Effect of Dietary Conjugated Linoleic Acid on the Composition of Egg Yolk Lipids[1]

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ABSTRACT Forty-eight 27-wk-old White Leghorn hens were assigned randomly to four diets containing 0, 1.25, 2.5, or 5.0% conjugated linoleic acid (CLA). Hens were fed the CLA diets for 2 wk before eggs were collected for the study. Classes of egg yolk lipids were separated, and fatty acid concentrations in total lipid, triglyceride (TG), phosphatidylethanolamine (PE), and phosphatidylcholine (PC) were analyzed to determine the incorporation of dietary CLA isomers into different classes of egg yolk lipids.

The amounts of CLA incorporated into lipid, PC, PE, and TG of egg yolk were proportional to the levels of CLA in the diet. However, more CLA was incorporated in TG than in PC and PE. The incorporation rates of different CLA isomers into different classes of lipids also were significantly different: *cis*-9, *trans*-11 and *cis*-10, *trans*-12 CLA were deposited more in TG, but *cis*-11, *trans*-13 CLA deposition in TG was significantly less. There were large differences in the concentrations of *cis*-8, *trans*-10 CLA in PC and PE. The inclusion of CLA into the diet influenced the metabolism of polyunsaturated fatty acids. The contents of 5,8,11,14-eicosatetraenoic, 9,12-octadecadienoic, and 9,12,15-octadecatrienoic acids were decreased as dietary CLA increased. Three isomers of hexadecadienoic acid were found in egg yolk lipids from hens fed 5% dietary CLA. The detection of hexadecadienoic acid isomers in lipid indicates that the utilization of CLA as an energy source after the first round of β-oxidation may be less favorable than that of 9,12-octadecadienoic acid.

(*Key words*: conjugated linoleic acid, egg yolk, fatty acid composition, lipid classes)

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INTRODUCTION

Results of several experiments show that dietary conjugated linoleic acid (CLA) has anticarcinogenic and antiartherogenic effects and enhances immune response in animals (Ip *et al.*, 1995; Lee *et al.*, 1995; Belury *et al.*, 1996). Conjugated linoleic acid is found in many foods, especially in ruminant products, but its concentration in these products is low (Chin *et al.*, 1992). On the basis of a rat model study, Ip *et al.* (1995) estimated that a 70-kg human should consume 3.0 g CLA/d to obtain beneficial effects from CLA. This amount is about three times that of the daily CLA consumption of average adults in the US. Therefore, it is necessary to heighten the CLA level in foods.

The content of CLA in foods can be increased by feeding animals with synthetic CLA sources, which contain more than 50% of various kinds of CLA isomers. The synthetic CLA sources can be prepared by alkali isomerization, and the contents of CLA isomers are influenced by isomerization conditions (Ackman, 1998). According to the bond location and *trans*/*cis* combinations, there should be 16 different forms of CLA isomers. Employing silver-ion high-performance liquid chromatography, Sehat *et al.* (1998) identified 12 peaks of CLA isomers. However, the *trans*/*cis* or reverse *cis*/*trans* forms of CLA isomers could not be separated well (Sehat *et al.*, 1998; Belury, 1995). *Cis*-9, *trans*-11 and *trans*-10, *cis*-12 isomers are main CLA isomers that exist naturally and also in synthetic CLA sources. There is no difference between naturally existing CLA and synthetic CLA in CLA isomers. The relative abundance of CLA isomers is different because natural CLA isomers exist mostly in foods of animal origin, and those isomers that have high absorption and deposition rates are expected to have a higher concentration in those foods. In synthetic CLA, however, the composition of CLA isomers mainly depends on isomerization conditions (Ackman, 1998). The different absorption and deposition rates of CLA are also related to their different biological effects (Chen and Sih, 1998). The *cis*-9, *trans*-11 isomer was first suggested to be a bioactive isomer (Chin

