Cardiometabolic health benefits of dairy-milk polar lipids

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Low-quality dietary patterns impair cardiometabolic health by increasing the risk of obesity-related disorders. Cardiometabolic risk relative to dairy-food consumption continues to be a controversial topic, due to recommendations that endorse low-fat and nonfat dairy foods over full-fat varieties despite accumulated evidence that does not strongly support these recommendations. Controlled human studies and mechanistic preclinical investigations support that full-fat dairy foods decrease cardiometabolic risk by promoting gut health, reducing inflammation, and managing dyslipidemia. These gut- and systemic-level cardiometabolic benefits are attributed, at least in part, to milk polar lipids (MPLs) derived from the phospholipid- and sphingolipid-rich milk fat globule membrane that is of higher abundance in full-fat dairy milk. The controversy surrounding full-fat dairy food consumption is discussed in this review relative to cardiometabolic health and MPL bioactivities that alleviate dyslipidemia, shift gut microbiota composition, and reduce inflammation. This summary, therefore, is expected to advance the understanding of full-fat dairy foods through their MPLs and the need for translational research to establish evidence-based dietary recommendations.

INTRODUCTION

A substantial proportion of the global population has poor cardiometabolic health. This is reflected by the high prevalence rates of numerous, interrelated cardiometabolic diseases and disorders (eg, obesity, prediabetes and diabetes, metabolic syndrome [MetS], and nonalcoholic fatty liver disease [NAFLD]).[1–3] Costs to manage these conditions also pose a substantial societal burden, with expenditures in the United States alone exceeding trillions of dollars annually for the direct and indirect costs of obesity, type 2 diabetes mellitus (T2DM), and cardiovascular disease (CVD).[4] Critical to improving human health is establishing effective dietary practices that can alleviate the risk of developing these disorders or that can reverse their progression toward causing premature death. This is especially important because many persons with impaired cardiometabolic health have subclinical conditions (eg, prediabetes, MetS), which are often left unmanaged other than to encourage lifestyle modification. Although numerous foods are rich in bioactive components and have the potential to improve cardiometabolic health, bovine-derived dairy foods have been touted for their low cost and nutrient-rich matrix of high-quality proteins and micronutrients.[5]

Although humans are the only mammal who drink dairy milk throughout their lifespan, its consumption is

known to decrease in an age-dependent manner.[6] Furthermore, recommendations support 3 daily servings of dairy foods to help achieve nutritional adequacy of several essential vitamins and minerals, but American adults are estimated to only achieve ∼50% of this amount.[7] Indeed, estimates indicate that if Americans were to meet dairy recommendations, billions of dollars could be saved annually from a reduction in several cardiometabolic diseases and certain cancers and neurological disorders.[7] Despite the potential of dairy foods to thwart cardiometabolic risks, controversy surrounds recommendations that encourage low-fat or nonfat varieties over full-fat dairy foods to reduce the intake of saturated fat and maintain energy balance.[8] This controversy exists because compelling evidence supports full-fat dairy foods to protect against cardiometabolic disorders (eg, obesity, MetS, T2DM), whereas outcomes of relatively few studies are neutral or suggest adverse effects.[9]

The specific components of full-fat dairy foods responsible for their putative health benefits are not well established, but the milk fat globule membrane (MFGM) has received significant attention.[10] The MFGM is a unique, trilayer membrane that contains the milk polar lipids (MPLs; ie, milk phospholipids and sphingolipids) and surrounds the lipids of the milk fat globule (MFG) core. MPLs are abundant in certain higher-fat dairy foods but can be reduced during the commercial processing that produces lower-fat dairy foods.[10] The reduced dietary availability of MPLs raises health concerns because they show promise to favorably influence intraluminal lipid emulsions,[11] gut barrier functions,[12] dyslipidemia,[13] and inflammation[14] toward improved cardiometabolic health. Therefore, in this review, we discuss the controversy surrounding full-fat bovine dairy-food consumption relative to cardiometabolic risk and the reported bioactivities of MPLs that protect against dyslipidemia, improve gut microbiota composition, and decrease gut and systemic inflammation. This summary should advance the understanding of the health benefits of full-fat bovine dairy foods mediated through their MPLs and the need for translational research to establish evidence-based dietary recommendations.

CARDIOMETABOLIC HEALTH

Cardiometabolic health is the term that describes the metabolic and cardiovascular health status of an individual.[15] Metabolic diseases are often characterized by mitochondrial dysfunction and altered host metabolism, which are implicated in insulin resistance, T2DM, NAFLD, obesity, and MetS. Whereas, CVD, including hypertension, cerebrovascular disease, and coronary artery disease, describes disorders of the heart and

circulation.[16] Importantly, cardiometabolic disorders precede overt CVD, consistent with evidence that persons with MetS are twice as likely to develop CVD than those without MetS.[17,18]

The high prevalence of cardiometabolic disorders is of substantial concern. Indeed, as is the case globally,[19] CVD is leading cause of death in the United States, with an estimated 121.5 million adults having some form of this disease.[20] The prevalence of obesity has increased by 20% from 1988 to 2018 and now affects 42.4% of Americans.[21] MetS afflicts ∼35% of all adults, and disproportionately affects those older than 60 y (48.6%) compared with 19.5% of those aged 20–39 y.[22] Consistent with NAFLD being the hepatic manifestation of MetS, an estimated 80 million to 100 million cases exist[23] and 10.8 million new cases are predicted by 2060, along with an economic burden of $350 billion.[24] Given the close association between MetS and diabetes, it is not surprising that age-related trends for T2DM are similar, with 4.2% of the 34.2 million diagnosed cases being among young adults (aged 18–44 y) vs 26.8% among those > 65 y of age.[25] Also, 34.5% of US adults have prediabetes,[25] suggesting that the incidence of T2DM will increase in the coming years. Although some overlap in the prevalence of these cardiometabolic disorders exists, it is unquestionable that a substantial proportion of the population has compromised cardiometabolic health, and this drives efforts to mitigate the risk for premature death.

Although CVD-related death has a high incidence, 90% of its known risk factors (eg, dyslipidemia, hypertension, obesity, insulin resistance) are modifiable by diet and lifestyle.[26] A common underlying etiology of insulin resistance and impaired cardiometabolic health is chronic, low-grade inflammation,[27] including that attributed to postprandial metabolism. For example, during a meal, the intestinal lumen is exposed to complex foodstuffs (eg, macronutrients, saturated fatty acids), gut hormones, and bacteria. Low-quality dietary patterns (eg, Western diet,[28] typical American diet[29]) are implicated in hyperglycemia and hyperlipidemia.[30] Chronic excursions in blood glucose provoke the formation of advanced glycation end-products that induce damage to the vascular endothelium and exacerbate insulin resistance.[31] In close association with hyperglycemia and hyperlipidemia is metabolic endotoxemia, which results from the translocation of gut-derived endotoxins (eg, lipopolysaccharides derived from gram-negative bacteria) to the systemic circulation where it can bind cellular Toll-like receptor-4 to initiate NF-κB–dependent inflammation[32] and provoke cardiometabolic risk. That poor quality diets drive chronic inflammation supports the concept that dietary modification could have substantial influence on mitigating cardiometabolic risks. Although numerous dietary factors can

positively influence this paradigm, we describe in this review benefits of bovine dairy foods, with a focus on their controversial aspects: fat-free and nonfat varieties of dairy are recommended over full-fat varieties for a healthy dietary pattern[6,33] despite evidence that full-fat dairy may reduce cardiometabolic risks.[34] Importantly, we highlight in this review the bioactivities of MPLs (Figure 1) found in the MFGM that surrounds the triglyceride-rich MFG.

## BOVINE DAIRY-MILK COMPOSITION

Bovine dairy milk is well recognized for its nutrient-rich matrix of macronutrients and essential micronutrients.[33] The principal components of dairy milk, including its water, fat, proteins, and carbohydrate, were characterized during the early 20th century.[37] Its proteins are primarily casein (80%) and whey protein (20%), and its carbohydrate mainly is lactose.[37] Milk proteins contain all 9 essential amino acids to make it a high-quality protein source.[33] Proteomic analysis has also revealed hundreds of milk proteins, which have been reviewed elsewhere.[38–40] Separate from the major proteins of casein and whey, bovine milk contains membrane proteins in the MFGM. These proteins constitute 1%–4% of the total protein content and exist in a 3:2 ratio with MFGM polar lipids.[41] These minor proteins include lactadherin (or MFG–epidermal growth factor-8 [EGF-8]) > xanthine oxidase > butyrophilin, and varying quantities of mucin 1, cluster of differentiation 36, adipophilin, fatty acid–binding protein, and protease peptone 3, which have been reviewed elsewhere.[42,43] Milk fat exists primarily as triglycerides (∼98%); the remaining 2% presents as diacylglycerol, phospholipids (∼1%), cholesterol, and free fatty acids.[44] Milk fatty acids are predominantly saturated fatty acids (70%), followed by monounsaturated fatty acids (25%) and polyunsaturated fatty acids (3.4%).[45] Dairy milk also contains macrominerals, trace elements, and water- and fat-soluble vitamins. Importantly, the nutrient-dense matrix of dairy milk can help achieve nutritional adequacy for several nutrients (namely, calcium, magnesium, phosphorous, vitamin A, vitamin B_{12}, zinc, potassium, and choline).[46]

The lipid profile of raw milk is variable, due to its dependence on the diet and the microbial populations in the rumen of the cow. The application of biochemical techniques in the early 1900s simplified the approach to assess the fat content of dairy milk, which enabled an understanding that its fat content varied between 2% and 5%.[47] Modern-day chromatographic and mass spectrometry techniques have permitted the identification of > 400 types of fatty acids from dairy milk, with only 12 fatty acids present at proportions > 1%.[48] Palmitic acid (C16:0; ie, the carbon chain of palmitic acid consists of 16 carbon atoms and there are no double bonds), the most abundant saturated fatty acid, contributes 28%–34% of the total fatty acid species. Thereafter, myristic acid (C14:0; 11%) and stearic acid (C18:0; 12%) are the next most abundant fatty acids.[48,49] Of the unsaturated fatty acids, oleic acid (C18:1) predominates, at 20%–25% of all fatty acids.[44,48]

