Maternal Supplementation with CLA Decreases Milk Fat in Humans

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ABSTRACT: CLA refers to isomers of octadecadienoic acid with conjugated double bonds. The most abundant form of CLA (rumenic acid (RA): *c*9,*t*11-18:2) is found in milk and beef fat. Further, CLA supplements containing RA and *t*10,*c*12-18:2 are now available. Consumption of commercially produced CLA has been shown to decrease adipose accretion in growing laboratory and production animals and cause milk fat depression in cows. We tested the hypothesis that CLA supplementation would increase milk CLA concentration and decrease milk fat content in humans. Breastfeeding women (n = 9) participated in this double-blind, placebo-controlled, crossover study divided into three periods: intervention I (5 d), washout (7 d), and intervention II (5 d). Women were randomized to treatment order. During each intervention period, women consumed 1.5 g of CLA supplement or placebo (olive oil) daily; during the washout period, no supplements were consumed. Milk was collected by complete breast expression on the final day of each period; milk output was estimated by 24-h weighing on the penultimate day of each intervention period. Milk RA and *t*10,*c*12-18:2 concentrations were greater ($P < 0.05$) during the CLA treatment period as compared to the placebo period. Milk fat content was significantly lower during the CLA treatment, as compared to the placebo treatment ($P < 0.05$). Data indicate no effect of treatment on milk output. Therefore, it would be prudent that lactating women not consume commercially available CLA supplements at this time.

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CLA refers to a mixture of positional and geometric isomers of linoleic acid (*c*9,*c*12-octadecadienoic acid). CLA is found naturally in dairy and beef foods and may provide protection against various types of cancers, heart disease, and diabetes (1–7). The most common, naturally occurring isomer is *c*9,*t*11-18:2 and is referred to as rumenic acid (RA) (8). RA is a potent anticarcinogen, especially in mammary tissues (9). Other isomers of CLA (e.g., *t*10,*c*12-18:2) may also be biologically active (10).

Feeding a commercially produced CLA mixture to lactating rats increases the concentration of CLA in milk and the growth and feed efficiency of the suckling pups (11). Data also indicate that human milk, but not infant formula, contains a substantial amount of RA (12), and that diet influences its content (13,14). Park *et al.* (14) reported a change in total milk lipid when a high-CLA (specifically, RA) diet was consumed by lactating women. In this study, milk fat content was higher during a period of high dairy intake then that observed during a period of low dairy intake (4.6 ± 0.5 versus 3.9 ± 0.2%, respectively; $P < 0.05$).

Commercially synthesized CLA, which is available in "health food" stores, contains a substantial amount of another form of a *t*10 FA isomer (*t*10,*c*12-18:2) as well as several other FA (including RA). Treatment with commercially available CLA mixtures (which contain *t*10,*c*12-18:2) decreases milk fat in cows (15,16). Furthermore, recent data confirm that it is the *t*10,*c*12-18:2 CLA isomer that causes milk fat depression (MFD) in lactating cows (17). Thus, human consumption of CLA supplements containing *t*10,*c*12- 18:2 might be of public health importance, especially in lactating women.

In summary, investigators have reported a decrease in milk fat following consumption of diets containing high levels of specific *trans* FA including commercially synthesized CLA and those in partially hydrogenated vegetable oils (PHVO). In several cases, MFD in cows has been associated with an increase in the consumption of 18-carbon FA containing a *trans* double bond at the 10th position (18). Thus, the study described here was designed to explore whether maternal consumption of a commercially produced CLA mixture containing *t*10,*c*12-18:2 decreases milk fat in humans. The following hypotheses were tested: (i) maternal CLA supplementation increases RA and *t*10,*c*12-18:2 concentrations of human milk and (ii) maternal CLA supplementation decreases milk fat content of human milk.

MATERIALS AND METHODS

Subjects. Healthy, lactating women (n = 10) from 1 to 12 mon postpartum were recruited from the Moscow, Idaho–Pullman, Washington, area. All procedures used in this study were approved by both the Human Assurances Committee of the University of Idaho and the Washington State University Institutional Review Board.