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Abbreviation Key: BHA = butylatedhydroxyanisole; CLA = conjugated linoleic acid; GC = gas chromatography; MS = mass selective; PC = phosphatidylcholine; PE = phosphatidylethanolamine; TG = triglyceride.

et al., 1992). But, most recently, the *trans*-10, *cis*-12 isomer was suggested to cause biological changes instead of the *trans*-9, *cis*-11 isomer (Park *et al.*, 1999a,b). Both of the suggested biologically active isomers are main CLA isomers, existing in foods and also in synthetic CLA sources, and are expected to have high absorption and deposition rates. Feeding animals synthetic CLA sources should be a good way to enrich biologically effective CLA in foods. Chamruspollert and Sell (1999) reported that feeding diets containing 5% CLA to laying hens resulted in an 11% CLA content in egg yolk lipids. Thus, such eggs could be valuable CLA sources for humans. However, the incorporation rates for CLA isomers into different classes of lipids are not known. The objective of this study was to determine the concentration of CLA isomers in different classes of egg yolk lipids produced by hens fed CLA.

MATERIALS AND METHODS

Hen Feeding and Sample Preparation

Twelve 27-wk-old White Leghorn hens kept in individual cages were assigned to each of four dietary treatments that consisted of diets containing 0, 1.25, 2.5, or 5% CLA. Hens were fed the diet shown in Table 1 for 2 wk before collecting eggs for this study. The CLA source, which contained 62% CLA, was obtained from a commercial company[3] (Table 2) and was included at 2.05, 4.10, or 8.2% of the diet to obtain 1.25, 2.5, or 5.0% CLA, respectively. The control diet contained 8.2% soybean oil, and the CLA source was substituted for the soybean oil on a weight:weight basis. Fatty acid compositions of experimental diets are presented in Tables 1 and 2. Eggs were collected for 3 consecutive d, and four eggs (from different hens) per treatment were randomly selected. Egg yolk was separated from white and used for lipid extraction. Fatty acid compositions of total egg yolk lipid and lipid classes were analyzed. Each egg was used as a replication, and the whole process was repeated after 3 wk.

TABLE 1. Percentage composition of diets fed to laying hens

Ingredients	Percentage
Corn	35.29
Soybean meal	18.17
Wheat middlings	22.85
Limestone	8.77
Dicalcium phosphate	0.40
Meat and bone meat	3.00
Dehydrated alfalfa meal	2.50
Mineral premix[1]	0.30
Vitamin premix[2]	0.30
DL-methionine	0.14
Sodium chloride (iodized)	0.08
Soybean oil	8.20 to 0[3]
CLA source	0 to 8.20[3]
Calculated analysis	
ME, kcal/kg	2,905
Protein	17.00
TSAA	0.70
Methionine	0.40
Lysine	0.90
Calcium	3.85
Nonphytate phosphorus	0.35
Sodium	0.20
Ether extract	10.31

[1]Mineral premix provides per kilogam of diet: Mn, 80 mg; Zn, 90 mg; Fe, 60 mg; Cu, 12 mg; Se, 0.147 mg; and sodium chloride, 2.247 g.

[2]Vitamin premix supplies per kilogram of diet: retinyl acetate, 8,065 IU; cholecalciferol, 1,580 IU; 25-hydroxy-cholecalciterol, 31.5 μg, dl-α-tocopheryl acetate, 15 IU; vitamin B_{12}, 16 μg; menadcre, 4 mg; riboflavin, 7.8 mg; pantothenic acid, 12.8 mg; niacin, 75 mg; choline chloride, 509 mg; folic acid, 1.62 mg; and biotin, 0.27 mg.

[3]In control group, soybean oil, 8.20%; CLA source, 0%. In 1.25% CLA group, soybean oil, 6.15%; CLA source, 2.05%. In 2.5% CLA group, soybean oil, 4.10%; CLA source, 4.10%. In 5.0% CLA group, soybean oil, 0%; CLA source, 8.20%.

