Low-Fat Milk Ingestion Prevents Postprandial Hyperglycemia-Mediated Impairments in Vascular Endothelial Function in Obese Individuals with Metabolic Syndrome

Kevin D. Ballard, Eunice Mah, Yi Guo, Ruisong Pei, Jeff S. Volek, and Richard S. Bruno

Abstract

Greater intakes of low-fat dairy foods are associated with a lower risk of cardiovascular disease. The objective of this study was to examine whether acute low-fat milk ingestion would limit postprandial impairments in vascular endothelial function by limiting oxidative stress responses that decrease nitric oxide (NO) bioavailability. A randomized, double-blind, crossover study was conducted in adults with metabolic syndrome (MetS) who ingested low-fat milk (475 mL) or an isocaloric volume of rice milk after an overnight fast. Brachial artery flow-mediated dilation (FMD), plasma glucose, malondialdehyde (MDA), arginine (ARG), and asymmetric dimethylarginine (ADMA) were assessed at 30-min intervals during the 3-h postprandial period. Participants (n = 19) postprandial FMD responses were unaffected by low-fat milk but transiently decreased (P < 0.01) from 6.2 ± 0.8% (mean ± SEM) at baseline to 3.3 ± 0.7% at 30 min and 3.9 ± 0.6% at 60 min following rice milk consumption. Glucose and MDA increased to a greater extent in the rice milk trial (P < 0.001). The MDA area under the 3-h postprandial curve (AUC0–3 h) was correlated with glucose AUC0–3 h (r² = 0.75, P < 0.01) and inversely related to FMD AUC0–3 h (r = −0.59; P < 0.01). ARG decreased following rice milk and increased with low-fat milk, whereas only rice milk increased ADMA/ARG. The ADMA/ARG AUC0–3 h was correlated with MDA AUC0–3 h (r = 0.55) and was inversely related to FMD AUC0–3 h (r = −0.52; P < 0.05). These findings suggest that low-fat milk maintains vascular endothelial function in individuals with MetS by limiting postprandial hyperglycemia that otherwise increases lipid peroxidation and reduces NO bioavailability. This trial was registered at clinicaltrials.gov as NCT01411293. J. Nutr. doi: 10.3945/jn.113.179465.

Introduction

Greater intakes of low-fat dairy foods are associated with a lower risk of cardiovascular disease (CVD)-related morbidity (1,2). Although the mechanism by which low-fat dairy protects against CVD remains unclear, bioactive milk constituents (1), including milk peptides and micronutrients, may lower CVD risk by attenuating hypertension (3,4) and improving vascular endothelial function (4,5). Indeed, whey protein isolate or sodium caseinate decreased blood pressure in overweight adults; arterial stiffness also decreased in those provided whey protein isolate (4). We showed that acute ingestion of a whey protein-derived peptide by college-aged (6) and middle-aged adults (7) improves postprandial responses in brachial artery flow-mediated dilation (FMD), a noninvasive index of vascular function that predicts future CVD (8). Other bioactive components of milk also exert antimicrobial, immunomodulatory, opioid, and mineral-binding activities (9), and milk contains angiotensin-converting enzyme inhibitory peptides that lower blood pressure (9).

Approximately 34% of Americans have metabolic syndrome (MetS) (10), a disorder that increases the risk of CVD-related morbidity and mortality in association with its hallmark characteristics, including dyslipidemia, hypertension, central obesity, and glucose intolerance (11). Epidemiological evidence indicates an inverse relation between the incidence of MetS and dairy consumption (12), suggesting that greater dietary inclusion of dairy foods may mitigate dysregulated metabolic responses in MetS that increase the risk of CVD.
Metabolic disturbances occurring postprandially contribute to vascular endothelial dysfunction (VED) and CVD-related mortality (13,14). For example, postprandial hyperglycemia (PPH) induced by a glucose challenge in healthy adults transiently lowers brachial artery FMD in association with increasing lipid peroxidation and the ratio of asymmetric dimethylarginine (ADMA):arginine (ARG) (15). This suggests that PPH impairs endothelial function by increasing oxidative stress and reducing NO bioavailability. In agreement with epidemiological studies suggesting that PPH better predicts CVD-related mortality than fasting glucose (14), clinical studies support dietary strategies to limit PPH and downstream responses leading to VED (16).

Low-fat milk not only contains bioactive components, but it also induces a relatively low glycemic response (17), suggesting that it would limit PPH-mediated impairments in endothelial function. We therefore hypothesized that low-fat milk, compared with an isocaloric volume of rice milk, would limit impairments in endothelial function in individuals with MetS by limiting PPH-mediated responses that induce oxidative stress and reduce NO bioavailability. Individuals with MetS were targeted for these studies because of their greater CVD risk (11) that is explained in part by lower FMD responses (18), greater oxidative stress (19,20), and exaggerated PPH responses known to occur with obesity (21). These studies utilized rice milk as a nondairy comparison beverage that closely approximates the energy and micronutrient content of low-fat milk but differs in its carbohydrate and protein content and composition. Although the carbohydrate concentrations of these beverages differ, they were specifically chosen without any reformulation to enhance translation of the study, as this best recapitulates the manner in which humans consume these beverages. Likewise, using test beverages of differing glycemic loads permits evaluation of low-fat milk as a potential alternative to higher glycemic beverages to limit PPH-mediated responses that otherwise impair vascular function. To define the vasoprotective activities of low-fat milk, a double-blind, randomized, cross-over study was conducted to examine postprandial FMD, biomarkers of oxidative stress, and the ratio of ADMA:ARG as an indirect index of NO bioavailability (22) in response to low-fat milk or rice milk.

