Dietary habits are a major determinant of cardiometabolic diseases including heart disease, stroke, and type 2 diabetes mellitus. In the United States, for example, poor diet contributes to nearly half of all cardiometabolic deaths, causing an estimated 1000 deaths each day. The resulting global health and economic burdens are staggering.

In recent years, nutritional science has been transformed by an explosion of evidence, shedding new light on key compounds and pathways for how diet influences health and well-being. Flavonoids, for example, have effects on skeletal muscle, adipocytes, liver, and pancreas, and myocardial, renal, and immune cells, for instance, related to 5′-monophosphate-activated protein kinase phosphorylation, endothelial NO synthase activation, and suppression of NF-κB (nuclear factor-κB) and TLR4 (toll-like receptor 4). Effects of dairy are similarly complex and may be mediated by specific amino acids, medium-chain and odd-chain saturated fats, unsaturated fats, branched-chain fats, natural trans fats, probiotics, vitamin K1/K2, and calcium, as well as by processing such as fermentation and homogenization. These characteristics of dairy foods influence diverse pathways including related to mammalian target of rapamycin, silent information regulator transcript-1, angiotensin-converting enzyme, peroxisome proliferator–activated receptors, osteocalcin, matrix glutamate protein, hepatic de novo lipogenesis, hepatic and adipose fatty acid oxidation and inflammation, and gut microbiome interactions such as intestinal integrity and endotoxemia. The complexity of these emerging pathways and corresponding biological responses highlights the rapid advances in nutritional science and the continued need to generate robust empirical evidence on the mechanistic and clinical effects of specific foods. (Circ Res. 2018;122:369-384. DOI: 10.1161/CIRCRESAHA.117.309008.)

Key Words: cardiovascular disease ■ cheese ■ diabetes mellitus ■ flavonoids ■ milk ■ review ■ yogurt
have systemic effects via interaction with the microbiota.9 Isoflavones and catechins have, in particular, been extensively studied (and covered by recent reviews) with regard to their cardiometabolic effects.10–14 As reviewed below, compelling experimental evidence suggests that flavonoids influence multiple physiological pathways related to cardiometabolic diseases (Figure 1).

**Microbial Generated Flavonoid Metabolites**

Colonized microbiota can enzymatically convert flavonoids into small phenolic acids and aromatic metabolites.15,16 Feeding studies that trace metabolic conversion suggest that flavonoids may be converted into small phenolic acids and aromatic metabolites.15,16

Such observations have increased interest in these microbiota-derived phenolic metabolites suppressed production of proinflammatory cytokines and vascular adhesion molecules, compared with their parent flavonoids.20–22 Several microbial derived flavonoid metabolites also protected against pancreatic β-cell dysfunction and death.23

Dietary flavonoids may also alter gut microbial composition, for example, because of probiotic-like properties and stimulation of growth of specific bacteria.24,25 In animal models of obesity, feeding of flavonoids altered gut microbiota community structure, including increased levels of Akkermansia muciniphila,26–28 which seem to confer metabolic benefits.29,30 Flavonoids may also influence the gut microbiota production of short-chain fatty acids (SCFA, up to 6 carbons in length).31 SCFA, predominantly acetic (2:0), propionic (3:0), and butyric (4:0) acids, are produced by large intestinal bacteria mainly from fermentation of nondigestible or poorly digestible carbohydrates (eg, dietary fiber).32 In addition to being an energy source, experimental studies suggest that microbial produced SCFA act as signaling molecules and can influence host energy metabolism, glucose-insulin homeostasis, production of endocrine hormones (eg, GLP-1 [glucagon-like peptide 1]), and inflammatory pathways. In some studies in mice and rats, dietary SCFA protected against weight gain, improved glucose tolerance, and increased insulin sensitivity.33–36 However, conflicting results have also been observed: in mice fed a high-fat/calorie diet, oral or intravenous acetate reduced food intake and weight gain,37,38 whereas intragastric infusion in rats fed a high-fat/calorie diet had the opposite effect.39 The reasons for these differences remain unclear, highlighting the need for further mechanistic studies including in humans. Experimental evidence suggests that physiological effects of SCFA are partly mediated by specific GPR (G-protein–coupled receptors) present in multiple cells and tissue types including colon, adipose, and the sympathetic nervous system.39 Specific SCFA may also act via different pathways: for example, in rats, metabolic benefits of dietary propionic acid required GPR activation, whereas butyrate did not.40 GPR signaling or other mechanisms such as epigenetic modification41 may account for antihypertensive and anti-inflammatory effects of SCFA in some cellular and animal studies.42,43 It remains unclear how much the variability of gut-produced SCFAs depends on flavonoids, and the clinical relevance

