α-Tocopherol bioavailability is lower in adults with metabolic syndrome regardless of dairy fat co-ingestion: a randomized, double-blind, crossover trial[1–3]

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ABSTRACT

Background: Increasing dietary fat intake is expected to improve α-tocopherol bioavailability, which could be beneficial for improving α-tocopherol status, especially in cohorts at high cardiometabolic risk who fail to meet dietary α-tocopherol requirements.

Objective: Our objective was to assess dose-dependent effects of dairy fat and metabolic syndrome (MetS) health status on α-tocopherol pharmacokinetics in plasma and lipoproteins.

Design: A randomized, crossover, double-blind study was conducted in healthy and MetS adults (n = 10/group) who ingested encapsulated hexadeuterium-labeled (d_6)–*RRR*–α-tocopherol (15 mg) with 240 mL nonfat (0.2 g fat), reduced-fat (4.8 g fat), or whole (7.9 g fat) milk before blood collection at regular intervals for 72 h.

Results: Compared with healthy participants, those with MetS had lower ($P < 0.05$) baseline plasma α-tocopherol (μmol/mmol lipid) and greater oxidized low-density lipoprotein (LDL), interleukin (IL)–6, IL-10, and C-reactive protein. Regardless of health status, d_6–α-tocopherol bioavailability was unaffected by increasing amounts of dairy fat provided by milk beverages, but MetS participants had lower estimated d_6–α-tocopherol absorption ($\pm$SEM) than did healthy participants (26.1% $\pm$ 1.0% compared with 29.5% $\pm$ 1.1%). They also had lower plasma d_6–α-tocopherol AUC from 0 to 72 h, as well as maximal concentrations (C_{max}: 2.04 $\pm$ 0.14 compared with 2.73 $\pm$ 0.18 μmol/L) and slower rates of plasma disappearance but similar times to C_{max}. MetS participants had lower d_6–α-tocopherol AUC from t = 0–12 h ($AUC_{0–t\ final}$) in lipoprotein fractions [chylomicron, very-low-density lipoprotein (VLDL), LDL, high-density lipoprotein]. Percentages of d_6–α-tocopherol $AUC_{0–t\ final}$ in both the chylomicron (r = -0.46 to -0.52) and VLDL (r = -0.49 to -0.68) fractions were inversely correlated with oxidized LDL, IL-10, IL-6, and C-reactive protein.

Conclusions: At dietary intakes equivalent to the Recommended Dietary Allowance, α-tocopherol bioavailability is unaffected by dairy fat quantity but is lower in MetS adults, potentially because of greater inflammation and oxidative stress that limits small intestinal α-tocopherol absorption and/or impairs hepatic α-tocopherol trafficking. These findings support higher dietary α-tocopherol requirements for MetS adults. This trial was registered at www.clinicaltrials.gov as NCT01787591. *Am J Clin Nutr* 2015;102:1070–80.

Keywords: α-tocopherol, bioavailability, metabolic syndrome, nonalcoholic steatohepatitis, pharmacokinetics

INTRODUCTION

Oxidative stress and inflammation are associated with metabolic derangements in metabolic syndrome (MetS),[7] which affects 34.7% of Americans (1). Weight loss effectively resolves central obesity, dyslipidemia, insulin resistance, and hypertension in MetS (2). However, most weight loss efforts fail (3), and those aiming to lose weight often restrict fat (4), which decreases intakes of the antioxidant vitamin, α-tocopherol (5). Thus, need exists to examine oxidative stress during MetS on α-tocopherol bioavailability while developing strategies that promote adequate α-tocopherol status.

Although vitamin E encompasses 8 lipophilic tocopherols and tocotrienols having chain-breaking antioxidant activity (6), α-tocopherol is the only form that meets human requirements (7). Despite its importance, >92% of Americans fail to meet the Estimated Average Requirement of α-tocopherol [12 mg/d (8)], with the lowest intakes observed in obese individuals (9). Oxidative stress increases the oxidative turnover of α-tocopherol (10), suggesting that oxidative stress and inflammation associated with MetS (11) may increase dietary α-tocopherol requirements. Although pharmacokinetic studies have been

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[2] The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Center for Advancing Translational Sciences or the NIH.

[3] Supplemental Table 1 is available from the "Online Supporting Material" link in the online posting of the article and from the same link in the online table of contents at http://ajcn.nutrition.org.

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[7] Abbreviations used: $AUC_{0–72\ h}$, AUC from 0 to 72 h; $AUC_{0–t\ final}$, AUC from t = 0–12 h; C_{max}, plasma maximal concentration; CRP, C-reactive protein; d_0, unlabeled; d_3, trideuterium labeled; d_6, hexadeuterium labeled; d_9, nonadeuterium labeled; MetS, metabolic syndrome; OSU, The Ohio State University; T_{max}, time to maximal concentration; α-TAC, α-tocopheryl acetate.

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performed in hyperlipidemic adults ingesting a single dose (150 mg) of α-tocopheryl acetate (α-TAC) (12), the absence of studies in MetS adults ingesting α-tocopherol at usual dietary amounts precludes any ability to establish specific dietary α-tocopherol recommendations for this population. Thus, a need exists to evaluate α-tocopherol pharmacokinetics in MetS and identify low-energy, nutrient-dense dietary approaches to improve α-tocopherol status.

Bovine milk delivers fortified vitamins A and D (13), but it is a poor source of dietary α-tocopherol [0.05–0.5 mg α-tocopherol/L milk (14)]. Little is known regarding dairy fat on α-tocopherol bioavailability. Microdispersion of 200 mg *all rac*–α-TAC in milk containing 1% fat increased plasma α-tocopherol to a greater extent when consumed daily for 4 wk compared with its microdispersion in orange juice (15). Plasma hexadeuterium-labeled (d_6)–α-tocopherol also increased to a greater extent when d_6–*RRR*–α-TAC (150 mg) was ingested with cereal with whole milk and cream compared with cereal with low-fat milk (16). Although these studies suggest that dairy fat promotes α-tocopherol bioavailability, confounding variables and large amounts of α-TAC administered limit an understanding of dose-dependent effects of dairy fat on α-tocopherol bioavailability when α-tocopherol is ingested at amounts of the Recommended Dietary Allowance (15 mg/d) (7).

Despite the lack of clear evidence, dairy fat is expected to dose-dependently increase α-tocopherol bioavailability consistent with our earlier work (17). Although no dose-response studies have examined bioavailability of unesterified α-tocopherol in response to dietary fat, adults ingesting apples fortified with d_6–*RRR*–α-TAC (22 mg) with 0, 2.4, and 11 g fat had fat-dependent increases in plasma d_6–α-tocopherol with absorption increasing to 33% with the highest fat-containing meal compared with 10% from the nonfat meal (17). Thus, we hypothesized that oxidative stress and inflammation in MetS adults would lower the bioavailability of unesterified *RRR*–α-tocopherol and that dairy fat would dose-dependently improve α-tocopherol bioavailability regardless of health status. We tested this hypothesis by conducting an α-tocopherol pharmacokinetics study in healthy and MetS adults who ingested d_6–*RRR*–α-tocopherol (15 mg) with nonfat milk (0.2 g fat), reduced-fat milk (4.8 g fat), or whole milk (7.9 g fat).

METHODS

Materials

All HPLC-grade solvents and most chemicals were from Fisher Scientific. The following were from Sigma: bile, ioxidanol (Optiprep), lipase, pancreatin, pepsin, and nonadeuterium-labeled (d_9)–α-tocopherol. d_6–*RRR*–α-tocopherol was kindly provided by DSM Nutritional Products.

