Graduate Student Literature Review: A scoping review on the impact of consumption of dairy products on phosphatidylcholine and lysophosphatidylcholine in circulation and the liver in human studies and animal models*

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ABSTRACT

Dairy consumption is inversely related to the risk of developing type 2 diabetes in epidemiological research. One proposed hypothesis is that phospholipid (PL) species associated with dairy consumption mediate this relationship. This scoping review aimed to identify the existing literature in animal and human trials investigating the impact of dairy products, including milk, yogurt, and cheese as well as dairy-derived PL supplementation on PL and its species in the circulation, summarizing the characteristics of these studies and identifying research gaps. A systematic search was conducted across 3 databases (PubMed, Scopus, and Web of Science) in March 2021. Of 2,427 identified references, 15 studies (7 humans and 8 animal studies) met the eligibility criteria and were included in the final narrative synthesis. The evidence base was heterogeneous, involving a variety of clinical and preclinical studies, metabolically healthy or obese/diabetic participants or animal models, and displayed mixed findings. Circulating postprandial concentrations of total PL were elevated acutely but unchanged after longer intervention with dairy products. The PL concentration remained stable even after a high dosage of milk supplemented with dairy-derived PL, which may be related to increased fecal excretion; however, certain phosphatidylcholine (PC) or lysophosphatidylcholine species were increased in circulation by interventions. These include several PC species with 32 to 38 total carbons in addition to the dairy biomarkers C15:0 and C17:0. The results of this scoping review demonstrate a small body of literature indicating that dairy products can influence blood concentrations of PC and lysophosphatidylcholine species in both rodents and humans without alteration of total PL and PC. There is a lack of well-designed trials in humans and animals that explore the potential differences between individual dairy foods on PL species. In addition, trials to understand the bioactive properties of PC and lysophosphatidylcholine species on cardiometabolic risk are needed. Key words: dietary intake, yogurt, diabetes, lipid metabolism

INTRODUCTION

Metabolic disorders such as insulin resistance, type 2 diabetes, metabolic dysfunction-associated fatty liver disease (also called nonalcoholic fatty liver disease), and dyslipidemia are related pathophysiological conditions in which normal metabolic processes of the body are disrupted (O’Neill and O’Driscoll, 2015). Investigations into identifying plasma metabolite signatures of obesity and metabolic dysfunction have uncovered crucial information on the molecular processes that cause cardiometabolic diseases. Strong evidence from MS-based lipidomic studies indicates that the pattern of circulating or tissue phospholipid (PL) is a unique biochemical signature associated with diagnosed metabolic disorders (Cai and Yang, 2018; Yin et al., 2020). Phospholipids, which have hydrophilic phosphate heads and hydrophobic lipid tails, are important molecules for signal transduction pathways and play important roles in membrane structure and cell division. Phosphatidylcholine (PC) is the most prevalent PL in mammalian cells. Lysophosphatidylcholine (LPC) is derived by hydrolytic cleavage of PC catalyzed by the phospholipase A2 enzymes (Kita et al., 1999). Phospholipid panels captured using high-throughput technology have been widely used in epidemiological studies to improve prediction and better biological understanding of diseases. Disturbances in
the proportion of PL subclasses and individual species may alter glucose and lipid homeostatic pathways leading to the development of glucose intolerance and to the progression of insulin resistance and type 2 diabetes (Zeng et al., 2019). Phospholipid species can modulate the activity of the peroxisome proliferator-activated receptors and the production of eicosanoids and other lipid mediators that exacerbate obesity and low-grade inflammation (Kim et al., 2013). Thus, some PL species have been identified as biomarkers of type 2 diabetes (Hsu et al., 2000; Guasch-Ferré et al., 2016). For instance, reduced levels of LPC 18:2 are consistently associated with obesity, dysglycemia, insulin resistance, and type 2 diabetes, and have been proposed as a predictive biomarker for metabolic dysfunction (Szynańska et al., 2012; Wang-Sattler et al., 2012; Ferranmini et al., 2013), whereas PC 38:3 has a positive association with obesity (Yin et al., 2020). However, whether normalizing PL profiles is associated with improvements in outcomes is unclear.

The liver plays a crucial role in lipid metabolism. Hepatic tissue is the primary site of PL production and metabolism (Nguyen et al., 2008). Accumulating evidence supports the hypothesis that hepatic lipid deposition as triacylglycerides may not be inherently harmful in people with obesity and obesity-related diseases (Alkhouri et al., 2009; Mashek, 2021). However, as metabolic abnormalities progress, a more unhealthy hepatic lipid composition profile may lead to poorer metabolic health and a more aggressive fatty liver disease trajectory (Musso et al., 2018). In this context, bioactive lipid intermediates such as PC, LPC, and their species proposed to be linked to the development of hepatic lipotoxicity or insulin resistance, or both (Gentile and Pagliassotti, 2008; Fridén et al., 2022). Therefore, rather than an absolute quantity, hepatic lipid composition may underpin hepatic and systemic metabolic dysfunction.