<table>
<thead>
<tr>
<th>Nonstandard Abbreviations and Acronyms</th>
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<tbody>
<tr>
<td>AMPK 5′-monophosphate-activated protein kinase</td>
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<tr>
<td>BP  blood pressure</td>
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<tr>
<td>CHD  coronary heart disease</td>
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<tr>
<td>CVD  cardiovascular disease</td>
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<tr>
<td>GLP-1  glucagon-like peptide 1</td>
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<tr>
<td>GPR  G-protein–coupled receptors</td>
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<tr>
<td>MCSFA  medium-chain saturated fatty acids</td>
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<tr>
<td>MFGM  milk fat globule membranes</td>
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<tr>
<td>MGP  matrix glutamate protein</td>
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<tr>
<td>MK  menaquione</td>
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<tr>
<td>mTOR  mammalian target of rapamycin</td>
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<tr>
<td>NF-κB  nuclear factor-κB</td>
</tr>
<tr>
<td>OCSFA  odd-chain saturated fatty acids</td>
</tr>
<tr>
<td>PDX  pancreatic duodenal homeobox</td>
</tr>
<tr>
<td>PGC-1α  peroxisome proliferator–activated receptor-γ coactivator-1α</td>
</tr>
<tr>
<td>PPAR  peroxisome proliferator-activated receptors</td>
</tr>
<tr>
<td>PREVDED  PREvention con Dieta MEDiterranea trial</td>
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<tr>
<td>RCT  randomized controlled trial</td>
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<td>SCFA  short-chain fatty acids</td>
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**Table. Subtypes of Flavonoids and Their Typical Food Sources**,†

<table>
<thead>
<tr>
<th>Flavonoids</th>
<th>Anthocyanidins</th>
<th>Flavonols (Flavan-3-ols)</th>
<th>Flavanones</th>
<th>Flavones</th>
<th>Flavonols</th>
<th>Proanthocyanidins</th>
<th>Isoflavones</th>
</tr>
</thead>
<tbody>
<tr>
<td>Major Flavonoids</td>
<td>Food Sources</td>
<td>Major Flavonoids</td>
<td>Food Sources</td>
<td>Major Flavonoids</td>
<td>Food Sources</td>
<td>Major Flavonoids</td>
<td>Food Sources</td>
</tr>
<tr>
<td>Cyanidin Delphinidin Malvidin Pelargonidin Peonidin Petunidin</td>
<td>Berries, red wine</td>
<td>Catechins epicatechins</td>
<td>Apricots, cocoa, chocolates, red grapes, red wine, tea</td>
<td>Eriodictyol Hesperitin naringenin</td>
<td>Citrus fruit, mint, tomatoes</td>
<td>Apigenin Luteolin</td>
<td>Celery, parsley</td>
</tr>
</tbody>
</table>

*Major flavonoids include the 6 major subclasses consumed in the United States (anthocyanidins, flavonols, flavanones, flavones, flavonols, and proanthocyanidins),4 as well as isoflavones, which are regularly consumed by populations with a high intake of soy and legumes.5†Typical food sources according to Gu et al4 for proanthocyanidins and Bondonno et al† for all other subtypes of flavonoids.
of microbiota-generated SCFA in humans is being elucidated. Yet, the overall emerging evidence supports bidirectional interactions between flavonoid consumption and gut microbiota composition and function that alter physiological pathways relevant to cardiometabolic health.

**Glucose-Insulin Homeostasis**

A large number of animal-experimental studies have tested the effects of purified flavonoid compounds or flavonoid-rich plant extracts across multiple tissues. Relevant molecular pathways seem to include (1) modulation of gene expression and signaling pathways. Enhancement of AMPK (5′-monophosphate-activated protein kinase) phosphorylation and activation appears to be a common mechanism affected by several types of flavonoids. Modulation of other signaling pathways has also been observed including increased expression of PPAR-γ (peroxisome proliferator-activated receptor-γ) and inhibition of NF-κB (nuclear factor-κB) activation; (2) interaction with gut microbiota. Dietary flavonoids may alter gut-microbial composition because of probiotic-like properties and stimulate growth of specific bacteria (e.g., Akkermansia muciniphila) that may confer metabolic benefits. Conversely, metabolism of dietary flavonoids by gut bacteria generates downstream metabolites (e.g., phenolic acids) that may possess unique properties and reach higher circulating and tissue concentrations compared with parent flavonoids, thus enhancing biological activity of flavonoids; (3) Direct flavonoid–protein interactions. Growing evidence suggests that flavonoids both stimulate and inhibit protein function, including of ion channels in the vasculature and liver and carbohydrate digestive enzymes (α-amylase and α-glucosidase) in the gastrointestinal tract. Such effects may partly contribute to regulation of vascular tone and glucose metabolism. ERK1/2 indicates extracellular signal-regulated kinases 1 and 2; GLUT4, glucose transporter type 4; IRS2, insulin receptor substrate-2; MAPK, mitogen-activated protein kinase; PGC-1α, peroxisome proliferator-activated receptor-γ coactivator-1α; PKA; protein kinase-A; SREBP-1c, sterol regulatory element–binding protein-1c; TG, triglycerides; and TLR4, toll-like receptor 4. (Illustration Credit: Ben Smith.)

In vitro studies suggest that a variety of flavonoids inhibit key enzymes involved in the digestion and absorption of dietary carbohydrates including α-amylase, α-glucosidase, and sodium-dependent glucose transporter, which may contribute to reduced postprandial glycemia. Flavonoids could also improve glucose–insulin homeostasis via multiple signaling pathways. Cell culture and animal studies have identified adenosine 5′-adenosine monophosphate-activated protein kinase (AMPK) and PPAR-γ (peroxisome proliferator–activated receptor-γ) as 2 of the key pathways via which some flavonoids enhance muscle glucose uptake and improve adipocyte function. Flavonoid treatment in animal models also led to reduced liver fat accumulation and improved hepatic insulin response.
sensitivity, which were related to reductions in de novo lipogenesis and increase in fatty acid β-oxidation. Finally, cellular and animal studies suggest that several types of flavonoids protected pancreatic β-cells against glucotoxicity and inflammation and enhanced insulin secretion. Activation of AMPK has again been implicated in mediating the effects of flavonoids on insulin secretion, but other mechanisms including modulation of intracellular calcium through activation of membrane ion channels have also been identified for specific flavonoids.

