Conjugated Linoleic Acid and Oxidative Stress

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ABSTRACT: At the present time, conjugated linoleic acid (CLA) is the subject of a growing number of studies since it has been demonstrated to possess anticarcinogenic and antiatherogenic activities in experimental animal models and to increase in some pathological states in humans. In both situations, CLA has been claimed to be involved in oxidative stress, as an antioxidant in the first case and as a primary product of a free-radical attack on polyunsaturated fatty acids (PUFA) in the other. The controversial results are due mostly to a lack of a suitable methodology because the presence of conjugated dienes (CD) in lipid moiety has been taken for years as evidence of lipid peroxidation due to the occurrence of this structure in fatty acid hydroperoxides. We have recently developed a new methodology that consists of the extraction of fatty acids, including CD fatty acid hydroperoxides, by mild saponification and their separation and identification by high-performance liquid chromatography with diode array detector. Fatty acid analyses of liver homogenate, oxidized *in vitro* either with Fe-ADP or *t*-butyl hydroperoxide (*t*-ButylHP), of lamb and rats fed CLA at levels known to prevent carcinogenesis, showed that CLA and its metabolites steadily decreased during oxidative stress and that they are more prone to oxidation than their corresponding methylene-interrupted fatty acids. No significant antioxidant effect of CLA was detected in any model tested. However, CD fatty acid hydroperoxides increased in the *t*-ButylHP model but not in the Fe-ADP model, owing probably to the degradation of CD fatty acid hydroperoxides induced by this oxidative agent. In conclusion, CLA and its metabolites seem to behave, under oxidative stress, as regular PUFA. Thus, it is highly unlikely that the peculiar effects of CLA are directly related to interference in lipoperoxidative processes.
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KEY WORDS: Conjugated dienes, conjugated linoleic acid, diode array detector, HPLC, hydroperoxides, oxidative stress, second derivative UV spectrophotometry.

The occurrence of conjugated dienes (CD) in biological systems is usually taken as evidence of an ongoing lipoperoxidative process (1,2). In fact, owing to their divinyl-methane structure, polyunsaturated fatty acids (PUFA) are particularly susceptible to hydrogen abstraction with subsequent formation of free-radical intermediates and rearrangement of double bonds to form conjugated diene fatty acid hydroperoxides (CDOOH) (3). The use of CD as markers of lipid peroxidation is due to their characteristic absorption at around 234 nm, which renders feasible their detection by ultraviolet (UV) spectrophotometry (1,2).

However, irrespective of the spectrophotometric technique used (1,2), it is not possible to obtain further structural information on the lipid molecules that contain the CD structure, i.e., CD nonhydroperoxides, which are naturally present in biological systems (4). CD nonhydroperoxides, mostly conjugated linoleic acid (CLA), can also be formed during partial hydrogenation, performed industrially (5) or by the anaerobic bacterium *Butyrivibrio fibrisolvens,* present in the intestinal flora of ruminants (6). This explains the presence of CD in ruminant meat, milk, and dairy products (7,8).

It has been shown that CLA is absorbed and assimilated in rat tissues (4) and that it is metabolized to conjugated linolenic and conjugated eicosatrienoic acids in the liver of rats fed for 1 wk a diet that contained about 0.04% CLA (9) and to conjugated arachidonic acid in lamb liver (8). These findings suggest that elongation and desaturation of CLA may occur in the liver similar to the parent compound, linoleic acid.

At the present time, CLA is the subject of a growing number of studies because it has been demonstrated to have anticarcinogenic (10–12) and antiatherogenic (13) activities in experimental animal models and to increase in some pathological states in humans (14,15). In both situations, CLA has been claimed to be involved in oxidative stress, as an antioxidant in the first case (10,11) and as a primary product of a free radical attack to PUFA in the other (14).

