Estimation of Conjugated Linoleic Acid Intake by Written Dietary Assessment Methodologies Underestimates Actual Intake Evaluated by Food Duplicate Methodology[1,2]

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ABSTRACT Conjugated linoleic acids (CLA) are conjugated isomers of linoleic acid, which may promote health with regard to cancer, heart disease, diabetes, bone formation, growth modulation and immunity. The *c*9,*t*11 isomer of CLA, rumenic acid (RA), is the major isomer present in the diet. However, dietary intakes of CLA and RA by humans have not been examined rigorously, nor has the relationship between dietary CLA or RA and health (e.g., body composition). Three-day dietary records (DR) were collected from adult men (n = 46) and women (n = 47) and analyzed using a nutrient database modified to contain total CLA and RA. Simultaneously, 3-d food duplicates (FD) were collected to determine analytically individual fatty acid intakes, including those of total CLA and RA. Chronic total CLA and RA intakes were estimated using a semiquantitative food-frequency questionnaire (FFQ). Body composition was estimated using body mass index and percentage of body fat. Total CLA intake was estimated from FD to be 212 ± 14 and 151 ± 14 mg/d (mean ± SEM) for men and women, respectively; RA intake was estimated to be 193 ± 13 and 140 ± 14 mg/d for men and women, respectively. In general, CLA and RA intakes estimated by DR and FFQ were significantly lower than those estimated by FD. Body composition was not significantly related to dietary total CLA or RA intake. In conclusion, results suggest that DR and FFQ methodologies are not reliable estimators of individual total CLA and RA intakes and may underestimate total CLA and RA intakes of groups. Intake of total CLA and RA was found to be significantly lower than that suggested previously by others. J. Nutr. 131: 1548–1554, 2001.

KEY WORDS: • *conjugated linoleic acid* • *CLA* • *rumenic acid* • *diet* • *humans*

The term "conjugated linoleic acid" (CLA)[4] refers to a group of conjugated fatty acid isomers of linoleic acid (C18:2) with double bonds in *cis-*, *trans-* or mixed configurations (Lavillonniere et al. 1998). The *c*9,*t*11-octadecadienoic acid, also referred to as rumenic acid (RA) (Kramer et al. 1998), is the predominant form found naturally in foods such as dairy and beef (Chin et al. 1992). Conjugated linoleic acid has been proven unequivocally to inhibit carcinogenesis in animal models (National Research Council 1996) as well as in in vitro cell cultures (Schonberg and Krokan 1995, Shultz et al. 1992). In addition, CLA appears to be involved in growth modulation (Ostrowska et al. 1999, Park et al. 1999a); control and CLA-supplemented animals in these studies exhibited similar body weights, but body fat percentages were reduced in CLA-supplemented animals. Intake of CLA may also influence diabetes (Houseknecht et al. 1998), bone health (Li et al. 1999) and the immune system (Hayek et al. 1999, Turek et al. 1998).

It is thought that the major source of CLA in human tissues is the diet (Kamlage et al. 1999). Typical human consumption of CLA estimated from 3-d written dietary record (DR) and semiquantitative food-frequency questionnaire (FFQ) methodologies (Herbel et al. 1998, Park et al. 1999b) ranges from 20 to 290 mg/d. Because these methods of estimating dietary intake may be somewhat inaccurate (Lee and Nieman 1996), validation against more direct methodologies (e.g., 3-d food duplicates; FD) seemed prudent. Thus, the major objective of this study was to estimate current and chronic CLA intakes in a representative sample of men and women, aged 18–60 y. In addition, recent work by Wolk et al. (1998) and Smedman et al. (1999) suggests that the content of pentadecanoic (C15:0) and heptadecanoic fatty acids (C17:0) present in subcutaneous adipose tissue as well as serum C15:0 are useful markers of dietary milk fat intake. Both fatty acids are synthesized by ruminant bacterial flora and not by humans; they have been

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[4] Abbreviations used: BD, body density; BF, percentage of body fat; BMI, body mass index; CLA, conjugated linoleic acid; CSFII, Continuing Survey of Food Intakes by Individuals; DR, 3-d written dietary record; FAME, fatty acid methyl esters; FD, 3-d food duplicates; FFQ, semiquantitative food-frequency questionnaire; RA, rumenic acid.

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suggested to be potential markers for dairy and beef intake and thus, perhaps, dietary CLA intake in humans. Consequently, we also investigated the relationship between intakes of CLA or RA and C15:0 or C17:0. Further, to our knowledge, no data investigating the relationship between CLA intake and body composition in humans have been published. Therefore, another objective of this study was to examine the relationship between CLA or RA intake and body composition in humans.