Subjects and study design

This protocol was approved by Institutional Review Board at The Ohio State University (OSU). Participants were recruited from the Columbus, Ohio, area and all aspects of the intervention were performed at the OSU Clinical Research Center from July 2013 to May 2014. In this double-blind, randomized, crossover study, sex- and age-matched healthy and MetS adults (n = 5 women and 5 men/group; aged 24–40 y) completed each of the trials separated by ≥2-wk washout. Before enrollment, height, weight, waist circumference, and blood pressure were measured and a fasting blood sample was obtained to assess blood chemistries (described below). Waist circumference was determined at the level of the umbilicus, and blood pressure was reported as the mean of 2 measurements taken 1 min apart. MetS was defined by the presence of ≥3 of the following risk factors (18): waist circumference ≥102 cm for men and ≥88 cm for women, fasting triglyceride ≥1.7 mmol/L, fasting glucose ≥5.6 mmol/L, resting systolic (≥130 mm Hg) and diastolic (≥85 mm Hg) blood pressure, and HDL cholesterol <1.0 mmol/L for men and <1.3 mmol/L for women. Participants also met the following inclusion criteria: stable body mass (±2 kg during past 3 mo), nondietary supplement user for >2 mo, no use of medications known to affect lipid metabolism, nonsmoker, <3 alcoholic drinks/d, <5 h of aerobic activity/wk, and no history of gastrointestinal disorders or lactose intolerance.

Participants arrived at the OSU Clinical Research Center after an overnight fast (10–12 h). They consumed 240 mL nonfat milk, reduced-fat milk, whole milk, or soy milk (**Table 1**) with encapsulated d_6–*RRR*–α-tocopherol (15 mg). Trial order was determined by simple randomization (computer-generated random numbers). Blood samples were collected from the antecubital vein before (0 h) and at 3, 6, 9, 12, 24, 26, 48, and 72 h after co-ingestion of test beverages and d_6–*RRR*–α-tocopherol. Blood was collected into evacuated tubes containing EDTA for most biochemical analyses except for measures of plasma vitamin E, vitamin C, and oxidized LDL that were performed in plasma prepared from sodium heparin–containing evacuated tubes. Plasma was isolated by centrifugation and snap-frozen in liquid nitrogen before storing at −80°C. Plasma obtained from EDTA-containing blood-collection tubes was stored at 4°C for ≤3 d before lipoprotein isolation (described below).

All foods were provided to participants for 3 d before and during the first 24 h of each 72-h trial. Daily menus providing 2000 kcal or 2500 kcal were prepared and assigned to each participant based on his or her estimated energy requirement,

TABLE 1
Nutrient composition of milk beverages (per 240-mL serving)[1]

	Nonfat	Reduced fat	Whole	Soy
Energy, kcal	83	122	149	100
Carbohydrate, g	12.2	11.7	11.7	10.0
Protein, g	8.26	8.05	7.69	6.00
Fat, g	0.20	4.83	7.93	3.50
Total saturated fat, g	0.14	3.07	4.55	0.5
Monounsaturated fat, g	0.05	1.37	1.98	1.00
Polyunsaturated fat, g	0.01	0.18	0.48	2.00
Cholesterol, mg	4.9	19.5	24.4	0.0
Vitamin A, IU	500	464	395	501
Vitamin D, μg	115	120	124	119
α-Tocopherol, mg	0.01	0.16	0.38	0.23
γ-Tocopherol, mg	0.00	0.01	0.01	0.30
Vitamin K, μg	0.00	0.49	0.73	3.4

[1]Nutrient composition was obtained from ProNutra (Viocare Inc.) and the USDA Nutrient Database (19), except for α- and γ-tocopherol, which were determined by liquid chromatography–mass spectrometry, described under Methods.

which was calculated by using the Harris-Benedict equation (20). Dietary α-tocopherol intakes were also controlled at 5 mg/d, consistent with median intakes in Americans (8), and vitamin C was controlled at amounts meeting sex-specific Recommended Dietary Allowance (7). Lunch with snacks and dinner were provided after blood collection at 6 h and 12 h, respectively. Participants were allowed to consume snacks at any time after lunch and before dinner. No foods other than water and those provided were allowed for 3 d before each trial and during the initial 24 h after ingestion of d_6–*RRR*–α-tocopherol with each test beverage.

Dietary analysis

Participants were instructed by a dietitian to consume all provided foods and beverages and were required to complete a food diary for the 3 d immediately preceding each trial. Food and beverage intakes during the first 12 h of each trial were recorded by study personnel. A review of food records by a dietitian indicated that participants consumed only the provided foods. Participants were required to return all food and beverage containers to quantify uneaten portions and calculate dietary intakes by using ProNutra analysis software (version 3.4; Viocare Inc.).

Biochemical analysis

Plasma triglyceride, total cholesterol, HDL cholesterol, glucose, alanine aminotransferase, and aspartate aminotransferase were measured by using clinical assays according to the manufacturer's instructions (Pointe Scientific). Plasma insulin was measured by ELISA (Alpco). LDL cholesterol was calculated with the Friedewald equation (21). Total plasma lipids represent the sum of plasma cholesterol and triglyceride. Insulin resistance (HOMA-IR) was calculated from fasting glucose and insulin as described (22).

Lipoprotein separation

Lipoprotein isolation was performed by density ultracentrifugation as described (16, 23, 24), with minor modifications. Ultracentrifuge tubes containing plasma were overlaid with saline (0.9% sodium chloride, wt:vol) and centrifuged with a SW 41 TI rotor in an Optima L-90K ultracentrifuge (100,000 $\times$ *g*, 30 min, 20°C; Beckman Coulter), and an aliquot of the upper triglyceride-rich fraction (defined as the chylomicron fraction) was stored at −80°C.

Isolation of VLDL, LDL, and HDL from chylomicron-free plasma was performed by using a continuous iodixanol gradient consisting of a 12% (wt:vol) iodixanol-plasma mixture, a 9% iodixanol-saline solution, and saline. The 12% (wt:vol) iodixanol-plasma mixture was prepared by diluting 60% (wt:vol) iodixanol in chylomicron-free plasma. The 9% iodixanol saline solution was prepared by diluting 60% (wt:vol) iodixanol in saline. The 12% iodixanol-plasma mixture was transferred to a polyallomer centrifugation tube, carefully overlaid with the 9% iodixanol-saline solution, and subsequently filled to capacity with saline. After centrifugation with a TLA110 near-vertical rotor (350,000 $\times$ *g*, 3 h, 16°C; Beckman Coulter), the resulting lipoprotein fractions, defined as VLDL, LDL, and HDL, were stored at −80°C.

In vitro digestion

In vitro digestion was performed through the small intestinal phase of digestion as described (25), with minor modifications, to examine the interaction between dairy fat and α-tocopherol quantity on α-tocopherol bioaccessibility. Bioaccessibility of α-tocopherol was assessed at 2 quantities of α-tocopherol across the 3 different dairy milk beverages: *1*) 15 mg α-tocopherol/240 mL milk consistent with the dosage administered in this study and *2*) 268 mg (equivalent to 400 IU) α-tocopherol/240 mL milk consistent with α-tocopherol supplements at ≥400 IU being the most common dosage (26). In brief, *RRR*–α-tocopherol was added to milk before acidifying to pH 2.5 with hydrochloric acid and adding porcine pepsin. After incubation (37°C, 1 h), sample pH was adjusted to 6.0 with sodium bicarbonate. Then, bile extract and a mixture of pancreatin and lipase prepared in sodium bicarbonate were added before adjusting to pH 6.5. After incubation (37°C, 2 h), an aliquot of chyme was centrifuged (5600 $\times$ *g*, 4°C, 45 min; Avanti J-E Centrifuge, Beckman Coulter). The supernatant was filtered to obtain the aqueous or bioaccessible fraction (i.e., mixed micelle) that was immediately extracted to determine α-tocopherol (described below). α-Tocopherol bioaccessibility was calculated as the percentage of α-tocopherol recovered from the filtered aqueous fraction relative to the initial mass of α-tocopherol in the predigested milk (n = 3/α-tocopherol dose and milk type).