In fact, it has been hypothesized that CLA could be formed in the presence of proteins by the reaction of the carbon-centered diene-conjugated lipid free radical before the CLA could interact with oxygen or on the reversal of this interaction (14). However, this hypothesis has been strongly criticized. In fact, Thompson and Smith (16) showed that the *in vitro* induction of lipid peroxidation in human and rat blood with either UV irradiation or phenylhydrazine failed to increase the plasma level of CLA. The induction of lipid peroxidation *in vivo* in rats, pretreated with either phenylhydrazine or bromotrichloromethane, also failed to increase the plasma

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level of these isomers. In support of these results Halliwell and Chirico (17) stated that, although some groups continue to maintain that CLA is produced by free radical activity, such a conclusion is extremely unlikely. First, the preferred reaction of carbon-centered radicals is with oxygen, not with protein, and second, lipid peroxidation produces a series of products from all PUFA and not one specific geometric isomer of a product from one fatty acid.

In regard to the antioxidant activity of CLA, Ha *et al*. (10) claimed that, in a test tube model, CLA is an effective antioxidant, more potent than α-tocopherol and almost as effective as butylated hydroxytoluene (BHT). Furthermore, Ip *et al*. (18) showed that feeding of CLA resulted in a decrease in the extent of lipid peroxidation (measured by the TBA test) in the mammary gland but not in the liver. In contrast, Van Den Berg *et al*. (19) tested whether CLA could protect membranes that are composed of 1-palmitoyl-2-linoleoyl phosphatidylcholine (PLPC) from oxidative modification under conditions of metal ion-dependent or -independent oxidative stress. Progress of oxidation was determined by measurement of CD formation and by analysis of fatty acids. The oxidative susceptibility of CLA was higher than that of linoleic acid, and comparable to arachidonic acid. When oxidation of PLPC (1.0 mM) was initiated with the lipid-soluble 2,2′-azobis(2,4-dimethylvaleronitrile) or the water-soluble 2,2′-azobis(2-amidinopropane) hydrochloride, the radical scavengers α-tocopherol and BHT at 0.75 μM efficiently inhibited PLPC oxidation, as evident from a clear lag phase. In contrast, 0.75 μM CLA did not have any significant effect on PLPC oxidation. Inhibition of PLPC oxidation by higher concentrations of CLA appeared due to competition, not to an antioxidant effect. When oxidation of PLPC was initiated by hydrogen peroxide/Fe^{2+} (500 μM/0.05–20 μM), both α-tocopherol (1 μM) and ethylene glycol-bis(aminoethyl ether) tetraacetic acid (50 μM) efficiently inhibited PLPC oxidation. However, CLA (1–50 μM) did not show a clear protective effect under any conditions tested. They concluded that CLA, under those test conditions, did not act as an efficient radical scavenger in any way comparable to vitamin E or BHT. CLA also does not appear to be converted into a metal chelator under metal ion-dependent oxidative stress, as had previously been suggested (10).

In this paper, the involvement in free radical reactions, if any, of CLA, physiologically incorporated in liver triglycerides (TG) and phospholipids (PL) of lambs or rats, has been reconsidered. We employed a new methodology that consists of the extraction of fatty acids, including CDOOH and non-hydroperoxides, by mild saponification, and their separation and simultaneous identification by high-performance liquid chromatography (HPLC) with diode array detector. The oxidative stress has been triggered by two different prooxidants, Fe-ADP or *t*-butyl hydroperoxide (*t*-ButylHP).

MATERIALS AND METHODS

All solvents used were of HPLC grade (Carlo Erba, Milan, Italy). The following fatty acids were purchased from Sigma Chemical Co. (St. Louis, MO): arachidonic, linolenic, linoleic, oleic, eicosatrienoic, vaccenic, and elaidic. Hydroperoxy-octadecadienoic acid (HPODE) was purchased from Cascade Biochem Ltd. (London, United Kingdom). Desferal (deferoxamine methanesulfonate), an iron chelator, was purchased from Ciba-Geigy (Basel, Switzerland). A mixture of standard CLA was obtained from Nu-Chek-Prep, Inc. (Elysian, MN). All other reagents and chemicals were of the highest available purity.

Autoxidation of linoleic acid. One milliliter of linoleic acid solution (0.1 mg/mL CH_3CN) was dried down in a round-bottomed test tube (i.d. 16 mm and length 180 mm) under vacuum. The samples were incubated at 37°C, exposed to air for 4, 8, 12, 16, 20, and 24 h in a shaking water bath, where samples were immersed 4 cm in the water; controls were kept at 0°C. Artificial light exposure was maintained throughout the experiment. However, no significant differences were noted between samples maintained in the dark or exposed to artificial light (20). Three independent experiments were performed, involving triplicate analyses for each sample/condition.