Methods

Participants. The protocol for this study was approved by the Institutional Review Boards at the University of Connecticut and The Ohio State University, and written informed consent was obtained from all participants before enrolling. Men (n = 14) and premenopausal women (n = 7) with MetS were enrolled on the basis of age (18–50 y), a BMI indicative of obesity (≥30 kg/m²), and nonsmoking status. All participants were recreationally active (<5 h/wk of exercise), nondiabetic, and not using any vasoactive medications or dietary supplements for ≥2 mo. MetS was defined by the presence of ≥3 of the following risk factors (23): waist circumference ≥102 cm for men and ≥88 cm for women, fasting TGs ≥1.7 mmol/L (≥150 mg/dL), fasting glucose ≥5.6 mmol/L (≥100 mg/dL), resting systolic (≥130 mm Hg) and diastolic (≥85 mm Hg) blood pressure, and HDL-cholesterol <1.0 mmol/L (<40 mg/dL) for men and <1.3 mmol/L (<50 mg/dL) for women. Body mass was measured to the nearest 0.1 kg on a calibrated scale and height (± 0.1 cm) was measured using a stadiometer. Waist circumference was determined at the level of the umbilicus to the nearest 0.1 cm. Participants’ blood pressure and heart rate were measured 2 times separated by 3 min using an automated blood pressure monitor (Omron BP760) after resting for 15 min in the supine position.

Study design. Participants completed a randomized, double-blind, cross-over study in which they visited the study center (University of Connecticut) in the fasting state (10–12 h) on 2 occasions. Test beverages were administered to participants by an individual not involved in vascular measurements or specimen analysis. During each visit, they ingested 475 mL of 1% low-fat milk (Big Y Foods) or an isocaloric volume of rice milk (435 mL; Rice Dream Enriched Original) (Table 1). Rice milk, due to its similar energy and micronutrient content but higher glycemic index compared with low-fat milk (17), was specifically chosen as a comparison test beverage consistent with our studies demonstrating that PPH transiently suppresses endothelial function (15). The volume of low-fat milk provided was consistent with recommendations of the U.S. Dietary Guidelines (25) and evidence demonstrating that 2 servings/d (480 mL/d) of milk for 4 wk lowers systolic blood pressure in healthy adults (26).

Blood samples were collected from a flexible catheter prior to (0 h) and following the ingestion of each test beverage at 30, 60, 90, 120, 150, and 180 min. Brachial artery FMD was determined at the same time intervals, whereas blood pressure and heart rate were determined at 0, 15, 45, 75, 105, 135, 165, and 185 min. To standardize responses between trials, participants were provided all foods and beverages for 3 d preceding each trial and then replicated their 3-d diet for the second arm of the study. Participants also abstained from caffeine and alcohol for 24 h, maintained their current level of physical activity, and avoided strenuous exercise for 48 h preceding each trial. Prior to enrollment, female participants completed a health questionnaire to assess their menstrual history and oral contraceptive use. Three of the 5 enrolled female participants reported taking daily oral contraceptives for ≥6 mo and all female participants reported having regular menstrual cycles (27 ± 3 d; mean ± SD) with a range of menses of 4–8 d (6 ± 2 d). Each arm of the study was therefore completed when women were 9–10 d beyond their menses. Thus, the washout period between trials was 1 wk for men and 1 mo for women to account for changes in vascular reactivity that occur throughout the menstrual cycle (27).

Dietary modifications. Participants’ energy requirements were estimated using the Harris-Benedict equation and adjusted for physical activity level (28). Participants were provided all foods and beverages and restricted from all dairy products for 3 d preceding each trial. Diets were planned to provide 55–60% of energy from carbohydrate, 12–15% of energy from protein, and <30% of energy from fat. Compliance with dietary modifications was verified from 3-d food records, which were reviewed for accuracy by a registered dietitian. Participants then received a copy of their food record along with the same foods and beverages in order to replicate their dietary pattern leading into the second trial of the study. Energy and nutrient intakes were analyzed using Nutrition Data System for Research (NDSR 2011).