Study design and sample collection. This 17-d study was carried out as a randomized, double-blind, crossover, placebo-controlled experiment and consisted of three periods: intervention I (5 d), washout (7 d), and intervention II (5 d). Upon

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Abbreviations: FFQ, food frequency questionnaire; MFD, milk fat depression; PHVO, partially hydrogenated vegetable oils, RA, rumenic acid.

enrollment, subjects completed a questionnaire concerning maternal and infant health and demographic information.

During each intervention period, subjects ingested either two capsules daily of Tonalin™ (PharmaNutrients, Inc., Lake Bluff, IL) Soft Gel CLA supplements (750 mg each) or two placebo supplements (olive oil; 750 mg each; Table 1). Both placebo and CLA supplements were of identical appearance. Tonalin™ supplements contained approximately 600 mg of CLA isomers and 150 mg of other fatty acids. Thus, each subject consumed approximately 560 mg of *t*10,*c*12-18:2, 547 mg of RA, and 93 mg of various other CLA isomers on a daily basis during the CLA treatment.

Milk samples were collected on the final day of each period by complete breast expression using an electric breast pump (Model SMR-B-R; Ameda-Egnell, Inc., Cary, IL). Subjects were asked to obtain all milk from one breast after at least 2 h had elapsed from the previous feeding and while nursing the infant on the other breast until the milk flow had ceased. All milk samples were collected from the same breast between 1300 and 1500 h and frozen at –70°C until biochemical analyses. Nonfasting maternal blood (30 mL) was collected on the last day of each intervention period by venipuncture into heparinized collection tubes. Blood samples were centrifuged immediately, and plasma was harvested and stored at –70°C until lipid and fatty acid concentrations were determined. Infant milk consumption was estimated by weighing the infant before and after each feeding session for a 24-h period on day 4 of each intervention period using an electronic infant scale (SECA Model 727, Columbia, MD).

Lipid extraction, preparation of FAME, and FA determination. Extraction of milk and plasma lipid was done using modified Folch methodology (19). Lipids were extracted from milk and blood samples in duplicate using chloroform/methanol (2:1), and percent lipid was determined gravimetrically. Total lipid was diluted with hexane to a concentration of 0.25 mg lipid/mL. Each sample was then methylated using a methanolic sodium methoxide solution (20,21).

TABLE 1
FA Profiles (% of total) of CLA and Placebo Supplements[a]

	Supplement type	
FA	Placebo	CLA
9:0	0.3	ND[b]
10.0	2.5	ND
16:0	10.9	2.4
10:1*n*-7	0.8	ND
18:0	3.6	0.3
18:1*n*-9	75.2	19.9
18:2*n*-6	6.7	0.4
*c*9,*t*11-18:2	ND	36.4
*t*10,*c*12-18:2	ND	37.2
*c*9,*c*11-18:2	ND	1.3
*c*10,*t*12-18:2	ND	1.1

[a]CLA mixture and placebo (olive oil) produced by Natural Lipids (Hovdebygda, Norway).
[b]ND, not detectable.

Analysis of the methyl esters was performed on a gas chromatograph (Hewlett-Packard 6890 Series with auto injector) fitted with an FID. FA profile was determined by split injection (20:1) onto a CP-Sil 88 fused-silica capillary column (100 m × 0.25 mm, Chrompack, Raritan, NJ) using a programmed temperature gradient method. The hydrogen carrier gas pressure was constant, and the injector and detector temperatures were 255°C. Initial oven temperature was 70°C. Following injection of sample, oven temperature was increased at 4°C/min to 175°C and held for 3 min. Oven temperature was then increased at 1°C/min to 185°C and held for 20 min. Oven temperature was then increased at 3°C /min to 215°C followed by an increase at 10°C/min to 255°C and held for 5 min, after which oven temperature was returned to 70°C. Individual FA were identified by comparison of retention times to those of pure standards (Matreya, Inc., Pleasant Gap, PA). A response correction factor for each FAME was used to convert peak area percentage to weight percentage. Correction factors were determined by analyzing butter oil of a known FA profile with certified values (CRM 164; European Community Bureau of Reference, Brussels, Belgium).