In summary, rigorous documentation of CLA intake in any human population has not been reported. Further, the relationship between CLA intake and body composition in humans has not been investigated. Therefore, this study was designed to test the following general hypotheses: *1*) Chronic and current CLA and RA intakes in the U.S. population do not exceed 500 mg/d. *2*) A positive relationship exists between CLA or RA intake estimated by 3-d DR and FD methodologies. *3*) There is no relationship between CLA or RA intake and body composition in humans.

SUBJECTS AND METHODS

Subjects. Men (n = 51) and women (n = 51) were recruited from the communities of Pullman, WA and Moscow, ID. To be eligible for inclusion, subjects had to be between the ages of 18 and 60 y, healthy (self-reported) and not suffering from eating disorders. All data were collected within a 1-y period (April 1997–March 1998). Physical activity levels of subjects were assessed using defined categories (sedentary or slightly, moderately, very and extremely active). The Human Subjects Institutional Review Board of Washington State University and the Human Assurances Committee at the University of Idaho approved all procedures used, and written informed consent was obtained.

Dietary assessment. Chronic total CLA and RA intakes were estimated using a FFQ that we developed (Park et al. 1999b); the FFQ assessed long-term intakes of 70 foods containing CLA. Collection of current dietary intake data occurred during a period that included two weekdays and one weekend day (Lee and Nieman 1996). Data collected by DR and FFQ were evaluated using a computerized nutrient database (Food Processor, Version 7.02; ESHA Research, Salem, OR), which we modified to contain quantities of total CLA and RA (mg/g fat) in ~190 foods (Chin et al. 1992, Fritsche and Steinhart 1998, Ha et al. 1989, Hanson and McGuire 1998, Lin et al. 1995, Dr. Michael Pariza (University of Wisconsin, Madison; personal communication), Shantha et al. 1992, 1994 and 1995, Werner et al. 1992). When individual CLA isomers were not reported for foods, the published CLA content was classified as "total CLA." However, for most food items, two categories were created, i.e., RA and total CLA. It is noteworthy that very few published manuscripts reported which CLA isomers were included in the estimate of total CLA. When possible, ingredients of complex food items were entered individually to ensure inclusion of total CLA and RA concentrations and a more complete fatty acid profile. Composite FD were collected simultaneously to the recording of DR during the study. All food collected was kept refrigerated by the subjects until the food was weighed by study personnel, homogenized and frozen at −20°C for later fatty acid analysis. We also created two categories related to CLA intake as estimated by FD, i.e., RA and total CLA, which was a sum of the RA and *t*10,*c*12–18:2 isomers. These were the only CLA isomers that were detectable in our analyses.

Anthropometric assessment. Anthropometric measurements were made in the morning after the last day of food collection and dietary assessment between 0600 and 0900 h while the subjects were fasting. All anthropometric measurements were made on the right side of the body by a single investigator while subjects were dressed in light clothing. Skinfold measurements were obtained in duplicate using a Lange skinfold caliper (Cambridge Scientific Industries, Cambridge, MD) for the estimation of body density (BD) and ultimately the percentage of body fat (BF). Standard procedures were used to measure the following 7 skinfold sites: pectoral, umbilicus, thigh, triceps, suprailiac, axilla and subscapular (Jackson and Pollock 1985). Body density was estimated using previously published equations (Jackson and Pollock 1985). The conversion of BD to BF was derived from the equation developed by Siri (1961). Weight (± 0.1 kg) was measured using an electronic scale (Seca Alpha, Model 770, Hamburg, Germany), and height (± 0.1 cm) was measured using a wooden height board (Seca Measure-All). Body mass index (BMI) was calculated as weight (kg) divided by height squared (m^2).

Lipid and fatty acid analyses. Methanol, chloroform, hexane (all HPLC grade) and diethyl ether (reagent grade) were obtained from Fisher Scientific (Santa Clara, CA). Sodium methoxide was purchased from Fluka (Milwaukee, WI); methyl acetate and oxalic acid were purchased from Sigma-Aldrich Chemical (St. Louis, MO), and the anhydrous milk fat reference standard was obtained from the Commission of the European Communities (CRM 164; European Community Bureau of Reference, Brussels, Belgium). Conjugated octadecadienoic acid (99% *c*9,*t*11–18:2) and a complex fatty acid methyl esters (FAME) mixture (C8:0-C22:1, KEL-FIM-FAME-5) were obtained from Matreya (Bellefonte, PA) to determine retention times for individual fatty acids.