Assessment of endothelial function. FMD was assessed by high-frequency ultrasonographic imaging as described (7). In brief, a blood

<table>
<thead>
<tr>
<th>TABLE 1 Nutrient composition of test beverages¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low-fat milk</td>
</tr>
<tr>
<td>Volume, mL</td>
</tr>
<tr>
<td>Energy, kcal</td>
</tr>
<tr>
<td>Fat, g</td>
</tr>
<tr>
<td>Carbohydrate, g</td>
</tr>
<tr>
<td>Protein, g</td>
</tr>
<tr>
<td>Saturated fat, g</td>
</tr>
<tr>
<td>Cholesterol, mg</td>
</tr>
<tr>
<td>Sugars, g</td>
</tr>
<tr>
<td>Fiber, g</td>
</tr>
<tr>
<td>Sodium, mg</td>
</tr>
<tr>
<td>Calcium, mg</td>
</tr>
<tr>
<td>Magnesium, mg</td>
</tr>
<tr>
<td>Vitamin D, μg</td>
</tr>
<tr>
<td>α-Tocopherol, mg</td>
</tr>
</tbody>
</table>

¹ Nutrient composition was obtained from the USDA Nutrient Database SR-25 (24). Participants ingested 475 mL of 1% low-fat milk (Big Y Foods) or an isocaloric volume of rice milk (Rice Dream Enriched Original) immediately prior to a 3-h postprandial trial.
pressure cuff was placed on the right forearm and the brachial artery was imaged longitudinally in the distal third of the upper arm using a 5- to 12-MHz multi-frequency linear array transducer connected to a high-resolution ultrasound (T3000; Terson). Continuous Doppler velocity was simultaneously assessed at an insonation angle of 60°. After recording end-diastolic preclosure brachial artery images for 1 min, the forearm cuff was inflated for 5 min using a rapid cuff inflator (Hokanson E20) and then rapidly released. Recordings of vessel diameter and blood velocity were obtained 1 min prior to cuff deflation and for 3 min thereafter at 5 frames/s (Camtasia Studio, TechSmith). A 3-frame smoothing average was used to determine maximal postocclusion diameter. Offline analyses were performed using automated edge-detection software with end-diastolic gating (Medical Imaging Applications). All vascular measurements and analyses were performed by the same technician who was unaware of the treatments. Relative FMD (%) was calculated as: [maximal postocclusion diameter (mm) – preclosure diameter (mm)]/preclosure diameter (mm) × 100. From end-diastolic synchronized diameter (D1; mm) and velocity data (V1; m · s⁻¹), the shear rate (an estimate of shear stress without viscosity; s⁻¹) was calculated as 4V1/D1 (29). To determine the hyperemic stimulus responsible for FMD, the shear rate AUC (AUCSR) was calculated from the time of cuff release until the time of maximal postocclusion diameter (30).

Blood sampling. Blood was collected into evacuated tubes containing EDTA, lithium heparin, or sodium heparin. Plasma was obtained by centrifugation (1500 × g, 15 min, 4°C), aliquoted into cryogenic tubes, snap-frozen in liquid nitrogen, and stored at −80°C until analyzed. For analyses of ascorbic acid and glutathione, a portion of plasma was mixed 1:1 with 10% perchloric acid (PCA) containing 1 mmol/L diethylenetriaminepentaacetic acid. The sample was centrifuged (15,000 × g, 5 min, 4°C) and the supernatant was collected, flash frozen in liquid nitrogen, and archived at −80°C for future analysis.

Materials. HPLC-grade solvents were purchased from Fisher Scientific as were the following chemicals: ascorbic acid, diethylenetriaminepentaacetic acid, methylmonooarginine, o-phthalaldehyde, PCA, potassium hydroxide, and potassium phosphate.

Clinical chemistries. Plasma TGs, glucose, total cholesterol, and HDL-cholesterol were measured using separate commercially available clinical assays (Pointe Scientific) on a SpectraMax M2 microplate reader (Molecular Devices). Plasma LDL-cholesterol was calculated using the Friedewald equation (31). Plasma insulin was measured using an ELISA kit in accordance with the manufacturer’s instructions (Alpco Diagnostics). HOMA-IR was calculated using fasting plasma glucose and insulin as described (32): [(glucose (mmol/L) · insulin (μU/mL))/22.5].