The MFGM contains 2 major classes of polar lipids that account for ∼1% of total lipids in milk (Figure 1). Milk phospholipids primarily contain a glycerol backbone as glycerophospholipids, whereas sphingolipids have a sphingoid base (eg, sphingosine).[35] Glycerophospholipids differ in their polar head groups, and, in order, the most abundant in dairy milk are phosphatidylethanolamine > phosphatidylcholine > phosphatidylinositol > phosphatidylserine. Sphingolipids also possess unique polar head groups. For example, sphingomyelin (SM), which is both a phospholipid and sphingolipid (ie, a phosphosphingolipid) and represents ∼25% of total MPLs,[10] can be formed by a phosphodiester bond with either phosphocholine or phosphoethanolamine. Glycolipids are another subclass of sphingolipids that are formed by glycosidic linkage of glucose at the C1 hydroxyl position of a sphingoid base to yield glucosylceramide; lactosylceramide is generated by the addition of a galactose residue. Glucosylceramide and lactosylceramide are also known as cerebrosides.[50] Phospholipids differ structurally from sphingolipids. They contain 2 esterified fatty acids, whereas sphingolipids are characterized by a single amide-linked fatty acid on the sphingoid base backbone.[36] The fatty acid substitutions on phospholipids are variable, but the sn-1 position tends to be a saturated fatty acid, whereas an unsaturated fatty is often at the sn-2 position.[51] Sphingolipids, with their single fatty acid, tend to contain saturated fatty acids. Morrison et al[52] reported that the predominant N-linked fatty acids on sphingosine are C18:0, C22:0, and C24:0, which is in alignment with recent reports that long-chain and very-long-chain saturated fatty acids (C16:0 to C24:0) are common in sphingolipids.[53] The fatty acid profile of milk triglyceride also differs from that of MPLs in that short-chain fatty acids (C4:0, C6:0, C8:0) are virtually absent in MPLs, whereas C16:0, C18:0, C18:1, and C18:2 are common.[53,54]

It is important to note that commercial processing substantially affects MFGM and its membrane-associated polar lipids. Fresh whole milk (3.5% fat with 35 mg of MFGM per 100 g) is used to produce cream (38% fat with 200 mg of MFGM per 100 g) by separating the milk-fat layer from milk before homogenization.[10] Cream can be churned into butter, but this mechanically vigorous process results in a butter product that has very little polar lipid content.[55] Buttermilk, the co-product of churning cream, has high MPL levels compared with other dairy products.[55] Indeed, buttermilk contains 2.03 g MPL per
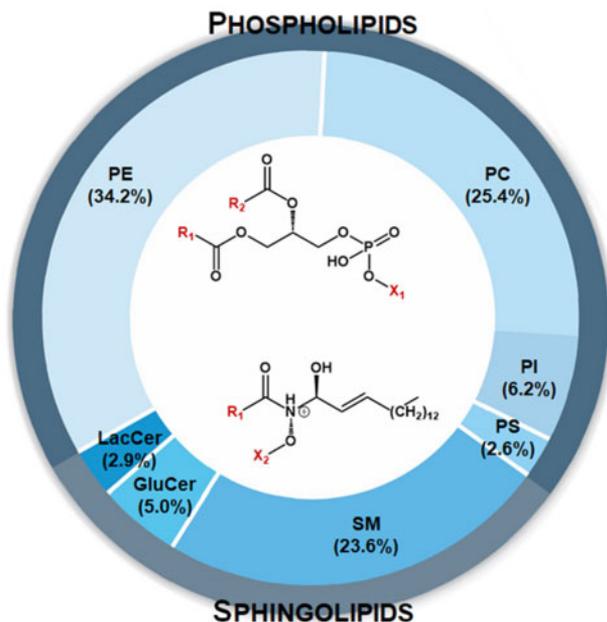
Figure 1 **Distribution of polar lipids in raw milk from cows.** The polar lipid content of bovine milk is 12.8–40.0 mg/100 g, with total phospholipid concentrations approximately doubling those of total sphingolipid concentrations.[35,36] Phospholipids contain a phospho-glycerol backbone, with a saturated fatty acid at R_1, an unsaturated fatty acid at R_2, and 1 of several alcohols (ie, ethanolamine, serine, inositol, or choline) at the polar head of X_1. Sphingolipids consist of an amide-containing sphingoid backbone (primarily sphingosine in milk). Similar to phospholipids, R_1 is a saturated fatty acid, whereas X_2 is substituted with phosphocholine, phosphoethanolamine, or a sugar. *Abbreviations*: GluCer, glucosyl ceramide; LacCer, lactosyl cer-amide; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PI, phosphatidylinositol; PS, phosphatidylserine; SM, sphingomyelin.

100 g dry matter compared with 0.17–0.26 g in butter.[10] That buttermilk is richer in MPLs than butter also explains why MFGM is often isolated from buttermilk for commercial use. Not only is the MFGM ingredient used to manufacture MFGM-enriched dairy products, it is also used in infant formula in an attempt to recapitulate the polar lipid content of breast milk.[56] Importantly, because the MFGM surrounds fat globules of milk, it is not sur-prising that the removal of dairy fat to manufacture low-fat and nonfat milk significantly reduces total MPL levels by up to 40% (Figure 2). Although the intent is to reduce dietary energy and saturated fat content, this practice of defatting milk may also contribute to the controversy concerning dairy foods and cardiometabolic health, be-cause it also depletes health-promoting MPLs.

CONTROVERSY SURROUNDING THE CONSUMPTION OF DAIRY AND DAIRY FAT ON CARDIOMETABOLIC HEALTH

The risk -benefit relationship between cardiometabolic disorders and dairy foods has received significant attention. This paradigm has been assessed in observa-tional studies, both retrospective and prospective, and evidence continues to accumulate from the relatively fewer controlled trials in humans. The work in this area has considered the potential benefits of whole dairy foods, including lower-fat and higher-fat varieties, as well as the macronutrient, micronutrient, and other bioactive components in whole dairy foods that are thought to influence cardiometabolic health. However, in this section, we focus on the ongoing scientific con-troversy of whether the consumption of full-fat dairy foods adversely affects cardiometabolic health.

In support of the benefits of dairy foods, but without consideration of fat content, results of a meta-analysis in-dicated that higher dairy intake among cohort studies (relative risk [RR] = 0.86; 95%CI: 0.79–0.92) and cross-sectional and case-control studies (odds ratio [OR] = 0.83; 95%CI: 0.73–0.94) was associated with a lower risk of MetS.[57] Dose-response analysis of prospec-tive studies also suggested a 6% lower risk of MetS with each 1-serving increment of dairy consumption (RR = 0.94; 95%CI: 0.90–0.98).[57] Another meta-analysis of cohort studies[58] that expanded upon earlier meta-analyses conducted by the same authors[59–61] confirmed that higher intake of total dairy (RR = 0.97; 95%CI: 0.95–1.00) and low-fat dairy (RR = 0.96; 95%CI: 0.92–1.00) corresponded to a 3%–4% lower risk of T2DM. Dairy food–specific associations were detected such that yogurt consumption was associated with a lower T2DM risk (RR = 0.86; 95%CI: 0.83–0.90; $P < 0.001$) and each 200 g intake of dairy milk was associated with an 8% lower risk of stroke (RR = 0.92; 95%CI: 0.88–0.97).[58] In a large, multicountry cohort study of > 136 000 individuals, greater intake of dairy food (> 2 servings/d and regardless of fat content) compared with diets devoid of any dairy foods were associated with a lower risk of cardiovascular-associated death (RR = 0.77; 95%CI: 0.58–1.01; $P = 0.029$), major cardiovascular events (hazard ratio [HR] = 0.78; 95%CI: 0.67–0.90; $P = 0.0001$), and stroke (HR = 0.66; 95%CI: 0.53–0.82; $P = 0.0003$); these rela-tions were observed without any altered risk of myocar-dial infarction.[62] In this study, associations were also influenced by intake of specific dairy foods. Greater milk (HR = 0.90; 95%CI: 0.82–0.99; $P = 0.053$) and yogurt consumption (for > 1 vs 0 servings/d, HR = 0.86; 95%CI: 0.75–0.99; $P = 0.0051$) corresponded with a lower risk of several cardiovascular-related end points, whereas neither cheese nor butter consumption correlated with these cardiovascular-related outcomes. Similarly, evidence from a cross-sectional study of Portuguese adolescents (aged 15–18 y; n = 494) showed that those with higher dairy-milk intake had significantly lower cardiometabolic risk scores (based on summing z-scores of all individual risk factors) than those with lower dairy-milk intake (10.6%
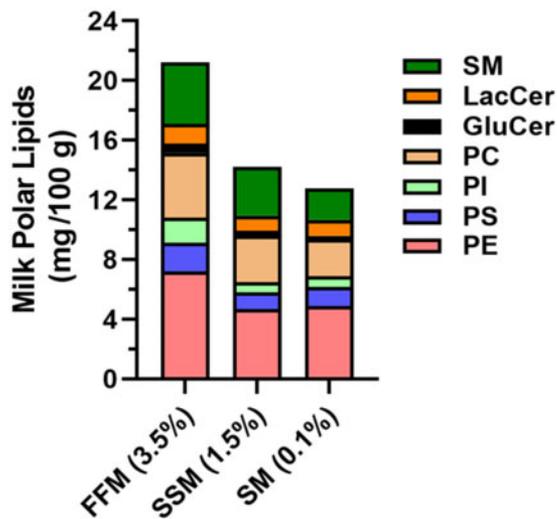
Figure 2 **Milk polar lipid content in fluid cow's milk.** Although the relative proportions of specific milk polar lipids are unaffected, the total quantity of milk polar lipid is reduced by ≤40% in SSM (1.5% fat, weight per weight) and skim milk (0.1%) compared with FFM (3.5%). Data from Rombaut et al.[55] *Abbreviations:* FFM, full-fat milk; GluCer, glucosyl ceramide; LacCer, lactosyl ceramide; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PI, phosphatidylinositol; PS, phosphatidylserine; SM, sphingomyelin; SSM, semi-skim milk

vs 18.1%).[63] Likewise, those with appropriate dairy intake (defined as being above the median for the population) were less likely to have elevated cardiometabolic risk scores (OR = 0.53; 95%CI: 0.30–0.93; $P < 0.027$). However, cardiometabolic risk scores of these persons were not associated with intake of total dairy, yogurt, or cheese. This finding suggests that the observed health benefits are milk specific and potentially reflect complex interactions in the overall dietary pattern.