Antioxidants, oxidative stress, and inflammation

Plasma vitamin C and uric acid were measured as described (27) with a Thermo Scientific Dionex UltiMate 3000 HPLC-electrochemical system. Plasma oxidized LDL (Mercodia Inc.) and high-sensitivity C-reactive protein (CRP; Biocheck) were measured by ELISA. Plasma IL-6, IL-10, and TNF-α were measured by using a fully automated, multianalyte immunoassay on a Simple Plex system (Protein Simple). α-Tocopherol and γ-tocopherol from plasma, lipoproteins, test milk beverages, and simulated digestions were extracted with hexane after alkaline saponification as described (27), with minor modifications. Extracted samples were injected onto a liquid chromatography–mass spectrometry system, and separation was performed by using 100% methanol on a Synergy Hydro-RP column (100 $\times$ 2.0 mm, 2.5 μm; Phenomenex). Detection was performed by using single-ion monitoring after negative ionization at the following mass-to-charge ratios: unlabeled (d_0)–γ-tocopherol, 415.4; d_0–α-tocopherol, 429.4; d_6–α-tocopherol, 435.4; and d_9–α-tocopherol, 438.4 (internal standard). The 2-wk washout effectively restored d_6–α-tocopherol to amounts below detection limits (<100 fmol on column) in most participants. For the few with detectable d_6–α-tocopherol (≤50 nmol/L), pharmacokinetics analysis was performed by subtracting 0-h concentrations from those during the 72-h trial as described (16). d_6–α-Tocopherol in lipoproteins was expressed as percentage of total α-tocopherol (% d_6–α-tocopherol) and normalized to protein (μmol/g protein), as determined by using a Bradford assay (Bio-Rad).

Power calculation and statistical analysis

Power calculations were performed by using Power and Sample Size Calculation (version 3.0.43; Vanderbilt University). Primary endpoints were differences in plasma d_6–α-tocopherol pharmacokinetic parameters among milk treatments and between

healthy and MetS participants. We hypothesized that α-tocopherol bioavailability would increase in a dairy fat–dependent manner and that α-tocopherol bioavailability would be lower in MetS adults compared with healthy adults regardless of the amount of co-ingested fat. Estimates of variability were based on a study demonstrating that plasma maximal concentrations (C_{max}) of α-tocopherol increased by 0.33 μM/g of co-ingested fat (17). Given an SD of 0.75 μM for plasma d_6–α-tocopherol C_{max} (17), 12 participants would be needed to detect a 1.02-μM difference in C_{max} between whole and reduced-fat milks with 90% power ($P < 0.05$). To also allow for comparisons by sex and health status, we recruited 10 healthy and 10 MetS participants (n = 5 men/5 women per group).

Pharmacokinetic parameters of plasma and lipoprotein d_6–α-tocopherol were determined by using PK Solutions (version 2.0; Summit Research Services). An estimate of fractional α-tocopherol absorption was calculated by extrapolation to 0 h by using a noncompartmental approach as described (28). AUCs for plasma and lipoprotein d_6–α-tocopherol were calculated by using the trapezoidal rule. Data were analyzed with the Statistical Analysis System (SAS, version 9.4; SAS Institute) and Statistical Package for the Social Sciences (SPSS, version 22; IBM Corporation). Dietary intakes, screening values, and plasma and lipoprotein pharmacokinetics of healthy and MetS subjects were compared by using an independent Student's t test. Plasma α-tocopherol pharmacokinetics were analyzed as a Latin square design in SAS PROC MIXED. Fixed effects were milk type, sex, healthy status, trial order, and their interactions. The variance-covariance structure of repeated measures within subjects was modeled by using an unstructured variance-covariance matrix. The Kenward-Roger approximation was used to obtain the correct df, and a priori comparisons were used to assess our hypothesis. Consistent with the study objective, findings from only the 3 dairy milk treatments of the study (i.e., nonfat, reduced fat, and whole milk) are reported herein. A future study is investigating a separate objective evaluating compositional differences between soy and dairy milks on α-tocopherol pharmacokinetics. Milk trial order did not affect plasma α-tocopherol pharmacokinetic responses. Main effects for sex indicated that women had greater plasma d_6–α-tocopherol AUC from 0 to 72 h ($AUC_{0–72\ h}$) and C_{max} but without any significant post hoc differences, nor were there any statistically significant sex interactions for plasma pharmacokinetics. Because the study specifically aimed to assess dose-dependent effects of dairy fat on α-tocopherol pharmacokinetics in healthy and MetS adults, data were collapsed for sex to assess milk type and health status on α-tocopherol pharmacokinetics. Relations between study variables were calculated by using Pearson correlation coefficients. Repeated-measures ANOVA was used to assess the effects of time and health status on plasma triglyceride. For in vitro digestions, α-tocopherol dose, dairy milk type, and their interaction on α-tocopherol bioaccessibility were analyzed by 2-factor ANOVA with Bonferroni correction. Data are reported as raw means ± SEMs. Statistical significance was set as $P \leq 0.05$.

RESULTS

Participants and dietary intakes

A total of 10 healthy and 10 MetS participants were enrolled and completed the study without any adverse events (**Figure 1**). MetS participants were obese on the basis of BMI and had blood pressure, waist circumference, and fasting concentrations of glucose, triglyceride, and HDL cholesterol in agreement with established clinical criteria of MetS (**Table 2**). Specifically, MetS participants had 3 (n = 7) or 4 (n = 3) of the MetS criteria; none met all 5 criteria. All had waist circumference consistent with MetS criteria (10/10 participants), followed by impaired fasting glucose (8 of 10) and low HDL cholesterol (8 of 10 participants), hypertriglyceridemia (5 of 10), and hypertension (2 of 10). MetS participants also had greater HOMA-IR than did healthy participants (Table 2), suggesting increased insulin resistance. Compared with healthy participants, MetS participants also had higher oxidized LDL and lower plasma vitamin C concentrations. At screening, plasma concentrations of d_0–α-tocopherol (μmol/L) were similar between healthy and MetS participants (Table 2) but were lower in MetS participants when corrected for plasma total lipids (μmol/mmol lipid) as recommended (29). In contrast, plasma d_0–γ-tocopherol (μmol/L) was greater in MetS participants compared with healthy participants, but this group difference did not remain statistically significant on normalization to total lipids. In addition, MetS compared with healthy participants had greater concentrations of the following circulating inflammatory markers: CRP, IL-6, and IL-10.

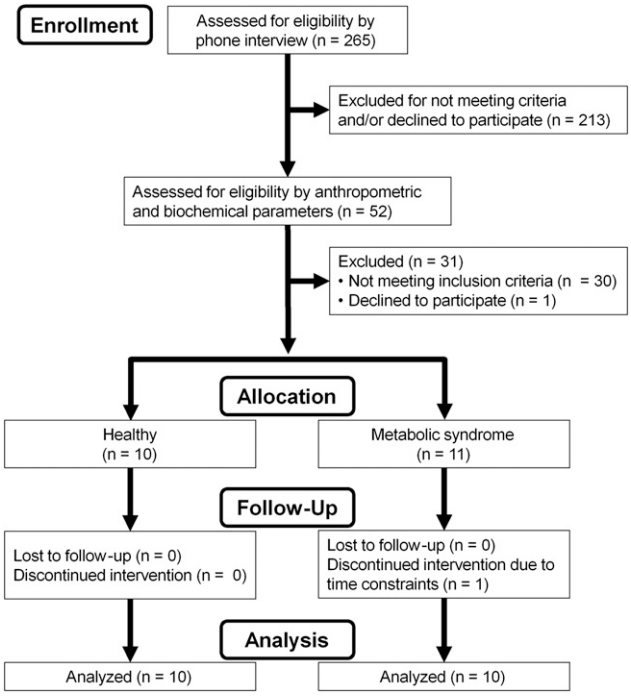

FIGURE 1 Enrollment and follow-up of healthy individuals and those with metabolic syndrome who participated in the randomized, crossover, double-blind study.