NO homeostasis. ARG, the amino acid required for NO biosynthesis (22), and ADMA, an endogenously produced competitive inhibitor of NO synthase (NOS), were measured by HPLC-fluorescence (HPLC-FL) with minor modifications (15). In brief, plasma was spiked with methylmonooarginine (0.6 mmol/L) and then extracted by solid phase extraction (Hypersep Retain-CX SPE column; 30 mg, 1 mL; Fisher Scientific). The extract was dried under nitrogen gas and the residue dissolved in water for derivatization using o-phthalaldehyde. HPLC analysis was performed on a Shimadzu LC-20XR system equipped with a RF-20A XL fluorescence detector programmed to 340/455 nm (excitation/emission) and a Kinetex XB-C18 column (50 × 3.0 mm i.d., 2.6 μm; Phenomenex). ARG and ADMA were separated at 1.4 mL/min using 50 mmol/L potassium phosphate buffer (pH 6.5) and 6.5% (v:v) acetic acid and then extracted with hexane. The extract was dried under nitrogen gas and the residue was dissolved in methanol:ethanol (1:1) prior to LC-MS analysis. Analysis was performed using a Prominance UFLC system (Shimadzu) consisting of a refrigerated autosampler (SIL-30AC), a degassing unit (DGU-20AS), a CTO-30A column oven, a communication module (CBM-20A), 2 LC-30AD pumps, and an MS detector (MS-2020) programmed to 350°C for the heating block, 250°C for the desolvation line, 1.5 L for nebulizing gas, and 15 L/min for the drying gas. Instrument control and acquisition were performed using Shimadzu LabSolutions (version 5.4). HPLC separation was at 0.2 mL/min on Kinetex C18 column (100 × 2.1 mm i.d., 2.6 μm; Phenomenex) using 100% methanol as the mobile phase. Detection was performed using single ion monitoring in negative electrospray ionization mode at the following mass-to-charge ratios (m/z): α-tocopherol, 429.4; γ-tocopherol, 415.4; nitro-γ-tocopherol, 460.4; and d6-α-tocopherol, 435.4 (internal standard).

Ascorbic acid and thiols. Ascorbic acid was analyzed by HPLC-Coularray (ES) from PCA-treated plasma as described (33). Plasma thiols, as reduced glutathione (GSH), glutathione disulfide, cysteine (CYS), and cystine (CYSS), were measured by HPLC-FL on the Shimadzu HPLC-FL system with minor modifications (35). Separation was performed at 1 mL/min on a 3-aminopropyl column (250 × 4.6 mm, 5 μm; Thermo Fisher Scientific) using the following binary gradient: 40% mobile phase B for 10 min, a linear increase to 85% B for 17 min and held for 33 min, then back to 40% B for 2 min, and equilibrated at 40% B for 13 min.

Malondialdehyde. Malondialdehyde (MDA), a marker of lipid peroxidation, was measured by HPLC-FL on the aforementioned Shimadzu HPLC-FL system as described (15).

Statistical analyses. Sample size was determined utilizing data from our previously reported postprandial study examining changes in FMD following the oral ingestion of simple carbohydrates (15). Data indicated that fasting FMD responses were normally distributed (6.8 ± 1.6%; mean ± SD) and decreased to 3.9% at 60 min following glucose ingestion. Our calculations indicated that a minimum of 9 participants would be needed in the present study to reject the null hypothesis with 90% power (P < 0.05). Data (means ± SEMs) were analyzed by SPSS version 15.0. Initial analyses were performed using 3-way repeated-measures ANOVA to examine effects due to gender, treatment, and time. No gender effects were detected and therefore 2-way repeated-measures ANOVA was used to evaluate differences due to treatment, time, and their interaction for most analyses. The Greenhouse-Geiser correction was used to adjust the df for study variables with unequal variance, which was identified using Mauchly’s test of sphericity. Bonferroni post-hoc tests were used to compare all postprandial time points to baseline (t = 0 min) within each treatment group and between trials at time-matched points. The area under the 3 h postprandial curve (AUC0–3 h) for each study variable was calculated using the trapezoidal rule. A Student’s paired t test was used to evaluate dietary intakes and AUC0–3 h data between trials. Multiple linear regression, controlling for within-subject repeated measures, was used to calculate correlation coefficients as described (36). An α-level of P ≤ 0.05 was considered statistically significant for all analyses.

Results

Participants and dietary intakes. There were 21 men and women who enrolled in this study (Fig. 1). Data from 19 participants (n = 14 males, 5 females) were included in the final analysis, because 1 participant disclosed taking herbal supplements during the second arm of the study and another participant contracted an acute illness (i.e., common cold) that required the use of an over-the-counter anti-inflammatory medication. Participants were obese on the basis of BMI (37), prehypertensive (38), and had waist circumference and fasting concentrations of glucose, TG, and HDL-cholesterol indicative of MetS (23) (Table 2). Specifically, participants had 3 (n = 9), 4 (n = 7), or all 5 MetS criteria (n = 3), with the most frequently occurring criterion being elevated waist circumference (19 of
levels no different from baseline by 90 min, whereas FMD responses throughout the low-fat milk trial were unaffected (P > 0.05) (Fig. 2A). Time-matched FMD responses at 30 min were higher (P < 0.01) in the low-fat milk trial compared with the rice milk trial and FMD AUC_{AUCSR} was 26% greater in the low-fat milk trial compared with the rice milk trial (908 ± 118 vs. 1150 ± 145% · min; P < 0.01).

Preocclusion brachial artery diameters did not differ between or within trials (Supplemental Table 2). Only a main effect for time (P < 0.001) occurred for maximal postocclusion diameter with specific time-dependent decreases occurring at 30 min relative to baseline during the rice milk trial only (P < 0.001) (Supplemental Table 2). Thus, within- and between-trial differences in FMD responses were independent of any changes in preocclusion vessel diameters and were likely due to decreases in postocclusion diameters during the rice milk trial only.

