Dietary Conjugated Linoleic Acid Did Not Alter Immune Status in Young Healthy Women[1]

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ABSTRACT: The purpose of this study was to examine whether conjugated linoleic acid (CLA) supplementation in human diets would enhance indices of immune status as reported by others for animal models. Seventeen women, 20–41 yr, participated in a 93-d study conducted in two cohorts of 9 and 8 women at the Metabolic Research Unit of Western Human Nutrition Research Center. Seven subjects were fed the basal diet (19, 30, and 51% energy from protein, fat, and carbohydrate, respectively) throughout the study. The remaining 10 subjects were fed the basal diet for the first 30 d, followed by 3.9 g CLA (Tonalin)/d for the next 63 d. CLA made up 65% of the fatty acids in the Tonalin capsules, with the following isomeric composition: *t*10,*c*12, 22.6%; *c*11,*t*13, 23.6%; *c*9,*t*11, 17.6%; *t*8,*c*10, 16.6%; and other isomers 19.6%. Most indices of immune response were tested at weekly intervals, three times at the end of each period (stabilization/intervention); delayed-type hypersensitivity (DTH) to a panel of six recall antigens was tested on study day 30 and 90; all subjects were immunized on study day 65 with an influenza vaccine, and antibody titers were examined in the sera collected on day 65 and 92. None of the indices of immune status tested (number of circulating white blood cells, granulocytes, monocytes, lymphocytes, and their subsets, lymphocytes proliferation in response to phytohemagglutinin, and influenza vaccine, serum influenza antibody titers, and DTH response) were altered during the study in either dietary group. Thus, in contrast to the reports with animal models, CLA feeding to young healthy women did not alter any of the indices of immune status tested. These data suggest that short-term CLA supplementation in healthy volunteers is safe, but it does not have any added benefit to their immune status.

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Conjugated linoleic acid (CLA) is a mixture of the isomers of the 18:2 fatty acids that have conjugated double bonds. One of the prominent isoforms of CLA is the 9-*cis* and 11-*trans* (9*c*,11*t*-18:2) isomer. This isomer, rumenic acid, is found naturally in beef and dairy products. Several other isomers of CLA are produced industrially during the processing of vegetable oils. The most abundant among these isomers include the 8*t*,10*c*-18:2, 10*t*,12*c*-18:2, and11*c*,13*t*-18:2. In addition, there are several other minor isomers.

Feeding a mixture of the CLA isomers was found to provide several health benefits in animal models, including anticancer (1–8), antiatherogenic (9,10), and antidiabetogenic actions (11). It has also been reported to decrease body fat while increasing muscle (12,13) and bone mass (14). Additionally, it has been reported to block the endotoxin-induced suppression of growth (15,16) and to alter several indices of immune status (17–19). For example, feeding diets containing CLA to experimental animals enhanced *ex vivo* splenocyte proliferation, IL-2 production, delayed-type hypersensitivity (DTH) response, and macrophage phagocytosis (16–18). In contrast, other investigators found no effect of CLA feeding on lymphocyte proliferation (17), DTH (18), and tumor necrosis factor α (TNF α) production (19). Others report a decrease in the production of IL-2, IL-6, and TNF α, and the macrophage phagocytic activity by the feeding of diets containing CLA (7,19). Overall, the observed effects of dietary CLA on immune functions in animal models have been variable.

Despite the variability in data from animals and the lack of data from humans, there are healthy people who are eager to supplement their diets with CLA. We do not know if supplementation of human diets with CLA has any beneficial health effects, or even if it is safe. Therefore, the purpose of this study was to determine whether it is safe to supplement human diets with CLA, and if it will enhance human immune status. We conducted this study with healthy adults rather than with a group having compromised immune status, because we wanted to know if there are any added benefits from CLA supplementation. We examined the effect of feeding a mixture of CLA isomers on a number of indices of immune status, such as lymphocyte subsets and proliferation, DTH, and influenza antibody titers in young healthy women.