Separation of Lipid Classes

Two grams of egg yolk from each egg were weighed into a test tube with 20 mL solvent (chloroform:methanol = 2:1; vol/vol, Folch *et al.*, 1957) and homogenized with a Brinkman polytron[4] (Type PT 10/35) for 10 s at high speed. Twenty-five micrograms of butylated hydroxyanisole (BHA, 10%) dissolved in 98% ethanol were added to the sample prior to homogenization. The homogenate was filtered through a Whatman No. 1 filter paper into a 100-mL graduated cylinder, and 5 mL 0.88% NaCl solution were added. After the cylinder was capped with a glass stopper, the filtrate was mixed well. The inside of the cylinder was washed twice with 10 mL Folch 2 (3:47:48/$CHCl_3$:CH_3OH:H_2O), and the contents were stored until the aqueous and organic layers clearly separated.

The upper layer was siphoned off, and the lower layer was moved to a glass scintillation vial and dried at 50 C under nitrogen. The dried sample was redissolved with chloroform to make the final concentration of lipid at 0.2 g/mL. The lipid-chloroform solution (150 μL) was loaded onto an activated (120 C for 2 h) silica gel plate[5] (20 $\times$ 20 cm). The plate was developed first in Solvent I, composed of chloroform:methanol:water (65:25:4), until the solvent line reached the middle of the plate. The plate was air dried and then redeveloped in Solvent II, composed of hexane:diethyl ether (4:1), until the solvent front reached 1 inch below the top of the plate. After air drying for 10 min at room temperature (22 C), the plates were sprayed with 0.1% 2′,7′-dichlorofluororescein in ethanol. Lipid classes were identified under UV light, and triglyceride (TG), phosphatidylethanolamine (PE), and phosphatidylcholine (PC), identified using standards,[3] were scraped into separate test tubes (Ahn *et al.*, 1995) and methylated.

Analysis of Fatty Acid Composition

One milliliter of methylating reagent (anhydrous methanolic-HCl-3*N*)[6] was added to the test tube containing TG, PE, or PC; capped tightly; and incubated in a water

[3]Conlinco, Inc. Detroit Lakes, MN 56502.
[4]Brinkman Instruments, Inc., Westbury, NY 11590-0207.
[5]Sigma-Aldrich, St. Louis, MO 63178.
[6]Supelco, Bellefonte, PA 16823.

TABLE 2. The fatty acid composition of soybean oil and conjugated linoleic acid (CLA) source fed to hens

Conjugated linoleic acid	Percentage	Soybean oil	Percentage
Hexadecanoic acid	4.16	Hexadecanoic acid	14.64
9,12-Octadecadienoic acid	6.65	9,12,15-Octadecatrienoic acid	8.07
9-Octadecenoic acid	16.97	9,12-Octadecadienoic acid	50.54
Octadecanoic acid	2.10	9-Octadecenoic acid	21.56
cis-9, *trans*-11 CLA	17.94	Octadecanoic acid	4.56
trans-10, *cis*-12 CLA	20.27	Others	0.63
cis-8, *trans*-10 CLA	4.41		
cis-11, *trans*-13 CLA	15.34		
Other CLA isomers	3.56		
Others	8.60		