Time to peak dilation of the brachial artery was unaffected within and between trials (Supplemental Table 2). AUC_{AUCSR} also did not differ between trials, but it similarly decreased in both trials at 30 min compared with baseline (P < 0.01) (Fig. 2B), which is known to occur with repetitive reactive hyperemia (7). Despite a decrease in the vasodilatory stimulus, low-fat milk but not rice milk maintained FMD responses at 30 min relative to baseline. This suggests that transient decreases in FMD responses mediated by rice milk occurred independent of shear rate, but no between-trial differences in AUC_{AUCSR} were observed (Fig. 2B).

Fasting mean arterial pressure (rice milk, 92 ± 2 mm Hg vs. low-fat milk, 94 ± 2 mm Hg) and heart rate (rice milk, 66 ± 1 bpm vs. low-fat milk, 67 ± 2 bpm) did not differ between trials. Although effects due to time (P < 0.05) and trial (P < 0.05) occurred for mean arterial pressure, no statistically significant pair-wise differences were detected. Heart rate did not differ throughout the postprandial period or between trials (P > 0.05).

**Plasma glucose, insulin, and TG.** Plasma glucose, insulin, and TG did not differ at baseline between trials (Fig. 3). Glucose
Increased by 30 min following the ingestion of either test beverage but to a greater extent during the rice milk trial (trial × time: \( P < 0.001 \)) (Fig. 3A). Glucose remained elevated through 120 min during the rice milk trial, whereas it returned to concentrations no different from baseline by 60 min during the low-fat milk trial. Insulin increased by 30 min during both trials and to a greater extent during the rice mice trial (trial × time: \( P < 0.001 \)) (Fig. 3B). Insulin returned to baseline concentrations by 150 and 180 min in the low-fat milk and rice milk trials, respectively. TG was unaffected by trial or time (Fig. 3C). Consistent with our prior work examining PPH on endothelial function (15), multiple linear regression controlling for within-subject repeated measures indicated that FMD AUC0–3 h and glucose AUC0–3 h were inversely related \((r = -0.57; P < 0.01)\), supporting that PPH impairs endothelial function (Table 3).

**Discussion**

This study in individuals with MetS demonstrates that low-fat milk maintains postprandial vascular endothelial function by limiting PPH-induced oxidative stress that otherwise reduces NO bioavailability. We show that postprandial FMD is unaffected by low-fat milk, whereas rice milk transiently decreases it. This transient decrease coincides with PPH-mediated increases in lipid peroxidation, but independent of any changes in antioxidant status, suggesting that vasoprotective activities of low-fat milk are attributed to lower glycemic responses that limit MDA generation. The ADMA:ARG ratio also increases following rice milk, an effect associated with increases in PPH, and is higher at all postprandial time points compared with low-fat milk. Thus, these data support that low-fat milk limits PPH that otherwise transiently impairs vascular function, likely by mitigating lipid peroxidation and decreases in NO bioavailability.

The prevalence of MetS continues to grow at an alarming rate (10), which is concerning, because it increases the risk of metabolic syndrome. Data are means ± SEMs, \( n = 19 \). *Different from 0 min within a trial, \( P < 0.01 \). †Different from time-matched points between trials, \( P < 0.01 \).

**Antioxidant status and oxidative/nitrative stress.** Ascorbic acid, α- and γ-tocopherols, and thiols did not differ between trials at baseline and no effects were observed for ascorbic acid, α-tocopherol, or glutathione disulfide in response to either test beverage (Supplemental Table 3). A main effect due to time \((P < 0.05)\) was observed for γ-tocopherol but without any statistically significant pair-wise differences. Time effects occurred for CYSS \((P < 0.001)\), CYS \((P < 0.05)\), and GSH \((P < 0.05)\) (Supplemental Table 3).

Relative to baseline, CYSS was lower at 120–180 min in the rice milk trial \((P < 0.01)\) and at 180 min in the low-fat milk trial \((P < 0.01)\; data not shown). No pair-wise differences were detected for CYS or GSH during either trial \((P > 0.05)\).

Nitro-γ-tocopherol did not differ between trials, nor was it affected by either beverage \((P > 0.05)\) (Supplemental Table 3). In contrast, a time × treatment interaction indicated that the lipid peroxidation marker MDA increased by 17–33% at 30–120 min in the rice milk trial but was unaffected throughout the low-fat milk trial (Fig. 4). Increases in MDA by rice milk were also reflected by a greater MDA AUC0–3 h \((P < 0.01)\) (Table 4). MDA AUC0–3 h was also correlated \((P < 0.01)\) with glucose AUC0–3 h \((r = 0.75)\) and inversely related to FMD AUC0–3 h \((r = -0.59)\), suggesting that VED mediated by PPH occurs in an oxidative stress-dependent manner (Table 3).

**NO status.** Plasma ARG increased at 30 min during the low-fat milk trial but decreased at 60 min in the rice milk trial (trial × time: \( P < 0.001 \)) (Fig. 5A). ARG was higher \((P < 0.01)\) in the low-fat milk trial at 30–180 min compared with time-matched responses in the rice milk trial. ARG AUC0–3 h was also higher in the low-fat milk trial \((P < 0.001)\) (Table 4). ADMA increased at 30 min during the rice milk trial and increased at 30–60 min during the low-fat milk trial (trial × time: \( P < 0.05 \)) (Fig. 5B).