Not all observational studies have detected positive outcomes for dairy-food consumption. In a prospective study of biracial urban adults (aged 30–64 y; n = 1371), researchers examined baseline and the annual change in dairy intake relative to cardiometabolic risk.[64] Cheese (HR = 1.13; 95%CI: 1.05–1.23; $P < 0.05$) and yogurt (HR = 1.21; 95%CI: 1.01–1.44; $P < 0.05$) consumption at baseline and the annual change in cheese (HR = 1.90; 95%CI: 1.47–2.47; $P < 0.05$) and yogurt (HR = 1.21; 95%CI: 1.01–1.44; $P < 0.05$) consumption were associated with an increased risk of central obesity. Central obesity risk, however, was unrelated to the consumption of total milk at baseline or its annual change. Baseline fluid-milk consumption, however, was associated with lower risk of MetS (HR = 0.86; 95%CI: 0.78–0.94; $P < 0.05$), with favorable associations specifically observed for high-density lipoprotein cholesterol–related dyslipidemia (HR = 1.10; 95%CI: 1.01–1.21; $P < 0.05$). In contrast, the annual change in dairy-fat consumption

from all foods (based on myristic acid [C14:0] intake relative to total fat consumption) was positively associated with MetS, specifically triglyceride and high-density lipoprotein cholesterol dyslipidemias, but not with other MetS criteria (ie, central obesity, hypertension, hyperglycemia).[64] Despite these disparate observations by dairy-food type, a strength of this study was its prospective assessment of dietary intake on 2 separate occasions, which would be expected to reduce measurement error of dietary variables. However, the authors acknowledged that dairy-food intake by the low-socioeconomic-status cohort in their study was approximately half a serving les than the national average. Furthermore, only 5% of these persons achieved the recommended 3 daily servings of dairy consumption, which may have influenced the observed relations.

Other cohort studies have also assessed the relationship of MetS risk with dairy-food intake but have considered whether full-fat dairy foods influence this association. The PURE study was a large-scale, multinational, prospective cohort study that examined the dietary habits of adults (aged 35–70 y) over a median follow-up of 9.1 years.[65] Findings suggested that ≥2 servings/d of dairy foods compared with zero daily servings corresponded to a 24% lower MetS risk (OR = 0.76; 95%CI: 0.71–0.80; $P < 0.0001$). Higher intake of whole-fat dairy alone (OR = 0.72; 95%CI: 0.66–0.78; $P < 0.0001$) or when consumed as part of a diet containing low-fat dairy (OR = 0.89; 95%CI: 0.80–0.98; $P = 0.0005$) was also associated with a lower prevalence of MetS. This finding was in contrast to observations indicating that low-fat dairy consumption was not associated with MetS risk, suggesting that milk-fat content mediates the health benefits of low-fat dairy. The PURE study also showed that higher dairy intake (≥2 servings/d vs 0) was associated with 11% (HR = 0.89; 95%CI: 0.82–0.97; $P < 0.02$) and 12% (HR = 0.88; 95%CI: 0.76–1.02; $P < 0.01$) lower incidence of hypertension and T2DM, respectively. Similar analyses also assessed the relationship of whole-fat and low-fat dairy foods on cardiometabolic risk. Although the consumption of whole-fat dairy alone exhibited similar directionality to lower cardiometabolic risk, the relationship was not statistically significant. Combined intake of whole-fat and low-fat dairy, however, corresponded with lower risks of hypertension (HR = 0.87; 95%CI: 0.79–0.96; $P = 0.02$) and diabetes (HR = 0.86; 95%CI: 0.73–1.02; $P = 0.01$). In another large-scale prospective study (n = 108 065 adults; 14.2 y follow-up), researchers also observed relationships between dairy-food intake and cardiometabolic risk.[66] Greater consumption of nonfermented milk among men was associated with an increased risk of developing diabetes (HR = 1.17; 95%CI: 1.03–1.34; $P = 0.018$) and myocardial infarction

(HR = 1.23; 95%CI: 1.10–1.37; $P < 0.001$). On the contrary, greater intake of butter, fermented milk, and cheese tended to correspond to a lower risk of diabetes and/or myocardial infarction. Those persons who consumed no dairy or chose lower-fat varieties of dairy had higher risks of diabetes, myocardial infarction, or stroke.[66] Although the effect sizes of these observations were small, the findings support the concept that dietary inclusion of dairy fat, at least from certain foods, may reduce cardiometabolic risk.

In agreement with others,[67,68] the totality of evidence from observational studies supports the concept that, at best, dairy foods have positive benefits on cardiometabolic risk and, at worst, neutral benefits. This perspective likely reflects the complexity of investigating whole foods on health outcomes, suggesting that stronger experimental approaches to assess dairy consumption could be helpful to resolve these questions. The odd-chain fatty acids C15:0 and C17:0 are highly abundant in dairy foods because they are derived from microbial biosynthesis in ruminants.[69] These odd-chain fatty acids have been used as biomarkers of dairy intake to study the association of dairy intake with cardiometabolic health.[67,70] The evidence supports an inverse relationship between cardiometabolic risk and odd-chain fatty acids, which reinforces the concept that higher-fat varieties of dairy foods may provide greater benefits than lower-fat varieties. For example, in a mouse model of NAFLD, supplementation of pentadecanoic acid (C15:0) reduced hepatic macrophage infiltration and decreased aspartate aminotransferase, suggesting that odd-chain fatty acids in dairy milk help protect against liver injury.[71] A study in adults with NAFLD and age- and sex-matched healthy adults also showed that plasma phospholipid C17:0 and free fatty acids C15:0 and C17:0 were inversely related with glucose tolerance and liver fat.[72] Similarly, findings of a meta-analysis of 16 prospective cohort studies identified that T2DM risk was lowest among people with the highest levels of C15:0 (HR = 0.80; 95%CI: 0.73–0.87), C17:0 (HR = 0.65; 95%CI: 0.59–0.87), and trans-16:1n-7.[73] It is conceivable, therefore, that dairy fat, regardless of its source (eg, milk, cheese, yogurt), may reduce cardiometabolic risk.

Despite the growing number of reports that identify favorable health associations of dairy foods and dairy fat, recommendations of the *Dietary Guidelines for Americans* continue to emphasize the consumption of fat-free or low-fat dairy milk, yogurt, and cheese over full-fat varieties of dairy foods.[33] This guidance aims to reduce excess dietary energy and saturated fat consumption to protect against CVD, obesity, and related disorders. In contradiction, evidence implicating dairy-food consumption in obesity risk is limited and

inconsistent, and some studies actually identify favorable associations of full-fat dairy with adiposity in adults.[74–78] Findings of a systemic review and meta-analysis of 10 prospective studies ($> 46\,000$ children and adolescents; 3-y follow-up) also suggest that dairy consumption is not associated with childhood obesity risk, although the researchers did not distinguish between foods of varying dairy-fat content.[79] Even when whole-fat dairy vs reduced-fat dairy (ie, some or all of the fat removed) was considered in a systematic review of studies in children aged 2–18 y (n = 29), it was concluded that cardiometabolic risk was not associated with whole-fat dairy consumption.[80] Similar neutral effects were also concluded in an earlier meta-analysis of aggregated data in children and adolescents, although a positive benefit of dairy on adiposity was observed specifically among adolescents.[81] In a cohort of preschool-age Latino children, a population with a disproportionate propensity for obesity, higher milk-fat consumption was associated with a lower risk of obesity (OR = 0.88; 95%CI 0.80–0.97; $P = 0.01$).[82] Thus, although the rationale for limiting full-fat dairy consumption in the *Dietary Guidelines for Americans* is well intended to limit obesity, including that in children, it is unclear if the evidence substantiates this position. This perspective has received support in a review of cross-sectional (n = 43), longitudinal (n = 31), and randomized controlled studies (n = 20).[83] The authors concluded that the majority of evidence suggests the risk of obesity was either unrelated to or inversely associated with dairy intake regardless of the type of milk or dairy product. Indeed, in $< 10\%$ of these studies was an unfavorable association observed between dairy foods and body weight.

