Supplementation with Seabuckthorn Oil Augmented in 16:1n–7*t* Increases Serum *Trans*-Palmitoleic Acid in Metabolically Healthy Adults: A Randomized Crossover Dose-Escalation Study

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ABSTRACT

Background: In animal models *cis*-palmitoleic acid (9-hexadecenoic acid; 16:1n–7*c*), a lipokine, improves insulin sensitivity, inflammation, and lipoprotein profiles; in humans *trans*-palmitoleic acid (16:1n–7*t*) has been associated with lower incidence of type 2 diabetes. The response to dose-escalation of supplements containing *cis*- and *trans*-palmitoleic acid has not been evaluated.

Objectives: We examined dose-escalation effects of oral supplementation with seabuckthorn oil and seabuckthorn oil augmented in 16:1n–7*t* on serum phospholipid fatty acids (PLFAs).

Methods: Thirteen participants (7 women and 6 men; age 48 ± 16 y, BMI 30.4 ± 3.7 kg/m^2) participated in a randomized, double-blind, crossover, dose-escalation trial of unmodified seabuckthorn oils relatively high in 16:1n–7*c* (380, 760, and 1520 mg 16:1n–7*c*/d) and seabuckthorn oils augmented in 16:1n–7*t* (120, 240, and 480 mg 16:1n–7*t*/d). Each of the 3 escalation doses was provided for 3 wk, with a 4-wk washout period between the 2 supplements. At the end of each dose period, fasting blood samples were used to determine the primary outcomes (serum concentrations of the PLFAs 16:1n–7*t* and 16:1n–7*c*) and the secondary outcomes (glucose homeostasis, serum lipids, and clinical measures). Trends across doses were evaluated using linear regression.

Results: Compared with baseline, supplementation with seabuckthorn oil augmented in 16:1n–7*t* increased phospholipid 16:1n–7*t* by 26.6% at the highest dose ($P = 0.0343$). Supplementation with unmodified seabuckthorn oil resulted in a positive trend across the dose-escalations (P-trend $= 0.0199$). No significant effects of either supplement were identified on blood glucose, insulin, lipids, or other clinical measures, although this dosing study was not powered to detect such effects. No carryover or adverse effects were observed.

Conclusions: Supplementation with seabuckthorn oil augmented in 16:1n–7*t* and unmodified seabuckthorn oil moderately increased concentrations of their corresponding PLFAs in metabolically healthy adults, supporting the use of supplementation with these fatty acids to test potential clinical effects in humans. This trial was registered at clinicaltrials.gov as NCT02311790. *J Nutr* 2020;150:1388–1396.

Keywords: seabuckthorn oil, *cis*-palmitoleic acid, *trans*-palmitoleic acid, dose-escalation, phospholipid fatty acids, glucose, insulin, cholesterol, triglyceride

Introduction

Growing research indicates that the conventional chemistry categorization of fatty acids (e.g., SFA, MUFA, PUFA) belies the biological complexity of potential health effects of individual fatty acids of varying structure and chain length, including those present at relatively low concentrations (1). Among these, palmitoleic acid (9-hexadecenoic acid; 16:1n–7), which exists in both *cis* (16:1n–7*c*) and *trans* (16:1n–7*t*) isomeric forms, is of particular interest. In in vivo animal and human observational studies, supplementation with 16:1n–7*c* improves insulin sensitivity (2–5), decreases inflammation and dyslipidemia (6), and slows the development of atherosclerotic lesions (5). Because 16:1n–7*c* is a relatively specific downstream product of hepatic de novo lipogenesis, it may reflect the conversion of excess dietary starch, sugar, and protein into fatty

acids. It has been hypothesized that exogenous administration of 16:1n–7c participates in a negative feedback loop to suppress hepatic de novo lipogenesis (2, 7). Other postulated mechanisms include stimulation of β-cell insulin secretion, elevation of hepatic fatty acid oxidation, and acceleration of macrophage differentiation into an anti-inflammatory phenotype (4).

Yet, these promising results in animal models have been challenging to test in humans, in part because of limited evidence on blood dose-response effects of 16:1n–7c supplements. Dietary sources are rare, including seabuckthorn oil and macadamia nuts (8). Because these foods are not commonly consumed, blood concentrations of 16:1n–7c largely reflect endogenous synthesis. Positive associations have been reported between higher blood concentrations and lower metabolic risk (9, 10). Thus, these observational studies of endogenously produced 16:1n–7c do not provide inference on the potential health effects of exogenously provided 16:1n–7c.

The *trans* isomer of palmitoleic acid, 16:1n–7t, naturally present in dairy fat, is a metabolic product of bacterial synthesis in the rumen of animals (9–11). In observational studies, higher blood concentrations of 16:1n–7t have been associated with greater insulin sensitivity and lower incidence of type 2 diabetes (T2D) (10–14), suggesting a potential role of this isomer in metabolic health. Yet, like 16:1n–7c, these results in observational studies have been challenging to confirm in intervention studies owing to limited data on the dose-response effect of 16:1n–7t supplementation on blood concentrations.

Thus, the dearth of information on the dose-response effect of oral supplementation with 16:1n–7c or 16:1n–7t on blood concentrations has limited the design and implementation of interventional studies. To address this gap in knowledge we investigated, in a randomized, double-blind, dose-escalation trial, the effects of unmodified seabuckthorn oil and seabuckthorn oil augmented in 16:1n–7t on serum phospholipid fatty acid (PLFA) profiles in generally healthy adults. We hypothesized that supplementation with unmodified seabuckthorn oil and seabuckthorn oil augmented in 16:1n–7t would result in a dose-dependent increase in their respective PLFA concentrations.