Time-matched ADMA concentrations were higher \((P < 0.01)\) at 90, 120, and 180 min during the low-fat milk trial compared with the rice milk trial, which is also reflected by higher ADMA AUC0–3 h in the low-fat milk trial \((P < 0.01)\) (Table 4). Upon calculating the ADMA:ARG ratio, a trial × time interaction indicated that it increased relative to baseline at 60–180 min during the rice milk trial \((P < 0.01)\) and decreased at 30 min during the low-fat milk trial \((P < 0.01)\) and that the ADMA:ARG ratio during the rice milk trial was higher at 30–180 min compared with time-matched responses in the low-fat milk trial \((P < 0.01)\) (Fig. 5C). The ADMA:ARG AUC0–3 h was also higher in the rice milk trial \((P < 0.001)\) (Table 4). The ADMA:ARG AUC0–3 h was correlated to MDA AUC0–3 h \((r = 0.55; P < 0.05)\) and was inversely related to MDA AUC0–3 h \((r = -0.52; P < 0.05)\), suggesting that oxidative stress-mediated decreases in NO status contribute to VED (Table 4). These findings suggest that low-fat milk preserves NO bioavailability by decreasing the proportion of ADMA relative to ARG, an effect that would be expected to decrease competitive inhibition of NOS (22).
CVD-related morbidity and mortality (11), likely by inducing VED (18), oxidative stress, and inflammation (19,20,39). VED is an early event leading to CVD (40) with oxidized LDL-cholesterol (oxLDL) implicated as an initiating insult (41). That individuals with MetS have greater lipid peroxidation (19,20,39) is important, because oxLDL inhibits NO synthesis (42), suggesting that impairments in endothelial function (18) occur at least in part in an oxidative stress-dependent manner that lowers NO bioavailability.

We hypothesized that low-fat milk, due to its relatively low glycemic response (17), would limit transient impairments in endothelial function. In this study, FMD was unaffacted following low-fat milk ingestion, an effect largely attributed to its lower glycemic response that limits lipid peroxidation and preserves NO status as suggested by a lower ADMA:ARG ratio (22). Rice milk was specifically chosen as a nondairy comparison beverage because of its higher carbohydrate content and glycemic impact (17) but similar total fat and micronutrient content. As expected, rice milk induces PPH to a greater extent than low-fat milk, and glucose AUC0–3 h is inversely related to FMD AUC0–3 h. Thus, consistent with our work examining FMD in response to glucose or fructose (15), this study shows decreases in endothelial function as blood glucose increases. Although insulin AUC0–3 h is also inversely related to FMD AUC0–3 h, this study shows decreases in endothelial function as blood glucose increases.

That individuals with MetS have greater lipid peroxidation (19,20,39) is important, because oxLDL inhibits NO synthesis (42), suggesting that impairments in endothelial function (18) occur at least in part in an oxidative stress-dependent manner that lowers NO bioavailability.

We hypothesized that low-fat milk, due to its relatively low glycemic response (17), would limit transient impairments in endothelial function. In this study, FMD was unaffected following low-fat milk ingestion, an effect largely attributed to its lower glycemic response that limits lipid peroxidation and preserves NO status as suggested by a lower ADMA:ARG ratio (22). Rice milk was specifically chosen as a nondairy comparison beverage because of its higher carbohydrate content and glycemic impact (17) but similar total fat and micronutrient content. As expected, rice milk induces PPH to a greater extent than low-fat milk, and glucose AUC0–3 h is inversely related to FMD AUC0–3 h. Thus, consistent with our work examining FMD in response to glucose or fructose (15), this study shows decreases in endothelial function as blood glucose increases. Although insulin AUC0–3 h is also inversely related to FMD AUC0–3 h, hyperglycemia suppresses endothelium-dependent dilation even after insulin secretion is inhibited (43), indicating that PPH, rather than hyperinsulinemia, induces transient VED as observed in the present study.

Differing carbohydrate contents between tests beverages preclude a full understanding of the extent to which dairy milk protects against PPH-mediated changes in FMD. However, providing isocaloric volumes of beverages matched for energy, fat, and micronutrients has considerable translational value, because this most closely recapitulates the manner in which humans are exposed to these beverages and this dose (~2 cups) is consistent with the recommendations of the U.S. Dietary Guidelines (25). Differences in protein content between beverages may have contributed to the outcome of this study, because adding whey protein to a carbohydrate challenge dose-dependently decreases PPH compared with a carbohydrate challenge alone (44). The higher protein content of low-fat milk may have also lowered PPH by delaying gastric emptying (45). Further study is needed to define the additive contributions of dairy components on vascular reactivity and whether dairy milk attenuates PPH otherwise induced by a glucose challenge.