Based on the weighed quantification of participants' food consumption, energy and nutrient intakes did not differ between trials (**Supplemental Table 1**) for the 3 d preceding and the initial 24 h of each 72-h pharmacokinetics trial. Regardless of health status, women had lower energy intakes than did men (healthy: 1963 ± 2 kcal compared with 2427 ± 137 kcal; MetS: 1958 ± 5 kcal compared with 2450 ± 28 kcal). Likewise, dietary intakes of female participants for all nutrients, with the exception of α-tocopherol and vitamin K, were lower than those of male participants (data not

TABLE 2
Participant characteristics according to health status[1]

	Healthy	MetS[2]	*P* value
Age, y	30.3 ± 1.3	32.8 ± 1.6	0.240
BMI, kg/m^2	22.6 ± 0.7	37.7 ± 3.0	0.001
Waist circumference, cm	75 ± 2	116 ± 7	0.001
Systolic blood pressure, mm Hg	119 ± 3	128 ± 7	0.260
Diastolic blood pressure, mm Hg	74.1 ± 2.5	79.0 ± 3.2	0.242
Glucose, mmol/L	4.95 ± 0.11	5.97 ± 0.24	0.001
Insulin, mU/L	4.0 ± 1.0	10.3 ± 1.7	0.010
HOMA-IR	0.91 ± 0.24	2.76 ± 0.54	0.008
HDL cholesterol, mmol/L	1.45 ± 0.08	1.07 ± 0.09	0.004
LDL cholesterol, mmol/L	2.14 ± 0.17	3.12 ± 0.36	0.024
Cholesterol, mmol/L	4.02 ± 0.15	4.98 ± 0.38	0.032
Triglyceride, mmol/L	0.93 ± 0.11	1.73 ± 0.24	0.008
Total lipid,[3] mmol/L	4.95 ± 0.23	6.70 ± 0.48	0.004
Alanine aminotransferase, U/L	12.3 ± 2.0	14.3 ± 2.4	0.528
Aspartate aminotransferase, U/L	12.5 ± 2.7	10.9 ± 0.9	0.576
Oxidized LDL, U/L	51.8 ± 3.5	69.1 ± 4.2	0.005
Vitamin C, μmol/L	72.6 ± 4.5	53.0 ± 4.9	0.008
Uric acid, μmol/L	305 ± 18	352 ± 27	0.168
α-Tocopherol, μmol/L	22.2 ± 1.2	23.9 ± 0.9	0.282
α-Tocopherol, μmol/mmol cholesterol	5.57 ± 0.31	5.06 ± 0.43	0.351
α-Tocopherol, μmol/mmol lipid	4.54 ± 0.27	3.71 ± 0.27	0.042
γ-Tocopherol, μmol/L	2.27 ± 0.11	3.70 ± 0.42	0.004
γ-Tocopherol, μmol/mmol cholesterol	0.57 ± 0.03	0.81 ± 0.14	0.113
γ-Tocopherol, μmol/mmol lipid	0.47 ± 0.03	0.59 ± 0.10	0.241
C-reactive protein, mg/L	0.85 ± 0.15	2.98 ± 0.34	0.001
TNF-α, pg/mL	9.1 ± 0.6	10.6 ± 0.6	0.086
IL-10, pg/mL	2.28 ± 0.10	2.82 ± 0.17	0.014
IL-6, pg/mL	0.73 ± 0.17	2.13 ± 0.50	0.016

[1]Values are raw means ± SEMs (n = 10 participants/group). Differences between healthy and metabolic syndrome participants for assessments performed during the screening period were compared using a Student's independent t test.
[2]MetS, metabolic syndrome.
[3]Total lipid was calculated as the sum of total cholesterol and triglyceride.

shown). There were no differences in energy and nutrient intakes between healthy and MetS participants, nor were there any changes in participants' body mass throughout the study (data not shown).

Dairy fat effect on α-tocopherols

Plasma d_0–α-tocopherol and d_0–γ-tocopherol concentrations were not different between milk trials at baseline (i.e., t = 0 h, **Table 3**) or throughout each of the 72-h pharmacokinetics trial (data not shown). After d_6–α-tocopherol ingestion, d_6–α-tocopherol C_{max} occurred at ~12 h (time of maximal concentration; T_{max}) regardless of milk type (**Figure 2** and Table 3). Contrary to our hypothesis that dairy fat would dose-dependently increase α-tocopherol bioavailability, d_6–α-tocopherol pharmacokinetic parameters (i.e., $AUC_{0–72\ h}$, C_{max}, estimated absorption) were unaffected by dairy fat content of co-ingested milk beverages (Figure 2). Likewise, plasma d_6–α-tocopherol elimination kinetics were unaffected by dairy fat as evidenced by lack of differences in half-lives or elimination rates during the milk trials (Table 3).

Bioavailability of α-tocopherol, at least when provided as α-TAC, occurs in a fat-dependent manner (17). To better understand our unexpected clinical findings showing that bioavailability of unesterified α-tocopherol was unaffected by dairy fat, we evaluated bioaccessibility of unesterified α-tocopherol at 15 mg and 268 mg per 240-mL serving of each dairy milk by using a simulated digestion system. Recovery of α-tocopherol in chyme after simulated digestion was 80.6% ± 2.1% and unaffected by milk type ($P > 0.05$; data not shown). Statistically significant main effects were observed for α-tocopherol dose, milk type, and the α-tocopherol dose × milk type interaction for α-tocopherol bioaccessibility (**Figure 3**A). Consistent with our clinical observations showing no effect of dairy fat on d_6–α-tocopherol bioavailability, α-tocopherol bioaccessibility was unaffected by milk type when simulated digestions were performed at 15 mg α-tocopherol/240-mL serving of milk. In contrast, a dose-dependent effect of dairy fat on α-tocopherol bioaccessibility was observed when digestions were performed at an α-tocopherol dose recapitulating that of dietary supplement users (i.e., 268 mg α-tocopherol/240 mL milk; Figure 3A), such that α-tocopherol bioaccessibility increased as the copresence of dairy fat during simulated digestion increased. Similarly, a dose × milk interaction indicated that quantity of α-tocopherol recovered in the

TABLE 3
Pharmacokinetic parameters of plasma α-tocopherol[1]

	Healthy			MetS			*P* value		
	Nonfat milk	Reduced-fat milk	Whole milk	Nonfat milk	Reduced-fat milk	Whole milk	H	M	H × M
Baseline d_0–α-tocopherol, μmol/L	22.8 ± 1.7	22.5 ± 1.4	22.3 ± 1.3	24.5 ± 1.6	24.5 ± 1.1	23.1 ± 0.9	0.285	0.723	0.724
Baseline γ-tocopherol, μmol/L	2.24 ± 0.16	2.27 ± 0.18	2.22 ± 0.15	3.71 ± 0.40	3.85 ± 0.47	3.58 ± 0.43	0.006	0.317	0.859
$AUC_{0–72\ h}$, μmol/L × h	103 ± 7	115 ± 8	102 ± 8	86 ± 8	84 ± 7	81 ± 7	0.009	0.321	0.454
C_{max}, μmol/L	2.67 ± 0.20	2.98 ± 0.18	2.55 ± 0.21	2.14 ± 0.20	2.01 ± 0.19	1.98 ± 0.16	0.001	0.315	0.387
T_{max}, h	12.0 ± 0.0	12.0 ± 0.0	13.2 ± 1.2	12.0 ± 0.0	12.0 ± 0.0	12.0 ± 0.0	0.331	0.378	0.378
Elimination rate, μmol/L per hour	0.022 ± 0.001	0.024 ± 0.001	0.023 ± 0.001	0.019 ± 0.001	0.020 ± 0.001	0.021 ± 0.001	0.033	0.267	0.667
Half-life, h	31.6 ± 1.2	29.8 ± 1.3	30.5 ± 1.7	38.3 ± 3.5	36.3 ± 2.1	35.2 ± 2.5	0.021	0.387	0.733
Estimated absorption, % dose	27.8 ± 1.4	32.4 ± 1.6	28.4 ± 1.3	26.6 ± 1.8	26.0 ± 2.5	25.6 ± 1.9	0.045	0.614	0.627

[1]Pharmacokinetics of plasma α-tocopherol (raw means ± SEMs) of healthy (n = 10) and MetS (n = 10) participants who ingested 15 mg d_6–*RRR*–α-tocopherol with 240 mL nonfat, reduced-fat, or whole milk. P values were calculated by using repeated-measures-in-time analysis in PROC MIXED. $AUC_{0–72\ h}$, AUC from 0 to 72 h; C_{max}, plasma maximal concentration; d_0, unlabeled; H, main effect of health status; H × M, health status × milk interaction effect; M, main effect of milk; MetS, metabolic syndrome; T_{max}, time to maximal concentration.