**NO Bioavailability, Redox Status, and Vasoregulation**

In animal experiments, administration of flavonoids exerted vasorelaxation effects and lowered blood pressure. A key pathway via which flavonoids regulate vascular health is through altered NO metabolism, with evidence for both direct and indirect mechanisms. Several flavonoids can directly increase endothelial NO synthase expression and activity (the main source of NO in the vasculature), leading to enhanced production of NO. Effects on endothelial NO synthase level could be mediated through activation of AMPK. Flavonoids could also indirectly enhance NO bioavailability through lowering the production or enhancing the removal of reactive oxygen species that are known to breakdown NO. Treatment with different subgroups of flavonoids increased the activity of endogenous antioxidant enzymes including sodium oxide dismutase and catalase, reduced superoxide radical generation by NADPH (nicotinamide adenine dinucleotide phosphate) oxidase, and lowered protein and lipid biomarkers of oxidative stress.

**Weight Maintenance**

Supplementation with flavonoids prevented diet-induced weight gain in several animal models of obesity. In these investigations, flavonoids did not seem to influence energy intake, suggesting that they may contribute to weight regulation by increasing energy expenditure. For example, luteolin (a flavonoid abundant in pepper, apple skins, and carrots) upregulated AMPK and PGC-1α (PPAR-γ coactivator 1α) signaling cascades, leading to elevated thermogenic gene expression in brown and subcutaneous adipose tissues and enhanced energy expenditure in C57BL/6 mice fed low- or high-fat diets. Other flavonoids have also demonstrated an ability to induce brown fat–specific genes and proteins in cultured adipocytes. Additional mechanisms via which flavonoids could increase energy expenditure have been observed in animal-feeding studies, including stimulation of the sympathetic nerve system and increased skeletal muscle mitochondrial biogenesis and function. Several types of flavonoids may also prevent fat accumulation via reduced lipogenesis and increased β-oxidation of fatty acids as demonstrated in cultured adipocytes and mice.

**Anti-Inflammatory Effects**

Some flavonoids have demonstrated anti-inflammatory properties in adipose and myocardial tissues in animal studies after varied inflammatory stimuli including ischemia–reperfusion, diabetes mellitus, medication use, and high-fat diet. In these models, oral supplementation with flavonoids led to reduced inflammatory cell infiltration, lowered levels of proinflammatory cytokines and tissue fibrosis, and improved cell survival and function. A central pathway that seemed to mediate the anti-inflammatory effect of several flavonoids was inhibition of signaling via NF-κB (nuclear factor-κB). However, other mechanisms are likely involved and have been identified for specific flavonoids—for example, hexameric procyanidins (present in high concentrations in cocoa, tea, and apples) inhibited the binding of tumor necrosis factor-α to its receptor and subsequent proinflammatory activation in cultured cells.

**Clinical Effects**

A growing body of laboratory studies and randomized trials support cardiometabolic benefits of flavonoid-rich foods such as cocoa, tea, and berries. Flavonoid-rich cocoa produces small but measurable benefits on blood pressure (BP), endothelial function, insulin resistance, and blood lipids. In a systematic review and meta-analysis of 42 randomized controlled trials, chocolate, cocoa, and flavan-3-ol significantly reduced mean arterial pressure (−1.64 mm Hg; 95% confidence interval [CI], −3.27 to −0.01 mm Hg), improved flow-mediated dilation (1.34%; 95% CI, 1%–1.68%), lowered HOMA-IR (homeostatic model assessment for insulin resistance; −0.67; 95% CI, −0.98 to −0.36), and marginally improved low-density lipoprotein cholesterol (LDL-C) and high-density lipoprotein cholesterol (−0.07 and +0.03 mmol/L, respectively). BP lowering has been seen with as little as 6.3 g/d of dark chocolate (≈30 kcal/d, ie, =1.5% of total daily energy added to habitual diets without recommendations for other dietary calories). These benefits seem related to improved endothelial production of NO, a fundamental pathway for vascular and metabolic health that suggests the potential for benefits beyond lowering of BP alone. Accumulating data suggest that green or black tea can also modestly reduce BP in prehypertensive and hypertensive individuals—a meta-analysis of 10 trials suggests reduction of systolic blood pressure and diastolic blood pressure by 2.36 and 1.77 mm Hg, respectively—although risk of bias in most of these trials could not be fully evaluated because of insufficient reported information. A systematic review and meta-analysis of 22 randomized controlled trials of berries found moderate improvements in systolic blood pressure (−2.72 mm Hg; 95% CI, −5.32 to −0.12) and small improvements in glycemic control (HbA1C [hemoglobin A1C], −0.20%), body mass index (−0.36 kg/m²), LDL-C (−0.21 mmol/L), and inflammatory biomarkers (tumor necrosis factor-α, −1 pg/mL). Yet, most of these randomized controlled trials were small and of limited duration (<6 months).