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Abbreviations: BMI, body mass index; CD, cluster differentiation; CLA, conjugated linoleic acid; CI, 95% confidence intervals; CT, cytotoxic T lymphocytes; DTH, delayed-type hypersensitivity; GM, geometric mean; IL, interleukin; LPS, lipopolysaccharide; MRU, metabolic research unit; NK, natural killer; PBMNC, peripheral blood mononuclear cells; PHA, phytohemagglutinin; PPAR, peroxisomal proliferation activation receptor; RDA, recommended dietary allowance; TNF α, tumor necrosis factor α; USDA, U.S. Department of Agriculture; WBC, white blood cells.

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MATERIALS AND METHODS

Subjects and study design. Seventeen healthy women were selected to participate in two cohorts of a 93-d metabolic research unit study after a physical and clinical evaluation by a physician. Nine women (5 CLA group and 4 control group) participated in the first cohort (January 25 to April 28, 1998), and eight women (5 CLA group and 3 control group) participated in the second cohort (September 8 to December 10, 1998). Subjects were randomly assigned to the two groups, but to increase the power in the CLA group, we purposely assigned more subjects to the CLA group. They were all nonsmokers and non–drug-users and had body weights within 110–120% of ideal body weight (1983 Metropolitan Life Insurance Co.). Mean body weights, age, and body mass index for the study subjects are given in Table 1.

The study protocol was approved by the human use committees of the University of California, Davis, and the U.S. Department of Agriculture (USDA), Houston, TX. The study lasted for 93 d, although because of other scheduled procedures, no immunological tests were conducted after study day 92. All subjects were immunized on day 65 with a trivalent 1997–1998 influenza vaccine (Connaught Laboratories Inc., Swiftwater, PA). All subjects remained on the premises of the metabolic suite of the Western Human Nutrition Research Center for the duration of the study, except when going for daily walks (2 mi, twice daily) or other scheduled outings. Subjects were under supervision when going out of the metabolic unit. They consumed only those foods prepared by the staff of the unit. As shown in Figure 1, all study participants were fed a basal diet supplemented with a placebo (sunflower oil 3.9 g/d) for the first 30 d, after which they were divided into two groups; 7 subjects consumed the basal diet for the entire period of the study (control group), while for the remaining 10 subjects, a mixture of CLA isomers (3.9 g/d) replaced the placebo supplement from study days 31 to 93 (CLA group). We used a mixture of CLA isomers because that is what has been used in animal studies, and because purified isomers of CLA for human feeding trials were not available at the time the study was conducted. The body weights of the subjects were maintained within 2% of their initial body weights throughout the study by adjusting their caloric intake if necessary.

The nutrient content of the diets was calculated using USDA Handbook 8 (20); all known nutrients were at or above the recommended dietary allowance (RDA) level, and were not different between the two diets. Diets contained 1 × RDA of vitamin E from natural foods. An additional 100-mg capsule of α-tocopherol (Bronson Pharmaceutical, St. Louis, MO) was supplemented every 5 d to reduce the increased oxidative stress from CLA supplementation. The proportion of energy from protein, fat, and carbohydrate in both diets was 19, 30, and 51%, respectively. Diets were organized according to a 5-d rotating menu, comprised of 3 meals and a post-dinner snack. Specific menus were designed so that the fat would consist of saturated, monounsaturated, and polyunsaturated types providing 10% energy for both the control and intervention groups. Tonalin (gift from Pharmanutrient, Inc., Lake Bluff, IL) was the source of CLA. It was served as capsules by replacing an equivalent amount of the placebo oil. Both Tonalin and placebo oil capsules were administered to the subjects before each meal (breakfast, lunch, and dinner) under the supervision of the kitchen staff. As shown in Table 2, CLA isomers made up 65 wt% of the total fatty acids present in Tonalin, while other fatty acids made up the remaining 35%. The fatty acid composition of Tonalin and the placebo oil (sunflower) is given in Table 2. The major isomers of CLA present in Tonalin, expressed as a percentage of total CLA were: *t*10,*c*12, 22.6%; *c*11,*t*13, 23.6%; *c*9,*t*11, 17.6%; *t*8,*c*10, 16.6%; and other isomers 19.6%. Placebo capsules were made from sunflower oil containing 72.6% linoleic acid and no detectable CLA.