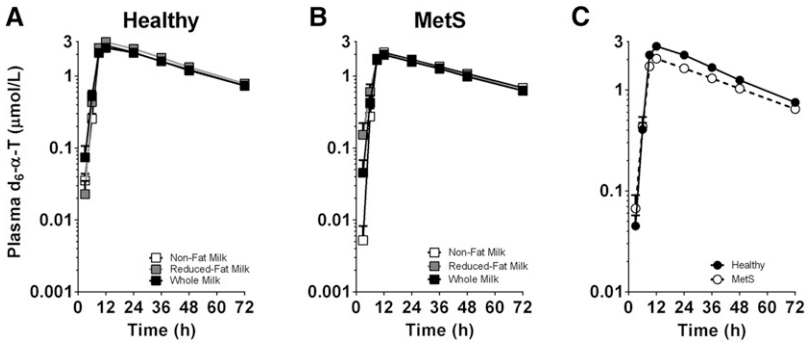

FIGURE 2 Plasma d_6–α-tocopherol concentrations in healthy (A) and MetS (B) participants (n = 10/group) who ingested 15 mg encapsulated d_6–*RRR*–α-tocopherol with nonfat, reduced-fat, and whole milk (240 mL). Each trial was separated by a ≥2-wk washout period. Plasma d_6–α-tocopherol concentrations (means ± SEMs) measured by liquid chromatography–mass spectrophotometry are shown at each time point for each trial. Panel C illustrates the mean pharmacokinetic response of plasma d_6–α-tocopherol in healthy and MetS participants irrespective of the type of dairy milk co-ingested with d_6–*RRR*–α-tocopherol. d_6, hexadeuterium labeled; MetS, metabolic syndrome; α-T, α-tocopherol.

aqueous fraction was unaffected when simulated digestions were performed at 15 mg α-tocopherol, whereas α-tocopherol recovery increased in a milk fat–dependent manner when simulated digestions were performed using 268 mg α-tocopherol per serving of milk (Figure 3B).

Alterations in α-tocopherol pharmacokinetics by health status

Consistent with the absence of any statistically significant differences in plasma d_6–α-tocopherol pharmacokinetic parameters between milks, data were collapsed to better define the influence of MetS health status on pharmacokinetic parameters. In comparison with healthy participants, plasma d_6–α-tocopherol C_{max} was 25% lower in MetS participants without any group differences in T_{max} (Figure 2C and **Table 4**). Consistent with their higher plasma lipids (28), elimination rates and corresponding half-lives of d_6–α-tocopherol were slower in MetS participants than in healthy participants. Bioavailability, determined on the basis of $AUC_{0–72\ h}$, reflects both absorption and elimination kinetics. Despite slower elimination rates, MetS adults had 21% lower $AUC_{0–72\ h}$ than did healthy participants. Estimated absorption of d_6–α-tocopherol was also 11% lower in MetS participants (Table 4). Group differences in these pharmacokinetic parameters persisted after normalization of plasma d_6–α-tocopherol to plasma total lipids, such that MetS participants had lower $AUC_{0–72\ h}$, C_{max}, and estimated absorption as well as a slower elimination rate, longer half-life, but similar T_{max} than did healthy participants (Table 4), suggesting that differences in α-tocopherol bioavailability by health status were not entirely hyperlipidemia dependent. Because lipid normalization did not affect the group differences of plasma pharmacokinetic parameters, nonlipid normalized data were used for all subsequent statistical analyses to facilitate interpretation. Clinical criteria of MetS (i.e., waist circumference, blood pressure, triglyceride, and glucose) along with BMI, plasma insulin, and HOMA-IR were negatively correlated ($P < 0.05$) with plasma d_6–α-tocopherol $AUC_{0–72\ h}$ and C_{max} (**Table 5**), whereas HDL cholesterol was positively correlated with these variables. $AUC_{0–72\ h}$ and C_{max} of plasma d_6–α-tocopherol were also inversely correlated with IL-10, IL-6, and CRP (Table 5), whereas plasma TNF-α and CRP were inversely correlated with estimated absorption ($r = -0.41$ and -0.40, respectively).

Postprandial lipid storage in enterocytes (30) occurs to a greater extent in obese individuals (31), suggesting that the lower C_{max} observed in MetS participants may be attributable to greater enterocyte retention of d_6–α-tocopherol. To assess this possibility, plasma triglyceride responses were considered an indirect marker of intestinal lipid flux. Plasma triglyceride at baseline and throughout the 72-h trial ($P < 0.001$) were greater in MetS adults

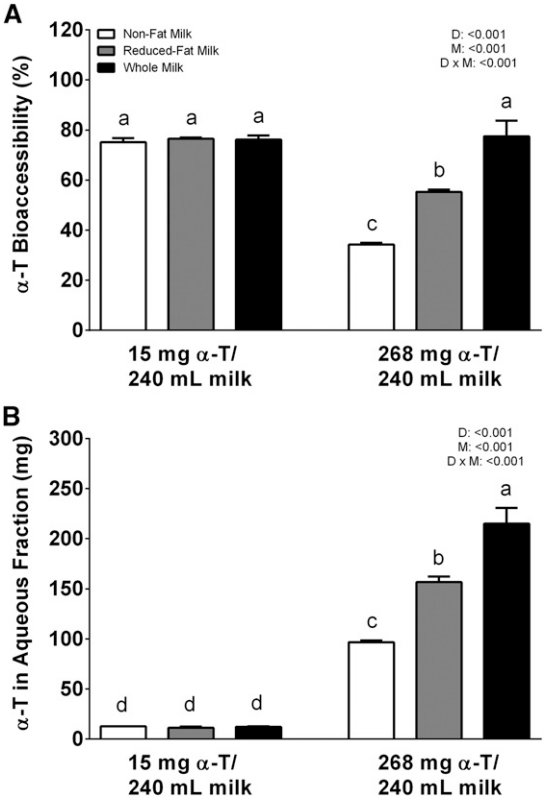

FIGURE 3 α-T bioaccessibility (A) and α-T recovery from the aqueous fraction (B) after in vitro digestions of nonfat, reduced-fat, and whole milks (240 mL) containing 15 or 268 mg *RRR*–α-T (n = 3/α-T D and M types). Bioaccessibility of α-tocopherol (means ± SEMs) was calculated as the percentage of α-T recovered from the aqueous fraction compared with the quantity subjected to in vitro digestion. α-T D, M, and D × M interactions were analyzed by 2-factor ANOVA. Bonferroni's posttest was used to evaluate all group mean differences following a statistically significant D × M interaction. D, dose; M, milk; α-T, α-tocopherol.