Autoxidation of linoleic acid in presence of CLA. CLA was dissolved in acetonitrile solution, along with linoleic acid, and processed as described above. Different concentrations, i.e., 20, 40, 80, and 160 nmoles per mg of fatty acids, were incubated at 37 or 0°C for 24 h. Three independent experiments were performed, involving triplicate analyses for each sample/condition.

Oxidation of lamb liver homogenates. Samples (six each) of liver from 1-mon-old lambs were obtained from a private breeder located in Sarroch, Sardinia, Italy. The tissues were promptly removed, after the animals were killed, and immediately processed. One gram of liver was homogenized in 8 mL of saline solution. The homogenate was brought to 80 mL with saline solution, and 20 mL of $FeCl_3$ (100 μM) and ADP (2.5 mM) solution or 5 mM of *t*-ButylHP was added. In control samples, 20 mL of saline solution was added. Twenty milliliters of each sample was incubated, and 1-mL aliquots were taken every 30 min and immediately extracted for total lipids.

Oxidation of rat liver homogenates. Six (per group) fasting male Wistar rats weighing 100 g were force-fed for 3 d with 2 g of extra virgin olive oil (EVO)/d or 80 mg of CLA in 1.92 g EVO/d (4% of total fat). EVO was used because, in contrast to refined vegetable oils (21), it is CLA-free. Water was offered *ad libitum* throughout the study. The animals were decapitated, their livers removed and processed without delay. One gram of liver was homogenized in 8 mL of saline solution. The homogenate was brought to 80 mL with saline solution, and 20 mL of $FeCl_3$ (100 μM) and ADP (2.5 mM) solution or 5 mM of *t*-ButylHP was added. In control samples, 20 mL of saline solution was added. Twenty milliliters of each sample was incubated, and 1-mL aliquots were taken every 30 min and immediately extracted for total lipids.

Lipid extraction and preparation of fatty acids. Total lipids were extracted from tissue by the Folch *et al*. (22) procedure. Preparation of free fatty acids was obtained by mild saponification as described by Banni *et al*. (8). Briefly, 3 mg of lipid

of each sample was dissolved in 5 mL ethanol, then 100 µL Desferal (25 mg/mL H_2O), 1 mL of a 25% water solution of ascorbic acid, and 0.5 mL of 10 N KOH were added. The solutions were left in the dark at room temperature for 14 h. After addition of 10 mL *n*-hexane and 7 mL H_2O, samples were acidified with 0.35 mL of 37% HCl to pH 3–4 and then centrifuged for 1 h at 900 × *g*. The hexane phase with the free fatty acids was collected, the solvent was evaporated, and the residue was dissolved in 0.5 mL of CH_3CN with 0.14% CH_3CO_2H (vol/vol). Aliquots (8 µL) of the sample were injected into the HPLC system. All solvent evaporations were performed under vacuum, and lipids were quantitated by the method of Chiang *et al*. (23). Recovery of unsaturated fatty acids, including CDOOH and CD fatty acid nonhydroperoxides (>85%), was calculated by using as an external standard a mixture of unsaturated fatty acid standards.

HPLC diode array detector analyses. Separation of unsaturated fatty acids was carried out with a Hewlett-Packard 1050 liquid chromatograph, equipped with a diode array detector 1040M (Hewlett-Packard, Palo Alto, CA), as described before (8). A C-18 Alltech (Deerfield, IL) Adsorbosphere column, 5 µm particle size, 250 × 4.6 mm, was used with a mobile phase of $CH_3CN/H_2O/CH_3COOH$ (70:30:0.12, vol/vol/vol) at a flow rate of 1.5 mL/min. Unsaturated nonconjugated fatty acids were detected at 200 nm, and CD fatty acids at 234 nm. Spectra (195 to 315 nm) of the eluate were obtained every 1.28 s and were electronically stored. Second-derivative UV spectra were generated with the Phoenix 3D HP Chemstation software (Hewlett-Packard). These spectra were taken to confirm identification of the peaks (8).