Diet is a key modifiable risk factor for cardiometabolic diseases, and altering the dietary composition is an effective option to achieve and maintain healthy metabolic function (Rychter et al., 2020). From a food group point of view, dairy products have beneficial effects on metabolic health (Timon et al., 2020; Yuzbashian et al., 2021a, 2021b) and are well recognized as nutrient-rich foods providing high-quality protein and calcium, among others (Drewnowski and Fulgoni, 2008). However, dairy foods have a complex influence on metabolism because of differences in their industrial processing as well as the complex matrix of nutrients of each food (Mozaffarian and Wu, 2018; Astrup et al., 2019). Several mechanisms have been evaluated to explain how dairy foods can influence metabolism and ameliorate metabolic risk factors (Fernandez et al., 2017; Gille et al., 2018; Unger et al., 2019), but a full understanding remains incomplete.

Milk and some by-products of dairy products are a considerable natural source of PL (Pertiwi et al., 2019; Unger et al., 2019; Timon et al., 2020). Milk-based foods consist of many products with clear differences in nutritional profile and matrix. In particular, processing alters dairy lipid composition and distribution, especially PL, in the final matrix (Pimentel et al., 2016). Compared with cows’ raw milk, cream, butter, and buttermilk contain 2 to 5-fold more PL by weight, but even skim milk retains PL comparable to whole milk, similar to hard cheeses and yogurt (Pimentel et al., 2016). However, cream and butter are naturally high in saturated fats and therefore have been limited by dietary guidelines to support a healthy diet (MyPlate, 2022). On the other hand, milk, yogurt, and cheese have been recommended by MyPlate (2022) as dairy products and account for 75 to 95% of total daily milk-based product intake in various populations (Hjartáker et al., 2002; Wang and Li, 2008; Rabiei et al., 2021). Thus, milk, cheese, and yogurt contribute the majority of dairy-derived PL in most diets. A hypothesis is proposed that the beneficial effects of dairy products on metabolic health may pertain to normalizing PL metabolism, mainly compensating circulating species of PC and LPC. Observational studies investigating modifications of the plasma metabolome associated with dairy consumption in individuals with metabolic disorders support this hypothesis (Nestel et al., 2014; Pertiwi et al., 2019; Santaren et al., 2019).

However, the effect of various kinds of dairy products or supplementation with dairy-derived PL on circulating PL subclasses and their potential relation with cardiometabolic health are still generally unknown. A more explicit description of the evidence base on human and animal studies is required to understand how dairy products or dairy-derived PL may alter the total or individual PL species in the circulation and their consentation in the liver. Therefore, we conducted a scoping review of the existing literature of both animal and human trials to synthesize the available data on the influence of dairy products and dairy-derived PL on circulating PL, its subgroups, and species to better understand their metabolic effects and, more specifically, their impact on cardiometabolic health. The specific research questions were as follows: (1) In adults, what is the impact of increasing dairy PL on circulating PL, PC, or LPC? (2) In rodent models, what is the impact of increasing dairy PL on circulating or hepatic PL, PC, or LPC? We also provide an overview of available data on the cardiometabolic risk factors in the included
studies. In this context, human intervention trials providing a diet modifying the amount of dairy PL (using dairy products with or without PL enrichment) were reviewed to provide evidence of the impact of dairy PL on circulating PL subclasses and individual species concentrations. Animal experiments were included to provide insights into the mechanisms of action of dairy on circulating PL. The effects of interventions on liver PL observed in animal studies were also analyzed as a secondary outcome of interest. The knowledge gap and research needs were also identified, and a theoretical basis for future research and practical applications were provided.

METHODS

Search Strategy

Three online databases, including PubMed (https://pubmed.ncbi.nlm.nih.gov/), Scopus (https://www.scopus.com), and Web of Science (https://www.webofscience.com), were searched in March 2021 to find relevant studies published between January 2000 to January 2021. Search terms were determined using the MeSH database (https://www.ncbi.nlm.nih.gov/mesh/) and relevant reviews. In addition to terms identified in the MeSH database, the following search terms were used to define specific search syntax (Supplemental File S1; https://figshare.com/articles/online_resource/Supplementary_docx/21397164; Yuzbashian et al., 2022) for each database: “dairy, milk, yogurt, and cheese” (MyPlate, 2022) combined with “phospholipid, phosphatidylcholine, and lysophosphatidylcholine.” After a careful manual search of all included studies’ reference lists, additional studies were added. We selected studies that were conducted on either humans or animals. A summary of the review and the reasons for excluding studies are presented in the PRISMA flowchart (Figure 1).

Eligibility Criteria

Studies included were interventional trials (animal and human) that investigated the effect of total or individual dairy products on the circulating or liver content of PL, PC, and LPC. Studies assessing dairy products based on MyPlate definition, including milk, skim, low-fat or full-fat milk, yogurt, and cheese custard, were included, but studies evaluating ice confections, ice creams, cream, sour cream, or butter were excluded. We also included studies that administered dairy-derived PL, for example, milk, or other types of dairy food enriched with dairy-derived PL. Studies that supplemented participants with PL from other sources were excluded. The outcome measures of interest were serum/plasma concentration and liver content of PL, PC, LPC, and their species. We excluded in vitro studies and narrative, nonsystematic reviews, conference abstracts, as well as those not published in English.

Selection Process

Relevant studies were stored in EndNote, and the title/abstract screening process initiated after removing duplicate articles. The full texts of the remaining studies were read to check inclusion and exclusion criteria, detailed above.