TABLE 4
Plasma d_6–α-tocopherol pharmacokinetic parameters averaged from nonfat, reduced-fat, and whole milk trials[1]

	Healthy	MetS
Without lipid normalization[2]		
Baseline d_0–α-tocopherol, μmol/L	22.5 ± 1.4	24.0 ± 0.9
Baseline d_0–γ-tocopherol, μmol/L	2.24 ± 0.15	3.71 ± 0.42*
$AUC_{0–72\ h}$, μmol/L × h	106 ± 7	84 ± 6*
C_{max}, μmol/L	2.73 ± 0.18	2.04 ± 0.14*
T_{max}, h	12.4 ± 0.4	12.0 ± 0.0
Elimination rate, μmol/L per hour	0.023 ± 0.001	0.020 ± 0.001*
Half-life, h	30.6 ± 1.1	36.6 ± 1.9*
Estimated absorption, % dose	29.5 ± 1.1	26.1 ± 1.0*
With lipid normalization		
Baseline d_0–α-tocopherol, μmol/mmol lipid	4.43 ± 0.30	3.53 ± 0.24*
Baseline d_0–γ-tocopherol, μmol/mmol lipid	0.44 ± 0.03	0.54 ± 0.06
$AUC_{0–72\ h}$, μmol/mmol lipid × h	18.2 ± 1.1	12.3 ± 1.1*
C_{max}, μmol/mmol lipid	0.47 ± 0.03	0.29 ± 0.03*
T_{max}, h	11.7 ± 0.3	11.7 ± 0.3
Elimination rate, μmol/mmol lipid per hour	0.023 ± 0.001	0.019 ± 0.01*
Half-life, h	31.4 ± 1.5	37.3 ± 2.1*
Estimated absorption, % dose	26.1 ± 0.7	23.5 ± 0.9*

[1]Baseline plasma d_0–α-tocopherol, d_0–γ-tocopherol, and pharmacokinetics of plasma d_6–α-tocopherol (means ± SEMs) of healthy (n = 10) and MetS (n = 10) participants who ingested 15 mg d_6–*RRR*–α-tocopherol with 240 mL nonfat, reduced-fat, or whole milk. *Statistically significant differences ($P < 0.05$) in pharmacokinetic values between healthy and MetS participants assessed by using a Student's independent t test. $AUC_{0–72\ h}$, AUC from 0 to 72 h; C_{max}, plasma maximal concentration; d_0, unlabeled; d_6, hexadeuterium labeled; MetS, metabolic syndrome; T_{max}, time to maximal concentration.
[2]Total plasma lipid was calculated as the sum of cholesterol and triglyceride.

than in healthy adults (**Figure 4**A). On expressing plasma triglyceride as change from baseline (Figure 4B), a peak was evident at the T_{max} of plasma d_6–α-tocopherol (i.e., 12 h) in both groups. To examine the post hoc hypothesis that lunch provided at 6 h stimulated the release of fat stored in the intestine (30, 31) to a greater extent in MetS participants at 12 h, the change in triglyceride from baseline to 12 h was compared between groups. The change in plasma triglyceride was statistically significant only in MetS adults ($P < 0.05$). To further explore this possibility, d_6–α-tocopherol enrichment was assessed in isolated chylomicrons (**Figure 5**). Chylomicron d_6–α-tocopherol enrichment, estimated as percentage d_6–α-tocopherol relative to total α-tocopherol or normalized to lipoprotein protein concentration, was lower in MetS adults, as indicated by their lower C_{max} of d_6–α-tocopherol (**Table 6**). Similarly, C_{max} of d_6–α-tocopherol for VLDL, LDL, and HDL fractions was lower in MetS adults. Consistent with their lower lipoprotein enrichment, MetS adults also had lower AUC from t = 0–12 h ($AUC_{0–t\ final}$) for all lipoprotein fractions than did healthy adults.

Plasma IL-10 and IL-6 were inversely correlated ($P < 0.05$) with % d_6–α-tocopherol $AUC_{0–t\ final}$ and C_{max} for chylomicron (r = −0.52 to −0.60), VLDL (r = −0.46 to −0.58), LDL (r = −0.59 to −0.66), and HDL (r = −0.60 to −0.64). Plasma CRP was inversely related ($P < 0.05$) to % d_6–α-tocopherol $AUC_{0–t\ final}$ and C_{max} for chylomicron (r = −0.67 and −0.75), VLDL (r = −0.68 and −0.69), LDL (r = −0.67 and −0.67), and HDL (r = −0.66 and −0.72). These correlations, with the exception of VLDL, persisted regardless of normalization of d_6–α-tocopherol to lipoprotein protein concentration (r = −0.49 to −0.58; $P < 0.05$). Last, baseline oxidized LDL was inversely correlated ($P < 0.05$) with the % d_6–α-tocopherol $AUC_{0–t\ final}$ and C_{max} for chylomicron (r = −0.46 and −0.45) and VLDL (r = −0.49 and −0.57).

DISCUSSION

Contrary to our hypothesis, α-tocopherol bioavailability regardless of health status was unaffected by co-ingesting increasing amounts of dairy fat (0.2–7.9 g fat) with 15 mg d_6–*RRR*–α-tocopherol. Likewise, estimated d_6–α-tocopherol absorption regardless of dairy fat amount was 29.5% in healthy adults compared with 26.1% in MetS adults ($P < 0.05$). These were higher than expected based on studies showing 10–33% absorption from 22 mg *RRR*–α-TAC in response to mixed meals containing 0–11 g fat (17) and 24% absorption from 1.2 mg *RRR*–α-tocopherol in collard greens ingested with 7 g fat (28).

TABLE 5
Correlations between d_6–α-tocopherol $AUC_{0–72\ h}$ and C_{max} and metabolic and inflammatory markers[1]

	$AUC_{0–72\ h}$	C_{max}
BMI	−0.61	−0.65
Waist circumference	−0.58	−0.63
Systolic blood pressure	−0.45	−0.45
Diastolic blood pressure	NS	NS
Triglyceride	−0.39	−0.40
HDL cholesterol	0.43	0.43
Glucose	−0.43	−0.52
Insulin	−0.45	−0.54
HOMA-IR	−0.46	−0.55
IL-10	−0.43	−0.43
IL-6	−0.45	−0.44
C-reactive protein	−0.50	−0.56

[1]Values are Pearson correlation coefficients (r) for statistically significant correlations ($P < 0.05$) for healthy and metabolic syndrome participants completing an α-tocopherol pharmacokinetics study (n = 10/group). $AUC_{0–72\ h}$, AUC from 0 to 72 h; C_{max}, plasma maximal concentration; d_6, hexadeuterium labeled.

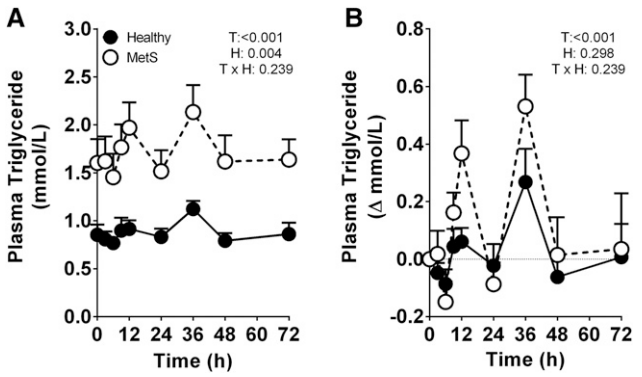

FIGURE 4 Plasma triglyceride concentrations (A) and plasma triglyceride concentrations expressed as change from baseline from healthy and MetS participants (n = 10/group; means ± SEMs). T, H, and T × H interactions were analyzed by repeated-measures ANOVA. H, health status; MetS, metabolic syndrome; T, time.

However, consistent with our findings that dairy milk promotes α-tocopherol absorption, 81% α-tocopherol absorption occurred when *RRR*–α-tocopherol (0.78 mg) was ingested with reduced-fat milk (32) containing an estimated 2.4 g fat (19). Although these findings support ingesting frequent but lower dietary α-tocopherol concentrations to achieve adequacy, we show that dairy milk promotes α-tocopherol bioavailability independent of its fat content, which could serve as an effective dietary strategy to promote α-tocopherol adequacy without delivering excess energy. However, consistent with our hypothesis, MetS lowers α-tocopherol bioavailability, possibly through a mechanism of reduced small intestinal absorption and/or impaired hepatic α-tocopherol trafficking, as evidenced by lower d_6–α-tocopherol absorption and enrichment in chylomicron and VLDL. The data reported herein support higher α-tocopherol requirements in MetS and that dairy milk promotes α-tocopherol bioavailability when ingested at the Recommended Dietary Allowance, an achievable dietary intake when incorporating α-tocopherol–rich foods (e.g., some nut varieties) (8).