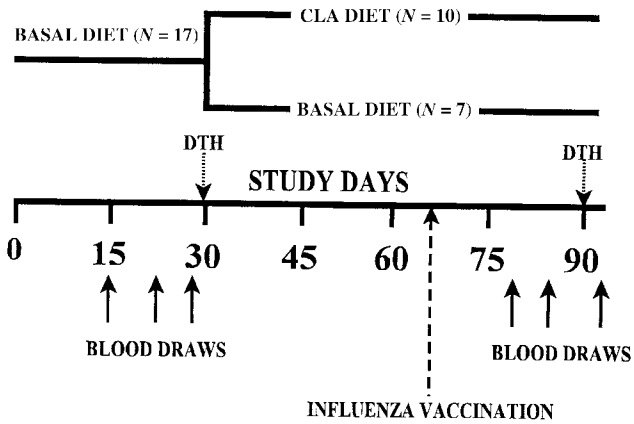

FIG. 1. Study design. Blood samples drawn on study day 15, 22, 29 (stablization period), and 78, 85, 92 (intervention period) were used to determine immune cell numbers and functions. DTH, delayed-type hypersensitivity response; CLA, conjugated linoleic acid.

Laboratory procedures. Blood samples were collected between 0700 and 0800 after an overnight fast on study days 15, 22, 29, 78, 85, and 92 by antecubital venipuncture into evacuated tubes containing heparin for cell culture experiments, EDTA for blood cell count and phenotypic analysis, and no anticoagulant for preparation of sera.

Blood cell count and lymphocyte phenotypic analysis. For each blood draw, a complete and differential cell count was performed using a Serono Baker Automated System (Model 9000 diff; Allentown, PA). Phenotypic analysis for B [cluster differentiation (CD) 19+ or CD19+], T (CD3+), helper (CD3+, CD4+), suppressor (CD3+,CD8+), natural killer (NK) (CD3–, CD16+,56+), and cytotoxic T (CT)(CD3+,CD16+, 56+) lym-

TABLE 1
Characteristics of Subjects[a]

	CLA group (n = 10)	Control group (n = 7)
Age (yr)	27.0 ± 1.8	29.3 ± 2.6
Weight (kg)	63.1 ± 2.1	63.2 ± 4.3
Body mass index (kg/m^2)	23.6 ± 0.5	21.9 ± 1.2

[a]Data shown are mean ± SEM. None of the characteristics were different between the two groups. CLA, conjugated linoleic acid.

TABLE 2
Fatty Acid Composition of Tonalin® CLA and Placebo (sunflower oil) Capsules

Fatty acid	Tonalin CLA[a]	Placebo[a]
14:0	0.25 ± 0.01	
16:0	4.71 ± 0.03	6.23 ± 0.10
16:1n-9	0.12 ± 0.01	
18:0	1.95 ± 0.02	4.09 ± 0.02
18:1n-9	24.70 ± 0.20	16.39 ± 0.02
19:0	0.20 ± 0.01	
18:2n-6	2.29 ± 0.03	72.57 ± 0.03
20:1n-9	0.24 ± 0.08	
9*c*,11*t*-18:2	11.43 ± 0.07	
8*t*,10*c*-18:2	10.79 ± 0.12	
11*c*,13*t*-18:2	15.29 ± 0.31	
10*t*,12*c*-18:2	14.69 ± 0.09	
8*c*,10*c*-18:2	1.38 ± 0.05	
9*c*,11*c*-18:2	1.59 ± 0.05	
10*c*,12*c*-18:2	2.45 ± 0.05	
11*c*,13*c*-18:2	1.32 ± 0.10	
11*t*,13*t* and 8*t*,10*t*-18:2	0.97 ± 0.04	
9*t*,11*t* and 10*t*,12*t*-18:2	5.02 ± 0.04	
20:2n-6	0.11 ± 0.03	
20:3n-6		0.29 ± 0.02
22:0		0.71 ± 0.06
Total	99.92 ± 0.08	100.00 ± 0.00
Unidentified	0.08 ± 0.08	