Complementary to interventional evidence, observational studies evaluating dietary flavonoids or flavonoid-rich foods have observed lower risk of cardiometabolic events. For example, among >90,000 middle-aged nurses followed for 18 years, those within the highest versus lowest quintile of estimated dietary anthocyanin intake had 32% lower risk of incident myocardial infarction (95% CI, 4%–51%; P=0.03); however, other major subclasses of...
flavonoids (flavanones, flavan-3-ol, flavonols, flavones, and flavonoid polymers) and total flavonoids were not associated with myocardial infarction. Subclass-specific associations were also observed in other population-based cohort studies, including for flavanones and ischemic stroke, and flavonols and type 2 diabetes mellitus. These findings suggest potential heterogeneous effects of different types of flavonoids in relation to specific cardiometabolic outcomes. Estimation of dietary flavonoid intake has important limitations including errors in self-recall and inaccuracies in food composition databases. Assessment of urine or blood flavonoid biomarkers is, therefore, a complementary approach to examining exposure but has only been used in a handful of long-term studies. These have predominantly focused on isoflavones and type 2 diabetes mellitus and suggest moderate inverse associations for daidzein and genistein, major isoflavones in soy foods. Data for other flavonoid biomarkers and risk of cardiometabolic diseases are scarce and inconsistent. Additional studies with larger sample sizes across population groups with diverse demographic and dietary habits are needed.

There is also evidence for cardiometabolic benefits of nuts and extravirgin olive oil, rich in other types of phenolic compounds (eg, phenolic acids and lignans). In the PREDIMED trial (PREvencion con DIeta MEDiterranea), participants at high risk of cardiovascular disease (CVD) were randomized to a Mediterranean dietary pattern and provided with daily extravirgin olive oil or mixed nuts (walnuts, hazelnuts, and almonds). Compared with the control diet (advice to reduce dietary fat), the intervention diets significantly improved CVD risk factor profiles including LDL-C, BP, and inflammatory biomarkers and result in a 30% lower risk of death, myocardial infarction, or stroke. Participants in the intervention groups also demonstrated less gain in central adiposity and decreases in body weight after 5 years of follow-up. Meta-analysis of prospective cohort studies provide further support of cardiometabolic benefits of higher nuts consumption: for each 1 oz (28 g) per day, a 30% lower risk of coronary heart disease (CHD; n=11 studies; relative risk=0.71; 95% CI, 0.63–0.80) and 39% lower risk of diabetes mellitus (n=4 studies; relative risk=0.61; 95% CI, 0.43–0.88).

Overall, growing evidence supports meaningful cardiometabolic benefits of foods rich in flavonoids and other phenolics. These findings support recommendations to increase dietary consumption of these foods and provide clear impetus for additional mechanistic trials, prospective cohorts, and clinical trials to better characterize the specific compounds of interest and their dose–response effects.

**Dairy Foods**

Dairy products constitute ~10% of all calories in the US diet. Yet, for such a major share of the food supply, relatively little research has evaluated the direct health impact of consuming dairy foods. Traditional dietary recommendations on dairy derive mostly from theoretical considerations about isolated nutrients (eg, eat 3 daily servings to obtain calcium or vitamin D for bone health; eat low-fat products to reduce calories for weight gain and reduce saturated fat for heart disease), rather than empirical evidence on actual mechanistic and clinical effects of consuming milk, cheese, yogurt, butter, or other dairy foods. Growing evidence suggests that different dairy foods have complex cardiometabolic effects based on potential inter-related influences of a range of nutrients and other characteristics such as probiotics, fermentation, and possibly homogenization (Figure 2). We do not discuss the potential role of vitamin D here, which has been extensively reviewed elsewhere.

**Calcium**

Cell culture and animal experiments have assessed calcium and cardiometabolic risk, alone or in conjunction with other dietary components. In several animal models of obesity, calcium supplementation inhibited weight gain, attenuated hepatic steatosis, and reduced hyperglycemia and insulin resistance. These effects were potentially mediated by correction of leptin and GLP-1 signaling, reduced levels of calcitriol (1,25-dihydroxyvitamin D3), suppression of hepatic and adipose lipogenesis, and alterations in gut microbiota composition. However, other animal models have not demonstrated such benefits. For example, in a mouse model of diet-induced obesity, calcium supplementation caused weight gain relative to control. In a meta-analysis of 20 trials including 2711 participants, calcium supplementation did not significantly lower body weight (−0.17 kg, 95% CI, −0.70 to 0.37) or body fat (−0.19 kg; 95% CI, −0.51 to 0.13). In comparison, dairy foods increase lean mass and reduce body fat, compared with control, in the presence of energy restriction for weight loss (see the section on Clinical Effects, below), suggesting that other components beyond calcium may be relevant.