Statistical analyses. INSTAT software (GraphPad software, San Diego, CA) was used to calculate the mean and standard deviation of samples from three independent experiments, involving triplicate analyses for each sample/condition. One-way analysis of variance was used to test whether the group means differed significantly. This program uses the Bonferroni method: the threshold for "significance" is the traditional value ($P < 0.05$) divided by the number of comparisons. Thus, we set a more strict (lower) threshold of significance for each comparison, so that there would be an overall probability of 5% that random chance could make any one (or more) of the differences "significant."

RESULTS

Figure 1 shows a typical chromatogram, taken at 234 nm, of HPODE and CLA. Both show the characteristic second-derivative UV spectrum of CD (1) but distinctly different retention times.

Figure 2 shows typical chromatograms of liver conjugated fatty acids after oxidation from rats fed CLA (A) and from lambs (B). It was possible to distinguish two main peaks in Figure 2A; one was CDOOH from PUFA oxidation, and the other was CLA of dietary origin. No other CD fatty acids were detected, showing that CLA elongation and desaturation of CLA did not occur under these experimental conditions. On the other hand, in Figure 2B, the CDOOH originate from PUFA oxidation; CLA and CD 18:3, CD 20:3 and CD 20:4 as metabolites of CLA are evident.

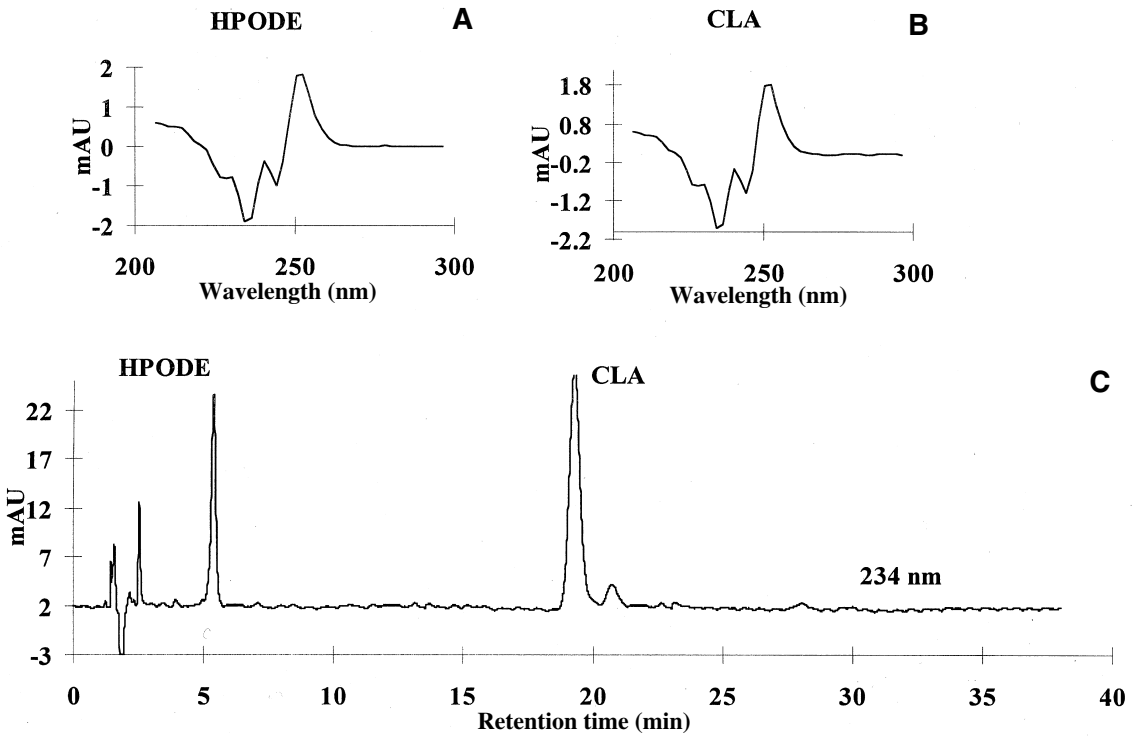

FIG. 1. Chromatogram, recorded at 234 nm, of hydroperoxy-octadecadienoic acid (HPODE) and conjugated linoleic acid (CLA) (C) and their second-derivative ultraviolet spectra (A,B).