[a]Values given as means ± SD (n = 5). See Table 1 for abbreviation. Tonalin was a gift from Pharmanutrient, Inc. (Lake Bluff, IL).

phocytes was done with a Becton-Dickinson FACStar flow cytometer (San Jose, CA) as previously reported (21). Briefly, whole blood collected in EDTA-containing vacutainer tubes was transferred into tubes containing fluorochrome-labeled monoclonal antibodies against specific CD antigens. After incubation for 30 min, the red cells were lysed with formaldehyde and removed by washing the leukocytes twice with Dulbecco's phosphate-buffered saline (PBS) without calcium and magnesium. The stained cells were fixed in 1% paraformaldehyde prior to analysis with the flow cytometer. A total of 10,000 lymphocytes were counted to determine their percentage distribution as lymphocyte subsets.

Isolation and culture of peripheral blood mononuclear cells (PBMNC). The PBMNC were isolated using Histopaque-1077 as previously reported (22). The culture medium used was RPMI-1640 (Gibco, Grand Island, NY) containing 10% autologous serum and L-glutamine (2 mmol/L), penicillin (100 KU/L), streptomycin (100 mg/L), and gentamicin (20 mg/L). One hundred microliters of the culture medium containing 1×10^5 PBMNC was added to each well of a 96-well flat-bottom culture plate, followed by an additional 100 μL of the culture medium with or without phytohemagglutinin (PHA; Sigma Chemical Co., St. Louis, MO), or influenza vaccine (Connaught Laboratories Inc.). Cells treated with PHA (0, 1.25, 2.5, 5.0, 10.0, and 20.0 mg/L) were cultured for 72 h, and those treated with influenza vaccine (0, 1/250, 1/500, 1/1000, 1/2000, and 1/4000) were cultured for 120 h. [^{3}H]Thymidine, 37 K Bq, in 50 μL, was added to each well during the last 24 h of cell culture. Cells were collected on filter strips, and the radioactivity was determined using a Packard β-gas counter (Hewlett-Packard Co., Palo Alto, CA). [^{3}H]thymidine incorporation into cellular DNA (Bq/1000 cell) was used as the index of PBMNC proliferation.

DTH skin testing. DTH was tested by intradermally injecting a battery of six recall antigens (tuberculin, mumps, tetanus, candida, streptokinase, and trichophyton) as previously described (22), and the induration diameters in mm were determined 48 h after the application of the antigens. An induration of 4 mm or more was considered positive. The total induration was determined by adding all the positive responses. The antigen score indicates the number of positive responses to the test antigens.

Serum antibody titers. The antibody titers were determined by using the hemagglutination inhibition assay (23) and the viral strains AH1N1, AH3N2, and B/Habrin (strains included in the vaccine). Results for antibody titers are expressed as the geometric mean of the antibody titers, with 95% confidence intervals.

Data analysis. The data from the two cohorts were combined for a repeated measures ANOVA using the SAS PROC MIXED procedure (24). Day, diet, and the interaction between them were considered the fixed effects, while cohort, diet × cohort, subjects within diet × cohort, and diet × cohort × day were the random effects. The period 1 (stabilization) vs. period 2 (intervention) × diet contrast was partitioned out of the day × diet effect. Pooled means ± SEM for the three measurements made at the ends of stabilization (day 15, 22, and 29) and intervention (day 78, 85, and 92) periods are shown in the Results section. Changes in the parameters examined are considered significant for $P < 0.05$ unless stated otherwise.

RESULTS

Both diets provided 19, 30, and 51% energy from protein, fat, and carbohydrates, respectively. The average daily caloric intake was approximately 2100 Kcal (2117 ± 191, mean ± SD) and was not significantly different between the two groups. Fatty acid composition of the diets is shown in Table 3. Fatty acids other than CLA and linoleic acid were not significantly different between the two diets. The control diet contained a nondetectable amount of CLA, while in the CLA diet, CLA isomers were 5.28% of the total fatty acids. The linoleic acid content of the control diet was about 5% higher than that in the CLA diet. This was because the placebo supplement contained sunflower oil, which contained 73 wt% linoleic acid.