Lipids of FD composites were extracted in triplicate using a modified Folch procedure (Clark et al. 1982). Each homogenized food composite (~2 g) was diluted with 38 mL of a 2:1 chloroform/methanol mixture, sonicated 40 s, centrifuged (400 × *g*) for 10 min and filtered into tubes containing 0.58 g/L saline. After a second centrifugation, the upper phase was removed and the lower phase was dried under nitrogen. Samples were diluted with hexane to contain 0.25 g lipid/L; triplicates were combined and stored at −20°C for analysis of FAME.

Samples were methylated using a methanolic sodium methoxide solution (Christie 1982). The methylation reagent, containing 400 mL sodium methoxide (5.4 mol/L) and 1.75 mL methanol, was prepared daily, and 40 μL was added after 40 μL methyl acetate. The reaction proceeded for 10 min at room temperature and was terminated by the addition of 60 μL of a mixture containing 1 g oxalic acid in 30 mL diethyl ether. After centrifugation for 2 min (400 × *g*), clear liquid supernatant was transferred into tubes containing ~2 g anhydrous calcium chloride and allowed to equilibrate for 60 min. The remaining liquid was transferred to injection vials, flushed with N_2 and capped.

Quantification of FAME was performed on a gas chromatograph (Hewlett-Packard 6890, Hewlett-Packard, Wilmington, DE) fitted with a flame ionization detector. Fatty acid profiles were determined by split injection (13.4:1; 2-μL sample) onto a CP-Sil 88 fused silica capillary column (100 m × 0.25 mm × 0.25 μm, Chrompack, Raritan, NJ) using a previously described temperature gradient (Grinari et al. 1998). The hydrogen carrier gas pressure was held constant (210 kPa), and the injector and detector temperatures were 255°C. Fatty acid concentrations were determined as percentages of total fatty acids. Correction factors for individual fatty acids were determined through use of the previously mentioned anhydrous milk fat reference standard with certified values.

Statistical analyses. Statistical analyses were conducted using MINITAB Statistical Software (Release 12.0; State College, PA). χ^2 tests were used to analyze the effect of gender on total CLA and RA food distribution. Pearson correlation coefficients (r) were calculated to determine the relationships between lipid and fatty acid intakes as estimated by DR and FD, and CLA/RA intakes as estimated by DR or FFQ and FD. Two-way, paired t tests were used to test for differences between means obtained using different dietary intake methodologies. Multiple regression analyses were performed to determine independent and interactive relationships between and among gender and lipid intakes estimated by DR or FFQ on total lipid and fatty acid intakes as estimated by FD. Fatty acid intake data obtained by FD were log transformed to meet the assumption of equal variance. In addition, data for CLA and RA intakes, age, energy intake (kJ) and activity level were centered to reduce the inflation of variance of the regression coefficients that resulted from multicollinearity (Myers 1990). A second series of multiple regression analyses was conducted to explore possible effects of gender, age, energy and CLA or RA intake (as estimated by FD), physical activity, and the interactions between the following: *1*) CLA or RA intake and age; *2*) CLA or RA intake and gender; *3*) CLA or RA intake and activity; and *4*) CLA or RA intake and energy intake on body composition (BF or BMI).

TABLE 1

Demographic variables and anthropometric measurements[1]

Variable	Gender	
	Male	Female
Age, *y*	32 ± 2	30 ± 2
Ethnicity, *n*		
Caucasian	42	40
Asian	3	5
Latino	1	2
Weight, *kg*	81 ± 2	62 ± 2
Height, *cm*	178 ± 1	164 ± 1
BMI,[2] *kg/m²*	25 ± 1	23 ± 1
Body fat, *g/100 g*	15 ± 1	21 ± 1

[1] Values represent means ± SEM (n = 46 for men and n = 47 for women).
[2] BMI, body mass index.

The final regression model was determined by best subset regression (Ott 1993). For t test and regression equations, individual and main effects were considered significant at $P \leq 0.05$. Interaction terms in the multiple regression analyses were considered significant at $P \leq 0.10$.

RESULTS

Description of study population and final data set. Data from 9 subjects were excluded from the analyses: 4 subjects (2 men, 2 women) withdrew for personal reasons, 1 subject (male) became ill during the study and 4 subjects (2 men, 2 women) were excluded due to incomplete data collection. Thus, the majority of data reported represent those of 46 men and 47 women. In addition, data from two women were excluded from the body composition analyses due to either inappropriate application of the body composition equation (lactating subject), or because the percentage of BF was outside the range of the method employed (extremely obese subject). Furthermore, in 4 subjects (2 men, 2 women), activity level was not documented. Descriptive statistics of our study population are shown in **Table 1**.