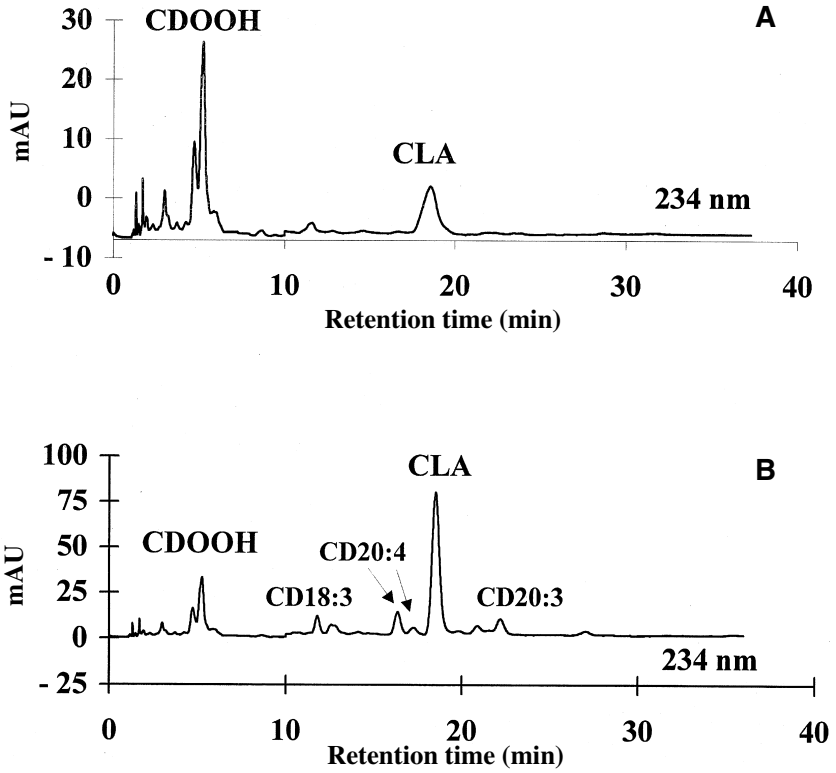

FIG. 2. Chromatograms of liver conjugated fatty acids after oxidation, from rats fed CLA (A) and from lambs (B). CDOOH, conjugated diene fatty acid hydroperoxides. See Figure 1 for other abbreviation.

Autoxidation of linoleic acid in presence of CLA. Figure 3 shows the decrease of linoleic acid and the formation of its hydroperoxides during 24 h of oxidation. As already described (20), the time course of autoxidation of linoleic acid has a characteristic pattern, with a nonlinear decrease of linoleic acid and a nonlinear increase of its hydroperoxides. Therefore, it is more accurate to measure the antioxidant activity of molecules incubated with linoleic acid for 24 h by taking the values of these two parameters and comparing them to the time course of the oxidation of linoleic acid alone for 24 h. Thus, it is possible to calculate "how many hours" the tested molecule has slowed down the oxidative process. The vertical lines represent the values of these two parameters during 24-h oxidation of 100 µg (356.6 nmoles) linoleic