Effect of CLA on circulating white blood cells (WBC). The number of total circulating WBC and their specific subgroups, at the ends of periods 1 (stabilization) and 2 (intervention), are shown in Table 4. This table shows that the number of total WBC, granulocytes, monocytes, and lymphocytes in both groups remained unchanged throughout the study. Also unchanged were the numbers of circulating B, NK, total T, helper, suppressor, and CT lymphocytes in both groups.

Effect of CLA on PBMNC proliferation. The influence of dietary CLA on the proliferation of lymphocytes cultured

TABLE 3
Fatty Acid Composition (wt%) of Experimental Diets

Fatty acid	Diet w/o suppl.[a]	Diet + placebo[a]	Diet + CLA[a]
11:00	0.38 ± 0.15	0.39 ± 0.18	0.40 ± 0.23
12:0	0.67 ± 0.40	0.70 ± 0.45	0.70 ± 0.43
14:0	3.29 ± 1.12	3.14 ± 1.25	3.11 ± 1.19
14:1n-7	0.18 ± 0.04	0.19 ± 0.03	0.18 ± 0.04
14:1n-5	0.32 ± 0.04	0.32 ± 0.05	0.30 ± 0.06
15:0	0.34 ± 0.13	0.33 ± 0.13	0.32 ± 0.13
16:0	19.04 ± 1.00	18.19 ± 1.23	17.99 ± 1.30
16:1*t*	0.13 ± 0.01		
16:1n-9	1.14 ± 0.25	1.07 ± 0.22	1.06 ± 0.23
17:0	0.38 ± 0.09	0.35 ± 0.08	0.35 ± 0.08
18:1n-7 DMA	0.28 ± 0.00	0.26 ± 0.00	0.26 ± 0.00
18:0	8.24 ± 0.99	7.91 ± 0.94	7.77 ± 0.93
18:1*t*, all isomers	5.23 ± 2.28	4.96 ± 2.24	4.58 ± 1.86
18:1n-9	25.13 ± 2.35	24.34 ± 2.40	25.23 ± 2.27
18:1n-7	1.24 ± 0.21	1.19 ± 0.20	1.21 ± 0.20
18:1n-5	0.98 ± 0.52	1.08 ± 0.35	1.06 ± 0.34
19:0			0.11 ± 0.00
18:2*tt*			0.25 ± 0.01
18:2n-6	29.85 ± 2.85	32.97 ± 2.31	27.56 ± 2.30
20:0	0.14 ± 0.02	0.15 ± 0.03	0.14 ± 0.02
18:3n-3	1.83 ± 0.31	1.74 ± 0.27	1.71 ± 0.28
20:1n-9	0.26 ± 0.00	0.26 ± 0.00	0.26 ± 0.00
9*c*,11*t* and 8*t*,10*c*-18:2	0.23 ± 0.00		2.22 ± 0.62
11*c*,13*t*-18:2			1.34 ± 0.42
10*t*,12*c*-18:2			1.30 ± 0.40
9*t*,11*t* and 10*t*,12*t*-18:2			0.44 ± 0.13
20:3n-6	0.25 ± 0.13	0.28 ± 0.15	0.30 ± 0.13
20:4n-6	0.19 ± 0.04	0.20 ± 0.00	0.20 ± 0.04
24:0	0.24 ± 0.00	0.25 ± 0.00	0.24 ± 0.00
Total	98.69 ± 0.52	98.71 ± 0.58	98.86 ± 0.39
Unidentified	1.31 ± 0.52	1.29 ± 0.59	1.14 ± 0.39

[a]Values given as means ± SD (n = 5). DMA, dimethylacetate; see Table 1 for other abbreviation.

with different concentrations of a T cell mitogen, PHA, and a B cell antigen, influenza vaccine are shown in Tables 5 and 6, respectively. Increasing the concentration of PHA increased lymphocyte proliferation, with the maximum attained at a PHA concentration of 10 mg/L. At all PHA concentrations, the proliferation of lymphocytes was not different between periods one and two in both dietary groups. B lymphocyte proliferation in response to the influenza vaccine increased with the increasing concentration of the vaccine, the maximum being attained at a dilution of 1/1000 in both groups (Table 6). B cell proliferation in response to influenza vaccine in period two was significantly higher ($P < 0.05$) than that in period one in both groups. This increase in proliferation was caused by the immunization with the same vaccine on day 65. Thus, CLA feeding did not alter proliferation of either T or B lymphocytes.