Our prior studies in healthy men indicate that PPH transiently impairs FMD by inducing oxidative stress responses that likely limit NO bioavailability (15). In contrast, low-fat milk, rice milk increases the lipid peroxidation marker MDA and the magnitude of lipid peroxidation is associated with decreases in FMD. The ADMA:ARG ratio also increases following rice milk, correlates with MDA, and inversely relates to FMD. This suggests that rice milk, a high-glycemic food, transiently impairs FMD by lowering NO bioavailability in a lipid peroxidation-dependent manner. Further work is needed to define the relation between other oxidative stress biomarkers and vascular function in response to dairy. Nonetheless, nitro-γ-tocopherol, a nitrative stress marker (34) that increases with inflammation (46), was unaffected in this study. This suggests endothelial function is impaired independent of inflammation, consistent with work showing that PHPP decreases FMD without affecting inflammatory cytokines (15). That inflammation is unaffected by low-fat milk is consistent with postprandial studies examining whey protein isolate or soy casein (47), or a whey-protein derived peptide (6), on proinflammatory mediators.

Oxidative stress mediated by PPH may induce VED by upregulating arginase-mediated catabolism of ARG, the substrate for NO biosynthesis. Treatment of glucose (24 h) to bovine coronary endothelial cells increases ARG activity and superoxide generation. These effects are abolished when NADPH oxidase is inhibited (48), supporting that hyperglycemia increases ARG activity in an oxidative stress-dependent manner. In the present study, rice milk decreases ARG relative to baseline beginning at 60 min. MDA AUC0–3 h is also inversely related to ARG and FMD AUC0–3 h, suggesting that PPH-mediated oxidative stress by rice milk increases arginase activity. That

---

### TABLE 3

Pairwise correlations between postprandial AUC0–3 h of study variables in individuals with MetS who ingested 475 mL of low-fat milk or an isocaloric volume of rice milk.1,2

<table>
<thead>
<tr>
<th></th>
<th>Glucose AUC0–3 h (nmol/L · min)</th>
<th>Insulin AUC0–3 h (pmol/L · min)</th>
<th>MDA AUC0–3 h (μmol/L · min)</th>
<th>ARG AUC0–3 h (μmol/L · min)</th>
<th>ADMA AUC0–3 h (nmol/L · min)</th>
<th>ADMA:ARG AUC0–3 h (nmol/μmol · min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>FMD AUC0–3 h (% - min)</td>
<td>-0.57**</td>
<td>-0.67**</td>
<td>-0.59**</td>
<td>0.57**</td>
<td>0.31</td>
<td>-0.52*</td>
</tr>
<tr>
<td>Glucose AUC0–3 h (μmol/L · min)</td>
<td>0.76**</td>
<td>0.75**</td>
<td>-0.69**</td>
<td>-0.43</td>
<td>0.68**</td>
<td></td>
</tr>
<tr>
<td>Insulin AUC0–3 h (μmol/L · min)</td>
<td>0.63**</td>
<td>0.79**</td>
<td>-0.42</td>
<td>0.84**</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MDA AUC0–3 h (μmol/L · min)</td>
<td>-0.56**</td>
<td>-0.45*</td>
<td>0.55*</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ARG AUC0–3 h (μmol/L · min)</td>
<td>0.74**</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ADMA AUC0–3 h (nmol/L · min)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>-0.52*</td>
</tr>
</tbody>
</table>

1 * P < 0.05; ** P < 0.01. ADMA, asymmetric dimethylarginine; ARG, arginine; AUC0–3 h, area under the 3 h postprandial curve; FMD, flow mediated dilation; MDA, malondialdehyde; MetS, metabolic syndrome.

2 Plasma concentrations for glucose, insulin, MDA, ARG, and ADMA.

3 Correlation coefficient.

---

**FIGURE 4** Plasma MDA concentrations during a 3-h postprandial period following the ingestion of 475 mL of low-fat milk or an isocaloric volume of rice milk by individuals with MetS. Data are means ± SEMs, n = 19. * Different from 0 min within a trial, P < 0.01.

† Different from time-matched points between trials, P < 0.01. MDA, malondialdehyde; MetS, metabolic syndrome.
Bioactive components that provide health benefits (54). Studies to the vasoprotective activities of low-fat milk observed herein. In risk (1, 2), it is possible that its bioactive components (1, 9) that pressure is unaffected.

that low-fat milk limits oxidative stress but that postprandial blood oxLDL, and inflammation in as few as 7 d (53). Similarly, we show that low-fat milk.

To clearly define the NO-dependent vasoprotective activities of using NOS inhibitors (e.g., NG-monomethyl-L-ARG) are needed suggested to reflect NO-dependent vasodilation (52), studies upregulation in protein ARG methyltransferase-I, which synthe-
sylaminohydrolase, the enzyme that degrades ADMA, and an lysophosphatidylcholine, a component of oxLDL. This resulted in oxidative stress-mediated suppression of dimethylarginine dimethyaminohydrolase, the enzyme that degrades ADMA, and an upregulation in protein ARG methyltransferase-I, which synthe-
sizes ADMA. Although the FMD technique utilized herein is suggested to reflect NO-dependent vasodilation (52), studies utilizing NOS inhibitors (e.g., N6-monomethyl-L-ARG) are needed to clearly define the NO-dependent vasoprotective activities of low-fat milk.