Methods

Study population

Study subjects ($n = 13$; 7 women and 6 men) were recruited from the greater Boston area using advertisements and the Human Nutrition Research Center on Aging at Tufts (HNRCA) volunteer database (Figure 1). Inclusion criteria were age 18–70 y; BMI (in kg/m^2) 25–40; fasting plasma glucose $\leq$120 mg/dL; normotensive (blood pressure <140/90 mm Hg) with or without medications; and normal thyroid (with or without medication for $\geq$6 mo), kidney, liver, and gastrointestinal clinical blood measures. Exclusion criteria were use of supplements containing fish oil or other lipid supplements within 3 mo of randomization; habitual consumption of $\geq$3 combined servings/d of cheese, full-fat milk, or full-fat yogurt (the major dietary source of 16:1n–7t); alcohol intake >7 alcoholic beverages/wk; inability or difficulty in taking pills; diagnosed type 1 or type 2 diabetes and/or taking glucose-lowering medications; use of medication known to affect lipid metabolism; and hormone replacement therapy. This study (NCT02311790) was conducted in accordance with the Declaration of Helsinki guidelines, all procedures were approved by the Institutional Review Board of Tufts University/Tufts Medical Center, and written informed consent was obtained from the study participants. The study was conducted between January 2016 and March 2017.

Recruitment and screening

Participants who responded to the study advertisements or were recruited from the HNRCA volunteer database were screened for eligibility. The participants were provided with information about the study design and procedures, and for those who were interested in this research, a prescreening telephone interview was administrated to assess potential eligibility. Participants who appeared to meet the inclusion criteria were scheduled for a prescreening in-person appointment to collect a fasting (12-h) blood sample. Based on the predetermined criteria, the qualified participant was invited for a subsequent screening visit to conduct a full health screen, obtain a blood sample, and assess their dietary habits with specific emphasis on the habitual consumption of full-fat dairy products. A total of 145 participants were screened for eligibility and 17 participants were enrolled into the study (Figure 1). Four of the participants did not complete the study because of the following reasons: time constraints ($n = 2$), noncompliance with study procedures ($n = 1$), and light sensitivity issues ($n = 1$).

Study design and interventions

We conducted a randomized, double-blind, crossover, dose-escalation study over 22 wk, including 2 randomly assigned supplementation periods consisting of escalating doses of either unmodified seabuckthorn oil (380, 760, and 1520 mg 16:1n–7c/d) or seabuckthorn oil augmented in 16:1n–7t (120, 240, and 480 mg 16:1n–7t/d). Each dose was provided for 3 wk, with a 4-wk washout phase between the 2 supplements (Supplemental Figure 1). The random assignment sequence for each participant was generated according to a block design and assigned at the time of study consent. The study statistician generated the random allocation sequence. Enrollment and assignment were conducted by the study coordinator. The 2 supplements were labeled A and B, and neither were aware of their identity. The appearance and characteristics of the 2 supplements were identical. Serum concentrations of the PLFAs 16:1n–7t and 16:1n–7c were the primary outcomes for this dose-escalation study, and the other fatty acids were exploratory outcomes. All participants, investigators, and laboratory personnel were blinded to the random assignment. For each of the 2 supplementation periods, participants were instructed to consume 2 capsules/d during the first 3 wk, 4 capsules/d during the second 3 wk, and 8 capsules/d during the third 3 wk, evenly divided between morning and evening meals. Compliance was evaluated using pill counts.

Unmodified seabuckthorn oil and seabuckthorn oil augmented in 16:1n–7t supplements

The seabuckthorn oil augmented in 16:1n–7t was developed and provided for this trial free of charge by Unilever, Netherlands. Both oils were derived from fresh seabuckthorn berries. The 16:1n–7c oil was
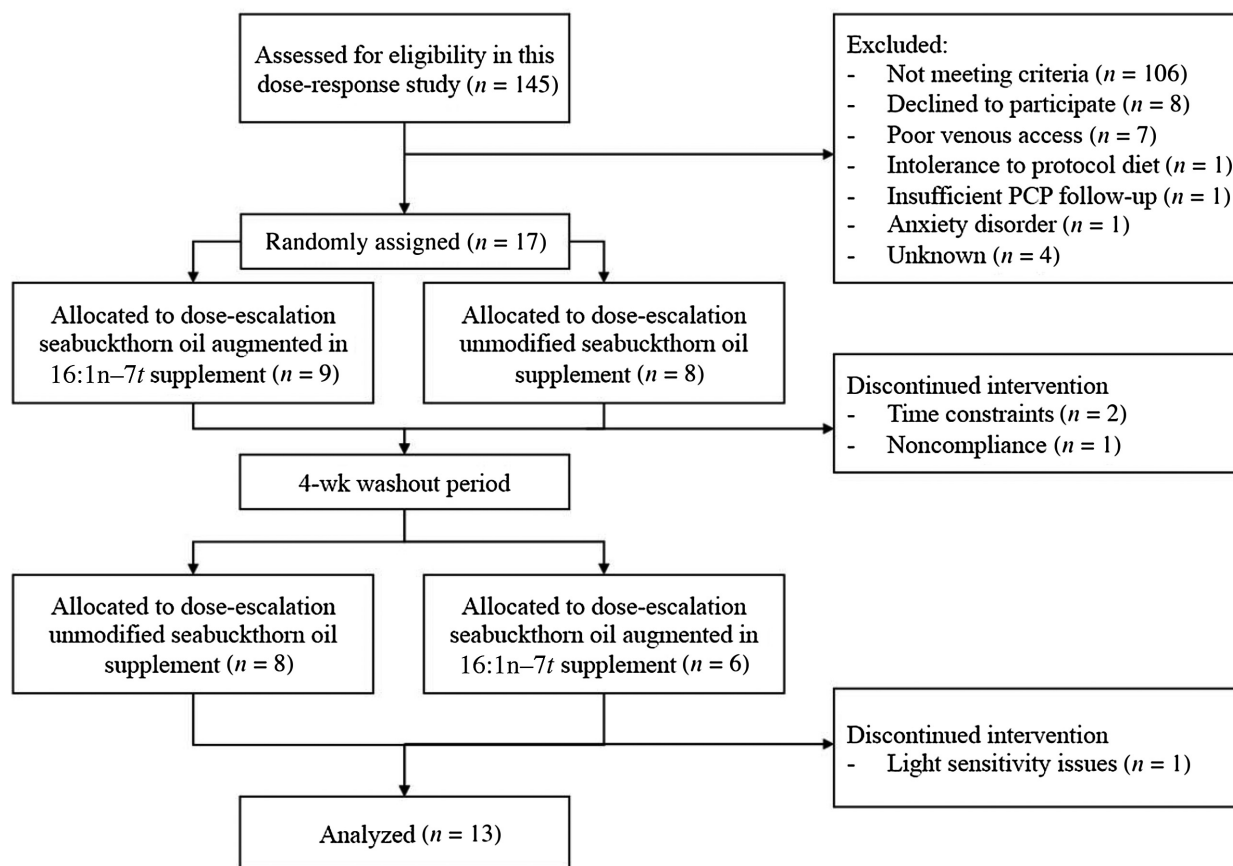
FIGURE 1 Flow diagram of the metabolically healthy adults supplemented with seabuckthorn oil augmented in 16:1n–7t and unmodified seabuckthorn oil. PCP, primary care provider.