Determination of maternal dietary intake. A semiquantitative food frequency questionnaire (FFQ) was completed by each subject to estimate "chronic" (past 3 mon) maternal RA intake (14). Furthermore, subjects completed written dietary records including data on weights of foods consumed during the last 3 d of each intervention period to determine current dietary intake. Information collected from the dietary records and FFQ were analyzed using a computer-based dietary assessment program (Food Processor® Version 7; ESHA Research, Salem, OR) modified to contain RA and *t*10,*c*12-18:2 contents of a variety of commonly consumed foods (14,22,23). We have shown that intakes of total CLA and RA estimated by these methods are significantly related to biochemically determined CLA intake ($P < 0.005$); however, they may somewhat underestimate an individual's intake of these FA (22). We were interested in using these methodologies to compare FA intakes between experimental groups, a purpose found to be well suited for the methodologies at hand (22). Dietary records were also analyzed for selected micro- and macronutrients and FA.

Statistical analyses. Data were analyzed as a repeated measures, crossover design using the Statistical Analysis System ANOVA procedure (SAS Institute Inc., Version 6.12, Cary, NC). Models used to test the stated hypotheses included period (intervention I, washout, or intervention II), subject, group, and treatment (CLA or placebo) as independent variables. Initially, models also included terms representing two carryover effects (short-term and long-term; 24). The full model implemented was as follows:

$$E(Y_{ijk}) = \mu_{ij} = \mu + \gamma_i + \pi_j + \tau_t + \lambda_\lambda + \theta s \qquad [1]$$

In this model, μ_{ij} represents the overall effect, γ_i represents the subject population, π_j represents the period, and τ_t represents the treatment. The parameter λ_λ represents a first-order (short-term) carryover effect, which would be that observed in the washout period from a treatment given in intervention period I. The pa-

rameter θs represents a second-order (long-term) carryover effect, which would be that observed in intervention period II from a treatment given during intervention period I. Each dependent variable was analyzed initially using the full model to test for carryover effects. If these were found to be significant ($P < 0.1$), the subject population was then broken into two groups: those that received CLA treatment during the first intervention period, and those that received the placebo treatment during the first intervention period. Data collected during intervention period I were then tested to see if they were different from baseline using a paired *t*-test. If carryover effects were found not to be statistically significant, these terms were removed from the model and data were combined across groups. Because maternal dietary information and plasma samples were not collected during the washout period, models used to analyze these data included only the following independent variables: group, subject, and treatment. Main effects and simple comparisons were considered statistically significant at the $P < 0.05$ level. All data presented in this manuscript represent means ± SEM. Finally, if the concentration of a FA in the plasma or milk was undetectable, then a value of zero was assigned for the purpose of statistical analyses.

RESULTS

Description of study population. Descriptive statistics of the subjects are shown in Table 2. Data from one subject were excluded from the analysis due to the subject's lack of compliance with the study protocol; thus, data presented here represent those of the remaining women ($n = 9$). Analyses indicated that there were no significant differences in any of the demographic or anthropometric variables between the women consuming CLA during intervention period I ($n = 5$) and those consuming the placebo during intervention period I ($n = 4$). Thus, data have been combined for purposes of presentation.

Maternal and infant dietary intakes. Information concerning current maternal dietary intake estimated by dietary records is shown in Table 3. There was no effect of group or treatment on any current dietary intake variable evaluated; data across groups were collapsed for purposes of presentation. Analyses of the FFQ indicated no significant effect of group on chronic total CLA or RA consumption (overall means: 351.2 ± 54.9 and 276.6 ± 47.3 mg/d, respectively). There was no effect of group or treatment on mean infant milk consumption (overall mean: 419.5 ± 47.4 g/d).