Description of dietary intakes. Chronic total CLA intake as estimated by FFQ was 197 ± 19 and 93 ± 11 mg/d and for RA 151 ± 15 and 72 ± 9 mg/d for men and women, respectively (**Fig. 1**). Information concerning dietary intake estimated by DR is presented in **Table 2** and Figure 1. Using DR, total CLA intakes were 176 and 104 mg/d and RA intakes 133 and 79 mg/d for men and women, respectively. Dietary intakes of total lipid and fatty acids as estimated by DR and FD methodologies are summarized in **Table 3**. Using FD, total CLA intakes were 212 and 151 mg/d and RA intakes 193 and 140 mg/d for men and women, respectively. In men, estimates of all fatty acid intakes except C18:3 were affected ($P < 0.05$) by the methodology used (i.e., DR vs. FD). Total lipid and C14:0, C16:0, C18:0 and C18:1 intakes were higher when estimated by DR; the opposite was found for C12:0, C18:2, RA and total CLA. In women, only intakes of C14:0, C16:0, C18:2, RA and total CLA differed between DR and FD methodologies and followed the same patterns as those seen in men. When expressed as proportion (%) of total lipid, only intakes of C18:1 in women and C18:3 in both genders were affected ($P < 0.05$) by the methodology used (data not shown).

When estimated by DR, total CLA intakes ranged from 3 to 486 and 1 to 399 mg/d for men and women, respectively; when estimated by FD, total CLA intakes ranged from undetectable to 454 and undetectable to 520 mg/d for men and women, respectively. When estimated by DR, RA intakes ranged from 1 to 358 and 1 to 336 mg/d for men and women, respectively; when estimated by FD, RA intake ranged from undetectable to 439 and undetectable to 500 mg/d for men and women, respectively. Chronic CLA intake, estimated by FFQ, ranged from 0 to 516 and 0 to 300 mg/d for men and women, respectively; chronic RA intake ranged from 0 to 412 and 0 to 223 mg/d for men and women, respectively. With the exception of total CLA for men estimated by FFQ, mean total CLA and RA intakes estimated by DR and FFQ were lower ($P < 0.05$) than those estimated by FD (Fig. 1). In men, the relationship between mean total CLA intakes estimated by DR or FFQ and FD was weak ($r = 0.38$ and 0.22, respectively), and the correlation was significant only for the relationship between DR and FD ($P = 0.05$). The relationship between RA intakes estimated by DR or FFQ and FD was weak but significant ($r = 0.42$ and 0.43, respectively, $P < 0.005$; **Fig. 2**). In women, relationships between total CLA intake estimated by DR or FFQ and FD were slightly stronger ($r = 0.56$ and 0.44, respectively) and significant ($P = 0.002$); similar results were found for RA (Fig. 2). Together, these data suggest a systematic underestimation of total CLA and RA intakes by DR and FFQ methodologies in both genders.

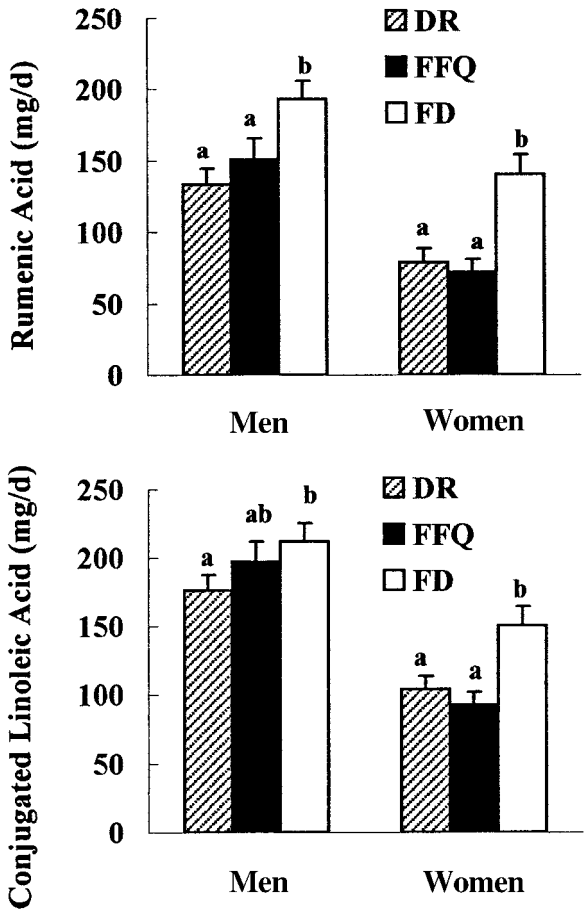

FIGURE 1 Rumenic acid (*upper panel*) and total conjugated linoleic acid (*lower panel*) intakes in men (n = 46) and women (n = 47) estimated by 3-d dietary record (DR), semiquantitative food-frequency questionnaire (FFQ) and 3-d food duplicate (FD) methodologies. Each bar represents the mean ± SEM. Means within a gender not sharing a letter are different, $P < 0.05$.