unmodified seabuckthorn oil, containing 35.5% 16:1n–7c by weight and no detectable 16:1n–7t. Each seabuckthorn oil supplement capsule (500 mg) contained 178 mg 16:1n–7c and no detectable 16:1n–7t. The seabuckthorn oil augmented in 16:1n–7t was produced by partial hydrogenation of naturally occurring seabuckthorn oil. The goal was to calibrate the process to augment the amount of 16:1n–7t produced and minimize the formation of other *trans* fatty acids. Owing to technical limitations the resulting product contained 18:1t others (12%), 16:1n–7t (9.6%), 16:1n–9t (5.7%), 18:1n–7t (2.78%), 18:1n–9t (1.93%), and 18:2t (0.36%) ([Table 1]). Each seabuckthorn oil augmented in 16:1n–7t supplement capsule (500 mg) contained 48 mg 16:1n–7t and 113 mg 16:1n–7c. The total PUFA content of the seabuckthorn oil augmented in 16:1n–7t was lower than that of the unmodified seabuckthorn oil.

Serum PLFA measurements

Overnight fasting blood was drawn from the antecubital vein at the beginning (baseline) and end of each 3-wk dosing period. Serum was immediately separated by centrifugation at $1100 \times g$ for 10 min at 4°C and stored at −80°C until analyses. All samples were analyzed in a single batch at the conclusion of the trial, using an established GC method ([15]). Briefly, lipids were extracted from serum using the Folch method ([16]) after addition of an internal standard (1,2 diheptadecanoyl-glycero-3-phosphocholine). The serum phospholipid (PL) fraction was isolated using solid-phase chromatography (aminopropyl columns), saponified, and methylated as previously described ([15]). The resulting FAMEs were analyzed using an Autosystem XL GC (Perkin Elmer) equipped with a 100 m × 0.25 mm capillary column (HP INNOWax, Agilent Technologies). Fatty acid peaks were identified by comparison with authenticated standards (NuCheck Prep) and expressed as mol% and μmol/L. The intra-assay CVs were 5.4% for 16:1n–7c, 6.8% for 16:1n–7t, 0.5%–4.3% for other fatty acids present at concentrations >5 mol%, 1.8%–7.1% for fatty acids present at concentrations between 1

and 5 mol%, and 2.8%–11.1% for fatty acids present at concentrations <1 mol%.

Clinical laboratory measures

Fasting blood samples were used to determine the secondary outcomes, including glucose homeostasis, lipids, and clinical measures. As safety measures, plasma total cholesterol, HDL-cholesterol, and triglyceride (TG) concentrations and serum glucose concentrations were measured using an AU400e automated analyzer (Beckman Coulter; assay CV <3%) with enzymatic reagents (Beckman-Coulter). LDL-cholesterol concentrations were calculated using the Friedewald formula ([17]). Serum insulin concentrations were determined by RIA (EMD Millipore). VLDL-cholesterol [TG (mmol/L)/2.2] concentrations, non-HDL-cholesterol (total cholesterol minus HDL-cholesterol) concentrations, and HOMA-IR [glucose (mmol/L) × insulin (mU/L)/22.5] ([18]) were calculated. Hematology and clinical chemistry measures were determined using a Horiba ABX Pentra 60c$^+$ Hematology Analyzer (HORIBA Instruments Inc.) and AU480 Clinical Chemistry Analyzer (Beckman Coulter Inc.), respectively.

Power calculation

In the absence of available 16:1n–7t supplementation data, sample size calculations were based on estimates of circulating fatty acid concentrations. The lowest dose for 16:1n–7t corresponded to the mean and the lowest dose of 16:1n–7c corresponded to the intermediate circulating concentrations. We took this approach because, compared with the concentration of 16:1n–7t, 16:1n–7c is relatively high and it can be synthesized endogenously. Based on observed associations between consumption of whole-fat dairy foods and PL 16:1n–7t concentrations, and the average content of 16:1n–7t in dairy foods, we hypothesized a potential 33%–50% increase in serum PL 16:1n–7t concentrations at the lowest dose, a 66%–100% increase at the middle

TABLE 1 Detectable FAs in seabuckthorn oil augmented in 16:1n–7t and unmodified seabuckthorn oil supplements[1]

| FAs | Seabuckthorn oil augmented in 16:1n–7t | | Unmodified seabuckthorn oil | |
	mg/500 mg capsule	% FA	mg/500 mg capsule	% FA
SFA total	182	36.3	168	33.5
16:0	168	33.5	160	31.9
MUFA total	314	62.9	301	60.2
cis total	154	30.9	301	60.2
16:1n–7c	113	22.5	178	35.5
16:1n–9c	12.5	2.50	ND	ND
18:1n–7c	22.5	4.5	38.6	7.71
18:1n–9c	6.85	1.37	84.5	16.9
24:1n–9c	0.1	0.02	0.05	0.01
trans total	160	32.0	ND	ND
16:1n–7t	48.0	9.6	ND	ND
16:1n–9t	28.5	5.7	ND	ND
18:1n–7t	13.9	2.78	ND	ND
18:1n–9t	9.65	1.93	ND	ND
18:1t, others	59.9	12.0	ND	ND
PUFA total	3.85	0.77	31.7	6.34
cis total	2.05	0.41	31.7	6.34
18:2n–6c	1.2	0.24	26.0	5.20
20:2n–6c	0.4	0.08	0.05	0.01
20:4n–6c	0.05	0.01	ND	ND
18:3n–3c	0.15	0.03	5.65	1.13
18:4n–3c	0.25	0.05	ND	ND
trans 18:2t	1.80	0.36	ND	ND

[1]Values are milligrams or percentages of FA. The unit of each supplement is mg/500-mg capsule. FA labeled as ND indicates a concentration <0.01%. FA, fatty acid; ND, not detectable.

dose, and a 100%–150% increase at the highest dose. Based on paired t testing, 90% power, and 2-sided $\alpha = 0.05$, a sample size of 11 subjects was calculated to detect a 33% increase between baseline and the lowest-dose group. For serum PL 16:1n–7c, we anticipated a potential increase in concentrations of 20%–30% at the lowest dose, 40%–50% at the middle dose, and 60%–70% at the highest dose. Performing sample size calculations as above, a sample size of 11 subjects was calculated to detect a 40% increase between baseline and the middle-dose group. Assuming a potential dropout rate of 15%–30% for a 22-wk dietary supplement study, we enrolled 17 participants to ensure that ≥12 participants completed the study (Figure 1).