TABLE 2
Demographic Variables and Anthropometric Measurements of the Women and Infants

Variable	Mean ± SEM
Maternal ($n = 9$)	
Age	31.5 ± 0.8
Weight (kg)	65.1 ± 2.2
Height (cm)	167.0 ± 2.2
BMI (kg/m^2)	23.4 ± 1.0
Time postpartum (mon)	5.3 ± 0.9
Parity (# children)	1.5 ± 0.3
Infant ($n = 9$)	
Weight (kg)	6.6 ± 0.6
Length (cm)	63.2 ± 2.4

TABLE 3
Intakes of Macronutrients, Selected Fatty Acids, CLA, and Rumenic Acid (RA) as Estimated by 3-d Dietary Records[a]

	Study period	
Dietary component	CLA	Placebo
Energy (kcal)	2561 ± 190	2361 ± 168
Protein (g)	90.9 ± 7.1	92.2 ± 8.7
Carbohydrate (g)	335.0 ± 47.0	324.9 ± 26.7
Lipid (g)	89.0 ± 14.7	79.4 ± 8.3
Saturated FA (g)	31.5 ± 5.4	31.1 ± 4.5
Monounsaturated FA (g)	27.5 ± 3.1	23.6 ± 2.9
PUFA (g)	13.3 ± 1.5	12.4 ± 1.8
18:1n-9 (g)	23.3 ± 2.8	19.5 ± 2.8
18:2n-6 (g)	10.8 ± 1.3	8.3 ± 1.3
18:3n-3 (g)	1.4 ± 0.2	1.2 ± 0.2
CLA (mg)	154.0 ± 40.9	168.7 ± 38.3
*c*9,*t*11-18:2 (RA; mg)	124.2 ± 32.6	139.5 ± 32.3

[a]Values represent means ± SEM ($n = 9$). Intakes of lipids, 18:1n-9, CLA, and RA represent only those from dietary sources; contributions from supplements are not included.

Plasma fatty acid composition. Maternal plasma FA profiles for each period are summarized in Table 4. Excluding RA and *t*10,*c*12-18:2, there was no effect of treatment on plasma FA concentrations. However, concentration of RA was greater during CLA treatment as compared to the placebo period (21.1 ± 2.9 and 12.8 ± 1.8 μmol/g lipid, respectively; $P < 0.05$). Similarly, plasma *t*10,*c*12-18:2 concentration was higher during the CLA treatment period as compared to the placebo period (3.6 ± 1.1 and 0.0 ± 0.0 μmol/g lipid, respectively; $P < 0.05$).

Milk composition. (i) FA composition. Milk FA profiles for each period are summarized in Table 5. Statistical analyses indicated a short-term carryover effect only for 10:0. Thus, the effect of CLA treatment on 10:0 could only be determined for those subjects consuming CLA during period I; data indicated no effect of CLA treatment on 10:0. As for all other FA,

TABLE 4
Plasma FA Composition During Each Intervention Period[a]

	Study period	
FA (μmol/g lipid)	CLA	Placebo
10:0	149.9 ± 45.3	224.9 ± 66.8
12:0	35.5 ± 16.0	16.5 ± 8.5
14:0	42.9 ± 10.5	45.6 ± 0.2
15:0	8.7 ± 2.9	9.5 ± 5.0
16:0	927.1 ± 28.5	926.3 ± 33.6
16:1n-7	53.3 ± 5.7	52.4 ± 7.9
18:0	395.5 ± 21.5	412.0 ± 21.5
18:1n-9	492.2 ± 27.6	507.1 ± 51.7
18:2n-6	733.0 ± 30.0	713.8 ± 30.3
18:3n-3	20.8 ± 3.6	34.1 ± 7.5
*c*9,*t*11-18:2 (RA)	21.1 ± 2.9[b]	12.8 ± 1.8
*t*10,*c*12-18:2	3.6 ± 1.1[b]	ND

[a]Values represent means ± SEM ($n = 9$).
[b]Significantly ($P < 0.05$) different from value during placebo period. For abbreviations see Tables 1 and 3.