bath at 60 C for 40 min. After cooling to room temperature, 2 mL hexane and 5 mL water were added, mixed thoroughly, and left at room temperature overnight for phase separation. The top hexane layer containing methylated fatty acids was used for gas chromatography (GC) analysis (Chin *et al.*, 1992). Analysis of fatty acid composition was performed with a GC[7] (HP 6890) equipped with an autosample injector[7] and flame ionization detector. A capillary column[7] (HP-5, 0.32 mm id, 30 m, 0.25 μm film thickness) was used. A splitless inlet was used to inject samples (1 μL) into the capillary column. Ramped oven temperature conditions (180 C for 2.5 min, increased to 230 C at 2.5 C/min, then held at 230 C for 7.5 min) were used. Temperatures of both the inlet and detector were 280 C. Helium was used as a carrier gas, and a constant column flow of 1.1 mL/min was used. Detector (flame ionization detector) air, H_2, and make-up gas (He) flows were 350, 35, and 43 mL/min, respectively. Fatty acids were identified using a Mass Selective (MS) Detector[7] (Model 5973). The GC-MS was performed with the same column conditions as described previously. The ionization potential of the MS was 70 eV, and the scan range was 45 to 450 m/z. Identification of fatty acids was achieved by comparing mass spectral data with those of the Wiley library.[5] Conjugated linoleic acid isomers in egg yolk lipids were identified by comparing with CLA standards purchased from Matreya[8] and Nuchek,[9] and also according to the report of Christie *et al.* (1997). The compositions of CLA isomers and fatty acids were reported as percentage composition. The area of each peak was integrated by using the ChemStation software,[7] and total peak area was used to calculate fatty acid composition.

Statistical Analysis

The effect of dietary CLA on the fatty acid composition of egg yolk lipids was analyzed statistically by ANOVA using SAS® software (SAS Institute, 1989). Student-Newman-Keuls multiple range test was used to compare differences among mean values ($P < 0.05$). Mean values and SEM are reported. Regression analysis also was conducted to determine the relationship between dietary CLA and the concentration of CLA isomers in egg yolk lipids. The correlation coefficients between CLA treatment and eicosatetraenoic acid content of yolk lipid also were determined.

RESULTS AND DISCUSSION

The CLA changed the fatty acid composition of total egg yolk lipid significantly (Table 3). The concentration of 9,12,15-octadecatrienoic acid was significantly decreased by dietary CLA. The CLA concentration was 0.12% in the control, but was not detectable in the 2.5 and 5.0% CLA treatments. There also were significant decreases in 9,12-octadecadienoic acid and 9-octadecenoic acid. The 5,8,11,14-eicosatetraenoic acid content markedly decreased with the increase of dietary CLA. This result indicated that polyunsaturated fatty acids of egg yolk lipids were more influenced by dietary CLA than monounsaturated fatty acids, and the degree of influence increased with the increasing degree of unsaturation. Because of the decreased concentrations of unsaturated fatty acids, the contents of octadecanoic acid and heptadecanoic acid increased as the dietary CLA level increased. Ahn *et al.* (1999) reported similar compositional changes of fatty acids in egg yolk lipids after feeding hens diets containing CLA.

In TG (Table 4), PC (Table 5), and PE (Table 6), there were similar decreases in unsaturated fatty acids as observed with total lipids. Several investigators have reported decreases of unsaturated fatty acids in tissue lipids after feeding a CLA diet. Lee *et al.* (1995) showed that the content of monounsaturated fatty acids in tissue decreased after CLA feeding. Thiel *et al.* (1998) reported that feeding CLA to pigs increased the hardness of fat in pork, which may be due to the increased melting point of fat because of decreases in polyunsaturated fatty acids and compensated increases in saturated fatty acids. Sugano *et al.* (1998) also reported a decrease in the concentration of 5,8,11,14-eicosatetraenoic acid and other unsaturated fatty acids after feeding CLA to mice. In the current study, the decrease of 5,8,11,14-eicosatetraenoic acid in different lipid classes varied; TG was more influenced by dietary

[7]Hewlett Packard Co., Wilmington, DE 16808-1610.
[8]Matreya, Inc., Pleasant Gap, PA 16823.
[9]Nuchek, Elysian, MN 56028.