In short-term trials in humans, calcium supplements modestly lower BP, with mean difference (95% CI) for systolic blood pressure, −1.43 mm Hg (−2.15 to −0.72 mm Hg; I²=0%) and for diastolic blood pressure, −0.98 mm Hg (−1.46 to −0.50 mm Hg; I²=49%). In some animal models of hypertension, reduction in BP after calcium supplementation were linked to improvement in both endothelial dependent and independent arterial relaxation, enhanced hyperpolarization of vascular smooth muscle, increased sodium excretion, and downregulation of renal angiotensin-converting enzyme. However, whether calcium intake has similar effects on these pathways in humans is not clear. Meta-analysis of long-term randomized trials found that calcium supplementation resulted in trends toward moderately elevated risk of myocardial infarction. For example, in the study by Mao et al, the odds ratio (95% CI) for the calcium-supplemented compared with the placebo group was 1.28 (0.97–1.68; P=0.08; I²=0%). Genetic variants related to higher serum calcium level also relates to elevated risk of myocardial infarction and coronary artery disease in Mendelian randomization studies. The potential for increased risk has been hypothesized to relate to postprandial hypercalcemia that occurs with supplements, in comparison to intake from foods, that may contribute to vascular calcification. Overall, calcium is not a convincing driver of cardiometabolic benefits of dairy foods, although effects could also depend on supplement versus dietary sources.
**Dairy Protein**

Bovine milk contains ≈32–34 g/L protein, largely casein (used to make curds during milk processing; ≈80% of dairy protein) and also whey protein (≈20%).

Both casein and whey protein include several smaller protein fractions and differ in amino acid composition. In some animal studies, enriching diets with casein, whey protein, or complete milk protein improved glucose–insulin and cardiometabolic risk factors. Such benefits might relate to specific dairy amino acids. For example, whey protein is rich in the branched-chain amino acids leucine, isoleucine, and valine, which activates important signaling pathways including mTOR (mammalian target of rapamycin) and silent information regulator transcript 1, which could contribute to enhanced thermogenesis and insulin secretion. However, branched-chain amino acid supplementation in animal studies has shown mixed results related to metabolic outcomes. Relatively few controlled trials of intact milk protein isolates have been performed in humans.

Several focused on casein-derived lactotripeptides, which significantly lowered systolic (mean difference, −2.95 mm Hg; 95% CI, −4.17 to −1.73 mm Hg) and diastolic BP (mean difference, −1.51 mm Hg; 95% CI, −2.21 to −0.8 mm Hg) based on pooled results across studies, although these findings should be interpreted cautiously because of substantial heterogeneity and potential for publication bias. Other short-term clinical studies (≤12 weeks) evaluated effects of milk protein on glucose–insulin homeostasis: overall favorable effects were observed, but long-term studies remain limited.

Bioactive peptides derived from dairy protein may also contribute, generated during fermentation (eg, in the production of cheese or kefir, sour milk) via action of bacterial proteolytic enzymes or during gastrointestinal (including microbiota-related) digestion. Several short peptides (3–4 amino acids in length) from casein and whey protein demonstrated inhibitory activity toward angiotensin-converting enzyme in vitro. Other dairy-derived peptides have also been shown...
to moderately inhibit dipeptidyl peptidase-4,152,153 which may contribute to increased half-life of incretin hormones (gastric inhibitory peptide and glucagon-like peptide-1) and improved glycemic control.143 On the other contrary, the relevance of such dairy-derived bioactive peptides has been challenged based on their low bioavailability, which produces circulating levels in the picomolar to nanomolar range.144

Overall, experimental and short-term human metabolic studies support potential cardiometabolic benefits of dairy protein, but the relative efficacy of casein versus whey protein, effects of individual amino acids versus peptide metabolites, and corresponding molecular mechanisms and relevant pathways remain understudied.

**Dairy Fats**

Dietary guidelines generally recommend low/nonfat dairy based on LDL-raising effects of myristic (14:0) and stearic (16:0) saturated fatty acids, underemphasizing positive effects of these fatty acids on very-low-density lipoprotein, chylomicron remnants, and high-density lipoprotein cholesterol155 and paying even less attention to potential health effects of the many other fatty acids that comprise the majority of dairy fat (eg, 14:0 plus 16:0 comprise ≤40% of total fatty acids in cow, sheep, and goat’s milk).156 These include medium-chain saturated fats (MCSFA) (between 6 and 12 carbons, ie, 6:0–12:0), odd-chain saturated fats (15:0, 17:0), monounsaturated and polyunsaturated fatty acids (18:1n-9, 18:2n-6, and 18:3n-3), branched-chain saturated fats, and trace amounts of natural (ruminant) trans fats (eg, transpalmitoleic acid, trans-16:1n-7).156–158 Dairy fat is also a source of phospholipids (milk fat globule membrane) and fat-soluble vitamins including D, K, and K2 (produced during fermentation; see below). MCSFA, representing ≈6% to 17% of dairy milk fatty acids, have different molecular and metabolic activities than longer chain fatty acids. For example, whereas longer chain saturated fats (16:0 and 18:0) activated NF-kB and decreased insulin sensitivity in cultured skeletal muscle cells, the MCSFA 8:0 and 12:0 did not.159 MCSFA also enhanced mitochondrial oxidative capacity and reduced lipid accumulation in cultured muscle cells relative to 16:0.160 These effects may account for observed reductions in body fat accumulation and insulin resistance in animals fed high MCSFA versus longer chain saturated fats.160,161 On the contrary, relative to a low-fat control diet, high-fat feeding with MCSFA enhanced hepatic de novo lipogenesis and triglyceride accumulation and reduced hepatic insulin sensitivity, in animal models.161,162 Induction of hepatic lipogenesis could be because of MCSFA activation and signaling via liver X receptor-α.163 Notably, many of the prior animal experiments examining MCSFA were obesity models and also focused on fruit (coconut) sources, and, thus, the metabolic effects of dairy-derived MCSFA under euglycemic conditions (eg, substituting for other types of dietary fatty acids) remain unclear.