Statistical analysis

All data were tested for normality using the D'Agostino–Pearson normality test with $\alpha = 0.05$, before statistical analysis (Prism 8, GraphPad Software). A paired-sample t test was conducted for the power calculation. Statistical significance for the primary outcome (serum PLFAs) and for the secondary outcomes (glucose homeostasis, serum lipids, and clinical measures) in both seabuckthorn oil augmented in 16:1n–7t and unmodified seabuckthorn oil across the 4 time points (baseline, phase 1, phase 2, phase 3) within each supplementation period was tested using a mixed-effects model ANOVA, and Tukey's test was conducted only when P values for ANOVA were <0.05 (Prism 8, GraphPad Software). Carryover effects across supplementation periods were assessed by Student's t test. The primary dose comparisons for PL 16:1n–7t and 16:1n–7c were baseline compared with 120 mg/d, and baseline compared with 760 mg/d, respectively, and the remaining dose comparisons were secondary dose comparisons. Labelled data with different superscripts indicate statistical differences from each other, and no additional adjustment was applied to individual PLFAs for multiple comparisons.

TABLE 2 Demographic profile of study participants[1]

Variables	Values ($n = 13$)
Age, y	48 ± 16
Women	7 (53.9%)
Height, m	167 ± 7.1
Weight, kg	85.7 ± 15.6
BMI, kg/m^2	30.4 ± 3.7
Waist circumference,[2] cm	98 ± 13
Women	93 ± 15
Men	102 ± 8
Hip circumference,[2] cm	106 ± 8
Waist-to-hip ratio[2]	0.9 ± 0.1
Systolic blood pressure, mm Hg	117 ± 13
Diastolic blood pressure, mm Hg	75 ± 10

[1]Values are means ± SDs or n (%).
[2]$n = 12$ owing to missing values.

In a secondary analysis, we evaluated trends in changes in PL 16:1n–7t and 16:1n–7c across the dose-escalation by modeling the fatty acid dose as a continuous dependent variable in a linear regression model with time as a continuous linear predictor. Correlation of repeated measures within subject was accounted for by including a random effect in the model. For all tests, $P < 0.05$ was considered statistically significant.

Results

Baseline characteristics of study participants

The mean ± SD age of the participants was 48 ± 16 y, 54% were women, and mean ± SD BMI was 30.4 ± 3.7 (Table 2). Consistent with the general US population, waist circumference measures were higher than optimal, especially in women. On the basis of pill counts, compliance was high: 98.9%.

Serum PLFA concentrations

Supplementation with the seabuckthorn oil augmented in 16:1n–7t resulted in a 26% increase in PL 16:1n–7t (mol%) (Figure 2A) at the highest dose (480 mg/d) compared with baseline ($P = 0.0343$ by ANOVA; P-trend = 0.0081). The seabuckthorn oil augmented in 16:1n–7t did not consistently alter PL 16:1n–7c concentrations (Figure 2C). The seabuckthorn oil augmented in 16:1n–7t also increased PL 18:1n–7c by 16.3%; observed effects on other PLFAs were smaller and not dose-dependent (Table 3).

There was no significant effect of supplementation with unmodified seabuckthorn oil on mean PL 16:1n–7c concentrations (0.49% to 0.53% to 0.56% to 0.60% for doses of 380, 760, and 1520 mg 16:1n–7c/d, respectively) (Table 4); however, there was a positive trend across increasing doses (P-trend: 0.0199) (Figure 2D). The unmodified seabuckthorn oil increased concentrations of PL 18:1n–7c by 10.2% at the middle dose (760 mg/d), with inconsistent and non-dose-dependent effects on the other PLFAs.

No significant carryover effect ($P > 0.05$) was observed in fasting PLFAs between randomization periods for either the seabuckthorn oil augmented in 16:1n–7t or the unmodified seabuckthorn oil.