TABLE 3. Influence of dietary conjugated linoleic acid (CLA) on the fatty acid composition of the total lipid of egg yolk

	Dietary CLA				
Fatty acids	0%	1.25%	2.5%	5.0%	SEM
Hexadecanoic acid	23.18[a]	25.15[a]	24.85[a]	25.12[a]	0.424
9-Hexadecenoic acid	1.25[a]	0.68[b]	0.63[b]	0.65[b]	0.038
9,12-Hexadecadienoic acid	0.24	0.38	0.23	0.38	0.040
Heptadecanoic acid	0.28[a]	0.28[a]	0.29[a]	0.23[b]	0.012
Hexadecadienoic acid (1)[1]	0.00[d]	0.05[c]	0.12[b]	0.27[a]	0.006
Hexadecadienoic acid (2)[1]	0.00[d]	0.04[c]	0.13[b]	0.28[a]	0.005
Hexadecadienoic acid (3)[1]	0.00[c]	0.00[c]	0.06[b]	0.18[a]	0.001
9,12,15-Octadecatrienoic acid	0.12[a]	0.06[b]	0.00[2]	0.00[2]	0.007
9,12-Octadecadienoic acid	20.08[a]	20.99[a]	19.25[a]	11.66[b]	0.400
9-Octadecenoic acid	35.53[a]	27.66[b]	25.21[c]	26.30[bc]	0.478
Octadecanoic acid	14.02[b]	16.57[a]	16.48[a]	15.56[a]	0.273
cis-9, *trans*-11 CLA	0.00[d]	1.39[c]	2.74[b]	5.41[a]	0.092
trans-10, *cis*-12 CLA	0.00[d]	1.04[c]	2.54[b]	5.87[a]	0.084
cis-8, *trans*-10 CLA	0.00[d]	0.33[c]	0.60[b]	1.24[a]	0.020
cis-11, *trans*-13 CLA	0.00[d]	0.45[c]	0.94[b]	2.37[a]	0.028
5,8,11,13-Eicosatetraenoic acid	5.19[a]	4.24[b]	3.91[b]	2.70[c]	0.156

[a–d]Means within a row with no common superscript differ ($P \leq 0.05$); n = 4.

[1]Corresponding to three hexadecadienoic acid isomer peaks identified by gas chromatograph-mass selective analysis.

TABLE 4. Influence of dietary conjugated linoleic acid (CLA) on the fatty acid composition of triglyceride of egg yolk lipid

	Dietary CLA				
Fatty acids	0.0%	1.25%	2.5%	5.0%	SEM
9-Hexadecenoic acid	2.34[a]	1.20[b]	1.16[b]	1.19[b]	0.061
Hexadecanoic acid	19.80[c]	25.85[b]	26.2[b]	28.43[a]	0.512
Hexadecadienoic acid (1)[1]	0.00[d]	0.07[c]	0.28[b]	0.83[a]	0.008
Hexadecadienoic acid (2)[1]	0.00[d]	0.07[c]	0.26[b]	0.62[a]	0.008
Hexadecadienoic acid (3)[1]	0.00[d]	0.02[c]	0.08[b]	0.23[a]	0.002
Heptadecanoic acid	0.25[c]	0.32[a]	0.28[b]	0.23[c]	0.011
9,12-Octadecadienoic acid	25.78[a]	26.01[a]	22.45[b]	12.74[c]	0.524
9-Octadecenoic acid	45.32[a]	33.02[b]	30.88[b]	28.55[b]	0.791
Octadecanoic acid	5.35[b]	10.04[a]	10.17[a]	9.90[a]	0.291
cis-9, *trans*-11 CLA	0.00[d]	1.60[c]	3.17[b]	5.51[a]	0.062
trans-10, *cis*-12 CLA	0.00[d]	1.40[c]	3.50[b]	7.64[a]	0.099
cis-8, *trans*-10 CLA	0.00[d]	0.22[c]	0.57[b]	1.54[a]	0.024
cis-11, *trans*-13 CLA	0.00[d]	0.32[c]	0.80[b]	2.20[a]	0.030
5,8,11,14-Eicosatetraenoic acid	0.53[a]	0.28[b]	0.18[c]	0.05[d]	0.011

[a–d]Means within a row with no common superscript differ ($P \leq 0.05$); n = 4.