The biological effects of trans-16:1n-7, branched-chain saturated fats, and odd-chain saturated fats have received relatively little attention. It has been hypothesized164 that dietary trans-16:1n-7 could exert similar effects as dietary cis-16:1n-7, which when consumed in the diet or produced outside the liver seems to act in a negative feedback loop to inhibit hepatic de novo lipogenesis, improve insulin sensitivity, and reduce inflammation,165-169 with corresponding risk factor improvements in one human trial.170 In cultured INS-1 β cells, treatment with trans-16:1n-7 activated PPAR-γ and the transcription factor PDX-1 (pancreatic duodenal homeobox-1).171 Yet, relevance of such effects on glucose–insulin homeostasis and other molecular effects of trans-16:1n-7 remain unknown. Potential mechanisms of branched-chain saturated fats also remain little explored. A branched chain FA (15-methyl-hexadecanoic acid) exhibited similar effects on PPAR-γ and PDX-1 as trans-16:1n-7 in cultured INS-1 β cells under basal conditions, and additionally countered high glucose mediated suppression of PDX-1.171 Intake of branched-chain saturated fats is not insubstantial—with estimated average at ≈500 mg/d in the United States (primarily from dairy and beef products),158 compared with between 125 and 160 mg/d for seafood-derived long-chain n-3 polyunsaturated fats.172 These findings highlight the potential quantitative importance of dietary intake of branched-chain saturated fats and the need to further assess their biological functions. Odd-chain saturated fats from dairy fat are incorporated into a range of tissues including blood, liver, and adipose.173,174 In addition to serving as an energy source via β-oxidation, other metabolic functions have been proposed such as enabling replenishment of the citric acid cycle and improving mitochondrial function,174 but such hypotheses remain to be tested in rigorous experimental investigations.

**Milk Fat Globule Membrane**

Milk fat is naturally bound by milk fat globule membranes (MFGM), a trilayered membrane rich in polar lipids (phospholipids and sphingolipids) and proteins, enclosing a triglyceride core (globule) of fatty acids.175 These polar lipids and proteins in MFGM seems to be bioactive. In mice, supplementation with sphingolipids and bovine milk phospholipids reduced serum cholesterol and hepatic lipid accumulation, attributed to reduced intestinal cholesterol uptake and changes in hepatic gene expression.176-178 Possible anti-inflammatory properties have also been reported—mice fed a MFGM-enriched diet exhibited decreased inflammatory responses to a systemic lipopolysaccharide challenge, possibly because of reduced gut permeability.179 Processing of dairy products can change the content and structure of MFGM—for instance, homogenization may destroy MFGM.180 A recent randomized trial among 57 overweight adults compared the effects on blood lipids and genetic expression of consuming ≈15% of calories from whipping cream (intact MFGM) versus butter (little MFGM because of homogenization), otherwise equivalent in contents of dairy fat and saturated fat. After 8 weeks, those consuming butter had predictable increases in LDL-C and apolipoprotein B:A-I ratio, whereas those consuming whipping cream showed no changes in their lipid profile.180 The whipped cream group demonstrated significantly lower expression of 19 genes in peripheral blood mononuclear cells, including USP45, MDM2, SNRPN, and CAPZA1, supporting effects of MFGM on genetic expression. Similar blunted effects on total and LDL-C have been seen in crossover trials comparing cheese to butter or nondairy saturated fat (see the section on Clinical Effects, below).181,182 These findings
suggest that MFGM and corresponding processing methods that preserve or destroy it may have important implications for cardiometabolic effects of dairy fat.

**Probiotics**

A growing body of evidence supports health effects of probiotics in foods, live microorganisms that can alter foods’ characteristics and host responses after consumption. Both yogurt and kefir (a fermented milk drink) often contain live bacteria (kefir can also contain yeasts). In several animal models of obesity and diabetes mellitus, dairy products with probiotics demonstrated cardiometabolic benefits compared to those without probiotics. For example, in C57BL/6 mice fed high-calorie/fat diets, animals given kefir had reduced weight gain, hepatic steatosis, LDL-C, and interleukin-6 levels compared with mice given unfermented milk. Such changes were accompanied by altered expression of hepatic and adipose tissue genes related to fatty acid oxidation (AOX, PPAR-α) and inflammation (MCP-1). Other studies suggest that efficacy is probiotic specific: eg, compared with unfermented milk, milk fermented with different strains of *Lactobacillus rhamnosus* probiotic specific: eg, compared with unfermented milk, milk fermented with different strains of *Lactobacillus rhamnosus* improved glucose tolerance and fasting glucose to varying extents in a diabetic rat model. The molecular mechanisms for probiotics’ health effects seem to involve changes in both composition and function of host gut microbiota. For instance, microbiota composition in animals was altered by probiotic dairy products such as yoghurt and kefir. Such compositional changes may enhance intestinal epithelial integrity and reduce low-grade inflammation because of endotoxemia (leakage of gut microbiota–derived lipopolysaccharide into systemic circulation), a putative contributor to obesity-related diseases. Probiotics also seem to influence host microbiota function, for example, altering production of functional mediators such as SCFA that may exert local and systemic effects on host metabolism. In sum, animal-experimental studies and human trials support a role for probiotics and probiotic–microbiome interactions in protective effects of yogurt for weight gain, obesity, and related metabolic conditions such as gestational diabetes mellitus.