Effect of CLA on DTH skin response. The induration responses were examined 48 h after the intradermal injection of six recall antigens on study days 28 and 90. All 17 subjects responded to mumps, tetanus, and candida antigens. The data from the other three antigens (trichophyton, streptokinase, and tuberculin purified derivative) were pooled, because fewer than 25% of the subjects responded to each of these antigens (data not shown). The induration score (sum of induration) and the antigen score (number of antigens testing positive) did not differ from study days 30 to 92 in either group (Table 7). The induration in response to individual antigens also did not change during the course of the study.

CLA feeding and serum influenza antibody titers. The pre- (day 65) and postimmunization (day 92) serum antibody titers against the three strains (AH1N1, AH3N2, B/Habrin) of influenza virus for both groups of subjects are shown in Table 8. For all three viral strains, the pre-immunization antibody titers were not different between the two groups. Immunization caused approximately 2-, 4-, and 10-fold increases in the concentration of circulating antibodies against AH3N2, B/Habrin, and AH1N1, respectively, in both groups. The increases in the antibody titers between the CLA and control group also were not different. The results shown regarding antibody titers in Table 8 were obtained with the hemagglutination inhibition assay; however, the results from enzyme-linked immunosorbent assay also lead to the same conclusion (data not shown).

DISCUSSION

The purpose of this study was to examine whether CLA supplementation of human diets would stimulate indices of human immunity as previously reported for animals (16–19).

TABLE 4
Effects of CLA Feeding on Circulating WBC[a]

	CLA group (n = 10)		Control group (n = 7)	
Cell type	Period 1	Period 2	Period 1	Period 2
WBC[1]	6.91 ± 0.68	6.51 ± 0.50	5.94 ± 0.37	5.50 ± 0.45
PMN[1]	4.08 ± 0.58	3.66 ± 0.38	3.28 ± 0.24	2.86 ± 0.32
% of WBC	57.57 ± 1.91	55.38 ± 2.02	55.21 ± 1.66	51.69 ± 3.12
Monocytes[1]	0.42 ± 0.04	0.44 ± 0.05	0.40 ± 0.04	0.38 ± 0.04
% of WBC	6.22 ± 0.43	6.45 ± 0.69	6.81 ± 0.73	7.03 ± 0.80
Lymphocytes[1]	2.42 ± 0.13	2.45 ± 0.16	2.25 ± 0.15	2.23 ± 0.21
% of WBC	36.21 ± 1.73	38.06 ± 2.00	37.98 ± 1.81	41.08 ± 2.88
B (CD19+)[1]	0.29 ± 0.04	0.30 ± 0.04	0.24 ± 0.03	0.27 ± 0.03
NK (CD3−, 16+,56+)[1]	0.25 ± 0.05	0.19 ± 0.03	0.26 ± 0.06	0.23 ± 0.05
T (CD3+)[1]	1.87 ± 0.10	1.98 ± 0.13	1.74 ± 0.13	1.76 ± 0.15
Helper (CD3+, 4+)[1]	1.21 ± 0.09	1.30 ± 0.12	1.08 ± 0.09	1.13 ± 0.10
Suppressor (CD3+,8+)[1]	0.59 ± 0.04	0.61 ± 0.06	0.60 ± 0.06	0.58 ± 0.06
CT (CD3+, 16+, 56+)[1]	0.07 ± 0.01	0.06 ± 0.01	0.09 ± 0.01	0.08 ± 0.01

[a]Abbreviations: WBC, white blood cells; PMN, polymorphonuclear leukocytes; CD, cluster designation; NK, natural killer; CT, cytotoxic T lymphocytes: for other abbreviations see Table 1. Superscript 1 indicates cell number x 10^{-9}/L. Data shown for period 1 are the pooled mean and SEM for study days 15, 22, and 29, and for period 2 are the pooled mean and SEM for study days 78, 85, and 92. None of these variables were different between the two groups, nor were they altered by CLA supplementation.