[1]Corresponding to three hexadecadienoic acid isomer peaks identified by gas chromatograph-mass selective analysis.

TABLE 5. Influence of dietary conjugated linoleic acid (CLA) on the fatty acid composition of phosphatidyl choline of egg yolk lipid

	Dietary CLA				
Fatty acids	0%	1.25%	2.5%	5.0%	SEM
Hexadecanoic acid	29.28[a]	29.46[a]	27.17[ab]	25.48[b]	0.765
9-Hexadecenoic acid	0.49[a]	0.23[b]	0.22[b]	0.29[b]	0.025
Heptadecanoic acid	0.23[a]	0.26[a]	0.25[a]	0.19[b]	0.013
9,12-Octadecadienoic acid	19.10[a]	18.79[a]	18.14[a]	12.31[b]	0.450
9-Octadecenoic acid	30.54[a]	28.24[b]	26.45[b]	27.41[b]	0.540
Octadecanoic acid	16.28	16.93	17.60	18.37	0.708
cis-9, *trans*-11 CLA	0.00[d]	0.97[c]	2.19[b]	4.78[a]	0.065
trans-10, *cis*-12 CLA	0.00[d]	0.66[c]	1.76[b]	4.01[a]	0.107
cis-8, *trans*-10 CLA	0.00[d]	0.1[c]	0.36[b]	0.66[a]	0.026
cis-11, *trans*-13 CLA	0.00[d]	0.44[c]	0.98[b]	2.67[a]	0.049
5,8,11,14-Eicosatetraenoic acid	3.91[a]	3.14[b]	2.78[b]	2.03[c]	0.120

[a–d]Means within a row with no common superscript differ ($P \leq 0.05$); n = 4.

CLA than other lipid classes. The 5,8,11,14-eicosatetraenoic acid content decreased from 0.53% in TG of the controls to near zero in TG of the 5.0% CLA group. The correlation coefficient between dietary CLA and 5,8,11,14-eicosatetraenoic acid content in total lipid of egg yolk was –0.93. These observations suggest that dietary CLA, especially at the 5% level in the diet, interfered with the metabolism of polyunsaturated fatty acids through the inhibition of biosynthesis or deposition of polyunsaturated fatty acid to egg yolk lipids. Because there were only limited amounts of 5,8,11,14-eicosatetraenoic acid in the diets, most of the 5,8,11,14-eicosatetraenoic acid in yolk lipid should be produced by biosynthesis *in vivo*. Because 9,12-octadecadienoic acid is the precursor of 5,8,11,14-eicosatetraenoic acid biosynthesis, absorbed CLA might have competed with 9,12-octadecadienoic or 9,12,15-octadecatrienoic acid for Δ6-desaturase, the rate-limiting enzyme for the conversion of these fatty acids into 5,8,11,14-eicosatetraenoic acids and 4,7,10,13,16,19-docosahexaenoic acid in liver microsomes (Belury and Kempa-Steczko, 1997).

Another reason for the reduced amount of 5,8,11,14-eicosatetraenoic acid in egg yolk produced with CLA diets could be related to the lower 9,12-octadecadienoic acid content in the CLA diet than in the control (Table 2). The amount of 9,12-octadecadienoic acid in the CLA diet was lower than that in the control diet because soybean oil, high in 9,12-octadecadienoic acid, was replaced with a CLA source that contained much less 9,12-octadecadienoic acid. Therefore, the reduced substrate concentration (9,12-octadecadienoic acid) for the biosynthesis of eicosatetraenoic acid in CLA-fed hens could have contributed to the low 5,8,11,14-eicosatetraenoic acid concentrations in egg yolk lipids. If this supposition is correct, then the biosynthesis of 4,7,10,13,16,19-docosahexaenoic acid should not be influenced. Our recent study indicated that CLA feeding did not inhibit the biosynthesis of 4,7,10,13,16,19-docosahexaenoic acid (D. U. Ahn, unpublished data). Therefore, CLA is not a competitive inhibitor of Δ6-desaturase that converts 9,12,15-octadecatrienoic acid to 4,7,10,13,16,19-docosahexaenoic acid.