Separate from excess dietary energy, the *Dietary Guidelines for Americans* recommends against full-fat dairy foods because of their high saturated-fat content.[33] For example, 1 serving of fluid full-fat milk (240 mL) contains 4.5 g of saturated fat and 7.9 g of total fat per 148.8 kcal vs 83.3 kcal and < 0.5 g of total fat per serving of nonfat milk.[84] Such guidance arises in part from evidence originating from the Seven Countries Study and similar investigations[85,86] that supports recommendations to limit total fat and saturated fat to $< 30\%$ and $< 10\%$ of total dietary energy, respectively.[87,88] These recommendations, especially those related to saturated-fat consumption, are the subject of much debate[89] and are inconsistent with findings of observational studies suggesting that all-cause mortality, CVD, stroke, and T2DM are unrelated to saturated-fat intake.[90] Others also have reported that dietary saturated fat increases circulating low-density lipoprotein cholesterol (LDL-C) but noted that the increase often corresponds with an increase in large LDL particles that

have a weaker relationship to CVD risk than do small LDL particles that have greater atherogenicity.[91] In agreement with those observations, higher consumption of saturated fatty acids typically present in dairy foods is associated with fewer small LDL particles.[92] Evidence from a rigorously controlled dietary intervention study in persons with MetS also examined how dietary saturated fat affects circulating saturated fatty acid levels.[93] Participants were provided controlled diets that progressively increased in carbohydrate while the total and saturated fat content was decreased. Data showed that dramatic shifts in saturated-fat intake, from high saturated-fat intake during the low-carbohydrate phase to low saturated-fat intake during higher carbohydrate intake did not affect the proportion of total saturated fatty acids in any plasma lipid fractions. Rather, palmitoleic acid, a biomarker that has been reported to predict MetS and T2DM,[94–99] increased during the high carbohydrate and low saturated-fat intervention phase but was lower during the low carbohydrate and high saturated-fat phase. Thus, evidence suggests that dietary saturated fats have limited detriment to health, at least when dietary carbohydrate is restricted, and that holistic dietary patterns need to be considered to fully assess the impacts of foods and their components.[91] The latter are important to consider because the typical American diet is relatively high in both saturated fat and carbohydrate.[29]

The challenge of resolving the debate about dairy foods and dairy-fat consumption and cardiometabolic health stems from the lack of randomized controlled trials, which provide the strongest evidence to establish causal inferences.[100] However, it is well recognized that not all diet- and health-related questions can be practically or ethically addressed through randomized controlled trials. Increased reliance, therefore, is placed on observations to formulate evidence-based dietary recommendations, especially prospective cohort studies in which dietary exposure can be assessed longitudinally before the onset of any clinical symptoms or disease pathology. Thus, although randomized controlled trials remain of critical importance, the preponderance of evidence from observational studies must be recognized. In this regard, it is difficult to conclude that dairy consumption adversely affects cardiometabolic health. To the contrary, evidence supports that it protects against the risk of poor cardiometabolic health. In part, these benefits appear to be influenced favorably by dairy fat.

Although removal of lipids, especially saturated fats, has been a primary focus of recommendations to consume nonfat or low-fat dairy foods,[33] MPL (ie, phospholipids and sphingolipids) have gained attention for their cardiometabolic benefits.[101] Importantly, lower-fat varieties of dairy foods often have decreased MPL content.[55,102] It is plausible, therefore, that well-intended efforts to limit dairy-fat intake have an unintended consequence of limiting intake of health-promoting MPLs. Thus, in the following sections, we highlight bovine-derived MFGM and its MPL in relation to cardiometabolic health. Whenever possible, controlled studies in humans are emphasized (Table 1[11,103–113]), and complementary evidence from preclinical models (Table 2[12–14,114–124]) offers insight on the biological efficacy of MPLs.

CARDIOMETABOLIC BENEFITS OF MFGM-DERIVED PHOSPHOLIPIDS

The MFGM originates from the apical surface of mammary epithelial cells and comprises a unique triple phospholipid and cholesterol layer embedded with proteins and glycoproteins that surround secreted milk-fat droplets.[125] The genes that synthesize MFGM are conserved across species and the composition of MFGM differs little between species. The MPL content accounts for 1% of total milk lipid, with approximately one-third each from SMs, phosphatidylcholines, and phosphatidylethanolamines.[126] MFGM is present in most dairy foods, but churned buttermilk is especially rich in the membrane fraction.[10] Churning of milk fat disrupts the membrane surrounding the fat globules and results in the coalescence of free fat that ultimately yields butter. In contrast, buttermilk is the aqueous phase (ie, MFGM-containing portion). Although various MFGM constituents (eg, polar lipids, gangliosides, proteins/enzymes) are reported to have health-promoting bioactivities,[101] we focus in this review on the cardioprotective activities of MPL, especially milk SM (MSM).

Lipid digestion and absorption

Dietary polar lipids are recognized for their function as natural emulsifiers in foods, with as much as 90% of the world market using soy lecithin.[11] However, MPLs are an alternative natural source of emulsifiers and notable for their high MSM content, which represents ~25% of total MPL. The chemical properties of MPLs make them highly effective to limit intestinal uptake of certain lipids,[123,127] with evidence indicating MSM but not egg phosphatidylcholine can disrupt micellar partitioning and absorption of cholesterol.[123] This finding supports that full-fat dairy foods, with their higher MPL content,[55] may reduce cardiometabolic risk by limiting dietary and/or biliary cholesterol absorption in the intestine.

The influence of polar lipids on dietary lipid absorption has been studied in lymph-cannulated rodents in which lipid emulsions were introduced to the upper duodenum via an indwelling catheter.[128] These studies

Table 1 **Controlled studies investigating MPL on cardiometabolic outcomes in humans**

Reference	Population (no.)	Design	Duration	Treatment	Major outcomes
Vors et al (2020)[11] Study 1:	Overweight, postmenopausal women (n = 58)	Double-blind parallel	4 wk	0, 3, or 5 g of buttermilk PL	↓ Serum and postprandial TC, LDL-C, apoB/apoA1, CMRF-C, and CMRF-TG at 5 g PL ↓ Serum TG at 3 g or 5 g PL ↓ Plasma PCSK9 at 3 g or 5 g PL ↑ Serum HDL-C at 5 g PL ↑ Fecal coprostanol at 3 g or 5 g PL ↔ Firmicutes/Bacteroides and SCFAs
Vors et al (2020)[11] Study 2:	Patients with ileostomy (n = 4)	Double-blind crossover	8 h postprandial	0, 3, or 5 g of buttermilk PL with meal containing with ^{13}C-triolein and ^{2}H-cholesterol	↓ Plasma and chylomicron iAUC ^{2}H-cholesterol at 3 g or 5 g PL ↑ Ileal efflux of TC
Ohlsson et al (2010)[103]	Healthy men (n = 20)	Single-blinded crossover	8 h postprandial	High-fat meal + 975 mg buttermilk SL vs comparator	↔ TG, TC, HDL-C, LDL-C, apoB/apoA1, insulin, glucose
Keller et al (2013)[104]	Healthy women (n = 14)	Unblinded crossover	10 d	3 g or 6 g buttermilk PL or 6 g buttermilk PL + 2 g PSt	↓ TC and HDL-C at 3 g PL (vs baseline) ↑ TC at 6 g PL (vs 3 g PL) ↑ Plasma MUFA (vs baseline) ↔ TG or LDL-C/HDL-C
Ramprasath et al (2013)[105]	Healthy men and women (n = 20)	Unblinded crossover	14 d	Prescribed diet + 0 or 1 g milk SM	↔ Serum LDL-C, VLDL-C, TG ↑ Serum HDL-C ↔ Cholesterol absorption or cholesterol FSR ↔ Luminal bile acids or solubilized TC
Weiland et al (2016)[106] Study 1:	Overweight/obese older adult men (n = 62)	Double-blind parallel	8 wk	0 or 2 g butter serum PL	↔ Serum TC, HDL-C, LDL-C, TG, PL, TC/HDL, apo1/apoB ↔ Serum glucose, insulin, HOMA-IR ↔ CRP, IL-6, sICAM-1, tHcy ↔ ALT, AST ↓ WC
Weiland et al (2016)[106] Study 2:	Overweight/obese older adult men (n = 57)	Double-blind parallel	7 wk	3 g MPL or 2.8 g soy PL	↔ Serum TC, HDL-C, LDL-C, TG, PL, TC/HDL, apo1/apoB. ↔ Serum glucose, insulin, HOMA-IR ↔ ALT, AST, GGT
Rosqvist et al (2015)[107]	Overweight, older adults (n = 57)	Single-blind parallel	8 wk	40 g whipping cream (intact MFGM with 19.8 mg PL) or 40 g butter oil (1.3 mg PL)	↓ LDL-C, TC, HDL-C, and apo1/apoB ↓ mRNA of cell cycle, apoptosis, protein degradation, and ER stress genes ↔ Plasma glucose, insulin, HOMA-IR

(continued)

Table 1 **Continued**

Reference	Population (no.)	Design	Duration	Treatment	Major outcomes
Beals et al (2019)[108]	Overweight/obese men and women (n = 36)	Double-blind crossover	6 h postprandial	Whipping cream + MFGM (PL not reported)	↓ iAUC insulin, iAUC IL-18 ↑ LPS, LBP ↓ iAUC EPHX2 and EPHX2 lymphocyte mRNA ↓ CD14 and LTBR lymphocyte mRNA in individuals with high baseline ratio of TC to HDL ↔ TG, HDL-C, LDL-C, IL-10, IL-1B, IL-2, IL-4, IL-6, IL-8, TNF-α, MCP-1, CRP, SAA, sICAM
Demmer et al (2016)[109]	Overweight/obese men and women (n = 36)	Double-blind crossover	6 h postprandial	Palm oil + MFGM	↑ TG, iAUC IL-10 ↓ TC, LDL-C, iAUC insulin, iAUC sICAM ↔ MCP1, cortisol
Conway et al (2013)[110]	Men and women with high LDL-C levels (n = 34)	Double-blind crossover	4 wk	Buttermilk (187.5 mg PL) or control (34.6 mg PL)	↓ Serum TC and TG ↔ HDL
Conway et al (2014)[111]	Men and women with high LDL-C levels (n = 34)	Double-blind crossover	4 wk	Buttermilk (187.5 mg PL) or control (34.6 mg PL)	↓ SBP, MAP, ACE ↔ Body weight, hip and waist circumference, aldosterone, ANG II
Ten Bruggencate et al (2016)[112]	Healthy young adults (n = 58)	Double-blind parallel	4 wk	Daily soy + MFGM (0 or 3.2 g PL) + Escherichia coli challenge at week 2	↓ GI distress ↔ Fecal wet weight, E. coli abundance, dry weight, or calprotectin ↔ Serum CFAII-specific IgG
Ohlsson et al (2010)[113]	Patients with ileostomy (n = 6)	–	8–9 h	250 mg SM	↑ C22:0 SM, C23:0 SM, C24:0 SM, sphingosine, and ceramide in ileostomy content compared with presupplementation ileostomy content