**Cheese, Fermentation, and Vitamin K**

There are 2 major forms of vitamin K: K1 (phyloquinone, rich in green-leafy vegetables and certain vegetable oils) and K2 (menaquinone, [MK], differentiated by the number of isoprene residues, MKn). Several vitamin K2–producing bacteria species are commonly used in industrial dairy fermentation, and cheese is a major source of vitamin K2 (especially MK-7, -8, and -9) in Europe and North America. All forms of vitamin K act as cofactors for post-translational carboxylation of protein glutamate residues into γ-carboxy glutamate, required for vitamin K–dependent proteins to become active. Although coagulation factors such as factors VII, IX, and X are well-known vitamin K–dependent proteins, growing evidence suggests that additional vitamin K–dependent proteins influence cardiometabolic health. This includes osteocalcin (made in bone cells) and MGP (matrix glutamate protein, primarily made in vascular smooth muscle cells and cartilage). Animal studies support a role of osteocalcin in improving β-cell proliferation, insulin expression, and upregulation of adiponectin in adipocytes. In several metabolic studies, vitamin K supplementation increased carboxylated osteocalcin concentrations and reduced insulin resistance. However, results were not always consistent in human studies, and opposing directions of associations between carboxylated/undercarboxylated forms of osteocalcin with insulin sensitivity have been observed in mice versus human, suggesting possible species differences. Levels of dietary vitamin K and proportions of osteocalcin that must be γ-carboxylated to improve glucose–insulin homeostasis also remain unclear. Similarly, although it has been hypothesized that vitamin K may reduce CVD risk by augmenting MGP, an inhibitor of vascular calcification, this has not yet been convincingly established. In several rats and mice studies, supplementation with vitamin K reduced arterial calcification, but whether such effects were mediated by MGP carboxylation or other mechanisms remains unclear.

Human metabolic studies demonstrate that specific types of vitamin K2 have longer half-lives and reach higher circulating levels than vitamin K1. For instance, compared with a half-life of 1 to 2 hours for vitamin K1, MK-7 and MK-9 have estimated half-lives of 2 to 3 days. These differences in bioavailability may have functional consequences—in one study among healthy adults, supplementation with MK-7 induced more complete carboxylation of osteocalcin. Such findings suggest that vitamin K2 moieties (representing ≈15% to 20% of total dietary vitamin K in Western diets, with the rest as vitamin K1) may disproportionately contribute to vitamin K activity in vivo. Furthermore, recent cohort studies suggest that K2, but not K1, is linked to lower CVD risk. For example, in a prospective cohort study among 16057 women aged 49 to 70 years, the hazard ratio for the risk of CHD per 10 μg/d (equivalent to ≈1 SD) of K2 intake was 0.91 (95% CI, 0.81–1.00; P=0.04), but K1 intake was not related to CHD risk. Given these findings and the specific links of cheese and fermented milk to clinical outcomes (see below), the potential role of fermentation and vitamin K2 in cardiometabolic risk represents a new area of promise for further research.

**Clinical Effects**

In short-term randomized trials, consumption of milk or overall dairy products increases lean mass and reduces body fat, especially in the setting of energy-restricted weight loss diets. Long-term effects are less clear and may vary by type of dairy. Observationally, several studies suggest that children who drink more low-fat milk gain more weight over time, whereas those who drink more whole-fat milk gain less weight. Few long-term trials have been performed in children, other than multicomponent dietary interventions that preclude inference on dairy per se. In longitudinal studies among adults, relationships between dairy intake and weight, CVD, and diabetes mellitus end points vary more by food type (eg, cheese, yogurt, milk, and butter) than fat content. For example, neither low-fat nor whole milk are appreciably related to long-term weight gain, perhaps related to subtle caloric compensation: when people eat more low-fat dairy, they on average increase their consumption of carbohydrates, whereas people who eat...
more full-fat dairy on average decrease their carbohydrate intake.\textsuperscript{202} Cheese consumption similarly seems relatively neutral for long-term weight gain, although this might be modified by carbohydrate intake: weight gain is seen when cheese is accompanied by refined carbohydrates, and relative weight loss is seen when cheese replaces refined carbohydrates.\textsuperscript{202} Yogurt seems consistently protective against long-term weight gain,\textsuperscript{196,202,203} even if sugar-sweetened (although in this case, only about half the benefit is seen, compared with unsweetened yogurt\textsuperscript{203}).