Data Charting

Charting forms were developed separately for human and animal studies, and 1 reviewer (E.Y.) charted information. The second reviewer (S.M.) checked all of the records. From each human study, the following data were charted: authors’ names, publication year, aim, sample size, age, study design, study duration, participants’ health conditions, results related to PL and its subclasses, and the main metabolic outcome of the study. The charted data for animal studies were authors’ names, publication year, aim, animal species, sex, age, number of animals in intervention groups and control groups, intervention characteristics, and main results, including metabolic outcomes. In the case of missing data or unclear pieces of information, it was considered that the authors did not report such variables.

Risk of Bias

The risk of bias (RoB) in human randomized trials was assessed using the revised Cochrane’s Risk of Bias (RoB2) assessment tools for randomized trials (Higgins et al., 2016). For animal studies, SYRCLE’s RoB tool was used to assess the RoB (Hooijmans et al., 2014). Both RoB results had been visualized by robvis (McGuinness and Higgins, 2020). Both tools assess the studies’ methodological quality by evaluating selection, performance, detection, attrition, reporting, or other bias. Evaluation of potential bias for each included study was done by 1 reviewer (E.Y.) and checked by a second (C.B.C.).

Summarization of Data

The data on the response of PL, PC, LPC, and their species to the dairy consumption were summarized as
significantly increased, decreased, or not changed by the intervention(s). We grouped the included studies by health conditions of the participants/animal models and duration of the intervention. The results were synthesized as a narrative summary of human and animal studies separately.

**RESULTS**

**Overview of Publications**

In this review, 2,427 articles were identified and screened after removing duplicates. After evaluation of the titles and abstracts, 133 papers were selected for
full-text assessment, of which 15 studies were identified that met the inclusion criteria. The citation lists of all included references were then reviewed to identify any additional relevant articles. Studies were classified according to their type, resulting in 7 human trials (Hlavatý et al., 2008; Tardy et al., 2009; Keller et al., 2013, 2014; Meikle et al., 2015; Weiland et al., 2016; Markey et al., 2017) and 8 animal studies (Ramaprasad et al., 2003; Higuchi et al., 2008; Wat et al., 2009; Kamili et al., 2010; Hanning et al., 2019; Millar et al., 2019; Zhou and Ward, 2019; Millar et al., 2020).

All human studies were randomized trials, either parallel (Hlavatý et al., 2008; Tardy et al., 2009; Weiland et al., 2016; Norris et al., 2017) or crossover design (Keller et al., 2013, 2014; Meikle et al., 2015; Markey et al., 2017) with duration ranging from 1 to 12 wk. Participant mean ages ranged from 25 to 63 yr. The participants of 3 studies were apparently healthy, or there was no restriction on the inclusion criteria (Keller et al., 2013, 2014; Meikle et al., 2015), whereas others selected individuals with high risk for cardiovascular disease (Markey et al., 2017), overweight, or obesity (Hlavatý et al., 2008; Tardy et al., 2009; Weiland et al., 2016; Norris et al., 2017).

Of 7 human studies, only 4 considered plasma concentration of PL as the primary outcome (Keller et al., 2013, 2014; Meikle et al., 2015; Weiland et al., 2016). Interventions varied from milk supplemented with 2, 3, or 6 g of milk-derived PL (Keller et al., 2013, 2014; Weiland et al., 2016) and manipulation of dairy product consumption in the regular daily diet of participants (Hlavatý et al., 2008; Tardy et al., 2009; Weiland et al., 2016; Norris et al., 2017). A summary of the included studies’ details and findings are presented in Table 1, sorted by year of publication.

Most animal studies (n = 7) were conducted in mice (Higuchi et al., 2008; Wat et al., 2009; Kamili et al., 2010; Millar et al., 2019; Zhou and Ward, 2019; Millar et al., 2020) except for 2 studies, which selected rats (Ramaprasad et al., 2003, Hanning et al., 2019; Table 2). Interventions included milk supplemented with milk-derived PL (Wat et al., 2009; Kamili et al., 2010; Millar et al., 2019; Zhou and Ward, 2019; Millar et al., 2020), cheese (Hanning et al., 2019), MUFA-enriched dairy foods (Higuchi et al., 2008), and 1 that used milk fat (Ramaprasad et al., 2003). Only 2 out of 7 studies considered PL or its subclasses as a primary outcome (Kamili et al., 2010; Hanning et al., 2019).

**Study Quality and RoB**

The results of the RoB evaluation for human and animal studies are shown in Figures 2 and 3, respectively. Three human studies were assigned high RoB (Hlavatý et al., 2008; Keller et al., 2013; Markey et al., 2017) with the main reason being a lack of information on randomization, concealment methods, and blinding of participants and investigators, which is a limitation of most nutrition interventions. The results for RoB were assessed as low for 2 studies (Tardy et al., 2009; Keller et al., 2014) and as some concerns in 2 randomized controlled trials (RCT; Meikle et al., 2015; Weiland et al., 2016) because of the randomization method.