Abbreviations: ACE, angiotensin-converting enzyme; ALT, alanine aminotransferase; ANG II, angiotensin II; apoA, apolipoprotein A; apoB, apolipoprotein B; AST, aspartate transaminase; BW, body weight; CD14, cluster of differentiation; CFAII, colonization factor antigen II; CMRF-C, chylomicron-rich fraction cholesterol; CMRF-TG, chylomicron-rich fraction triglyceride; CRP, C-reactive protein; EPHX2, epoxide hydrolase 2; ER, endoplasmic reticulum; FSR, fractional synthesis rate; GGT, γ-glutamyl transferase; GI, gastrointestinal; HDL-C, high-density lipoprotein cholesterol; HOMA-IR, homeostatic model assessment of insulin resistance; iAUC, incremental area under the curve; Ig, immunoglobulin; IL, interleukin; LBP, lipopolysaccharide-binding protein; LDL-C, low-density lipoprotein cholesterol; LPS, lipopolysaccharide; LTBR, lymphotoxin β receptor; MAP, mean arterial pressure; MCP-1, monocyte chemoattractant protein-1; MFGM, milk fat globule membrane; MPL, milk phospholipid; MUFA, mono-unsaturated fatty acid; PCSK9, protein convertase subtilisin kexin-9; PL, phospholipid; PSt, plant sterol supplementation; SAA, serum amyloid A; SBP, systolic blood pressure; SCFA, short-chain fatty acid; sICAM-1, soluble intercellular adhesion molecule-1; TC, total cholesterol; TG, triglyceride; tHcy, total homocysteine; TNF-α, tumor necrosis factor-α; VLDL-C, very-low-density lipoprotein cholesterol; WC, waist circumference.

have revealed that infusion of a lipid emulsion containing phosphatidylcholine (as either 1,2-dipalmitoyl phosphatidylcholine or 1,2-dilinoleoyl phosphatidylcholine) inhibits the absorption of fat-soluble vitamin E (α-tocopherol) by ∼50%, whereas the absorption of triolein (ie, triglyceride) increased by 42%–43%. Similar studies in this model system also show that 1,2-dioleoyl phosphatidylcholine, but not 1-oleoyl-2-hydroxy- phosphatidylcholine (lysophosphatidylcholine), reduced the absorption of both α-tocopherol and cholesterol. These findings support the concept that polar phospholipids affect the absorption of certain lipids. However, whether MPLs exert a similar effect was not evaluated in these studies. Indeed, in contrast to the polar lipids of

Table 2 **Controlled studies investigating MPL on cardiometabolic outcomes in rodents**

Reference	Model (no.)	Duration	Treatment	Major outcomes
Millar et al (2020)[114]	Male LDLr$^{-/-}$ mice (n = 45)	14 wk	HFD + 0, 1% or 2% (w/w) MPL	↓ Serum TC , NEFA, CCL2 at 2% MPL ↔ Serum HDL-C, TG, ALT, AST, insulin, glucose, TNF-α, IL-1B, adiponectin, resistin, or hepatic TG ↓ Hepatic TC and CE at 1%–2% MPL ↓ Hepatic *Scd1, Ccl4, Adgre1* mRNA at 1%–2% MPL ↓ Adipose and aortic *Ccl2* mRNA at 2% MPL ↑ Bacteroidetes, Actinobacteria, and *Bifidobacterium* at 2% MPL ↓ Atherosclerotic plaque at 2% MPL ↔ mRNA of adipose macrophage, cytokines, or metabolic genes
Milard et al (2019)[115]	Male C57Bl/6 mice (n = 45)	8 wk	LFD vs HFD + 0, 1.1%, or +1.6% (w/w) butter serum MPL	↓ Body mass and liver mass at 1.1% and 1.6% ↓ mRNA of hepatic *F4/80* at 1.6% ↑ ZO-1 mRNA at 1.1% and 1.6% ↑ Colonic crypt depth, *Akkermansia muciniphila* abundance at 1.1% and 1.6% ↔ Serum LPS, *Lbp, sCd14, Mcp-1*, and *Cxcl1* ↔ *Reg3γ, Pla2g2*, α-defensin mRNA, FITC-dextran test, Firmicutes/Bacteroidetes
Milard et al (2019)[116]	Male C57BL/6 mice (n = 40)	4-h	MSM (5 or 10 mg) or MPL (5 or 10 mg)	↑ Ileum *Cxc1, Cxcl2* mRNA at 5 mg MSM ↑ Ileum *Cxcl2* mRNA at 5 mg MPL ↑ Duodenal *Occ* and *Zo-1* at 5 and 10 mg MSM
Zhou et al (2019)[12]	Male *ob/ob* mice (n = 18)	2 wk	HFD vs HFD + milk GG (0.2 g/kg) or MPL (10 g/kg of diet)	↑ *Acat2* mRNA and plasma IL-6 with MPL ↓ *Acaa2* and *Acacb* mRNA with GG or MPL, ↓ % Mesenteric fat with GG or MPL ↓ Jejunal *ZO-1* protein with GG or MPL ↔ Subcutaneous or visceral adipose depots ↔ Intestinal permeability or plasma LPS
Norris et al (2017)[117]	Male C57BL/6 mice (n = 52)	10 wk	LFD vs HFD + 0, MSM (0.1%), or egg sphingomyelin (0.1%)	↓ Liver TG, TC, CE ↓ mRNA liver Scd1, Pparγ2, ↔ Serum ALT, AST ↔ Body mass, adipose mass, liver mass ↔ Adipose crown-like structures ↓ mRNA adipose F4/80, Tnfα ↓ Serum CCL2

(continued)

Table 2 **Continued**

Reference	Model (no.)	Duration	Treatment	Major outcomes
Lecomte et al (2016)[118]	Male C57BL6 mice (n = 22)	8 wk	LFD vs HFD + 1.2% soy PL or 1.2% MPL	↓ mRNA muscle Acox1, Cpt1b, Cd36 ↓ Weight gain, WAT mass, and liver weight at 1.2% MPL ↓ Fecal lipid content at 1.2% MPL ↓ Adipocyte hypertrophy and adipose tissue inflammation at 1.2% MPL (vs 1.2% +soy PL) ↑ Jejunum *FATP4 and FABP2* gene expression at 1.2% MPL ↑ Colonic goblet cells per crypt at 1.2% MPL
Wat et al (2009)[119]	Male C57BL6 mice (n = 40)	8 wk	Control diet (0 or 1.2% milk PL) or HFD (0 or 1.2% milk PL)	↓ Liver weight and hepatic lipid content at 1.2% HFD-PL (vs HF diet) ↓ Serum TG, PL, TC, non–HDL-C, HDL-C, apoA-I, and glucose in HF+PL at 1.2% HFD-PL (vs HFD) ↓ Hepatic fatty acid synthesis genes at 1.2% HFD-PL (vs HFD) ↓ expression of HMG CoA reductase and bile acid gene in at 1.2% control diet and 1.2% HFD-PL (vs control and HFD) ↔ serum insulin, *Acat2*, *Ldlr*, or SR-BI gene expression
Kamili et al (2010)[120]	Male C57BL/6 mice (n = 20)	3,5, or 8 wk	HFD + 0, 1.2% milk PLs	↓ Liver weight after 3 wk ↓ Total liver lipid, cholesterol, and TG ↑ Fecal cholesterol
Mathiassen et al (2015)[121]	Female BALB/C mice (n = 36)	30, 60, or 120-min	Soy PLs or milk PLs with ³H-inulin	↓ Absorption of milk PL-enriched TG ↔ Gastric-emptying measured by intestinal retrieved ³H-inulin or gut fatty acid clearance
Norris et al (2016)[13]	Male C57BL/6J mice (n = 30)	4 wk	HF, + 0.25% milk SM, or + 0.25% egg SM	↓ BW, serum TC, and NEFA ↓ Hepatic TG ↓ ABGC5 mRNA expression ↑ Proximal small intestine NPC1L, hepatic SREBP2, HMG-CoA reductase, and skeletal muscle GLUT4 expression ↓ Serum LPS and fecal abundance of Bacteroidetes ↑ Abundance of fecal Firmicutes, Actinobacteria, and *Bifidobacterium* ↔ Genes related to chylomicron formation, TG absorption, tight junction proteins in distal intestine, or genes related to lipid metabolism (*SREBP1c, LXRα, ACOX,* and *PPARα*), lipoprotein uptake (*LDLr and PCSK9*) or inflammation (*MCP1*)

(continued)

Table 2 **Continued**

Reference	Model (no.)	Duration	Treatment	Major outcomes
Lecomte et al (2015)[122]	Female Swiss mice (n = 7 per group)	1, 2, or 4-h	Water and oil emulsion enriched with 5.7 mg soy PLs or milk PLs	↓ Plasma apoB48 at 4 h ↑ Chylomicron size at 2 h ↑ SM chylomicron enrichment ↓ Duodenal mucosa *Apob* expression ↑ Jejunal MTTP gene expression ↔ Plasma TG, NEFA, duodenal mucosa *Cd36, Fabp2*, MTTP gene expression
Eckhardt et al (2002)[123]	Male C57L/J mice (n = 6)	4 d w/crossover	Control diet or milk PL + intragastric bolus of 0.15 mL medium-chain triglyceride containing ^{14}C-cholesterol and ^{3}H-sitostanol at day 4	↓ Cholesterol absorption in PL
Li et al (2020)[124]	Male C57BL/6 mice (n = 40)	8 wk	HFD + 100 mg, 200 mg, or 400 mg/kg bodyweight MFGM	↓ Glucose, insulin, HOMA-IR ↑ UCP1, PGC-1α, PRDM16 protein and mRNA
Norris et al (2017)[14]	Male C57BL/6J mice (n = 28)	10 wk	HFD or + 0.1% milk SM	↓ Serum IL-6, TNF-α, IFN-γ, and MIP-1β ↑ *Acetatifactor* abundance ↔ Firmicutes/Bacteroidetes, α-diversity, or β-diversity