We evaluated PLFA measures in absolute concentrations (μmol/L), which are jointly influenced by fatty acid composition as well as absolute concentrations in the fasting state. Supplementation with the oil augmented in 16:1n–7t produced
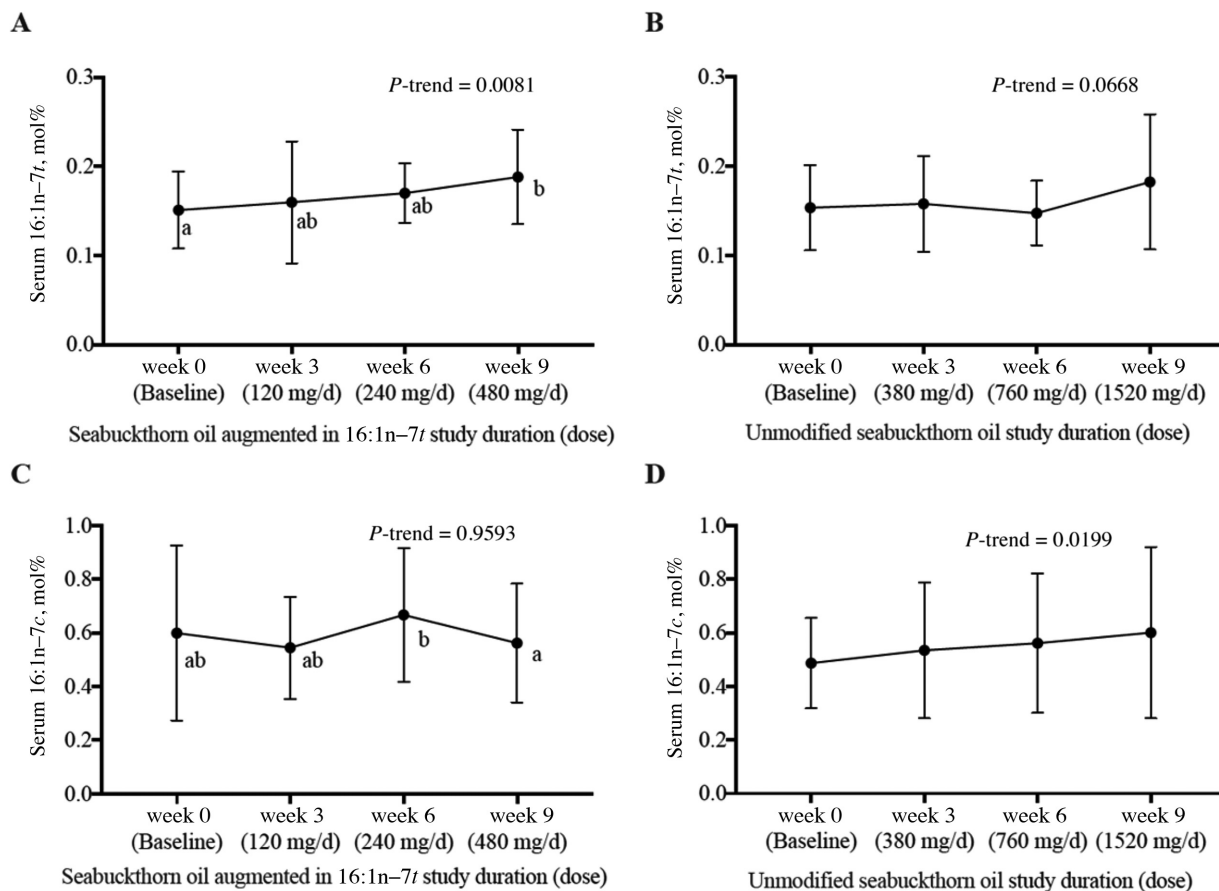
FIGURE 2 Fasting serum phospholipid fatty acid concentrations at the end of each supplement phase in metabolically healthy adults. (A) 16:1n–7t in the seabuckthorn oil augmented in 16:1n–7t. (B) 16:1n–7t in the unmodified seabuckthorn oil. (C) 16:1n–7c in the seabuckthorn oil augmented in 16:1n–7t. (D) 16:1n–7c in the unmodified seabuckthorn oil. Values are means ± SDs in mol% (A, C: $n = 13$; B, D: $n = 13$ in weeks 0, 6, and 9; $n = 12$ in week 3 owing to limited sample volume). Labeled means without a common letter differ, $P < 0.05$.

dose-dependent increases in PL 16:1n–7t concentrations, 15% at the highest dose, compared with baseline ($P = 0.0471$) (**Supplemental Table 1**). Change in serum PL 18:3n–6c was not consistent or dose-dependent. Supplementation with the unmodified seabuckthorn oil did not significantly alter PL 16:1n–7c concentrations (by ANOVA, $P = 0.27$; P-trend = 0.06) and produced inconsistent and non-dose-dependent changes in other fatty acids (**Supplemental Table 2**).

Safety outcomes

No significant effects of either supplement were observed in serum glucose, serum insulin, and lipid concentrations (**Supplemental Figure 2**). Likewise, no significant effect of the supplements was observed in clinical chemistry measures, with 1 exception: a 4% increase in serum lymphocytes ($P = 0.0298$) after the highest 16:1n–7t supplement dose (480 mg/d) (**Supplemental Tables 3** and **4**). All the markers were within their normal ranges, and no carryover effect on the basis of random assignment sequence was observed. No adverse events were reported by any of the participants.

Discussion

Dietary 16:1n–7c and 16:1n–7t are fatty acid isomers that occur naturally in foods at relatively low concentrations. Supplementation with 16:1n–7c in experimental animal models inhibits

hepatic de novo lipogenesis and improves insulin sensitivity, inflammation, and plasma lipid profiles ([19]). Experimental studies of 16:1n–7t in humans have not, to our knowledge, been reported. In this randomized dose-escalation intervention trial, supplementation with seabuckthorn oil augmented in 16:1n–7t modestly increased PLFA concentrations. Supplementation with unmodified seabuckthorn oil had smaller effects that were only statistically significant in a secondary analysis of the trend across escalating doses. Based on the clinical chemistry measures and subject reports, no adverse effects or supplement intolerance were identified.

These findings build upon and expand the prior literature by assessing the dose–response relations of unmodified seabuckthorn oil or seabuckthorn oil augmented in 16:1n–7t with PLFA concentrations. An understanding of these relations is a critical foundation for pursuing a more thorough evaluation of biological effects of these 16:1n–7 isomers in human studies.

Few prior trials have assessed potential effects of 16:1n–7c in humans. Among 60 adults with dyslipidemia and mildly elevated high-sensitivity C-reactive protein (hs-CRP) concentrations, 220 mg/d 16:1n–7c for 30 d, compared with placebo, was reported to reduce concentrations of hs-CRP, TG, and LDL cholesterol by 44%, 15%, and 8%, respectively, and increased HDL cholesterol by 5% ([5]). This dose was below the lower dose used in the current study. These findings could have been confounded, however, by the placebo formulation, which was not inert but contained 1000 mg

TABLE 3 Fasting serum phospholipid fatty acid concentrations during the dose-escalation course of the seabuckthorn oil augmented in 16:1n–7t in metabolically healthy adults[1]

