscle mass augmentation, which is surprising considering CC is regarded among the public as a source of high-quality, slow releasing protein source that could facilitate skeletal muscle repair during the sleeping window. This perception is likely driven by the high content of the slow digesting protein casein in CC, which would culminate in a more gradual and sustained release of amino acids into systemic circulation compared to faster digesting proteins such as whey. These characteristics may aid in reducing muscle breakdown and promoting muscle protein synthesis during longer durations without short intermittent protein feeding (i.e., during sleeping hours), which is highly desirable for those seeking to increase and/or maintain skeletal muscle mass (Jäger et al. 2017). However, there were no RCTs addressing this research question. It is therefore arguable that human studies elucidating the impact of CC on metrics of skeletal muscle mass augmentation and protein synthesis kinetics in the acute and subsequent postprandial window are warranted to inform recommendations to athletic populations seeking to improve performance, as well as health-conscious consumers desiring to improve body composition via skeletal muscle mass augmentation.

Amongst human studies identified in the present review, there have been no investigations into the impact of CC on gut health, including gut microbiome modulation as well as clinical outcomes such as gastrointestinal symptoms and bowel movements. CC production can differ, however, when curds are manufactured by the acid coagulation of skim milk through a starter culture rather than direct acidification, this would render CC a cultured dairy product and fermented food (FF). FF are increasingly gaining attention for the capacity to modulate the gut microbiome due to the intrinsic microorganisms and metabolites (Mukherjee et al. 2024; Valentino et al. 2024). Compared to other FF, CC may contain fewer live microorganisms, which would be similar to FF such as sourdough bread (Sakandar et al. 2019), beer (Zimmermann, Van Hoorde, and Butler 2018) and wine (Venturini Copetti 2019). Therefore, RCTs evaluating the impact of CC consumption on the gut microbiota and gut environment are warranted to address this gap in knowledge and develop the evidence base on CC, as well as FF as an overall food group. Another avenue of interest for gastrointestinal health is that due to the chemical structure of CC (pH ~5.1) and short storage time between manufacture and consumption, it represents an environment for the survival of probiotic species (Abadía-García et al. 2013; Blanchette et al. 1996). The *in vitro* work included in the present review also suggests that CC may be an efficacious carrier of bioactive compounds which could enhance health. While the primary interest of the present review is to establish the health impacts of CC as a standalone food, this concept would also be of interest as addition of probiotics, prebiotics, and/or other bioactive compounds within the matrix of CC may provide an additive impact in (and out of) the gut, via modulation of the gut environment.

In conclusion, despite global consumption due to culinary versatility and advocacy for putative health benefits amongst the fitness and health-conscious community, there is a dearth of evidence from high quality RCTs as to the health impact of CC. CC may be an impactful cultured dairy product that can impose a myriad of benefits across health outcomes including skeletal muscle, cardiometabolic, gastrointestinal, appetite regulation, weight loss and nutrient status. There is a need for high quality RCTs with dedicated research methodologies to address these research areas to develop a well-grounded evidence base for the impact of CC in human health.

Author contributions

The authors confirm contribution to the paper as follows: study conception: DNF; study design: DNF and PDC; data collection: DNF, HM, TB and PDC; analysis and interpretation of the data: DF, HM, TB and PDC; drafting of the paper: DF, HM, TB and PDC; development of full draft of manuscript: DF; critically revising manuscript for intellectual content: DF, HM, TB and PDC. All authors gave final approval of the version to be published and agree to be accountable for all aspects of the work.

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