Abbreviations: ABGC5, ATP binding cassette subfamily G member 5; Acaa2, acetyl-CoA acyltransferase 2; Acacb, acetyl-CoA carboxylase β; Acat2, acetyl-CoA Acetyltransferase 2; ACOX, acyl-CoA oxidase 1; Adgre1, adhesion G protein-coupled receptor E1; ALT, alanine aminotransferase; Apob, apolipoprotein B; AST, aspartate transaminase; CCL2, C-C motif chemokine ligand 2; ccl4, macrophage inflammatory protein-1 β; cd36, cluster of differentiation 36; CE, cholesteryl ester; Cpt1b, carnitine palmitoyltransferase-1b; CXCL1, C-X-C motif chemokine ligand 1; FABP2, fatty acid binding protein 2; FATP4, fatty acid transport protein; FITC-dextran, fluorescein isothiocyanate-dextran; GG, milk gangliosides; GGT, γ-glutamyl transferase; GLUT4, glucose transporter 4; HDL-C, high-density lipoprotein C; HFD, high-fat diet; HMGCoA, 3-hydroxy-3-methylglutaryl-CoA reductase; IFN-γ, interferon γ; IL, interleukin; LFD, low-fat diet; LXRa, liver X receptor α; MIP-1β, macrophage inflammatory protein-1 β; MPL, milk phospholipid; MSM, milk sphingomyelin; MTTP, microsomal triglyceride transfer protein; NEFA, nonesterified fatty acid; NPC1L, Niemann-Pick C1 like 1; PGC-1α, peroxisomal proliferator-activated receptor coactivator-1α; PL, phospholipid; Pla2g2, phospholipase A2g2; PPARα, peroxisome proliferator-activated receptor-α; PRDM16, PR domain-containing 16; Reg3y, regenerating islet-derived protein 3 gamma; Scd1, stearoyl-CoA desaturase; sCd14, soluble cluster of differentiation 14; SM, sphingomyelin; SR-B1, scavenger receptor class B type 1; SREBP1c, sterol regulatory element-binding protein 1c; Srebp2, sterol regulatory element-binding protein 2; TC, total cholesterol; TG, triglyceride; TNF-α, tumor necrosis factor-α; UCP1, uncoupling protein 1; WAT, white adipose tissue; w/w, weight per weight; ZO-1, zonula occluden-1.

nondairy origin that were used in this study,[128] milk phospholipids have a different fatty acid composition, with a saturated fatty acid (C14:0–18:0) typically present at the sn-1 position and an unsaturated fatty acid (C18:1-C18:2) at sn-2.[51]

Dietary sphingolipids in the Western diet consist mainly of SM that originates from dairy foods (∼25% of total MPLs), meat (∼5%–10% of total polar lipid), and eggs (∼2% of total polar lipid).[129] Of interest is that the SM species in dairy differ from those in other foods. MSM has fatty acids of very long chain lengths (C22:0-C24:0) compared with egg SM, which commonly has palmitic acid (C16:0).[13] The distribution of sphingoid bases (d16:0 to d19:0) is also more varied in MSMs and likely contributes to their favorable bioactivity to differentially interact with and desorb cholesterol from cellular membranes.[130] The fatty acid composition of MSMs also more greatly inhibits intestinal lipid absorption than that of egg SM, which is potentially due to stronger

hydrophobic interactions in the intestinal lumen by MSM.[127]

Structural differences between egg SM and MSM prompted studies[127] in the aforementioned lymph-cannulated rat model.[128] Interestingly, MSM compared with egg SM decreased the absorption of cholesterol, α-tocopherol, and triolein while reducing lymphatic output of phosphatidylcholine and SM.[127] These effects were attributed partly to an intraluminal interaction in which hydrogen bonding occurs between cholesterol and SM to decrease micellar solubilization and/or promote a slower transfer of cholesterol from the micellar matrix to the enterocyte. Also, MSM may have limited pancreatic lipase activity, consistent with radiotracer studies in vitro and in mice indicating that MPL attenuates free fatty acid release from emulsified triglyceride.[121] The differential inhibition of MSM and egg SM on lipid absorption is likely due to their unique fatty acid chain lengths such that MSM has a greater

abundance of very-long-chain saturated fatty acids (22:0, 23:0, and 24:0; 66% in total) in lieu of C16:0 that is highly abundant in egg SM (87%). Nonetheless, this lymph-cannulated rodent model provided strong evidence to support that MSM is a potent absorptive inhibitor of cholesterol and other lipids, suggesting that chronic MPL ingestion could have a cardiometabolic benefit.

Lipid-lowering effects in circulation and liver

In agreement with the cardiometabolic health benefits of milk phospholipids that decrease intestinal lipid absorption, chronic supplementation with milk phospholipids also attenuated hepatic cholesterol and triglyceride accumulation in preclinical rodent models.[131] This was demonstrated in mice provided a high-fat diet containing 1.2% cow's milk phospholipid.[120] Others have reported similar effects of MSM or MPL in reducing hepatic triglyceride and/or cholesterol ester in LDL-receptor knockout mice or wild-type mice fed a high-fat diet.[13,114,117] Evidence for an intraluminal effect was provided by data showing greater recovery of fecal ^{14}C-cholesterol and decreased recovery of hepatic ^{14}C-cholesterol. Hepatic cholesterol and triglyceride were also correlated with hepatic ^{14}C-cholesterol ($P < 0.001$), whereas hepatic cholesterol and triglyceride were inversely related to fecal ^{14}C-cholesterol ($P < 0.01$–0.05). It was also reported that milk-derived ceramide levels in liver tended to be lower despite substantially higher levels of dietary and fecal ceramides in mice supplemented with milk phospholipids. These findings suggest that milk phospholipids protect against liver steatosis, an asymptomatic but potentially debilitating cardiometabolic disorder,[23] consistent with a mechanism of limiting intestinal lipid absorption. Although liver steatosis was not evaluated, findings of a placebo-controlled trial in middle-aged adults showed that MFGM alleviates hyperlipidemia by limiting lipid absorption.[110] Adults provided 45 g/d buttermilk for 4 weeks had lower total cholesterol ($P = 0.019$) and LDL-C ($P = 0.057$) levels. The surrogate biomarker of intestinal cholesterol absorption, β-sitosterol, was also correlated with circulating total cholesterol and LDL-C. Whether these cardiometabolic benefits are attributed entirely to MPL could not be established in this study. This is because buttermilk closely reflects the composition of MFGM, including MPL and membrane proteins (eg, MFG-EGF-8); the latter are also known to exhibit health-promoting bioactivities.[132,133]

Nonetheless, these protective effects against hypercholesterolemia are consistent with earlier evidence in vitro showing that buttermilk limits cholesterol micellar solubility[134] and recent investigations in humans showing that MPLs decrease cholesterol absorption.[11] Thus, evidence in humans and rodents supports that MPLs promote cardiometabolic health by lowering levels of circulating and hepatic cholesterol, likely due to a gut-level effect that limits lipid absorption. Similar benefits of MPLs to reduce hepatic triglyceride are also apparent in rodents, but these health effects have yet to be studied adequately in humans.

Lipid digestion and lipemia

Studies in mice and in vitro have examined whether the intraluminal emulsification activities of MPLs affect postprandial lipemia and lipid digestion, respectively.[122] Mice gavaged with an emulsion stabilized with either MPL or soy polar lipids, which differ primarily in SM content (29.9% vs 0%), had a more rapid increase, but more rapid clearance, of plasma triglyceride in response to MPL; similar effects occurred for plasma nonesterified fatty acids. Plasma ApoB48 was also initially higher at 1 hour after MPL administration compared with soy polar lipids, but this phenomenon was the converse by 4 hours postadministration of polar lipids. Similarly, duodenal *Apob* mRNA expression was lower at 4 hours post-gavage among mice receiving MPLs, whereas a main effect for emulsion type indicated that *Mttp* expression was higher in response to MPL, with a specific between-treatment effect observed at 1 hour post-gavage. In agreement, chylomicron lipid content was higher at 1 hour after MPL administration but was significantly lower by 4 hours compared with the soy polar lipid treatment. However, chylomicron particle size among mice indicated MPLs were larger and there was a greater proportion of 24:1 ceramide, which has been suggested to be protective against MetS.[135]

To help explain these observations in mice,[122] in vitro digestion studies assessed the differential effects of MPL vs soy polar lipids on triglyceride digestive hydrolysis. No difference between polar lipid treatments occurred during the gastric phase of digestion, whereas MPLs during intestinal digestion increased triglyceride hydrolysis. The intestinal-level benefits of MPL are attributed at least in part to MSM. This is consistent with SM being a precursor to ceramide formation following intestinal digestion, and that the hydrolysis of ceramides promotes the production of large chylomicrons.[136] Interestingly, MPL-mediated triglyceride hydrolysis also may depend on the presence of milk glycerophospholipids, based on evidence that the addition of MSM to soy lecithin emulsions inhibits pancreatic lipase activity and combined hydrolysis from gastric and pancreatic lipases.[121] Taken together, MPLs uniquely protect against postprandial lipemia compared with soy polar lipids, which is consistent with a mechanism of

inhibiting chylomicron assembly; increasing their particle size, which would be expected to enhance their clearance[137]; and increasing triglyceride hydrolysis in the intestinal lumen. These findings also help explain that dietary SM and ceramides of MPL reduce fasting triglyceride of rodents.[119,138] Similarly, 4-week supplementation of an MPL-rich emulsion, but not a formula containing egg polar lipids, prevented an increase in plasma triglyceride levels in humans,[139] and phospholipid-rich buttermilk ingestion decreased fasting triglyceride levels in a placebo-controlled study.[110] However, in a separate study in humans in which the effect was compared of MPL and egg polar lipids on postprandial hypertriglyceridemia following a high-fat breakfast challenge, researchers found no between-treatment effects.[103] Two studies of parallel design conducted with men who consumed either drink enriched with 2 g of MPL compared with a control for 8 weeks, or test drinks formulated with MPL vs soy polar lipids for 7 weeks, also showed no effects on circulating lipids, apolipoproteins, biomarkers of inflammation, or insulin resistance.[106] These studies contradict the findings by Rosqvist et al,[107] who showed in an 8-week intervention that supplementation of MFGM with dairy fat prevents the increase in plasma lipids (namely, total cholesterol and LDL-C) and the ratio of apolipoprotein B to apolipoprotein A-I that are otherwise observed in response to butter oil. These findings suggest the underlying food matrix of dairy foods must be carefully considered when evaluating their cardiometabolic benefits.