TABLE 5
Milk FA Composition During Each Intervention Period and Washout Period[a]

FA (μmol/g lipid)	Study period		
	CLA	Washout	Placebo
8:0	106.9 ± 94.4	12.5 ± 2.1	14.6 ± 1.4
10:0	83.1 ± 16.9	66.8 ± 13.9	94.1 ± 20.9
12:0	227.9 ± 27.0	273.3 ± 31.0	227.9 ± 18.0
14:0	232.2 ± 23.2	256.0 ± 22.8	239.3 ± 24.1
15:0	14.3 ± 2.3	12.8 ± 2.1	15.7 ± 2.1
16:0	828.7 ± 63.2	781.9 ± 47.2	803.7 ± 65.9
16:1n-7	94.6 ± 11.9	84.5 ± 9.7	87.2 ± 13.6
18:0	225.5 ± 36.9	190.0 ± 36.9	259.3 ± 32.7
18:1n-9	1055.3 ± 72.3	1002.1 ± 52.1	1060.6 ± 61.3
18:2n-6	468.6 ± 61.7	450.7 ± 28.6	454.0 ± 42.1
18:3n-3	41.7 ± 6.8	38.8 ± 5.0	43.9 ± 6.8
*c*9,*t*11-18:2 (RA)	30.0 ± 2.1[b]	13.2 ± 2.5	15.3 ± 1.8
*t*10,*c*12-18:2	11.1 ± 2.1[b]	1.4 ± 1.4	ND

[a]Values represent means ± SEM (n = 9).
[b]Significantly ($P < 0.05$) different from that during placebo period. For abbreviation see Table 1.

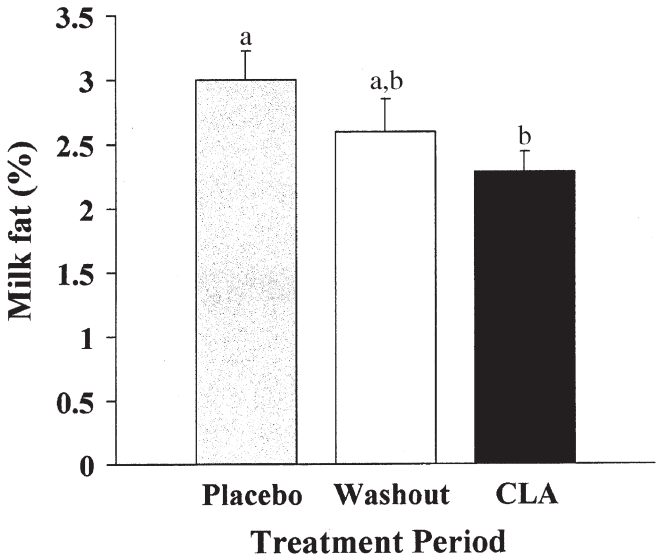

FIG. 1. Milk fat (mean ± SEM) content of human milk samples collected from women (n = 9) consuming a placebo supplement (1.5 g/d; olive oil), no supplement (washout period), or a commercially available CLA supplement (1.5 g/d; containing a mixture of CLA isomers). Bars not sharing a common superscript differ significantly ($P < 0.05$).

excluding RA and *t*10,*c*12-18:2, neither carryover nor treatment effects were significant. Thus, data for all other FA were combined across groups, and the effect of treatment was tested. RA concentration was greater during CLA treatment as compared to placebo (30.0 ± 2.1 and 15.3 ± 1.8 μmol/g lipid, respectively; $P < 0.05$). Similarly, milk *t*10,*c*12-18:2 content was greater during CLA treatment as compared to placebo treatment (11.1 ± 2.1 and 0.0 ± 0.0 μmol/g lipid, respectively; $P < 0.001$).

(ii) Total fat. Data indicated no carryover effect of previous treatment on milk fat content. Therefore, groups were combined for further analyses. Mean milk fat percentages for each group during each period are illustrated in Figure 1. Milk fat was lower ($P < 0.05$) during the CLA treatment as compared to the placebo treatment (2.3 ± 0.2 vs. 3.0 ± 0.2%, respectively).