Fatty acid, mol%	Baseline	Week 3 (120 mg 16:1n–7t/d)	Week 6 (240 mg 16:1n–7t/d)	Week 9 (480 mg 16:1n–7t/d)	ANOVA P values
SFA	47.5 ± 2.81	47.3 ± 3.76	47.4 ± 2.65	46.4 ± 1.88	0.358
10:0	0.01 ± 0.007	0.01 ± 0.004	0.01 ± 0.004	0.01 ± 0.004	0.179
12:0	0.04 ± 0.01	0.04 ± 0.02	0.05 ± 0.03	0.05 ± 0.02	0.265
14:0	0.50 ± 0.12	0.45 ± 0.11	0.48 ± 0.11	0.41 ± 0.14	0.052
15:0	0.24 ± 0.05	0.25 ± 0.06	0.24 ± 0.04	0.23 ± 0.05	0.688
16:0	28.9 ± 2.36	28.8 ± 2.87	28.8 ± 2.07	28.2 ± 1.41	0.520
18:0	15.8 ± 1.79	15.7 ± 1.83	15.9 ± 1.63	15.5 ± 1.77	0.575
20:0	0.22 ± 0.16	0.24 ± 0.18	0.23 ± 0.13	0.22 ± 0.17	0.668
22:0	1.03 ± 0.16	1.06 ± 0.23	0.99 ± 0.18	1.02 ± 0.19	0.496
24:0	0.76 ± 0.12	0.81 ± 0.20	0.73 ± 0.11	0.78 ± 0.14	0.215
MUFA	11.7 ± 1.67	11.8 ± 1.60	12.3 ± 1.50	11.7 ± 1.85	0.405
cis					
14:1n–5	0.01 ± 0.003	0.01 ± 0.002	0.01 ± 0.002	0.01 ± 0.002	0.107
16:1n–9	0.12 ± 0.03	0.12 ± 0.03	0.13 ± 0.03	0.11 ± 0.04	0.995
16:1n–7	0.60 ± 0.33[a,b]	0.54 ± 0.19[a,b]	0.67 ± 0.25[b]	0.56 ± 0.22[a]	0.045
18:1n–9	8.61 ± 1.43	8.62 ± 1.46	9.00 ± 1.20	8.35 ± 1.64	0.395
18:1n–7	1.23 ± 0.16[a]	1.31 ± 0.19[a,b]	1.35 ± 0.23[b]	1.43 ± 0.26[b]	0.001
20:1n–9	0.11 ± 0.03	0.12 ± 0.03	0.11 ± 0.02	0.11 ± 0.03	0.178
22:1n–9	0.02 ± 0.01	0.01 ± 0.01	0.02 ± 0.01	0.02 ± 0.01	0.501
24:1n–9	1.03 ± 0.30	1.04 ± 0.19	0.99 ± 0.12	1.11 ± 0.21	0.465
trans					
16:1n–9	0.03 ± 0.009	0.03 ± 0.025	0.03 ± 0.009	0.03 ± 0.011	0.442
16:1n–7	0.15 ± 0.04[a]	0.16 ± 0.07[a,b]	0.17 ± 0.03[a,b]	0.19 ± 0.05[b]	0.034
18:1n–10–12	0.03 ± 0.02	0.03 ± 0.03	0.03 ± 0.01	0.02 ± 0.01	0.688
18:1n–9	0.15 ± 0.05	0.16 ± 0.06	0.14 ± 0.03	0.15 ± 0.05	0.832
18:1n–7	0.11 ± 0.04	0.13 ± 0.07	0.13 ± 0.02	0.11 ± 0.04	0.413
n–6 PUFA	35.9 ± 2.60	35.8 ± 3.30	35.2 ± 3.15	36.6 ± 2.64	0.297
cis					
18:2n–6	22.6 ± 2.59	22.4 ± 2.86	22.0 ± 3.17	22.3 ± 2.57	0.743
18:3n–6	0.10 ± 0.04	0.09 ± 0.04	0.11 ± 0.08	0.08 ± 0.05	0.179
20:2n–6	0.28 ± 0.05	0.27 ± 0.04	0.27 ± 0.05	0.26 ± 0.05	0.624
20:3n–6	2.68 ± 0.71	2.55 ± 0.57	2.62 ± 0.73	2.45 ± 0.71	0.556
20:4n–6	9.69 ± 1.56[a]	9.91 ± 2.66[a,b]	9.7 ± 1.79[a]	10.9 ± 2.08[b]	0.014
22:2n–6	0.02 ± 0.008	0.02 ± 0.005	0.02 ± 0.007	0.02 ± 0.008	0.567
22:4n–6	0.31 ± 0.07	0.30 ± 0.08	0.32 ± 0.09	0.32 ± 0.08	0.519
22:5n–6	0.23 ± 0.04	0.22 ± 0.07	0.23 ± 0.08	0.24 ± 0.09	0.501
trans					
18:2n–6	0.16 ± 0.10	0.17 ± 0.15	0.16 ± 0.10	0.11 ± 0.10	0.505
18:2-CLA	0.02 ± 0.01	0.02 ± 0.02	0.02 ± 0.01	0.02 ± 0.01	0.352
n–3 PUFA	4.19 ± 0.67	4.43 ± 1.38	4.41 ± 1.15	4.72 ± 0.75	0.501
18:3n–3	0.22 ± 0.07	0.22 ± 0.09	0.21 ± 0.08	0.19 ± 0.06	0.619
18:4n–3	0.10 ± 0.07	0.11 ± 0.07	0.10 ± 0.07	0.08 ± 0.05	0.457
20:3n–3	0.04 ± 0.02	0.04 ± 0.01	0.04 ± 0.02	0.04 ± 0.01	0.084
20:5n–3	0.71 ± 0.3	0.70 ± 0.40	0.75 ± 0.40	0.67 ± 0.30	0.084
22:5n–3	0.68 ± 0.13	0.70 ± 0.21	0.72 ± 0.22	0.74 ± 0.10	0.716
22:6n–3	2.45 ± 0.69	2.66 ± 0.91	2.60 ± 0.68	3.00 ± 0.63	0.168

[1] *n* = 13. Values are means ± SDs. Findings were analyzed by a mixed-effects model ANOVA followed by Tukey's test. Labeled means in a row without a common letter differ, $P < 0.05$. CLA, conjugated linoleic acid.

medium-chain saturated fats, predominantly caprylic acid and capric acid. Among 20 patients with ulcerative colitis, provision of 720 mg/d 16:1n–7c for 8 wk, compared with placebo, was reported to reduce hs-CRP concentrations by 25% and to increase erythrocyte sedimentation rate levels by 29%, with accompanying upregulation of hepatocyte nuclear factor 4 gamma (*HNF4γ*) and hepatocyte nuclear factor 4 alpha (*HNF4α*) gene expression and downregulation of interleukin 6 (*IL6*) gene expression (20). The dose was in the mid-range of that used in the current study.