The ARRIVE guidelines for reporting animal studies (https://arriveguidelines.org) have not been as widely adopted as the CONSORT guidelines for reporting RCT. Thus, assessing their RoB based on methodological quality is more difficult. Two studies (Wat et al., 2009; Kamili et al., 2010) did not report the randomized allocation of animals, leading to high RoB, whereas other authors (Ramaprasad et al., 2003; Higuchi et al., 2008; Hanning et al., 2019; Millard et al., 2019; Zhou and Ward, 2019; Millar et al., 2020) stated that the allocation was randomized, but procedural details were not reported. Baseline characteristics between groups were poorly reported; therefore, we selected the initial reported BW to evaluate RoB. Only 4 studies received low RoB (Higuchi et al., 2008; Wat et al., 2009; Kamili et al., 2010; Millar et al., 2020) based on no BW differences between groups at baseline, whereas the rest were unclear. None of the studies reported data on blinding of caregivers or investigators or on the random selection of animals for outcome assessment, leading to the assignment of unclear RoB. No blinding of the outcome assessor was reported; however, the outcome of interest (PL) was an objective measurement less subject to bias than subjective measures. Regarding attrition bias, only 1 study reported that a considerable number of animals died during the study (Zhou and Ward, 2019); for other studies, information about the animal loss was adequate, and a similar number of analyzed animals in each group of the studies was reported. On this basis, all studies were assigned a low RoB for attrition. The assessment of PL and its subclasses was the primary outcome of 2 included studies, so the RoB for this domain was judged as low (Kamili et al., 2010; Hanning et al., 2019).

**Human Studies**

In Table 1, the main study characteristics and results of the effect of dairy consumption on PL concentration are summarized. Overall, circulating PL ranged from 1.93 to 2.23 mmol/L in all human subjects, regardless of their health. In the following sections, the studies are described in detail based on the initial health of participants.

**Healthy Participants.** Only 2 studies focused on healthy participants. One trial evaluated the acute
<table>
<thead>
<tr>
<th>Author, year</th>
<th>Participant&lt;sup&gt;1&lt;/sup&gt; (age/n/risk factors/gender)</th>
<th>Design&lt;sup&gt;2&lt;/sup&gt;</th>
<th>Intervention&lt;sup&gt;3&lt;/sup&gt;</th>
<th>Duration (wk)</th>
<th>Main result&lt;sup&gt;4&lt;/sup&gt;</th>
<th>Outcome of interest&lt;sup&gt;5&lt;/sup&gt;</th>
</tr>
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<tbody>
<tr>
<td>Markey et al. (2017)</td>
<td>25–70 yr/54/high risk for CVD/both</td>
<td>Crossover RCT</td>
<td>Isocaloric HF diet (38% total energy) with SFA-reduced, MUFA-enriched dairy products (modified) or regular dairy (control)</td>
<td>12</td>
<td>Modified vs. control: ↓ PC species including 14:0, 15:0, 17:0, 20:3, total SFA, ↑ PC species including 16:0, 18:1, total MUFA</td>
<td>There was no significant treatment effect for anthropometric measurements.</td>
</tr>
<tr>
<td>Weiland et al. (2016) Trial 1</td>
<td>50–76 yr/62/overweight or obese/men</td>
<td>Double-blind, parallel-group RCT</td>
<td>Participants consumed 200 mL/d LF milk enriched with 2 g of milk-PL (PL group) or 200 mL/d of milk enriched with 2 g of milk fat</td>
<td>8</td>
<td>Plasma total PL not affected by the intervention (1.97 ± 0.32 vs. 1.93 ± 0.37)</td>
<td>GGT activity decreased.</td>
</tr>
<tr>
<td></td>
<td>Trial 2</td>
<td>50–76 yr/57/overweight or obese/men</td>
<td>Double-blind, parallel-group RCT</td>
<td>Participants consumed 250 mL/d of LF milk enriched with 3 g of milk-PL (PL group) or 250 mL/d of milk enriched with 2.8 g of soy-PL</td>
<td>7</td>
<td>Plasma total PL not affected by the intervention (2.27 ± 0.41 vs. 2.22 ± 0.35)</td>
</tr>
<tr>
<td>Meikle et al. (2015)</td>
<td>40–46 yr/16/healthy/men</td>
<td>Crossover RCT</td>
<td>Participant consumed a breakfast containing HF dairy products or a breakfast containing soy oil-based foods</td>
<td>Postprandial (1–4 h after eating)</td>
<td>Dairy meal vs. baseline: No change in total LPC, ↑ plasma total PC, ↑ PC species including 28:0, 29:0, 30:0, 32:0, 32:1, 32:2, 32:3, 33:2, 33:3, 34:2, 34:3, 35:2, 35:3, 36:1, 36:3, 36:5, 36:6, 38:3, and LPC species including 18:3, 24:0, ▼ LPC species including 20:1, 22:5</td>
<td>No metabolic parameters were measured.</td>
</tr>
<tr>
<td>Keller et al. (2014) Adults/39/atopic dermatitis-metabolically healthy/both</td>
<td>Double-blind, crossover RCT</td>
<td>Participants consumed 250 mL/d of LF milk enriched with 3 g of milk-PL (PL group) or 250 mL/d of whole milk (control)</td>
<td>6</td>
<td>Plasma total PL (2.13 ± 0.36 vs. 2.09 ± 0.29 in PL group and 2.12 ± 0.33 vs. 2.09 ± 0.35 in control), PC, and LPC not affected by the intervention</td>
<td>There was no treatment effect on lipid profile and inflammation parameters.</td>
<td></td>
</tr>
<tr>
<td>Keller et al. (2013) Adults/14/healthy/women</td>
<td>Double-blind, crossover RCT</td>
<td>Participants consumed LF milk enriched with 3 g of milk-PL (low-PL group) or LF milk enriched with 6 g of milk-PL (high-PL group) or LF milk enriched with 2 g of milk fat (control)</td>
<td>10 d</td>
<td>Plasma total PL (2.27 ± 0.44 vs. 2.35 ± 0.36 vs. 2.29 ± 0.41 and PC not affected by the interventions)</td>
<td>TC in plasma was lower after low-PL in comparison with baseline. TC and LDL cholesterol rose significantly compared with low-PL PS-PL, resulted in lower LDL cholesterol compared with high PL.</td>
<td>continued</td>
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</table>
impact of dairy products on circulating postprandial PL. Compared with soy-based foods, a breakfast meal comprised of high-fat (HF) dairy foods (consisting of cheddar cheese, butter, and extra creamy whole milk) increased postprandial total plasma PC and PC containing dairy-derived fatty acids C15:0 and C17:0, but not LPC or circulating triglycerides (Meikle et al., 2015). After the dairy meal, postprandial plasma PC species including 29:0, 33:2, 33:3, 35:2, 35:3, 28:0, 30:0, 32:0, 32:2, 32:3, 34:2, 34:3, 36:1, 36:3, 36:5, 36:6, 38:3, and LPC 18:3 and 24:0 increased significantly. However, plasma LPC species including 20:1, 22:5, and 18:1 decreased after a HF dairy meal (Meikle et al., 2015). In healthy women, a short-term (10 d) intervention with milk enriched with milk-derived PL of 3- or 6-g dose had no effect on plasma PL, PC, and LPC (Keller et al., 2013). Decreased high-density lipoprotein cholesterol compared with baseline after 3 g of milk-PL intervention was noted, but no correlations with plasma PL were presented. Interestingly, measurements in feces indicated a slight milk-PL dose-dependent increase in PL excretion (Keller et al., 2013). Similarly, a randomized crossover trial with 6-wk intervention arms found no effect of consuming LF milk supplemented with 3 g of milk-PL compared with whole milk on either plasma total PL, PC, or LPC or on lipid profile or inflammatory markers (Keller et al., 2014). However individual PC and LPC species were not reported in the latter 2 studies (Keller et al., 2013, 2014).