TABLE 2

Daily intake of macronutrients, fatty acid classes, total conjugated linoleic acids (CLA) and rumenic acid (RA) as estimated by 3-d dietary record methodology[1,2]

Variable	Gender		SEM
	Male	Female	
Energy, *kJ*	10,885[a]	7420[b]	120
Protein, *g*	102[a]	68[b]	7
Carbohydrate, *g*	355[a]	265[b]	21
Lipid, *g*	90[a]	55[b]	5
Saturated fatty acids, *g*	32[a]	19[b]	2
Monounsaturated fatty acids, *g*	34[a]	20[b]	2
Polyunsaturated fatty acids, *g*	17[a]	11[b]	1
Percentage of energy			
Fat	30[a]	27[b]	2
Protein	15	15	1
Carbohydrate	53[a]	57[b]	2
Alcohol	0.8	0.8	0.3
Total CLA, *mg*	176[a]	104[b]	20
RA, (c9,*t*11-18:2) *mg*	133[a]	79[b]	15

[1] Total CLA is the sum of RA and *t*10,*c*12-18:2.
[2] Values represent means ± pooled SEM ($n = 46$ for men and $n = 47$ for women). Different superscripts within a row denote significant ($P < 0.05$) difference.

Relationship between C15:0/C17:0 and CLA/RA. Dietary intakes of C15:0 from FD were 174.3 ± 19.5 and 91.0 ± 16.2 mg/d for men and women, respectively. Dietary intakes of C17:0 were 89.4 ± 16.4 and 116.9 ± 28.0 mg/d for men and women, respectively. A weak but significant correlation was found between C15:0 and total CLA intake estimated by FD for both men and women ($r = 0.37$ and 0.36, respectively; $P < 0.05$) and for RA intake ($r = 0.30$ and 0.36, respectively; $P < 0.05$). No correlation was apparent between intake of C17:0 and total CLA or RA.

Dietary sources of CLA and RA. The distribution of foods that provided dietary total CLA and RA was determined using data collected by DR (**Fig. 3**). Because both total CLA and RA distributions of food sources were similar between genders, data were combined for purposes of presentation. Dairy products contributed the majority of CLA and RA followed by beef. Among dairy products, cheese was the primary source, contributing 30 and 33% of total CLA and RA intakes, respectively.

Relationship between total CLA or RA intake and body composition. After elimination of nonsignificant interactions, the final regression model predicting BF from total CLA intake (adjusted $R^2 = 0.41$) was as follows: BF = 11.1 + 7.59 (gender) − 0.18 (CLA by FD) + 1.97 (age) + 0.75 (energy intake) − 2.34 (activity level). The final model using RA intake was as follows: (adjusted $R^2 = 0.38$): BF = 14.0 + 7.13 (gender) − 0.07 (RA by FD) + 1.88 (age) + 0.7 (energy intake) − 1.24 (activity level). As expected, gender, age and activity level were significantly ($P < 0.05$) related to BF, suggesting that older, female and less active individuals tend to have a higher percentage of BF. Statistical analysis suggested no significant relationship between total CLA or RA intake and BF. When BMI was used instead of BF as an indicator of body composition, similar results were obtained for total CLA: BMI = 24.3 − 1.85 (gender) − 0.40 (CLA by FD) + 0.79 (age) + 0.79 (energy intake) − 0.66 (activity level) + 0.91 (age · CLA by FD) and RA: BMI = 25.0 − 2.01 (gender) − 0.15 (RA by FD) + 0.86 (age) + 0.82 (energy intake) − 0.45 (activity level) + 0.56 (age · RA by FD). Gender and age were again significant ($P = 0.01$ and 0.004, respectively; adjusted $R^2 = 0.30$) in the model using RA, whereas only age was significant when using total CLA in the model ($P = 0.01$; adjusted $R^2 = 0.23$). However, total CLA and RA intake were not significantly related to BMI.