Three isomers of hexadecadienoic acid also were detected in the total lipid and TG. These hexadecadienoic acid isomers probably were derived from the CLA isomers after two carbons were removed by β-oxidation. In PC and PE, there also were small peaks corresponding to the hexadecadienoic acid isomers in the eggs from the hens fed a 5.0% CLA diet, but these data are not shown because they were too low to calculate accurately. The accumulation of hexadecadienoic acid isomers in lipid indicates that further catabolism of these isomers after the first round of β-oxidation was more difficult than that of 9,12-octadecadienoic acid. Conjugated linoleic acid isomers might be unfit substrates for Δ6-desaturase or elongase and catabolic enzymes. It also is possible that CLA is a competitive inhibitor of Δ6-desaturase or elongase, which might be the reason for the low content of polyunsaturated fatty acids in lipids after CLA feeding. The decreased amount of 5,8,11,14-eicosatetraenoic acid in egg yolk lipids suggested that the liver synthesized less 5,8,11,14-eicosatetraenoic acid, which would account for the modulation of immune and inflammatory responses because 5,8,11,14-eicosatetraenoic acid is a precursor of many cell factors involved in these responses. Sugano *et al.* (1997, 1998) reported that CLA feeding lowered the concentration of prostaglandin E_2 and leukotriene 4 in serum and spleen of rats. Cook *et al.* (1993) and Miller *et al.* (1994) reported that CLA feeding reduced weight loss in chickens and rats after catabolic immune stimulation with endotoxin injection. These findings could be interpreted as showing that CLA reduced the synthesis of immune-related cell factors and made animals less sensitive to endotoxin. However, Sebedio *et al.* (1997) showed that CLA isomers could be elongated to 5,8,11,13-eicosatetraenoic acid because there were higher quantities of 5,8,12,14-eicosatetraenoic acid and 5,8,11,13-eicosatetraenoic acid in liver lipids of rats fed CLA than in liver lipids of controls.

The total amounts of CLA transferred into egg yolk lipids were highly related to dietary CLA, and the dietary CLA was deposited in all three egg yolk lipid classes (PE, TG, and PC) with no preference. Regression analysis of four CLA isomers in egg yolk lipids showed that the concentration of CLA in egg yolk lipids was proportional to that of the diets (Table 7), with a slope of 3.49 for *cis*-9, *trans*-11 CLA and 3.18 for *trans*-10, *cis*-12 CLA in total

TABLE 6. Influence of dietary conjugated linoleic acid (CLA) on the fatty acid composition of phosphatidyl ethanolamine of egg yolk lipid

	Dietary CLA				
Fatty acids	0.00%	1.25%	2.50%	5.00%	SEM
Hexadecanoic acid	13.02^{b}	16.37^{a}	16.42^{a}	17.58^{a}	0.630
9,12-Hexadecadienoic acid	0.77	0.65	0.69	0.67	0.038
Heptadecanoic acid	0.30	0.42	0.45	0.31	0.043
9,12-Octadecadienoic acid	13.44^{b}	15.60^{a}	15.41^{a}	12.01^{c}	0.385
9-Octadecenoic acid	19.27^{b}	19.15^{b}	20.86^{ab}	22.66^{a}	0.634
Octadecanoic acid	34.28^{a}	31.01^{b}	28.59^{c}	23.18^{d}	0.720
cis-9, *trans*-11 CLA	0.00^{d}	0.80^{c}	2.16^{b}	5.62^{a}	0.072
trans-10, *cis*-12 CLA	0.00^{d}	0.50^{c}	1.50^{b}	4.76^{a}	0.058
cis-8, *trans*-10 CLA	0.00^{d}	0.20^{c}	0.74^{b}	1.55^{a}	0.084
cis-11, *trans*-13 CLA	0.00^{d}	0.67^{c}	1.32^{b}	3.42^{a}	0.075
5,8,11,13-Eicosatetraenoic acid	17.14^{a}	14.12^{b}	11.34^{b}	8.37^{c}	0.703

$^{a–d}$Means within a row with no common superscript differ ($P \leq 0.05$); n = 4.