Although increased intake of saturated fat from dairy products would be expected to increase LDL-C,\textsuperscript{236} recent randomized controlled trials support heterogeneity in such effects depending on the type of dairy foods consumed. For instance, in a randomized crossover trial among 49 men and women, consuming equivalent amounts of fat and saturated fat from cheese, compared with butter, lowered total, LDL, and high-density lipoprotein cholesterol concentrations.\textsuperscript{181} Similar blunted effects on total and LDL-C were seen in a randomized controlled crossover trial comparing saturated fat and different dairy ingredients and mechanisms by which foods influence health. Numerous in vitro and animal studies support pleiotropic effects of flavonoids on multiple risk factors and pathways relevant to cardiometabolic diseases. Although molecular mechanisms continue to be clarified, identified signaling pathways include AMPK, PPAR-\gamma, PGC-1\alpha, and NF-\kappaB. Existing experimental studies also have methodologic limitations and the potential for publication bias, and the relevance of their findings to humans remains unclear. In addition, with >5000 naturally occurring flavonoids identified to date,\textsuperscript{235} observed effects on molecular pathways for some flavonoids are unlikely to be generalizable to others. Many mechanistic studies to date have focused on parent aglycone forms of flavonoids and frequently at supraphysiological concentrations (eg, 25–100 \(\mu\)mol/L, whereas systemic circulating concentrations in vivo are unlikely to reach \(\geq 10\ \mu\)mol/L).\textsuperscript{21,252} Although findings based on supraphysiological doses may be relevant for the development of flavonoids as pharmacological agents, they are less generalizable to cardiometabolic effects of flavonoids at usual dietary levels of intake. Furthermore, prior experimental studies have generally not accounted for complexities in flavonoid bioavailability and metabolism. For instance, most dietary flavonoids (except for flavon-3-ol) are found as glycosides, bound to one or more sugar moieties,\textsuperscript{7} which generally require hydrolysis before intestinal absorption.\textsuperscript{8} After absorption, flavonoids undergo phase-I and phase-II metabolism and are transformed into diverse glucuronidated, sulfated, and methylated metabolites.\textsuperscript{64} Unabsorbed flavonoids are also catabolized by colonic bacteria into many phenolic acids. Compared with their parent compounds, many flavonoid metabolites have longer half-lives and achieve much higher concentrations in circulation.\textsuperscript{22} Cardiometabolic effects of flavonoids observed in animal studies may, therefore, be largely attributable to their metabolites, rather than the pre-metabolized flavonoids. Yet, relatively few investigations have evaluated potential biological effects of flavonoid metabolites, partly limited by lack of available synthetic standards.\textsuperscript{20,22} On the basis of the promise of these compounds for physiological health, future mechanistic, experimental, and clinical studies are needed that take into account the diversity of types, bioavailability, and metabolism of flavonoids and their metabolites to better understand the most appropriate form and pathways for clinical benefits.

Similarly, for dairy foods, a variety of ingredients and processing methods seem to influence cardiometabolic health. Potentially relevant ingredients include specific amino acids, medium-chain and odd-chain saturated fats, unsaturated fats, branched-chain fats, natural \textit{trans} fats, probiotics, vitamin K1/K2, and calcium, as well as by processing techniques such as fermentation and homogenization. Corresponding pathways of effects include those related to mTOR, silent information regulator transcript-1, angiotensin-converting enzyme, peroxisome proliferator–activated receptors, osteocalcin, matrix glutamate protein, hepatic de novo lipogenesis, hepatic and adipose fatty acid oxidation and inflammation, and gut microbiome interactions such as intestinal integrity and endotoxemia.

For both flavonoids and dairy foods, the complexity of the emerging mechanistic pathways and responses is remarkable. This new evidence highlights the tremendous growth in knowledge, as well as the extent of what remains to be learned, on how different dietary factors influence health. In cohorts using objective biomarkers, higher blood biomarkers of diabetes mellitus also vary by food type: yogurt, but not milk, is consistently associated with lower risk, whereas consumption of cheese, which has highest calorie, fat, and saturated fat content, also associates with lower risk in several although not all studies.\textsuperscript{232,234,235,238,239} These differences may be partly elucidated by the divergent associations of total milk (generally unassociated with diabetes mellitus) versus fermented milk (linked to lower risk),\textsuperscript{234,238,240} suggesting a potential role for metabolic benefits of fermented products such as cheese (see above).

Interestingly, dairy fat itself may promote cardiometabolic health. In cohorts using objective biomarkers, higher blood levels of dairy fatty acids consistently associate with lower incidence of diabetes mellitus\textsuperscript{241–245} and perhaps CHD.\textsuperscript{246–248} With mixed findings for stroke,\textsuperscript{249} As described above, mechanistic explanations for these observations remain unclear, which could include metabolic effects of fermented foods (especially cheese, a major source of dairy fat), links of such biomarkers to MFGM, specific fatty acids (eg, branched-chain fatty acids, MCSFA, and specific ruminant \textit{trans} fats), other lipid-soluble factors, or unknown endogenous (nondietary) determinants of these blood biomarkers.\textsuperscript{250}

\textbf{Future Directions and Conclusions}

Modern nutritional science is elucidating the diversity of ingredients and mechanisms by which foods influence health. In vitro and animal studies support pleiotropic effects such as intestinal integrity and endotoxemia.
health. Given the prime importance of nutrition for cardiovascular and metabolic health, these results support the need for vigorous further investigation on the relevant components, biological pathways, and clinical effects of these and other foods.

Sources of Funding

The research reported in this article was supported by The National Heart, Lung, and Blood Institute, National Institutes of Health (2R01-HL085710, PI Dr Mozaffarian).

Disclosures

Dr Mozaffarian reports ad hoc honoraria or consulting from Astra Zeneca, Acasti Pharma, Boston Heart Diagnostics, GOED, DSM, Nutrition Impact, Haas Avocado Board, and Pollock Communications; scientific advisory board, Omada Health; and chapter royalties from UpToDate. Dr Wu reports research support from Unilever.

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Flavonoids, Dairy Foods, and Cardiovascular and Metabolic Health: A Review of Emerging Biologic Pathways
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Circ Res. 2018;122:369-384
doi: 10.1161/CIRCRESAHA.117.309008
Circulation Research is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0009-7330. Online ISSN: 1524-4571

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circres.ahajournals.org/content/122/2/369

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