DISCUSSION

Lipid and fatty acid intakes of our study population as estimated by FD are similar to those reported by the 1994–1996 Continuing Survey of Food Intakes by Individuals (CSFII) (USDA/Agriculture Research Service 1997), with the exception of stearic acid intake in men as estimated by FD, which was only 50% of the mean CSFII estimate. Thus, we conclude that the mean total CLA and RA intakes (as esti-

TABLE 3

Daily lipid and fatty acid intakes as estimated by 3-d dietary record and food duplicate methodologies[1,2]

	Men			Women		
	Dietary record	Food duplicate	SEM	Dietary record	Food duplicate	SEM
Total lipid, *g*	89[a]	84[b]	3	55	53	2
C12:0; lauric acid, *g*	0.8[a]	1.3[b]	0.2	0.6	0.7	0.1
C14:0; myristic acid, *g*	2.9[a]	2.2[b]	0.2	1.8[a]	1.4[b]	0.1
C16:0; palmitic acid, *g*	16.8[a]	14.5[b]	0.6	10.0[a]	8.9[b]	0.5
C18:0; stearic acid, *g*	7.9[a]	4.7[b]	0.3	4.6	4.2	0.3
C18:1; oleic acid, *g*	30.6[a]	26.2[b]	1.2	18.2	16.8	0.8
C18:2; linoleic acid, *g*	15.1[a]	18.6[b]	1.1	9.6[a]	12.4[b]	0.9
C18:3; linolenic acid, *g*	1.5	1.7	0.1	1.1	1.2	0.1
C18:2 *c*9,*t*11; RA,[3] *mg*	133[a]	193[b]	13	79[a]	140[b]	12
Total CLA,[4] *mg*	176[a]	212[b]	17	104[a]	151[b]	13

[1] Data presented are means ± pooled SEM; $n = 46$ for men and $n = 47$ for women. Different superscripts within a row and gender denote significant ($P < 0.05$) differences.
[2] These fatty acids accounted for 85 and 84% of fatty acids in dietary records from men and women, respectively, and 83 and 87% of fatty acids in food duplicates from men and women, respectively.
[3] Rumenic acid.
[4] Conjugated linoleic acid, sum of RA and *t*10,*c*12-18:2.

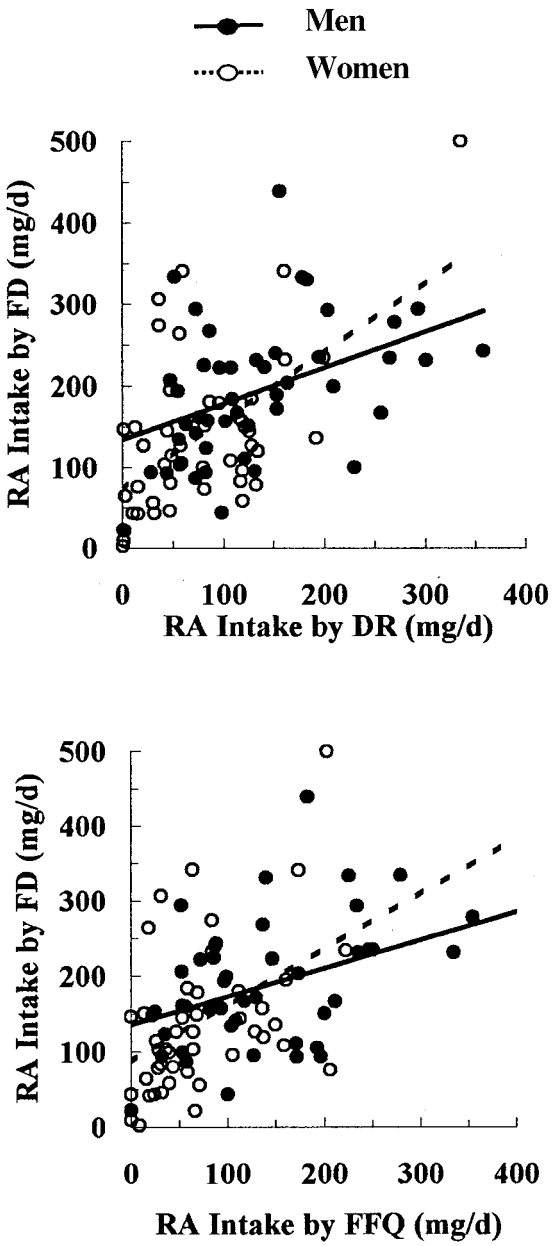

FIGURE 2 Relationships between rumenic acid intake estimated by 3-d dietary record (DR) and 3-d food duplicate (FD) methodology (*upper panel*) and food-frequency questionnaires (FFQ) and FD (*lower panel*) in men ($n = 46$) and women ($n = 47$). For men, the equation for the relationship between DR and FD was $y = 0.44x + 134.15$ ($r = 0.42$, $P < 0.05$), and for women, $y = 0.850x + 72.85$ ($r = 0.57$, $P < 0.01$). For FD vs. FFQ, the equation for men was $y = 0.371x + 136.46$ ($r = 0.43$, $P < 0.05$), and for women, $y = 0.748x + 86.41$ ($r = 0.45$, $P = 0.001$).

mated by FD) in this study may be representative of the average U.S. population intake. Results confirm our first hypothesis that total CLA and RA intake is < 500 mg/d. Furthermore, intakes of all commonly consumed fatty acids studied here were significantly correlated when DR and FD methodologies were compared, suggesting that these methodologies were properly utilized.