Participants with Cardiometabolic Risk Factors. No acute studies were identified. In women with obesity, a 3-wk intervention with regular yogurt showed that, compared with baseline, PC 16:0 and 18:1n-7 increased, whereas PC species including 14:0, 18:0, 20:2n-6, 20:3n-6, 20:4n-6, 20:5n-3, total SFA decreased, compared with the baseline values without alterations in total PL (Hlavatý et al., 2008). Participants’ body mass index, LDL cholesterol, and C-reactive protein were lower after a yogurt in comparison with baseline. However, this result was confounded by prescribed 30% reduction in energy intake as part of the intervention.

The impact of dairy products on circulating PL was consistent in 4 studies with interventions ≥4 wk. Three studies (2 trials) were conducted on people with obesity (Tardy et al., 2009; Weiland et al., 2016), with 1 considering additional cardiovascular risk factors (Markey et al., 2017). One of the parallel-arm RCT (using milk-PL enriched LF milk) found no effect on total plasma PL (Weiland et al., 2016). This study also found no intervention effect on most metabolic outcomes. However, Weiland et al. found either increased gamma-glutamyl transferase in the milk fat control group (trial 1) or reduced gamma-glutamyl transferase in the PL group.
<table>
<thead>
<tr>
<th>Author, year</th>
<th>Model</th>
<th>Intervention¹</th>
<th>Duration (wk)</th>
<th>Main result²</th>
</tr>
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<tbody>
<tr>
<td>Millar et al. (2020)</td>
<td>Mice LDLr⁻/⁻ C57BL/6J</td>
<td>HFPL1: HF diet + milk-PL 1% by wt; HFPL2: HF diet + milk-PL 2% by wt; Control: HF diet</td>
<td>14</td>
<td>No change in PC and PL of liver</td>
</tr>
<tr>
<td>Zhou and Ward (2019)</td>
<td>Mice C57BL/6J ob/ob</td>
<td>GG: standard diet + milk gangliosides at 0.2 g/kg of diet; PL: standard diet + with milk-PL at 10 g/kg of diet; Control: standard diet</td>
<td>2</td>
<td>↑ Total serum PL in the PL group compared with the GG and control groups; No change in PC and PL of liver</td>
</tr>
<tr>
<td>Hanning et al. (2019)</td>
<td>Rat Sprague Dawley</td>
<td>REG: HF diet + regular-fat cheese diet; Low: HF diet + LF cheese diet; HF diet: HF diet; LF: LF diet</td>
<td>8</td>
<td>Low vs. HF diet: ↑ LPC species including 14:0, 16:0, 16:1, 17:0, 18:1, 20:3, and 24:0 and PC species including 30:0, 32:1, 34:1, 34:3, 34:4, 36:3, 36:5, 38:3, 38:0; REG vs. HF diet: ↑ LPC species including 14:0, 16:0, 16:1, 17:0, 18:1, 20:3, and 24:0 and PC species including 30:0, 32:1, 34:1, 34:3, 34:4, 36:3, 36:5, 38:3, 38:0, 42:0; REG vs. low: ↑ PC species including 30:0 and LPC species including 14:0; ↓ PC species including 32:1, 34:3, 30:0, 36:5, 42:0; LPC species including 16:0; Plasma and liver total PL, PC, LPC were not affected by intervention</td>
</tr>
<tr>
<td>Milard et al. (2019)</td>
<td>Mice C57Bl/6</td>
<td>HFPL1: HF diet + milk-PL 1.9% (wt/wt); HFPL2: HF diet + milk-PL 3.8% (wt/wt); HF: HF diet; LF: LF diet</td>
<td>8</td>
<td>Liver content in PL, total SFA, MUFA, and PUFA did not differ among groups; HFPL1 vs. HF: ↑ PL species including 20:2, 23:0, 24:0; HFPL2 vs. HF: ↑ PL species including 20:2, 23:0, and 24:0; ↓ PL species including 18:1(n-7); HFPL2 vs. HFPL1: ↑ PL species including 20:2, 23:0; ↓ PL species including 18:1(n-7); HFPL1 vs. LF: ↓ PL species including 18:1(n-9), 22:0, 20:3(n-3), 20:4(n-6), 23:0, 24:0; ↓ PL species including 14:0, 15:0, 16:1, 18:1(n-7), 18:2(n-6), 20:1, 20:5, 24:1; HFPL2 vs. LF: ↑ PL species including 18:1(n-9), 22:0, 20:4, 23:0, 24:0; ↓ PL species including 14:0, 15:0, 16:1, 18:1(n-7), 18:2(n-6), 20:1, 20:5, 24:1; Plasma and liver total PL, PC, LPC were not affected by intervention</td>
</tr>
<tr>
<td>Kamili et al. (2010)</td>
<td>Mice C57BL/6</td>
<td>HFPL: HF diet + milk-PL; Control: HF diet</td>
<td>8</td>