Contrary to the findings of the present study, fat-dependent increases in α-tocopherol bioavailability in studies providing α-TAC (16, 17) may be due to lower micellarization efficiency of α-TAC relative to unesterified α-tocopherol such that more bile salt is required to solubilize α-TAC to facilitate uptake in Caco-2 cells (33). The extent to which fat quantity affects the bioavailability of α-TAC compared with α-tocopherol is unclear. Adults ingesting a 100-mg equimolar mixture of trideuterium-labeled (d_3)–α-tocopherol and d_6–α-TAC with a mixed meal had a d_3– to d_6–α-tocopherol ratio of ∼1.0, suggesting similar α-tocopherol bioavailability regardless of its esterification (34). However, dietary fat content was not reported, thereby limiting an understanding whether the similar bioavailability between α-tocopherol forms occurred in response to fat that exceeded quantities used in our study and others (16, 17). This study (34) also did not examine bioavailability of these α-tocopherol forms in response to varying fat quantity. Our simulated digestion studies indicate a dairy fat × α-tocopherol dose interaction on α-tocopherol bioaccessibility. Indeed, bioaccessibility is dependent on dairy fat only when digestions are performed using

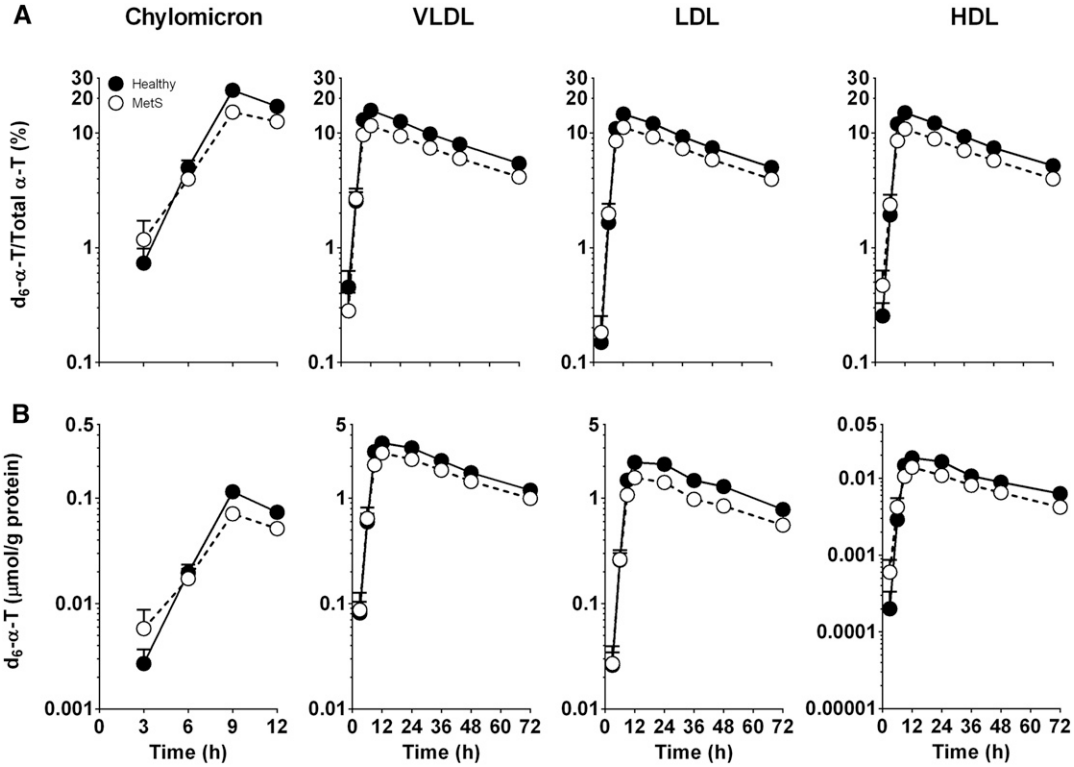

FIGURE 5 Labeled d_6–α-T enrichment expressed as percentage d_6–α-T relative to total α-T (A) and d_6–α-T normalized to protein (B) in chylomicron, VLDL, LDL, and HDL fractions isolated from plasma of healthy and MetS participants (n = 10/group; means ± SEMs). d_6, hexadeuterium labeled; MetS, metabolic syndrome; α-T, α-tocopherol.

TABLE 6
Pharmacokinetic parameters of α-tocopherol in plasma lipoprotein fractions expressed as percentage of total α-tocopherol or normalized to lipoprotein protein concentration[1]

Lipoprotein/variable	Healthy	MetS	*P* value
Chylomicron			
$AUC_{0-t\ final}$, % × h	113 ± 7	79 ± 8	0.006
C_{max}, %	23.6 ± 1.5	15.4 ± 1.2	0.001
$T_{max\ \%}$, h	9.1 ± 0.1	9.4 ± 0.4	0.509
$AUC_{0-t\ final}$, μmol/g protein × h	0.52 ± 0.06	0.36 ± 0.04	0.039
C_{max}, μmol/g protein	0.12 ± 0.01	0.07 ± 0.01	0.016
$T_{max\ \mu mol/g\ protein}$, h	9.1 ± 0.2	9.1 ± 0.3	1.000
VLDL			
$AUC_{0-t\ final}$, % × h	641 ± 28	484 ± 35	0.003
C_{max}, %	15.8 ± 0.8	11.6 ± 0.8	0.001
$T_{max\ \%}$, h	12.4 ± 0.4	12.4 ± 0.4	1.000
$AUC_{0-t\ final}$, μmol/g protein × h	145 ± 8	117 ± 9	0.030
C_{max}, μmol/g protein	3.51 ± 0.25	2.80 ± 0.19	0.036
$T_{max\ \mu mol/g\ protein}$, h	16.7 ± 1.6	14.1 ± 1.0	0.180
LDL			
$AUC_{0-t\ final}$, % × h	597 ± 24	466 ± 34	0.006
C_{max}, %	14.7 ± 0.6	11.2 ± 0.9	0.004
$T_{max\ \%}$, h	12.4 ± 0.4	12.0 ± 0.0	0.343
$AUC_{0-t\ final}$, μmol/g protein × h	97.3 ± 7.7	66.4 ± 6.5	0.007
C_{max}, μmol/g protein	2.36 ± 0.20	1.63 ± 0.19	0.015
$T_{max\ \mu mol/g\ protein}$, h	17.3 ± 2.0	16.0 ± 1.6	0.611
HDL			
$AUC_{0-t\ final}$, % × h	606 ± 29	457 ± 32	0.003
C_{max}, %	15.0 ± 0.7	10.8 ± 0.7	0.001
$T_{max\ \%}$, h	12.3 ± 0.4	12.4 ± 0.4	0.866
$AUC_{0-t\ final}$, μmol/g protein × h	0.75 ± 0.05	0.54 ± 0.06	0.011
C_{max}, μmol/g protein	0.02 ± 0.00	0.02 ± 0.00	0.051
$T_{max\ \mu mol/g\ protein}$, h	15.8 ± 1.6	20.3 ± 2.9	0.201

[1]Pharmacokinetics of lipoprotein α-tocopherol expressed as a percentage of d_6–α-tocopherol of total α-tocopherol or normalized to lipoprotein protein concentration (raw means ± SEMs) of healthy (n = 10) and MetS (n = 10) participants who ingested 15 mg d_6–*RRR*–α-tocopherol with 240 mL nonfat, reduced-fat, or whole milk. Differences between healthy and MetS participants were compared by using Student's independent t test. $AUC_{0-t\ final}$, area under the curve from t = 0–12 h for chylomicrons and 72 h for VLDL, LDL, and HDL; C_{max}, plasma maximal concentration; d_6, hexadeuterium labeled; MetS, metabolic syndrome; T_{max}, time to maximal concentration.

a high concentration of α-tocopherol (268 mg) but not at a lower concentration (15 mg), thereby corroborating our clinical observations. This suggests that the influence of dietary fat on α-tocopherol bioavailability is interdependent on α-tocopherol dose, consistent with higher α-tocopherol absorption occurring at a lower α-tocopherol dose (32).