Results from Herbel et al. (1998) who reported a mean dietary CLA intake of 139 mg/d in young men and women, and Park et al. (1999b) who estimated mean CLA intake in lactating women during periods of low and high dairy consumption (20 and 290 mg/d, respectively) support our data concerning CLA intake. The FFQ used in this study was developed initially and used previously by Park et al. (1999b) who reported a mean chronic CLA intake in lactating women of 227 ± 180 mg/d; this value is higher than the mean chronic CLA intake in the men (150 ± 15 mg/d) but more than threefold that of the women (72 ± 9 mg/d) studied here. The lower dietary lipid intake in our study compared with that of the lactating women studied by Park et al. (1999b) may explain in part the lower chronic CLA intake in the women reported in this study.

Additional data suggest that dietary CLA intakes in other countries are somewhat comparable to that of the U.S. population. Among older Swedish men, mean CLA intake was 160 mg/d (Jiang et al. 1999) as estimated by 7-d weighed DR and 24-h recalls. Further, Fritsche and Steinhart (1998) using a national consumption survey estimated that German men and women consume 430 and 350 mg RA/d, respectively. These estimates are approximately twice those estimated for the U.S. population. However, Germans consume ~10% more energy from fat than do Americans (Adolf et al. 1994).

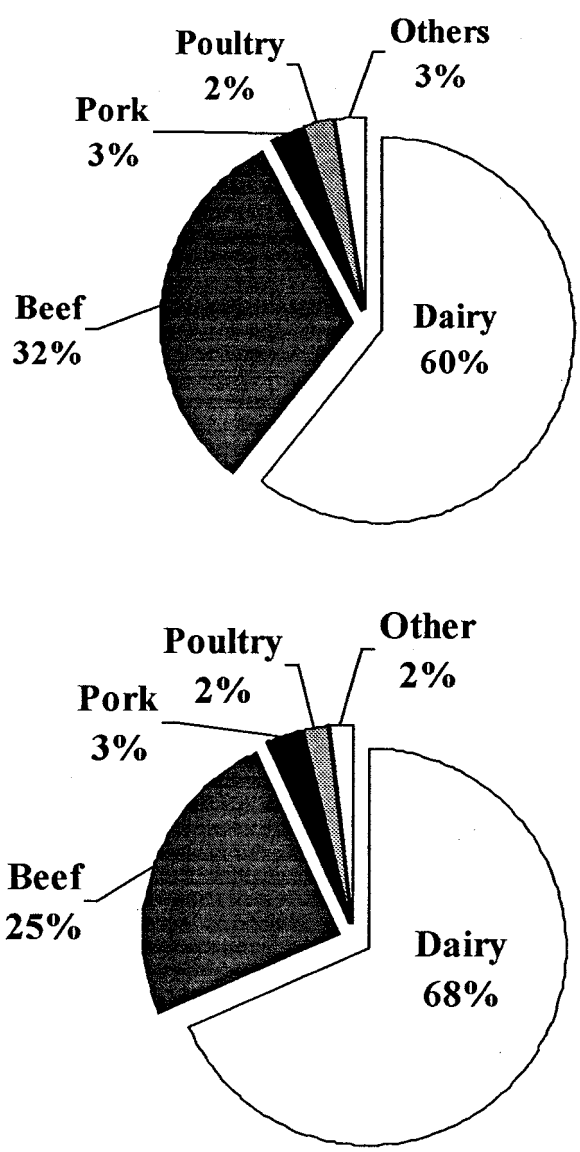

FIGURE 3 Distribution of sources of conjugated linoleic acid intake (*upper graph*) and rumenic acid intake (*lower graph*) estimated by 3-d dietary record methodology in men ($n = 46$) and women ($n = 47$).

A number of factors may have potentially influenced the accuracy of the data collected in this study. For example, the incompleteness of individual fatty acid quantities in the nutrient database may have resulted in an underestimation of the calculated data obtained from DR and FFQ. This concern may be especially pertinent for the total CLA and RA data. It is important for future investigators to report individual CLA isomers when documenting the CLA content of foods. Moreover, the use of a variety of oils and fats by food manufacturers introduces additional variation (Worley 1994). Furthermore, although the FD methodology may provide a more accurate measurement of actual dietary nutrient intake compared with calculations based on food composition tables, the additional time and effort invested by the subjects associated with this method may result in underestimation of usual nutrient intake (Lee and Nieman 1996). Nonetheless, because our FD methodology resulted in total CLA and RA intake values that were *greater* than those obtained by DR or FFQ, we believe that these values probably do not appreciably underrepresent true intakes.

