Null Results in Brief

Conjugated Linoleic Acid Content in Breast Adipose Tissue Is Not Associated with the Relative Risk of Breast Cancer in a Population of French Patients[1]

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Introduction

CLA[3] refer to a group of octadecadienoic acid isomers that contain two conjugated double bonds. CLA can be found in natural food sources, such as dairy products or meat of ruminant animals. Considerable attention has been directed recently to CLA based on their ability to act as preventive agents in experimental rat mammary carcinogenesis, irrespective of the type or level of fat present in the diet (1). This suggest that dietary CLA might also be used in humans for chemoprevention of breast cancer. The feasibility of increasing CLA content of food is already being considered to increase CLA intake in humans (2). Despite such a potential, few data are available in humans, likely as a consequence of the difficulty to obtain accurate estimates of dietary CLA intake. One study conducted in Finland examined the relationship between dietary or serum CLA in women and the risk of breast cancer, and found dietary or serum CLA to be significantly lower in cases than in controls, suggesting a protective effect of CLA on breast cancer risk but only in postmenopausal patients (3). Because CLA accumulates in body fat stores, we used the CLA content of breast adipose tissue, obtained at the time of surgery, as a qualitative biomarker of CLA dietary intake, and we conducted a case-control study among 297 women treated for breast cancer or benign breast disease at the University Hospital of Tours, France, to evaluate the hypothesis that CLA protect against breast cancer.

Subjects and Methods

This study was carried out from a population of 329 patients who was initially selected at the University Hospital of Tours, France, between 1992 and 1996, for a study on fatty acids and breast cancer risk, and was described previously (4). It included 241 patients with invasive breast carcinoma (cases) and 88 patients with benign breast pathologies (controls). Patients had surgery at first treatment step, during which a specimen of adipose tissue was retained and kept frozen in liquid nitrogen until analysis. For this present study, 28 cases and 4 controls were excluded from this population because there was no sufficient lipid extracts left for CLA analysis. The coded samples of cases and controls were disposed in a random sequence. The laboratory was blinded to links between samples and subjects. Total lipids were extracted from adipose tissue samples, triglycerides were purified by adsorption chromatography and fatty acids, and CLA were converted to fatty acid methyl esters with sodium methoxyde. CLA were first concentrated by a high performance liquid chromatography step. Then, the fatty acid methyl ester composition of adipose tissue was determined by GC (4). Total CLA was calculated as the sum of the isomers and was expressed as percentage of total fatty acids. The dimethyloxazoline derivatives were analyzed by GC-mass spectrometry. Patients were categorized into tertiles according to the percentage composition of CLA. ORs and 95% CIs were calculated for each tertile using an unconditional logistic regression analysis; estimates were adjusted for age, BMI, and menopausal status.

Results

GC analyses showed the presence of different isomers of CLA and the identification of the conjugated C18 dimethyloxazoline derivatives by GC-mass spectrometry indicated that the 9-*cis*,11-*trans* isomer represented the major isomer. Considering the sum of isomers, we found that mean CLA level was 0.44% of total fatty acids in cases (range, 0.19–0.75; SD, 0.10) and 0.43% in controls (range, 0.14–0.70; SD, 0.11). No significant difference in mean CLA levels between control and case patients was found ($P = 0.35$ by Student's t test). Within patients, partial Spearman correlation coefficients were calculated between CLA levels and some clinical characteristics. We found that CLA levels were not associated with age at diagnosis ($n = 297$; $r = 0.05$; $P = 0.35$) but were inversely associated with BMI ($n = 297$; $r = -0.13$; $P = 0.02$). No significant association was found between CLA levels in breast adipose tissue and breast cancer risk (Table 1).

Statistical Power. Given the sample size in the study, a 33% of exposed controls, and a 5% significance level, the power to detect a risk ratio of 1.8 is ~65%.

Study Limitations. There are several potential limitations. First, the study has been carried out in a geographically limited area in central France, where the ethnic diversity is low and cultural habits are very homogeneous. This might be reflected by the narrow range of CLA distribution among the population. This narrow range may be insufficient to detect a significant

Received 9/21/01; revised 3/22/02; accepted 4/3/02.
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[3] The abbreviations used are: CLA, conjugated linoleic acid; OR, odds ratio; CI, confidence interval; GC, gas chromatography; BMI, body mass index.

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Table 1 Estimated relative risk (ORs, crude, and adjusted[a]) of breast cancer and 95% CIs by adipose tissue CLA levels from the whole population ($n = 297$)

Fatty acids	ORs[a] (95% CIs)			*P* for trend
	Tertile 1 (low)	Tertile 2	Tertile 3 (high)	
CLA	1.00	1.29 (0.68–2.45)	1.42 (0.77–2.65)	0.27
		1.65 (0.80–3.37)[a]	1.83 (0.90–3.71)[a]	0.10

[a] Adjusted for age at diagnosis, BMI, and menopausal status.

association with breast cancer risk. Second, the validity of adipose tissue CLA levels as a biomarker of its past dietary intake is not known because no dietary questionnaires are available. Another limitation is that using patients with benign breast pathologies might bias results toward the null because benign breast disease increases breast cancer risk and may be related to the same cancer-related alterations, such as changes in diet and metabolism. Lastly, experimental studies on animals have shown that the feeding of CLA only during pubertal development of the mammary gland, before carcinogen administration, led to a reduction of 7,12-dimethylbenz(*a*)anthracene- or *N*-nitroso-*N*-methylurea-induced mammary carcinogenesis (5). Thus, the timing of CLA exposure may be inappropriate in our study.

Conclusions

In contrast to previous data derived from a case-control study based on serum CLA content (3), we were not able to document a negative association between adipose tissue CLA and the risk of breast cancer. Before any lack of association can be concluded, other studies based on an identical approach should be carried out in more heterogeneous populations or countries.

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