Others have examined the influence of MFGM on postprandial metabolism relative to inflammatory responses consistent with the recognition that the daily accumulation of metabolic and inflammatory insults contributes incrementally to CVD risk.[140,141] Demmer et al[109] conducted a randomized, double-blinded, cross-over trial to examine whether MFGM supplementation in overweight and obese middle-aged adults would attenuate postprandial inflammation and hyperlipidemia otherwise induced by a high-saturated fat meal (high in palmitate). MFGM in the high-fat meal challenge lowered total cholesterol and LDL-C levels but increased triglyceride levels while reducing insulin over the 6-hour postprandial period. Postprandial soluble intercellular adhesion molecule-1 levels also were lowered by MFGM, whereas the levels of the anti-inflammatory cytokine interleukin (IL)-10 were increased; numerous other pro-inflammatory cytokines were unaffected (eg, tumor necrosis factor (TNF)-α, IL-8). Although these findings support MPLs as limiting adverse metabolic and inflammatory effects caused by a high-fat meal, the MFGM ingredient used in this study also contains membrane proteins that potentially contribute to the observed benefits, consistent with a report of anti-

inflammatory effects of MFG-EGF-88 in a colitis model.[132] Additional studies are warranted to define the independent benefits of MPL and/or its specific lipid species on lipemia.

Inflammation and gut barrier permeability

Cardiometabolic health, including obesity and insulin resistance, is linked to gut health and the resistance of the intestinal barrier to permit the translocation of endotoxins that provoke Toll-like receptor-4/nuclear factor (NF)-κB inflammation.[142–144] In a neonatal rodent model, buttermilk-derived MPL improved gut barrier function by downregulating intestinal inflammation otherwise induced by endotoxin-Toll-like receptor-4-NF-κB.[145] Membrane-rich milk fat also protected mice against lipopolysaccharides-induced impairments in gut barrier integrity.[146] Likewise, studies in vitro using cell-based assays on neutrophils or monocytes suggested promise for select phospholipid- or ganglioside-rich fractions of MFGM to attenuate inflammatory responses (eg, neutrophil elastase activity, superoxide production, nitric oxide production, IL-1β release, cyclooxygenase-1 and -2 activities).[147]

Milard et al[116] examined the effects of MSM and MPL on gut physiology. MSM in human epithelial intestinal Caco-2/TC7 cells increased expression of the tight junction proteins occludin and zonula occluden-1 without affecting claudin-1. Analogous studies with an MPL-rich ingredient did not show a similar benefit. Follow-up studies specifically with MSM showed no benefit on transepithelial electrical resistance or cellular permeability to chemical probes, suggesting no functional improvement in gut integrity. MSM also did not affect the secretion of IL-6, IL-17, IL-22, TNF-α, or MCP-1, but it unexpectedly increased the expression and secretion of IL-8. These findings were extended by showing that IL-8 treatment causes tight junction protein overexpression, suggesting that MSM regulates barrier function through an indirect mechanism. Parallel studies in C57BL6 mice supported this mechanism, consistent with MSM increasing the expression of homologs of IL-8. Additional study is warranted to better define the indirect "pro-inflammatory" activities of MSM on gut barrier function.

Researchers hypothesized that in leptin-deficient (ob/ob) mice, which are obese in association with impaired gut barrier integrity and elevated circulating endotoxin,[148,149] MPL (provided as phospholipids or gangliosides) would protect against gut barrier function in association with inhibiting inflammation and liver steatosis.[12] Treatment with either MPL did not affect 2 separate indices of gut permeability nor did either MPL attenuate circulating endotoxin. Tight junction protein

expression was largely unaffected by either treatment with the exceptions that ganglioside supplementation increased colonic occludin, and supplementation with ganglioside or phospholipid decreased jejunal zonula occluden. Hepatic triglyceride was also unaffected, but phospholipid supplementation decreased hepatic cholesterol ester. Plasma cytokines and adipokines were also unaffected, except that levels of circulating IL-6 increased in response to phospholipid supplementation. These unexpected outcomes were potentially attributed to the severity of preexisting obesity in *ob/ob* mice and/ or they indicate that intact leptin signaling is required to observe the benefits of MPLs. Indeed, MSM supplementation in wild-type mice provided a high-fat diet prevented diet-induced increases in serum endotoxin levels.[13] However, this protective effect occurred without any improvement of gut barrier permeability or expression of intestinal tight junctions. These findings support that MSM reduced availability of gut-derived endotoxins from the microbiota, as discussed in the next section.

Gut microbiota and cardiometabolic risk

The gut microbiota is recognized to influence cardiometabolic health. To explore this, preclinical and clinical models were used in studies to define the potential prebiotic and/or antimicrobial activities of MPLs that alleviate cardiometabolic risk. C57BL6 mice were provided a high-fat diet supplemented with milk phospholipids (primarily as phosphatidylcholine and phosphatidylethanolamine), milk sphingolipids (primarily as MSM), or total MPLs.[150] Sphingolipid supplementation tended to reduce hepatic triglyceride biosynthesis ($P = 0.06$), whereas supplementation of phospholipids or MPLs significantly ($P < 0.05$) decreased hepatic de novo hepatic fatty acid biosynthesis. These effects occurred without any notable changes in gut microbiota composition, and the few, significant, pair-wise associations between bacterial populations and mouse phenotype (eg, body mass, liver mass, hepatic triglyceride or cholesterol, biomarkers of lipogenesis) were not consistent across treatments.[150] These findings suggest that unique species of MPL function differentially to regulate hepatic lipogenesis and protect against metabolic dysfunction but independently of any improvements in gut microbial community structure or function. Indeed, overweight, postmenopausal women (n = 58) who were provided MPL supplementation for 4 weeks in a parallel study design (0, 3, or 5 g/d; ~25% as SM) had no notable changes in major gut bacterial populations or fecal short-chain fatty acids.[11] The limited dietary control and lack of crossover design that can assess within-subject effects could have strengthened the approach to

potentially observe treatment effects. Nonetheless, MPLs at 5 g/d decreased the number of intestine-derived chylomicron particles in association with favorable changes in fasting and postprandial total cholesterol concentrations, the ratio of total cholesterol to high-density lipoprotein cholesterol, and the ratio of apolipoprotein B to apolipoprotein A1. Consistent with the premise that MPLs can decrease cholesterol absorption, fecal coprostanol, the gut-derived metabolite of cholesterol, was increased. A complementary study also showed that the ingestion of MPLs by patients with ileostomy decreased cholesterol absorption,[11] a finding that agrees with those indicating dietary MSM is incompletely absorbed and inhibits cholesterol absorption.[113] Of particular interest are the secondary analyses performed with obese postmenopausal women that showed MPL decreased atherogenic species of SM (C16 + 18 SM) and ceramide (C24:1) species in the serum,[151] and that C16 + 18 SM was correlated with favorable changes in total cholesterol, LDL-C, and ApoB levels. Despite high-dose supplementation of SM from MPLs, SM and ceramide concentrations in intestine-derived chylomicrons were decreased, whereas their levels in fecal contents increased. This suggests that an intestinal-level mechanism involving SM metabolism was responsible for these cardiometabolic benefits. These studies in rodents and humans also suggest that metabolic benefits from MPLs can occur independently of prebiotic effects on gut microbiota to limit hepatic lipogenesis and cholesterol absorption. However, recent studies in vitro indicate that MPLs enhance the cellular adhesion of lactic acid bacteria to Caco-2 cells,[152] suggesting that researchers should consider whether MPLs selectively influence mucosa-associated and luminal microbial communities that are distinct and differentially affected by environmental exposures.[153]

Consistent with evidence that MPLs protect against dyslipidemia, studies in LDL-receptor knockout mice were conducted to examine their potential to ameliorate atherosclerosis development in relation to altering gut microbial structure.[114] Supplementation of MPL at 1% or 2%, which corresponds to 0.2% or 0.4% MSM, as part of a high-fat and high-cholesterol atherogenic diet alleviated diet-induced increases in serum total cholesterol and very-low-density lipoprotein cholesterol and LDL-C; these effects were more pronounced at the higher MPL dose. Strikingly, MPLs (2%) reduced atherosclerotic plaque lesions in association with reduced markers of inflammation (ie, circulating CCL2, hepatic CCL4, hepatic Adgre1 [$P = 0.08$], and adipose [CCL2]). These anti-atherogenic benefits corresponded with putative improvements in gut microbiota composition, specifically increased abundances of Bacteroidetes, Actinobacteria, and *Bifidobacterium*, and a decreased

abundance of Firmicutes. Thus, the findings of studies contrasted with those of earlier preclinical and clinical studies by suggesting that prebiotic activities of MPLs help to protect against CVD.