Results also confirm our second hypothesis, i.e., that a positive relationship exists between total CLA or RA intake estimated by 3-d DR and FD methodologies. Although the correlations between total CLA or RA intakes as estimated by DR or FFQ and those estimated by FD were strong in both genders, mean total CLA and RA intakes estimated by these methods were quite different and suggested a systematic underestimation of total CLA and RA intake by FFQ and DR. The weak correlation between total CLA or RA intake estimated by DR and FD methodologies suggests that high natural variation in the CLA concentration of similar food products may result in major difficulties in constructing an adequate database of CLA contents in food. Natural variation in food CLA is likely to occur by differences in, for example, diet, season, maturity and breed of animal (Bauman et al. 2000). Therefore, food CLA concentrations used for computation of dietary intake represent mere estimates. Nonetheless, we believe that the data presented here, especially those collected by FD methodology, quite accurately reflect CLA intake in the population investigated. In conclusion, DR and FFQ methodologies underestimate the mean group intake of total CLA and RA, and researchers should use them with caution when estimating CLA intakes of individuals.

Animal data have suggested that consumption of a diet containing as little as 0.1 g CLA/100 g dry diet is sufficient to significantly reduce tumors (Ip et al. 1994) and may be useful as a reference for dietary intake recommendations in humans. Recently, Ip et al. (1999) demonstrated that RA is the biologically active anticarcinogenic CLA isomer. Noteworthy is the fact that this suggestion is based on an animal model that involves the administration of massive doses of a chemical carcinogen. Because humans are typically exposed to much lower concentrations of carcinogens, the effective RA dose for human cancer protection is likely to be less than what animal models suggest. Nonetheless, when calculated on a dry weight basis, both men and women in our study consumed diets containing approximately 0.03 g/100 g RA. Further, our data suggest that RA intake must be 620 and 441 mg/d for men and women, respectively, to exhibit a cancer protective effect (i.e., 0.1 g/100 g diet). Due to the lack of experimental human data, extrapolation from animals to humans provides the only means of dietary intake estimates. However, these represent very rough estimates and should be interpreted cautiously.

Increasing dietary CLA consumption to these levels could be accomplished by altering the consumption of CLA-containing foods. However, an increase in CLA consumption should be promoted with caution because a diet rich in CLA is also often high in fat. Alternatively, food products rich in CLA can be substituted for similar foods containing lower amounts of CLA (e.g., butter vs. margarine). An alternative approach to increasing dietary CLA intake is to naturally enhance CLA concentrations in food. For example, administration of sunflower oil to the lactating cow increases CLA concentrations approximately fivefold (Kelly et al. 1998). An increase of this magnitude would raise daily dietary RA intake in the present study to 551 and 491 mg/d in men and women, respectively.

A secondary objective of this study focused on the putative growth modulating characteristics of CLA and RA (Ostrowska et al. 1999, Park et al. 1997 and 1999a, Sisk et al. 1998). Results of the present study suggest, however, that total CLA and RA intakes were not related to the percentage of BF or BMI. This finding supports our initial hypothesis.

Finally, to investigate the relationship between total CLA or RA and C15:0 or C17:0, we determined intakes of both fatty acids from FD. Results indicate that there was a weak correlation between C15:0 and total CLA or RA, and an even weaker relationship between C17:0 and total CLA or RA. Thus, C15:0 or C17:0 intakes are not reliably related to either total CLA or RA intakes.

In summary, our data suggest that chronic as well as current total CLA and RA intakes in men and women do not exceed 500 mg/d. Dairy products were the primary source of total CLA and RA, followed by beef. A positive relationship existed between individual total CLA and RA intakes estimated by DR and FD. However, both DR and FFQ methodologies underestimated mean total CLA and RA intakes. In both genders, neither FFQ nor DR methodology can be used to predict individual CLA or RA intake, but may be used as indicators of relative CLA or RA intake among groups of people. In the future, more accurate and reliable methods to estimate individual total CLA as well as CLA isomer intakes must be investigated when the use of FD methodology is not appropriate or possible. For example, the relationship between CLA isomer concentrations in blood or adipose stores and dietary CLA isomer intake may be considered. Finally, we found no relationship between body composition and CLA or RA intake, suggesting that dietary CLA has little effect on body composition in humans.

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