<td>Plasma PL was not affected by the intervention; HFPL vs. control: Feces: ↑ all PL species and PC; Plasma: ↑ PC species including 32:1, 34:1, 32:0, 34:0; Liver: total PL was not affected by the intervention; ↓ total PC, PC species including 34:1, 34:2, 38:6; PC 36:3</td>
</tr>
<tr>
<td>Wat et al. (2009)</td>
<td>Mice C57BL/6</td>
<td>HFPL: HF diet + milk-PL 2.5% (wt/wt); HF diet: HF diet; NPL: standard diet + milk-PL 2.5% (wt/wt)</td>
<td>8</td>
<td>HFPL vs. HF diet: Liver: ↓ PL; Serum: ↓ PL</td>
</tr>
<tr>
<td>Higuchi et al. (2008)</td>
<td>Mice Crlj:CD-1 (ICR)</td>
<td>10% yogurt; 30% yogurt; N: normal diet</td>
<td>12</td>
<td>Plasma: 10% yogurt and 30% yogurt had lower PL concentrations than control group; No change in PC and PL of liver</td>
</tr>
<tr>
<td>Ramaprasad et al. (2003)</td>
<td>Rats wistar</td>
<td>Standard diet + milk fat; GNO: standard diet + groundnut oil; High-cholesterol diet + milk fat; GNO: high-cholesterol diet + groundnut oil</td>
<td>8</td>
<td>Standard diet: Plasma: ↑ PL in milk fat; Liver: no difference between groups; Cholesterol-enriched diet: Plasma: no difference between groups; Liver: no difference between groups</td>
</tr>
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</table>

¹HFLP = high-fat diet plus phospholipid; HF = high-fat; LF = low-fat; PL = phospholipid; GG = gangliosides; REG = regular; NPL = standard (normal) diet with phospholipid; N = normal diet; GNO = groundnut oil.

²PC = phosphatidylcholine; LPC = lysophosphatidylcholine; up arrows = increased; down arrows = decreased.
(compared with a soy-PL control, trial 2), which was interpreted as a benefit of milk-PL on fatty liver (Weiland et al., 2016).

Dairy-derived biomarkers PL 15:0 and 17:0 in the blood can indicate intervention compliance (Pranger et al., 2019; Azab et al., 2020). Two trials examined the incorporation of specific fatty acids into the plasma PL fraction. Tardy et al. (2009) found that provision of ruminant trans fats increased their abundance in circulating PL while also increasing total MUFA and reducing total SFA and PUFA in PL. Markey et al. (2017) likewise found that feeding dairy enriched in MUFA and depleted in SFA (decreased amount of PC 15:0 and 17:0) led to a similar pattern in fatty acids incorporated into plasma PL. Thus, although most studies show null effects on total PL, differences can be observed when PL species are quantified. In addition to being a measure of compliance when C15:0 and C17:0 are measured, it is possible that certain species may exert biological activities. However, Tardy et al. (2009) did not find differences in BW or metabolic outcomes in women with abdominal obesity, and Markey et al. (2017) performed no metabolic measurements.

## Animal Studies

Eight studies assessed the effect of dairy intake on PL metabolism in animals, with 6 employing obese rodent models or HF background diet to induce obesity. Regarding total plasma PL in obese mice and rats, Hanning et al. (2019) and Kamili et al. (2010) found no difference, whereas Zhou and Ward (2019) reported an increase and Wat et al. (2009) reported a decrease after dairy consumption. Hanning et al. (2019) was the only study to use cheese, whereas the other groups employed milk-PL or milk gangliosides; in fact, all studies intervened for 8 wk. Total hepatic PL was likewise unchanged by the intervention in 5 studies (Kamili et al., 2010; Hanning et al., 2019; Milard et al., 2019; Zhou and Ward, 2019; Millar et al., 2020), with Wat et al. (2009) noting a decrease. Interestingly, fecal PL excretion increased by 2.8-fold in the latter study (Wat et al., 2009) and Kamili et al. (2010) also noted increased excretion of PL. On a standard LF diet background, enrichment with milk-PL had no effect on plasma or hepatic PL in mice with a mutation of the obese (ob) gene encoding leptin (Zhou and Ward, 2019).