TABLE 7. The incorporation rate of dietary conjugated linoleic acid (CLA) isomers to egg yolk lipid

CLA isomers	Incorporation rate[1]				Significance of difference[2]
	Lipid	PC	PE	TG	
					P
cis-9, *trans*-11 CLA	3.49	3.10	3.74	4.23	<0.01
trans-10, *cis*-12 CLA	3.18	2.24	2.84	3.30	<0.01
cis-8, *trans*-10 CLA	3.14	1.70	4.97	2.88	<0.01
cis-11, *trans*-13 CLA	1.77	2.00	2.87	1.09	<0.01

[1]Incorporation rate is expressed as the slope of the linear relationship between the content of CLA isomers in feed and their content in lipid classes of egg yolk (PC = phosphatidylcholine; PE = phosphatidylethanolamine; TG = triglyceride).

[2]Significances were determined by considering lipid types as split-plot treatments.

yolk lipid. Chin *et al.* (1992) reported that only *cis*-9, *trans*-11 CLA was found in phospholipids and suggested that this isomer was the biologically active form. However, we found four CLA isomers present in the phospholipids of egg yolk. More recently, Park *et al.* (1999a,b) suggested that *trans*-10, *cis*-12 was the biologically effective CLA isomer, which, in part, supports this result. The results of Park *et al.* (1999b) and Chin *et al.* (1992) indicated that different CLA isomers might have different activity *in vivo*. Data presented in Table 7 show that *cis*-9, *trans*-11 CLA had a higher, but *cis*-11, *trans*-13 CLA had a lower, incorporation rate to TG than other lipid classes. Interestingly, *cis*-8, *trans*-10 CLA had a very high incorporation rate to PE, but had a low incorporation rate to PC (Tables 5 and 7). The reason for the large differences in incorporation rates of CLA isomers to various lipid classes is not known but could be related to their stereospecificity. Both PC and PE are positively charged. However, three methyl groups surrounding the positively charged nitrogen in PC make the charge weaker but spatially larger than the PE (Voet and Voet, 1995). The space limitation could make incorporation of *cis*-8, *trans*-10 CLA in PC difficult, but the strong positive charge of PE could attract electrically dense double bond of *cis*-8, *trans*-10 CLA, and the *cis*-8 structure might provide a suitable distance for it to be incorporated in PE. Because of the structural differences among TG, PC, and PE, we hypothesize that CLA isomers have different preferences for the positions to be incorporated in glycerol backbone. In total lipids, the deposition of dietary *cis*-11, *trans*-13 CLA in egg yolk lipids is significantly less than that of other isomers. Kramer *et al.* (1998) fed CLA to pigs and found that the incorporation of different CLA isomers was different. But the isomer composition of the back fat of pigs was similar to that of the diet, which corresponds to our observation with egg yolk lipids. Kramer *et al.* (1998) also showed that the proportion of *cis*-9, *trans*-11 CLA in phospholipids from liver was higher than that of the diet.

This study showed that dietary CLA isomers could be incorporated into various lipid parts with high efficiency. Diets containing 5% CLA resulted in about 15% of CLA in yolk lipid. Therefore, a large egg (60 g) from hens fed 5% CLA diet would provide over one-third of the daily CLA recommendation (3 g) for an adult human. The incorporation rates of CLA isomers into lipid classes were different, and the catabolism of CLA was not as efficient as linoleic acid. Not much information on the metabolism of CLA isomers *in vivo* is available, and some results are conflicting. More research is needed to determine the metabolic pathways of CLA *in vivo* and their implications for human health.

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