α-Tocopherol absorption occurs, albeit to a limited extent, in the absence of co-ingesting fat (17). However, we show an estimated α-tocopherol absorption at >26% when it is co-ingested with nonfat milk (0.2 g fat) compared with 10% absorption after ingestion of α-TAC with 0 g fat (17), supporting that physicochemical properties of milk independent of its fat content promote α-tocopherol bioavailability. In support, lymphatic vitamin D_3 absorption in pancreatic duct-ligated rats doubled after intraduodenal administration of a vitamin D_3 emulsion prepared with taurocholate and milk fat globule membrane compared with taurocholate alone (35). Milk fat globule membrane is the membrane that encircles milk lipid droplets, which allows their dispersion. Phospholipids, the major lipid of milk fat globule membrane, is released into the aqueous phase during milk processing (36). Thus, nonfat milk, despite having <0.5 g fat/240 mL milk, retains ~50% of its phospholipid (36) and may facilitate α-tocopherol solubilization and absorption. Whey protein and casein also stabilize emulsions to promote milk fat hydrolysis by pancreatic lipase (37, 38). In comparison with nonhomogenized milk, homogenization increases postprandial chylomicron production and clearance (39). Thus, future studies examining interactions between dairy milk components and α-tocopherol dose and form on α-tocopherol bioavailability should be considered.

After small intestinal incorporation of α-tocopherol into chylomicrons, α-tocopherol is taken up at the liver and repackaged in an α-tocopherol transfer protein-dependent manner for hepatic secretion as part of VLDL (6). In MetS adults, who are often afflicted with nonalcoholic steatohepatitis (40), hepatic α-tocopherol trafficking may be disrupted by α-tocopherol sequestration in steatotic hepatocytes. Our MetS adults had lower VLDL α-tocopherol enrichment, which may reflect dilution of α-tocopherol in VLDL lipids consistent with overproduction of VLDL triglyceride in MetS adults (41). Nonalcoholic fatty liver disease also increases hepatic α-tocopherol accumulation (42) despite greater VLDL secretion (43), further supporting that hepatic α-tocopherol trafficking may be impaired in MetS.

Decreased α-tocopherol bioavailability in MetS may also be mediated at the small intestine, consistent with our MetS adults having lower d_6–α-tocopherol $AUC_{0-72\ h}$ and chylomicron enrichment, which would limit α-tocopherol availability for subsequent hepatic secretion. In support, electron microscopy studies of human enterocytes after a high-fat meal indicated that a proportion of an oral fat load remains both within the enterocyte as neutral lipid droplets within the cytosol and as preformed chylomicrons retained in the lymph (30). This storage depot of dietary triglyceride was then mobilized by ingesting glucose at a later meal, as evidenced by enterocyte secretion of new chylomicron particles and a rapid increase in plasma triglyceride (30). That plasma triglyceride increased to a greater extent at 12 h in our MetS participants, despite similar carbohydrate intakes during lunch at 9 h, suggests their greater intestinal lipid storage compared with healthy participants. Indeed, adults with greater body fat have greater intestinal fat storage (31). Thus, lower α-tocopherol absorption in our MetS adults could reflect decreased enterocyte uptake from the intestinal lumen but also "trapping" of α-tocopherol within enterocytes and delayed secretion into the lymphatic system. This would also explain their lower d_6–α-tocopherol enrichment in VLDL, LDL, and HDL. Unfortunately, chylomicron particle size and quantity were not assessed, precluding an ability to define chylomicron production and α-tocopherol incorporation into chylomicron particles. Also, despite using an established protocol for isolating chylomicrons (16), the chylomicron fraction may not have been devoid of other lipoproteins, as suggested by a delay in chylomicron T_{max} in our study compared with others (i.e., 9 compared with 6 h) (12). Further study is warranted to discriminate between small intestinal and hepatic responses mediating impaired α-tocopherol bioavailability in MetS.

We show that inflammatory (IL-6, IL-10, CRP) and oxidative stress (oxidized LDL) markers were inversely related to chylomicron and VLDL % d_6–α-tocopherol $AUC_{0-t\ final}$. Thus, lower

α-tocopherol enrichment of chylomicron and VLDL in MetS may be mediated by inflammation and/or oxidative stress that limits intestinal absorption and hepatic secretion of α-tocopherol. Supporting intestinal inflammation in our MetS adults, insulin-resistant obese patients compared with those who were insulin sensitive had increased intestinal inflammation and oxidative stress consistent with greater duodenal inflammatory proteins and lipid peroxidation (44). Intestinal inflammation is expected to limit intestinal lipid secretion consistent with proinflammatory cytokines decreasing triglyceride-rich lipoprotein secretion in Caco-2 cells (45, 46). Similarly, greater hepatic lipid peroxidation is associated with advanced severity of nonalcoholic steatohepatitis in humans (47), and inadequate hepatic α-tocopherol status may prevent VLDL secretion consistent with α-tocopherol protecting apolipoprotein from degradation and restoring VLDL production by lowering hepatocyte lipid peroxidation (48). Thus, although oxidative metabolites of α-tocopherol at intestines and liver have not been studied, MetS adults may have had lower α-tocopherol bioavailability because of its greater oxidation within the enterocyte or hepatocyte, but further work is needed to define localized oxidative stress responses on α-tocopherol bioavailability.

In conclusion, MetS lowers α-tocopherol bioavailability regardless of the amount of co-ingested dairy fat. These findings could have important application to improve hepatic α-tocopherol status consistent with α-tocopherol supplementation improving histologic evidence of nonalcoholic steatohepatitis and liver injury biomarkers in humans (49). This study could not determine absolute α-tocopherol bioavailability, which requires comparative oral and intravenous studies, and may underestimate absorption (32). Faster α-tocopherol elimination rates suggest higher α-tocopherol requirements (10). In contrast, MetS lowers α-tocopherol bioavailability but delays α-tocopherol elimination. This may reflect slower lipoprotein catabolism and tissue uptake (50) but is likely multifactorial consistent with α-tocopherol bioavailability being inversely related to inflammatory markers and MetS parameters. Studies that independently assess MetS-related risk factors on α-tocopherol bioavailability are needed, and heterogeneity of MetS status highlights a limitation of solely using α-tocopherol elimination kinetics to assess α-tocopherol status, which may be overcome by using emerging biomarkers of α-tocopherol status (51). Longer term studies reflecting both fast (plasma, liver) and slow (adipose) turning over pools of α-tocopherol (32) also should be considered in contrast to our study and others focusing on rapidly turning over α-tocopherol pools (12, 16, 52). Potential sex differences in α-tocopherol bioavailability also require attention consistent with our female participants, regardless of health status having greater plasma d_6–α-tocopherol $AUC_{0–72\ h}$ and C_{max}, but in contrast to sex differences previously identified for the metabolism of γ-tocopherol but not α-tocopherol (53).

The authors' responsibilities were as follows—EM, MGT, and RSB: were responsible for the study design; SKC: provided medical oversight during the study intervention; GB: assisted with the statistical analysis; EM, TNS, CC, MLF, KES, and RSB: were responsible for collecting and analyzing the data; EM and RSB: wrote the initial draft of the manuscript; and all authors: contributed to the editing and review, as well as read and approved the final manuscript. None of the authors reported any conflicts of interest.

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