TABLE 5
Effects of CLA Feeding on Proliferation (Bq/1000 cells) of PBMNC Cultured with PHA[a]

	CLA group (n = 10)		Control group (n = 7)	
PHA (mg/L)	Period 1	Period 2	Period 1	Period 2
0.00	0.04 ± 0.01	0.04 ± 0.01	0.05 ± 0.01	0.05 ± 0.01
1.25	2.39 ± 0.97	2.32 ± 0.80	3.35 ± 1.26	3.08 ± 0.94
2.50	14.38 ± 2.50	15.53 ± 1.93	12.21 ± 2.13	13.85 ± 2.20
5.00	21.55 ± 2.39	22.92 ± 2.16	18.47 ± 1.74	19.32 ± 2.59
10.00	24.31 ± 2.57	27.07 ± 2.67	21.49 ± 1.93	23.03 ± 2.62
20.00	24.06 ± 2.41	26.21 ± 2.48	21.51 ± 1.41	21.86 ± 1.81

[a]Abbreviations: PBMNC, peripheral blood mononuclear cells; Bq, Becquerel; PHA, phytohemagglutinin. See Table 1 for other abbrevation. Data for periods one and two represent the same study days as in Table 4. At all PHA concentrations, PBMNC proliferation in both groups did not change during the study.

The concentration of CLA in the basal diet used in our study was nondetectable, while in the CLA-supplemented diet, it was 5.3 wt% of the total fatty acids (providing approximately 1.5% of daily energy intake). Thus, the CLA concentration used in our study was comparable to that used in many of the animal studies. However, the isomeric composition of the CLA used in our study was different from that reported for the animal studies. Most of the animal studies report that *c*9,*t*11-18:2, and *t*10,*c*12-18:2 made up about 80% of all the CLA isomers (3,8). In our study, these two isomers were only about 40% of all isomers. The CLA used in our study was prepared by refluxing a natural oil (safflower oil), while a mixture of the synthetic isomers of CLA was used in many of the animal studies. At the time we conducted this study, synthetic isomers of CLA for human consumption were not available. The difference in isomeric composition may also be due to inaccurate identification of CLA isomers in some of the other studies. The acid-catalyzed methylation causes isomerization of CLA isomers (25,26). In our study, we used sodium-methoxide for fatty acid methylation, thus avoiding this problem (25,26).

None of the indices of immunity tested (number of circulating

TABLE 6
CLA Feeding Does Not Alter the Proliferation (Bq/10^6 cells) of PBMNC Cultured with Influenza Vaccine[a]

	CLA group (n = 10)		Control group (n = 7)	
Vaccine concentration	Period 1	Period 2	Period 1	Period 2
0.00	54 ± 15	52 ± 15	62 ± 16	53 ± 10
1/250	563 ± 163	1183 ± 225	527 ± 252	1110 ± 324
1/500	1226 ± 290	2297 ± 439	1439 ± 408	2499 ± 615
1/1000	1519 ± 345	2255 ± 477	1897 ± 502	2544 ± 554
1/2000	1307 ± 314	1866 ± 432	1610 ± 488	2070 ± 507
1/4000	812 ± 230	1382 ± 345	1134 ± 390	1584 ± 522

[a]See Tables 1 and 5 for abbreviations and data presentations. *In vivo* immunization with the vaccine on day 65 caused a significant ($P < 0.05$) increase in the proliferation of PBMNC cultured with all concentrations of the same vaccine *in vitro* during period two; however, there was no effect of CLA on this response.