In non-obese rodents, PL increased in plasma but not liver in a rat study using standard LF diet plus milk fat compared with LF control diet (Ramaprasad et al., 2003). However, PL concentration and liver content were stable after adding a certain amount of PL in their standard diet (Wat et al., 2009). Conversely, on a high-cholesterol diet background, milk fat had no effect on PL in plasma or liver (Higuchi et al., 2008). Even in the absence of effects on total PL, multiple 8-week
trials in obese rodent models that measured PL species reported alterations (Kamili et al., 2010; Hanning et al., 2019; Milard et al., 2019). A combination of HF diet with 1.9 or 3.8% (wt/wt) milk-PL increased hepatic PL 20:2, 23:0, and 24:0 compared with the HF diet group (Milard et al., 2019), but plasma concentrations were not reported. Plasma PC species 32:1, 34:1, 32:0, and 34:0 increased, but liver total PC and species 34:1, 34:2, 38:6, and 36:3 decreased (Kamili et al., 2010). In a study in rats, several PC and LPC species decreased in the HF diet group compared with the LF diet. However, intervention with regular- or reduced-fat cheese increased their concentration toward normal. For example, HF diet-induced reductions in LPC species such as 14:0, 16:0, 16:1, 17:0, 18:1, and 20:3 and PC species including 30:0, 34:1, 34:3, 34:4, 36:3, 36:5, and 38:3 were increased to the LF diet values by the cheese interventions (Hanning et al., 2019).

**DISCUSSION**

This scoping review synthesized the research on the effects of dairy intake or milk-PL on circulating PL and its subclasses, including LPC and PC. Although the design and quality of included intervention studies were heterogeneous, and some were not specifically designed to investigate the effects of dairy intake, the findings were relatively consistent. The main finding is that, in contrast to acute intervention, chronic human studies 10 d to 12 wk long generally demonstrate that total circulating PL, PC, and LPC are not affected by intervention with dairy products, remaining stable even after a high dosage of milk-PL. However, the serum concentration of PC and LPC species can be altered by dairy products or dairy-derived PL. This finding was consistent with the results of rodent studies. Effects on total PL may be limited by increased fecal excretion. To date, associations with cardiometabolic outcomes are weak and poorly supported by mechanistic studies.

Associations of PC and LPC species with metabolic dysfunctions have been reported in several studies (Hsu et al., 2000; Szymańska et al., 2012; Ferrannini et al., 2013; Cai and Yang, 2018; Semba et al., 2018; Suvitaival et al., 2018; Yin et al., 2020). In a recent cross-sectional study using a targeted metabolomics approach, some PC species (32:0, 32:1, 32:2, 34:1, 34:2, 34:3, 36:2, 36:3,
40:5, 40:6, 42:3, 42:4, and 42:5) were associated with a lower risk of insulin resistance (Sembra et al., 2018). In addition, LPC 18:0, 18:1, and 17:0 have been identified as negative predictors of type 2 diabetes (Suvitaival et al., 2018). In a human study, circulating PC and LPC species rapidly change after even 1 meal that contains a high amount of specific dairy-derived PL, and these extensive alterations remain up to 4 h (Meikle et al., 2015). The PL content in dairy can possibly compensate for those PC or LPC species that change in the context of metabolic dysfunction. Although mice exhibit significant differences in lipid metabolism versus humans (Bergen and Mersmann, 2005; Takahashi et al., 2016), some animal trials provide data regarding PC and LPC in serum that associate with benefits on insulin sensitivity (Hanning et al., 2019) or liver associated with reduced weight gain (Milard et al., 2019). The circulating PC species including 32:0, 32:1, 32:2, 34:1, 34:3, and 36:3 were considerably elevated after a course of dairy intervention in rodents (Kamili et al., 2010; Hanning et al., 2019). Furthermore, LPC species including 18:0, 18:1, and 17:0 also tended to increase (Hanning et al., 2019). Other studies found that milk consumption was associated with lower adiposity, increased insulin sensitivity, and improved glucose homeostasis among diabetic and prediabetic rats (Matsumoto et al., 2009; Yoshimura et al., 2018). In addition to milk intake, yogurt consumption enhanced insulin sensitivity in rodents (Johnson et al., 2007; Qu et al., 2018; Lasker et al., 2019). However, these studies did not report PC and LPC, and hence the degree to which long-term dairy food intervention increase food-specific PC and LPC species is only reported by Hanning et al. (2019).

Furthermore, plasma PL species are highly sensitive markers of the fatty acid composition of acute interventions (Meikle et al., 2015), persisting up to 3 wk (Hlavatý et al., 2008). However, the circulating PC and LPC species that were increased following the intervention were not all specifically dairy-derived (Meikle et al., 2015; Markey et al., 2017). Untargeted metabolomic analyses also indicated a great many more PL species were altered that might not be directly attributable to intake of dairy products or milk-PL (Meikle et al., 2015; Zheng et al., 2016). The capacity of meal-derived lipids to alter plasma PL pools is determined by absorption and subsequent metabolic processes, as well as the quantity of lipid in the meal and the size of the plasma PL pools (Lambert and Parks, 2012). Extensive processing or differences in the food matrix likely affect the PL content of dairy foods and their absorption; thus, it is tempting to speculate that individual dairy products have a specific effect on the serum concentration of PL species. Therefore, short- and long-term human studies are needed to elucidate how PL and its species are regulated in response to different types of dairy products. In addition, animal studies are also required to determine the mechanism and improve our understanding of how dairy foods change PL species, particularly those not attributable to dairy sources (Meikle et al., 2015; Hanning et al., 2019).