A study by Norris et al[13] further supports that MPLs exert cardiometabolic benefits by favorably altering populations of commensal and pathogenic bacteria while protecting against dysfunctional lipid metabolism implicated in NAFLD. In wild-type mice provided a high-fat diet (anhydrous milk fat) alone or supplemented with MSM or egg SM (0.25% weight per weight), serum total cholesterol and triglyceride levels increased in response to egg SM, whereas total cholesterol decreased without any changes in circulating triglyceride in response to MSM. Notably, hepatic triglyceride levels increased with egg SM supplementation in contrast to the favorable decreases induced by MSM that were associated with lowered expression of stearoyl-CoA desaturase-1. Additional gene expression measures suggested that cholesterol depletion occurred in intestinal and liver tissue. Of interest was that only MSM supplementation improved metabolic endotoxemia, but this was not explained by changes in gut permeability. Rather, MSM decreased total gram-negative bacteria (ie, endotoxin-containing) including Tenericutes, while increasing commensal populations (eg, Actinobacteria, *Bifidobacterium*) that have been suggested to have cardiometabolic benefits. On the basis of these findings, the antimicrobial activities of MSM likely decreased pyrogenic bacteria but apparently did not improve gut barrier permeability to limit endotoxin translocation. It is also possible that the known effects of MSM on cholesterol absorption and chylomicron assembly also limited chylomicron-dependent endotoxin absorption. More study in this area is needed to clarify the mechanisms by which MSM protects against endotoxemia-associated NAFLD and whether these benefits can be translated to humans.

Adiposity and inflammation

To our knowledge, no strong evidence in humans supports that MPL lowers the risk for obesity. However, findings from preclinical rodent models show promise that the anti-inflammatory activities of MPL may help reduce adiposity. For example, dietary supplementation of egg SM, but not MSM, increased adiposity in mice fed a high-fat diet.[13] Similar studies with MPL and soybean phospholipid have been conducted in mice fed a high-fat diet for 8 weeks.[118] Increased adipose mass and larger adipocytes were observed in mice supplemented with soybean phospholipid but not MPLs; the soybean phospholipid treatment also exacerbated hepatic triglyceride content. Interestingly, the worsening of obesity due to soybean phospholipid supplementation corresponded with increased expression levels of adipose pro-inflammatory markers (eg, *Tnfα*, *Mcp-1*, *F4/80*, lipopolysaccharide-binding protein), whereas none of these genes was upregulated by MPL supplementation in contrast to the lowered level of adipose *Cd68*. It is possible that MPLs are not anti-obesigenic per se but instead have a role to protect against dietary insults that provoke adiposity. However, recent studies have shown near dose-dependent effects of MPL at 1.1% and 1.6% to protect against body mass gain in mice fed a high-fat diet.[115] Plasma pro-inflammatory proteins were largely unaffected by MPLs, but hepatic expression of the macrophage marker *F4/80* tended to be downregulated ($P = 0.06$), and populations of gut bacteria of metabolic interest were altered by MPL. However, it is noteworthy that MSM blocks lipopolysaccharide-induced increases in pro-inflammatory gene expression in RAW264.7 macrophages, and these anti-inflammatory effects are lost when hydrolysis of MSM is inhibited.[14] A separate line of investigation in obese mice and in cultured cells showed that MFGM, likely mediated by its phosphatidylcholine content, protects against obesity by inducing adipose browning.[124] Collectively, emerging evidence in this area suggests that MPLs may limit obesity risk by lowering adipose inflammation, but mechanistic studies are needed to define the mechanisms by which MFGM- and MPL-enriched dairy products protect against obesity and whether the effects are mediated by the gut microbiota. Clinical validation is also required to translate these promising cardiometabolic benefits to improve human health.

CONCLUSION

This review was conducted to summarize the benefits of milk fat, specifically those attributed to MPLs, relative to their potential dietary role in managing the growing trends of poor cardiometabolic health. Although higher-quality dietary patterns have potential to thwart cardiometabolic risk, a critical need exists to establish the mechanisms by which bioactive food components regulate pathogenic responses. Bovine milk is recognized for its nutrient-rich profile, but debate continues about whether its total fat content adversely affects cardiometabolic health. Much of this controversy is attributed to the significant body of evidence derived from observational studies that can only establish associations. This supports a need for controlled studies, especially in humans, that are designed to establish causality. In addition, overall dairy consumption has declined in the United States, and the limited milk that is consumed is of lower-fat varieties that have reduced MPL content.[154] This is of potential concern in terms of
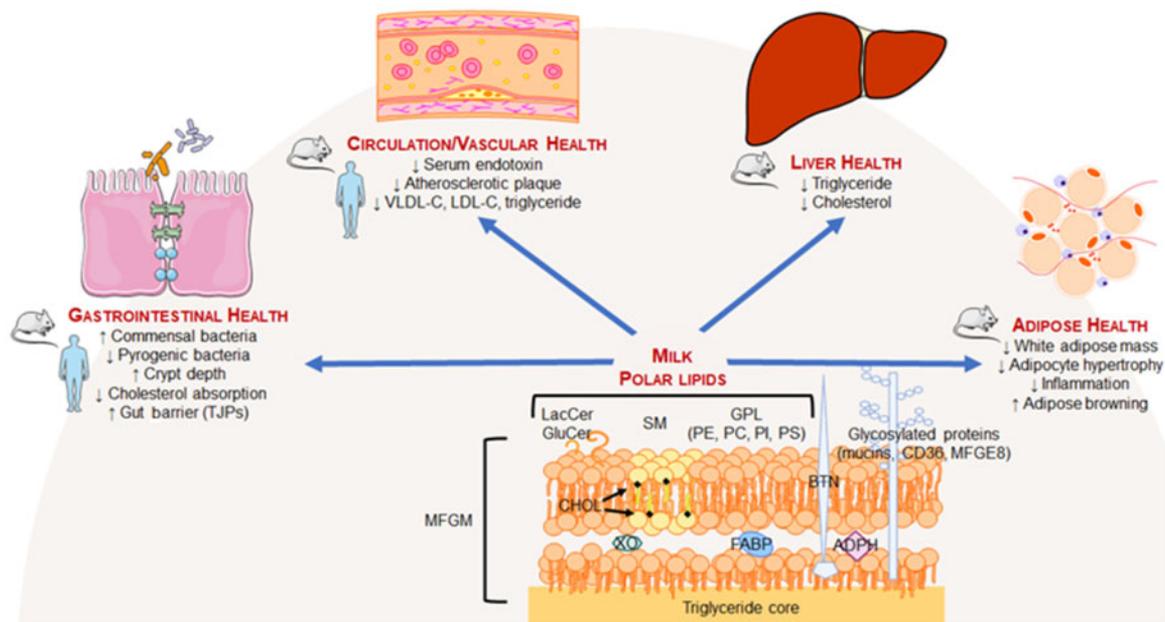
Figure 3 Summary of cardiometabolic activities of milk polar lipids (MPLs) in humans and rodent models. MPLs consisting of LacCer, GluCer, SM, and glycerophospholipids (PE, PC, PI, and PS) and several membrane-associated proteins are present in the MFGM trilayer. PC, SM, and PS are generally localized to the outer membrane; PE, PC, SM, and PS are more enriched in the inner membrane; and PI and PS compose the majority of the inner monolayer.[155] MPLs, especially SM, were noted in this review to have significant cardioprotective activities; the appearance of a rodent and/or human image in the figure denotes model system-specific benefits of MPL on cardiometabolic health outcomes. The most compelling evidence in humans, and supported by rodent studies, indicates that MPLs reduce circulating cholesterol levels by intraluminal emulsification activities that limit intestinal cholesterol absorption. Although studies in humans are lacking, evidence from rodent models support MPLs as improving liver health and gastrointestinal health, potentially involving prebiotic and/or antimicrobial activities on gut microbiota. Although relatively more limited in study regardless of model system, MPL in rodents reduces adiposity, possibly through a mechanism involving the browning of adipose tissue. Note: Schematic representation of MFGM is not drawn to scale. *Abbreviations:* ADPH, adipophilin; BTN, butyrophilin; CD36, cluster of differentiation 36; CHOL, cholesterol; FABP, fatty acid–binding protein; GPL, glycerophospholipid; GluCer, glucosyl ceramide; LacCer, lactosyl ceramide; LDL-C, low-density lipoprotein cholesterol; MFG-EGF8, milk fat globule–epidermal growth factor 8 protein; MFGM, milk fat globule membrane; MPL, PC, phosphatidylcholine; PE, phosphatidylethanolamine; PI, phosphatidylinositol; PS, phosphatidylserine; SM, sphingomyelin; TJP, tight junction protein; VLDL-C, very-low-density lipoprotein cholesterol; XO, xanthine oxidase

cardiometabolic health, because the MPL content, especially MSM, has demonstrated benefits in humans, with mechanistic support from preclinical rodent models, to protect against hypercholesterolemia by influencing intraluminal emulsions and limiting intestinal cholesterol absorption (Figure 3).

At least in rodents, MPLs regulate diverse cardiometabolic responses. These include gut-level benefits to exert prebiotic and antimicrobial activities on gut microbiota as well as limiting metabolic endotoxemia, inflammation, and NAFLD risk. However, such benefits in humans have not been established, due to a lack of study, and reflect a need for controlled clinical studies. Efforts will require large-scale intervention studies, ideally with prescribed basal diets, to isolate the independent effects of MPLs on gut bacteria community structure and health-promoting functions. Such studies would also be expected to yield considerable interindividual variability. Thus, the integration of multiomics approaches may help define personalized approaches for effective nutritional management of cardiometabolic risk.[156] The needed rigorous experimental approaches, especially randomized controlled trials, coupled with omics workflows may also help answer a critical question: do the emerging and confirmed cardiometabolic benefits of MPL outweigh any unresolved concern about the higher saturated fat and energy content of full-fat dairy milk?

Acknowledgments

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