With regard to 16:1n–7t, no published human trials were identified. This is partly due to the lack of currently available dietary supplements containing 16:1n–7t. To our knowledge, 16:1n–7t has never before been developed nor administered as a dietary supplement. In our study, partial hydrogenation of seabuckthorn oil increased the 16:1n–7t level from undetectable to 9.6%, 16:1n–9t from undetectable to 5.7%, and total 18:1t from undetectable to 16.7%. These increases were at the expense of 16:1n–7c, which declined from 35.5% to 22.5%; 18:1n–7c, which declined from 7.7% to 4.5%; and 18:

TABLE 4 Fasting serum phospholipid fatty acid concentrations during the dose-escalation course of the unmodified seabuckthorn oil supplement in metabolically healthy adults[1]

Fatty acid, mol%	Baseline[2]	Week 3[3] (380 mg 16:1n–7c/d)	Week 6[2] (760 mg 16:1n–7c/d)	Week 9[2] (1520 mg 16:1n–7c/d)	ANOVA P values
SFA	47.4 ± 2.81[b]	47.5 ± 2.72[b]	44.9 ± 1.75[a]	46.3 ± 2.18[a,b]	0.008
10:0	0.01 ± 0.004	0.01 ± 0.004	0.01 ± 0.004	0.01 ± 0.004	0.691
12:0	0.04 ± 0.03	0.04 ± 0.01	0.03 ± 0.01	0.04 ± 0.02	0.373
14:0	0.39 ± 0.12	0.44 ± 0.12	0.35 ± 0.09	0.43 ± 0.15	0.103
15:0	0.24 ± 0.04	0.24 ± 0.06	0.21 ± 0.03	0.23 ± 0.06	0.099
16:0	28.8 ± 2.41	29.0 ± 2.38	27.8 ± 1.96	28.4 ± 1.84	0.273
18:0	15.7 ± 1.66[b]	15.8 ± 1.96[b]	14.6 ± 1.93[a]	15.3 ± 2.0[a,b]	0.004
20:0	0.35 ± 0.16[b]	0.23 ± 0.16[a]	0.24 ± 0.15[a]	0.24 ± 0.14[a]	0.004
22:0	1.05 ± 0.16	1.03 ± 0.21	0.92 ± 0.17	0.98 ± 0.16	0.056
24:0	0.78 ± 0.16	0.80 ± 0.19	0.72 ± 0.14	0.74 ± 0.15	0.171
MUFA	11.0 ± 1.11[a]	11.4 ± 1.52[a]	12.3 ± 1.47[b]	11.8 ± 1.72[a,b]	0.003
cis					
14:1n–5	0.01 ± 0.004	0.01 ± 0.002	0.01 ± 0.003	0.01 ± 0.005	0.676
16:1n–9	0.11 ± 0.03	0.11 ± 0.04	0.13 ± 0.03	0.12 ± 0.03	0.094
16:1n–7	0.49 ± 0.17	0.53 ± 0.25	0.56 ± 0.26	0.60 ± 0.32	0.159
18:1n–9	7.86 ± 0.97[a]	8.25 ± 1.49[a]	9.05 ± 1.18[b]	8.50 ± 1.29[a,b]	0.004
18:1n–7	1.28 ± 0.22[a]	1.29 ± 0.17[a,b]	1.39 ± 0.24[b]	1.41 ± 0.23[a,b]	0.033
20:1n–9	0.11 ± 0.03	0.11 ± 0.03	0.11 ± 0.02	0.12 ± 0.03	0.603
22:1n–9	0.02 ± 0.01	0.02 ± 0.01	0.01 ± 0.01	0.01 ± 0.01	0.130
24:1n–9	1.09 ± 0.35	1.08 ± 0.33	1.06 ± 0.20	1.04 ± 0.17	0.837
trans					
16:1n–9	0.02 ± 0.01	0.03 ± 0.01	0.03 ± 0.01	0.03 ± 0.01	0.115
16:1n–7	0.15 ± 0.05	0.16 ± 0.05	0.15 ± 0.04	0.18 ± 0.08	0.323
18:1n–10–12	0.02 ± 0.02	0.03 ± 0.01	0.02 ± 0.01	0.03 ± 0.02	0.236
18:1n–9	0.14 ± 0.05	0.15 ± 0.05	0.14 ± 0.06	0.17 ± 0.07	0.341
18:1n–7	0.11 ± 0.04	0.12 ± 0.05	0.10 ± 0.04	0.14 ± 0.07	0.079
n–6 PUFA	36.5 ± 2.79	35.9 ± 2.74	37.3 ± 2.15	36.6 ± 2.51	0.373
cis					
18:2n–6	22.6 ± 2.63	22.3 ± 3.42	23.3 ± 2.36	22.8 ± 2.14	0.633
18:3n–6	0.08 ± 0.04	0.11 ± 0.07	0.09 ± 0.04	0.10 ± 0.05	0.299
20:2n–6	0.26 ± 0.05	0.28 ± 0.05	0.27 ± 0.06	0.27 ± 0.05	0.694
20:3n–6	2.64 ± 0.71	2.60 ± 0.76	2.57 ± 0.84	2.56 ± 0.68	0.904
20:4n–6	10.3 ± 2.45	10.2 ± 2.51	10.6 ± 1.87	10.4 ± 2.00	0.714
22:2n–6	0.02 ± 0.01	0.02 ± 0.01	0.02 ± 0.01	0.02 ± 0.01	0.669
22:4n–6	0.33 ± 0.12	0.32 ± 0.08	0.33 ± 0.07	0.33 ± 0.09	0.697
22:5n–6	0.22 ± 0.07	0.23 ± 0.05	0.22 ± 0.06	0.23 ± 0.07	0.748
trans					
18:2n–6	0.13 ± 0.12	0.15 ± 0.09	0.07 ± 0.05	0.13 ± 0.12	0.106
18:2-CLA	0.02 ± 0.01	0.02 ± 0.01	0.02 ± 0.01	0.02 ± 0.01	0.466
n–3 PUFA	4.59 ± 1.03	4.50 ± 1.04	4.93 ± 0.78	4.54 ± 0.63	0.456
18:3n–3	0.17 ± 0.06	0.19 ± 0.07	0.19 ± 0.08	0.19 ± 0.05	0.461
18:4n–3	0.09 ± 0.07	0.09 ± 0.03	0.08 ± 0.04	0.08 ± 0.06	0.646
20:3n–3	0.04 ± 0.01	0.04 ± 0.02	0.04 ± 0.01	0.04 ± 0.01	0.897
20:5n–3	0.79 ± 0.30	0.78 ± 0.34	0.88 ± 0.50	0.71 ± 0.25	0.633
22:5n–3	0.76 ± 0.24	0.70 ± 0.14	0.78 ± 0.13	0.72 ± 0.17	0.353
22:6n–3	2.74 ± 0.84	2.69 ± 0.75	2.96 ± 0.58	2.80 ± 0.71	0.554