Human trials of healthy participants using dairy enriched in PL (Keller et al., 2013, 2014) found alterations in circulating cholesterol, but because total circulating PL and PC were not changed, the mechanism is unclear. Possibly, measuring incorporation of dairy-derived PL into membranes could shed additional information. Studies of people with cardiovascular disease risk yielded variable cardiometabolic outcomes, but these could not be attributed to dairy interventions in most cases (Weiland et al., 2016). In contrast, in trials where the intervention increased dairy-derived fatty acids in the PL fraction, no metabolic improvements including insulin sensitivity or plasma lipids were observed (30) and 1 study did not report metabolic outcomes (Markey et al., 2017). A current meta-analysis of 30 human RCT of dairy interventions showed a beneficial effect on homeostatic model assessment for insulin resistance (Sochol et al., 2019); however, the effect of individual dairy foods was not presented nor were PL measured. Furthermore, based on findings from clinical studies (Pietiläinen et al., 2007; Wahl et al., 2012; Martínez-Ramírez et al., 2016), an RCT in humans with impaired PL subclasses is required to measure the efficacy of dairy PL from different dairy foods on PL subclasses and species and any associated benefits on glucose and lipid metabolism or body composition.

In the current scoping review, we focus on the most highly consumed dairy products, milk, yogurt, and cheese, as the greatest source of dairy-derived PL in the diet. For example, in a nationally representative sample of adult Canadians, these 3 products plus frozen dairy products contribute >95% of total dairy intake (Auclair et al., 2019). There are also various milk-based products with high content of PL that are consumed in lower amounts, such as buttermilk, cream, and butter that were excluded from this review. In this regard, an 8-wk single-blind, parallel-group RCT with 1 dL per day of whipping cream (139 to 190 mg of PL per 100 g) did not affect plasma total PL and PL species. However, increases in LDL cholesterol, non-high-density lipoprotein cholesterol, ApoB, and total cholesterol were observed compared with a control group consuming milk protein isolate (Rosqvist et al., 2015), which might be attributable, at least in part, to the saturated fat content. Another double-blinded randomized crossover placebo-controlled study indicated that 4 wk of buttermilk (93.7 mg of PL per day) consumption significantly reduced serum total cholesterol and triglyceride con-
centrations compared with the placebo (17.3 mg of PL) group, but effects on circulating PL were not reported (Conway et al., 2013). Results of these 2 studies do not alter our conclusion that consumption of dairy PL does not alter total circulating PL. However, further studies comparing individual dairy foods’ effects, including those with naturally high PL concentrations, on specific species of PL and potential biological outcomes are warranted.

This review has identified other limitations and gaps in the current literature. Heterogeneity in designs, methodology, and reporting in the included studies, makes conclusions challenging. Lipid metabolism differs between species limiting the transposition of findings from rodents to humans; however, some general similarities between humans and rodents support using rodents as a model for many aspects of human lipid metabolism (Gordon et al., 2015, Kaabia et al., 2018). Questions requiring further consideration are the extent to which PC or LPC species are raised after intervention by dairy products and the involvement of metabolic pathways that regulate the balance of PL species. In the current scoping review, we included studies that intervened with milk, yogurt, and cheese, all classified according to dairy products on MyPlate, the USDA dietary guidelines. However, other milk-based foods high in fat but still rich in PL, such as cream and buttermilk, were excluded. It is a possible limitation of the current work, and the effect of PL concentration might also be interesting. Another limitation was the potential confounding effect of participants’ habitual dietary intake before and during the intervention in human trials. Dairy foods may be consumed in processed or other foods, leading to underestimating the actual intake of dairy products, especially in the control groups. Because PL or its subclasses was not the primary outcome in most studies, results should be interpreted with caution because nonsignificant changes might result from lack of power rather than actual effect. The physiological basis for benefits of dairy PC and LPC on metabolism remains unclear, with evidence to the contrary also reported (Liu et al., 2020). Future research is needed to fill up the gaps and limitations in order to explore the effect of dairy foods on PL metabolism. Nonetheless, the current scoping review covered research using a variety of methodologies, allowing us to compile data from human and animal studies evaluating dairy-derived PL under various settings and, as a result, identify features that could be improved in future studies. As a strength of this study, the application of multiple databases provides a broader range of published papers and contribution of 2 researchers to conduct the literature search and study assessments for quality control.

CONCLUSIONS

Experimental and clinical studies provide evidence that total circulating PL is tightly regulated, even in interventions with a high milk-PL dosage. Data regarding the impact of dairy on total PC and LPC remain inconclusive. However, their species in the blood or liver have been repeatedly altered in response to dairy products without significant changes in total circulating PC. Our scoping review identified the need for additional human studies with a large number of participants and with a specific focus on individual dairy products, detailed dietary data, and strict intake control to provide stronger evidence and overcome the limitations previously discussed. Additional animal trials are warranted to describe better the PL-related metabolic pathways linked to the individual dairy products.

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