DISCUSSION

Supplementation with CLA increased total milk RA and *t*10,*c*12-18:2 concentrations and supports data from previous studies reporting that milk CLA concentration is influenced by diet (13,14). During the placebo and washout periods, mean milk RA values reported here are similar to those reported previously by Fogerty *et al.* (13) and McGuire *et al.* (12). Similarly, milk fat concentrations are also within the normal range reported previously by others (25). Thus, we can conclude that our hypotheses concerning the effects of CLA supplementation on milk total fat and FA composition are supported by these data.

We recognize several limitations to the present study. First, the sample size used in this investigation is somewhat limiting. Although *a priori* power calculations suggested that we enroll 20 participants, analysis of our early results (with nine women; required by the institutional review board) indicated a clear and statistically significant MFD and potential resultant decrease in lipid consumption by the infant caused by CLA supplementation. Thus, we decided that enrolling more women was not ethically justifiable. However, we would like to point out that all nine of the women studied had lower milk fat during the CLA supplementation period (as compared to the placebo) period, regardless of treatment order. This suggests that time postpartum and/or degree of breastfeeding did not influence the response to supplementation. We recommend that further studies investigating this phenomenon enroll only women in later lactation who are feeding substantial amounts of other foods to their infants, thus decreasing the risk of energy deficiency in the suckling child of the study participant. Second, the study design would have been stronger had pure supplements of RA and *t*10,*c*12-18:2 been available to us. Third, the FA composition of our placebo did not match our treatment (CLA) as closely as would have been considered ideal. Nonetheless, there is no evidence, to our knowledge, that consumption of olive oil or its principal FA influences milk fat. Thus, we believe that the difference observed in milk fat between CLA and placebo periods was due to CLA consumption. Future studies should endeavor to utilize more similar placebo supplements.

To our knowledge, this represents the first report of a decrease in human milk fat in response to CLA supplementation, although numerous animal studies have indicated MFD after the infusion or feeding of commercially prepared CLA or other *trans* FA to other species (15–18,21,26–28). We hypothesize that the MFD caused by CLA supplementation was in response to the increased consumption of the *t*10,*c*12-18:2 isomer of CLA. Recent work from our group investigating the effect of maternal dairy consumption on milk fat content (14) supports our hypothesis that RA is not the CLA isomer responsible for the MFD caused by CLA supplementation. Data from Park *et al.* (14) document a higher milk fat percentage during a period of high dairy

consumption as compared to a period of low dairy intake. During high dairy intake (corresponding to the period of increased human milk fat), human milk RA concentration was higher than during the low dairy intake period. This again suggests that RA consumption does not cause MFD. Further, data from Baumgard and colleagues (17) demonstrate that *t*10,*c*12-18:2, not *c*9,*t*11-18:2, is the CLA isomer responsible for inhibition of milk fat synthesis. Clearly, further work will be required to confirm this hypothesis in humans.

Similarly, consumption of diets fortified with commercially produced CLA or other *trans* fats has been associated with MFD in numerous animal models (15–18,21,27,29,30). For example, Loor and Herbein (15) investigated the response of bovine milk fat to infusion of linoleic acid plus CLA or linoleic acid only and found that CLA infusion resulted in a substantial reduction in milk fat. Similarly, Chouinard *et al.* (16) infused four doses (0, 50, 100, and 150 g/d) of commercially produced CLA over a 120-h period and reported a marked dose-response decrease in milk fat synthesis. Numerous studies have shown that feeding cows a diet rich in PHVO also causes MFD (26,30). A decrease in milk fat content in response to PHVO consumption has also been reported in mice (28). Overall, data suggest that *trans* FA, including those present in industrially produced mixtures of CLA or PHVO, can cause MFD in both bovine and murine species. These findings have not been reported in humans.

These findings indicate that consumption of currently available CLA supplements decreases milk fat content in women. Because FA in the form of TAG provide a significant proportion of the suckling human infant's energy, these findings may be of public health importance. Thus, we recommend that lactating women not consume currently available CLA supplements. We hypothesize that this effect is due to the *t*10,*c*12-18:2 CLA isomer and not the naturally occurring form (*i.e.*, RA). The testing of this hypothesis will require further investigation utilizing pure isomers. Finally, these data provide the first evidence, to our knowledge, that maternal FA consumption can modulate milk fat content in humans.

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