Given that dietary consumption is associated with lower CVD risk (1, 2), it is possible that its bioactive components (1, 9) that improve blood pressure (3, 4) and vascular function (4, 5) contribute to the vasoprotective activities of low-fat milk observed herein. In individuals with MetS, higher intakes of dairy foods decrease blood pressure and markers of oxidative stress, including MDA and oxLDL, and inflammation in as few as 7 d (53). Similarly, we show that low-fat milk limits oxidative stress but that postprandial blood pressure is unaffected.

Whey comprises ~20% of total milk proteins and contains bioactive components that provide health benefits (54). Studies show that FMD increases following acute ingestion of a whey protein-derived peptide in young and overweight middle-aged adults (6, 7), suggesting that whey improves endothelial function. In support, 15-min incubation of mesenteric arteries from hypertensive rats with whey-derived peptides improves endothelium-dependent relaxation, an effect abolished by inhibiting NOS (55). Thus, whey-derived peptides may improve endothelial function in an NO-dependent manner. The present study shows that low-fat milk maintains postprandial FMD, likely by preserving NO bioavailability, as suggested on the basis of a lower ADMA:ARG ratio and an inverse relation between the ADMA:ARG ratio and FMD AUC0–3 h. Further study is warranted to define the bioactive components responsible for the vasoprotective effects of low-fat milk on endothelial function and NO homeostasis. Also, because this study was limited to young individuals with MetS, additional work is warranted in other populations of high-CVD risk and to determine the contribu-
tion of specific MetS criteria on vascular health.

In conclusion, this study advances existing knowledge that dairy lowers the risk for MetS (12) and CVD (1, 2) by showing that low-fat milk limits PPH-mediated increases in lipid peroxidation and VED otherwise induced by rice milk. This is of public health importance consistent with evidence indicating exacerbated glycemic responses in obese individuals (21) and epidemiological findings suggesting that PPH better predicts future CVD risk compared with fasting glucose (14). That low-fat milk prevents postprandial increases in the ADMA:ARG ratio, which is inversely related to FMD, suggests that low-fat milk maintains vascular function in an NO-dependent manner. Thus, low-fat milk is an effective dietary strategy to attenuate postprandial VED by limiting PPH-dependent oxidative stress responses in individuals with MetS.

Acknowledgments

The authors thank Dr. Christopher Masterjohn for his assistance conducting the study intervention and Sarah Kranz and Catherine Lainas for their assistance in analyzing dietary intake.

TABLE 4 Baseline concentrations and AUC0–3 h for plasma markers of oxidative stress and NO status in individuals with MetS who ingested 475 mL of low-fat milk or an isocaloric volume of rice milk1

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th></th>
<th>AUC0–3 h</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Low-fat milk</td>
<td>Rice milk</td>
<td>Low-fat milk</td>
</tr>
<tr>
<td>MDA, μmol/L</td>
<td>1.25 ± 0.04</td>
<td>1.22 ± 0.04</td>
<td>235 ± 8</td>
</tr>
<tr>
<td>ADMA, nmol/L</td>
<td>487 ± 18</td>
<td>492 ± 21</td>
<td>91,900 ± 2470</td>
</tr>
<tr>
<td>ARG, μmol/L</td>
<td>76.3 ± 3.5</td>
<td>78.0 ± 3.9</td>
<td>15,100 ± 710</td>
</tr>
<tr>
<td>ADMA-ARG, nmol/μmol</td>
<td>6.58 ± 0.31</td>
<td>6.43 ± 0.25</td>
<td>1130 ± 51</td>
</tr>
</tbody>
</table>

1 Data are means ± SEMs, n = 19. AUC0–3 h was calculated using the trapezoidal rule for each participant during each trial. There were no differences between trials at baseline (P > 0.05). *Different from low-fat milk trial, P < 0.01. ADMA, asymmetric dimethylarginine; ARG, arginine; AUC0–3 h, area under the 3 h postprandial curve; MDA, malondialdehyde; MetS, metabolic syndrome.

FIGURE 5 Postprandial plasma ARG (A), ADMA (B), and the ADMA:ARG ratio (C) following the ingestion of 475 mL of low-fat milk or an isocaloric volume of rice milk by individuals with MetS. Data are means ± SEMs, n = 19. *Different from 0 min within a trial, P < 0.01. 1Different from time-matched points between trials, P < 0.01. ADMA, asymmetric dimethylarginine; ARG, arginine; MetS, metabolic syndrome.
data; R.S.B. and J.S.V. designed the research; K.D.B., E.M., Y.G., R.P., and R.S.B. conducted research and analyzed data; and K.D.B. and R.S.B. wrote the paper. All authors read and approved the final manuscript.

Literature Cited