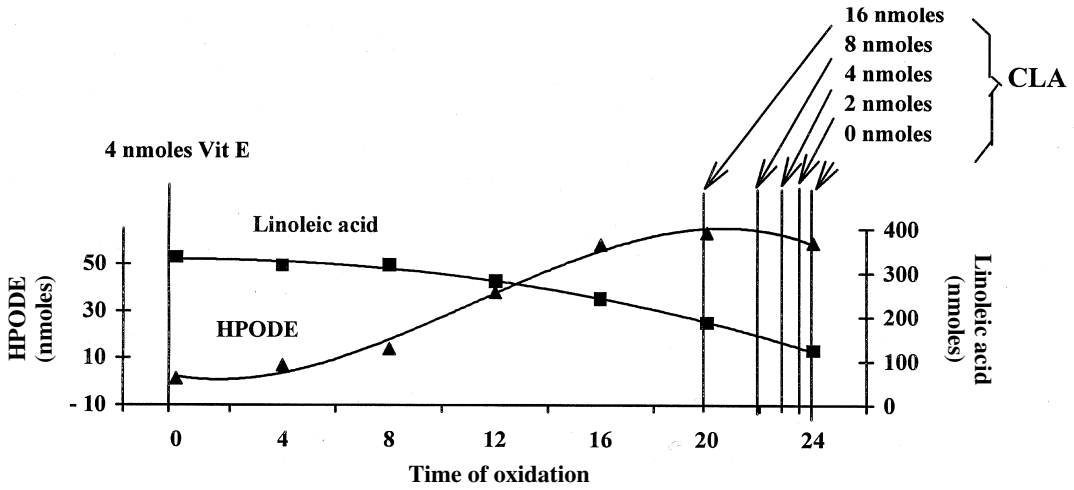

FIG. 3. Decrease of linoleic acid and formation of its hydroperoxide during 24 h of autoxidation of 100 mg (356.6 nmoles) of linoleic acid. The vertical lines represent the values of linoleic acid and its hydroperoxide during the oxidation in the presence of different concentrations of CLA. See Figure 1 for abbreviations.

TABLE 1
Decrease of 22:6 and 20:4, and Formation of CDOOH During 4 h Oxidation Induced by ADP-Fe or *t*-ButylHP of Liver Homogenate of Rats Treated with EVO (control) or EVO with 4% of CLA[a]

Hours of incubation	ADP-Fe		*t*ButylHP	
	Control[b]	CLA[b]	Control[b]	CLA[b]
	22:6			
0	8588.42 ± 1035.86	8268.21 ± 1076.82	7280.57 ± 756.96	8261.57 ± 639.65
4	6305.99 ± 1003.64*	6613.74 ± 1060.54*	3765.16 ± 264.16**	3621.47 ± 356.95**
	20:4			
0	20903.33 ± 920.32	21241.53 ± 1044.85	19146.73 ± 66.57	20885.52 ± 960.56
4	18038.18 ± 810.15*	18549.28 ± 1029.73*	13211.74 ± 326.89**	12667.32 ± 985.23**
	CDOOH			
0	565.40 ± 135.68	503.57 ± 120.45	925.68 ± 107.29	631.79 ± 108.19
4	656.99 ± 224.75	440.00 ± 111.77	1580.06 ± 178.24**	1402.65 ± 467.09**

[a] $*P < 0.05$, $**P < 0.01$; CLA, conjugated linoleic acid; CDOOH, conjugated diene fatty acid hydroperoxides; EVO, extra virgin olive oil; *t*-ButylHP, *t*-butyl hydroperoxide.
[b] Results given in terms of nanomoles/g of liver, ± standard deviation.

acid in the presence of different concentrations of CLA. CLA shows modest antioxidant activity, even at the highest concentration tested (4 h protection as shown in the time scale of Fig. 3 when the ratio linoleic acid/CLA is 20:1), while in a previous paper (20), it was shown that α-tocopherol was able to block linoleic acid oxidation at a linoleic acid/α-tocopherol ratio of about 100:1. The weak antioxidant activity of CLA may be explained by competition of CLA with linoleic acid for oxidation due to the higher susceptibility of CLA to oxidation, as already suggested by Van Den Berg *et al.* (19). This is demonstrated by the decrease of CLA during oxidation of rat liver homogenate, similar to that showed by 20:4, while 18:2 did not change significantly (data not shown).

Oxidation of rat liver homogenates. As shown in Table 1, 20:4 and 22:6 significantly ($P < 0.05$) decrease during rat liver homogenate oxidation induced by ADP-Fe or *t*-ButylHP, irrespective of the treatment (EVO, the control group, or EVO with 4% CLA, the CLA group). CDOOH formation did not increase when oxidation was induced by ADP-Fe, probably due to the breakdown of CDOOH induced by iron. On the other hand, CDOOH significantly ($P < 0.01$) increases when liver homogenate is oxidized with *t*-ButylHP. No significant differences were seen between control and CLA groups, which showed no antioxidant activity.