TABLE 7
Effect of CLA Feeding on Delayed-Type Hypersensitivity Response (DTH)[a]

	CLA group (n = 10)		Control group (n = 7)	
	Study day 30	Study day 90	Study day 30	Study day 90
Mumps (mm)	16.8 ± 3.4	22.3 ± 3.1	17.1 ± 2.5	15.6 ± 2.5
Tetanus (mm)	10.4 ± 3.1	12.1 ± 1.3	10.4 ± 2.6	13.4 ± 2.4
Candida (mm)	18.5 ± 1.4	18.5 ± 2.6	16.3 ± 3.8	17.9 ± 4.4
Other antigens (mm)	3.2 ± 0.7	6.2 ± 1.3	6.4 ± 1.2	16.5 ± 3.0
Sum induration (mm)	48.9 ± 5.4	59.1 ± 6.1	50.2 ± 6.2	63.4 ± 11.4
Antigen score	2.8 ± 0.3	3.6 ± 0.3	3.4 ± 0.2	3.9 ± 0.3

[a]DTH response to six recall antigens (mumps, tetanus, candida, trichophyton, streptokinase, and PPD) was determined 48 h after their intradermal injections. Data shown are mean ± SEM. CLA feeding did not alter the DTH response. See Table 1 for abbreviation.

TABLE 8
Effect of CLA Feeding on Serum Influenza Antibody Titers[a]

		CLA group (n = 10)		Control group (n = 7)	
Viral strain	Study day	GM	CI	GM	CI
AH1N1	65	53	30–92	47	25–90
	92	520	297–911	510	268–971
AH3N2	65	70	24–202	37	13–110
	92	149	51–433	144	49–425
B/Habrin	65	46	29–74	72	41–127
	92	160	100–257	290	165–510

[a]GM, geometric mean; CI, 95% confidence intervals. See Table 1 for other abbreviation. All subjects were immunized with influenza vaccine on day 65. Immunization significantly ($P < 0.05$) increased the antibody titers for all three viral strains in both groups. However, the increase in antibody titers was not significantly different between the two groups.

WBC, lymphocytes within different subsets, T and B lymphocyte proliferation, DTH, and serum antibody titers) were stimulated by CLA supplementation of the diets. Our results, showing no change in human lymphocyte proliferation with CLA supplementation are at variance with those of others who found increases in this response in mice, rats, and chickens (7,16,18). However, our results are in agreement with those of other investigators (17) who failed to detect an increase in splenocyte proliferation in mice fed CLA-containing diets for 6 wk. Similarly, our results regarding antibody production, DTH, and lymphocyte phenotypic analysis are consistent with the results from animal studies (16,18,27). While CLA supplementation did not improve any of the indices of immune status tested, it did not have any adverse effects either.

The discrepancy between our results and those obtained from animal models may simply be due to species' differences. Perhaps humans do not respond to CLA the same way rats, mice, and chickens do. One of the perceived mechanisms by which CLA exerts its health effects in animal models is through the induction of peroxisomal proliferation activation receptor (PPAR)-responsive genes in the liver (28). Humans appear to be resistant to peroxisome proliferation, and the promotor region of the human acetyl-CoA oxidase is resistant to transcriptional regulation by PPAR (29). Thus, inadequacy of the PPAR and their response elements may be why CLA failed to stimulate immune response in our human study. Even in the animal models, the effects of CLA on immune response have been only modest and have ranged from inhibition to stimulation. Based on these results, CLA does not seem to be a strong modulator of the immune response. Most of the animal experiments with CLA have involved growing animals, and in one study, the effects of CLA were less pronounced in the older mice than in the younger mice (18). It is possible that CLA did not stimulate indices of immune status in our study subjects because they were healthy adults, but it may stimulate them in growing human subjects, or in those with a compromised immune status. Another explanation may be that different isomers of CLA have opposing effects, and they may neutralize the effects of each other. Studies with purified isomers of CLA are needed to address this issue.

Other results from this study demonstrated that CLA supplementation did not affect body weight, fat-free mass, fat mass, percent body fat, or energy expenditure (30). Nor did it alter appetite, or the serum concentrations of insulin, glucose and lactic acid (31). There was a transient decrease in the concentration of serum leptin; however, it was not maintained until the end of the study (31). Taken together, the results from our study suggest that short-term supplementation of the diets of healthy adult humans with a mixture of CLA isomers is safe; however, there is no added benefit to the immune status and body composition. It is possible that CLA isomers have some health benefits to humans with a compromised immune status, or that one or more of the purified isomers may be beneficial to healthy adults. Until such information is obtained, CLA supplementation to improve human immune status or body composition should not be recommended.

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