[1] Values are means ± SDs. Findings were analyzed by a mixed-effects model ANOVA followed by Tukey's test. Labeled means in a row without a common letter differ, $P < 0.05$. CLA, conjugated linoleic acid.
[2] $n = 13$.
[3] $n = 12$ owing to limited sample volume in week 3.

1n–9c, which declined from 16.9% to 1.4%. This amount of enrichment of 16:1n–7t allowed for a dose-escalation of 0 to 480 mg/d. At the highest dose, we observed a 15.0% increase in PL 16:1n–7t concentrations, achieving a mean concentration of 0.19 mol%, although some participants achieved much higher values (≤0.27%). In observational cohorts, those with PL 16:1n–7t concentrations at the 90th percentile, between 0.10% and 0.26%, were at significantly lower risk of T2D (11, 14).

16:1n–7t is naturally present in very small quantities in dairy products. Most prior reports assessing *trans* fat in dairy products have focused on *trans*-18 isomers. One report found that total *trans*-16 isomers represented a range of between 0.20% and 0.65% of total milk fatty acids (14). Of these, one-third (33%) were 16:1n–7t, or ~0.066%–0.21% of total milk fatty acids. Assuming the value may be as high as 0.5%, our highest dose of 480 mg/d could correspond to about 96 g dairy fat, or ~12 glasses of full-fat milk per day. However, this

estimated correspondence has considerable uncertainty, because the quality and precision of these prior dairy compositional data are not high. Thus, amounts of 16:1n–7*t* in dairy foods and in the diet are not well established, and the present dosing study represents an advance in determining the circulating fatty acid response to 16:1n–7*t* supplementation doses at and possibly above dietary ranges.

Naturally occurring seabuckthorn oil contains ~35.5% 16:1n–7*c*. This allowed for a dose-escalation from 0 to 1520 mg/d, considerably higher than doses used in 2 prior human trials (220 mg/d and 760 mg/d) (11, 14). Also present in the seabuckthorn oil were 7.7% 18:1n–7*c*, 16.9% 18:1n–9*c*, and no detectable 16:1n–9*c*, 16:1n–7*t*, 16:1n–9*t*, or 18:1*t*. After 9 wk escalating supplementation with the unmodified seabuckthorn oil, only a modest trend in mean PL 16:1n–7*c* concentrations was seen. PL 18:1n–7*c* also modestly increased, consistent with the seabuckthorn oil content of this fatty acid. As a percentage of baseline concentrations, at the highest supplement dose, PL 18:1n–7*c* increased by 10.2% and PL 16:1n–7*c* by 22.4%. This suggests that the absence of statistical significance using ANOVA for the latter could relate to larger variation in background PL 16:1n–7*c* concentrations, perhaps related to varying hepatic de novo lipogenesis or other determinants. Our results highlight the need for further investigation of the pharmacokinetics and dose–response relations of 16:1n–7*c* supplementation.

In in vitro studies, 16:1n–7*c* upregulates gene expression of ELOVL5, the gene encoding the enzyme fatty acid elongase 5, which plays an important role in the metabolism of long-chain unsaturated fatty acids (21, 22). In the present study, PL 18:1n–7*c*, the product of the reaction for elongation of 16:1n–7*c*, was significantly increased after the participants received both supplements, albeit not in a dose-dependent manner. Similar increases in PL 18:1n–7*t* were not observed in response to either supplement, consistent with an absence of significant de novo synthesis of 16:1n–7*t* (23).

Our investigation has several strengths. We used a standard dose-escalation design, with randomization of the *cis* and *trans* study periods as well as an intervening washout period, to diminish potential carryover effects. A broad range of doses was utilized, including doses of 16:1n–7*c* higher than those in the few prior trials. Pill count suggested a high level of compliance with the study protocol.

Potential limitations include the variation in background PL 16:1n–7*c* concentrations among participants, which may have limited the statistical power to identify a significant response in this fatty acid using ANOVA. In addition, no adjustment was applied to account for multiple testing across fatty acids in order to minimize type II error, but there is an increased risk of type I error.

The serum phospholipid fraction was selected for analysis because the majority of available data for circulating fatty acids and cardiovascular disease risk/diabetes risk is for this lipid fraction. Although it is unlikely, the possibility cannot be ruled out that the results may have been different had another lipid fraction or RBCs been chosen.

Owing to the technical limitations of partial hydrogenation and existing natural substrates, we could not directly compare the effects of 16:1n–7*t* and 16:1n–7*c* on the basis of a one-to-one molar dose-escalation. However, we demonstrated the feasibility of assessing the PLFA response to the preparations available. Because whole full-fat dairy products contain 16:1n–7*t*, a change in dietary intake during the intervention period could have confounded the study results. To evaluate this

possibility, concentrations of PL 15:0 and conjugated linoleic acid (18:2-CLA) (24, 25), 2 biomarkers of full-fat dairy intake, were monitored: no significant changes were observed over the 9-wk supplementation period.

In conclusion, supplementation with a seabuckthorn oil augmented in 16:1n–7*t* increased its corresponding PLFA in a dose-dependent manner. Supplementation with unmodified seabuckthorn oil also modestly increased its corresponding PLFA, although this observation was statistically significant as a trend only. No adverse effects were apparent. Given the evidence linking palmitoleic acid and metabolic health, these findings provide important dose-response information on 16:1n–7 isomers to inform the design of future interventional trials in humans.

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