Oxidation of lamb liver PUFA. Figure 4 shows that CLA and its metabolites CD 18:3, CD 20:3, and CD 20:4 decrease during oxidation and show a higher susceptibility to oxidation with respect to their nonconjugated parent compounds. No formation of any CD fatty acids was noted during oxidation.

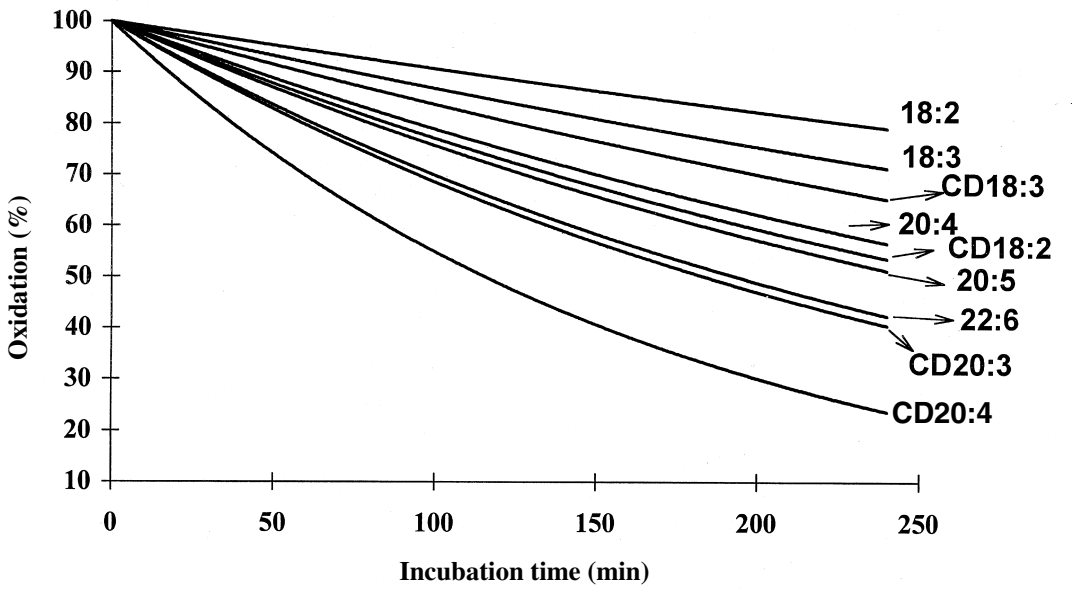

FIG. 4. Percentage of decrease during oxidation of the major conjugated dienes (CD) and nonconjugated diene fatty acids of lamb liver.

DISCUSSION

Oxidative stress is thought to be involved in the pathogenesis of several human diseases (24); a phenomenon that has come to be called "oxidative stress" involves a shift in the balance between anti- and prooxidants in favor of prooxidants. A specific role is played by free radicals and antioxidant status in the pathogenesis of tumors (24). The assessment of oxidative stress in humans is difficult owing to the lack of standardized methods for measuring it (25).

CD detection by spectrophotometric methods, one of the most frequently used markers of oxidative stress, is not always suitable because CLA is absorbed and assimilated in human and animal tissues. Therefore, our new experimental approach is envisaged to have value in those cases where the presence of CLA or CDOOH may be misinterpreted.

Although the involvement of CLA in oxidative stress, either as a product of such a process or as an antioxidant, was an intriguing hypothesis, our data demonstrate that CLA is not formed during free-radical attack on PUFA nor does it possess antioxidant activity. Furthermore, CD fatty acids are more susceptible to oxidation than their parent nonconjugated fatty acids. Alternatively, the increase of CLA in certain disease states might be related to the difficulty found by cells to metabolize this isomer under pathological conditions (26), and the anticarcinogenic and antiatherogenic activity of CLA might be related to an interference of its metabolites, such as conjugated eicosatrienoic and conjugated arachidonic acids, with eicosanoid biosynthesis (27).

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REFERENCES

1. Corongiu, F.P., and S. Banni, Detection of Conjugated Dienes by Second Derivative Ultraviolet Spectrophotometry, *Meth. Enzymol. 233*:303–310 (1994).
2. Glende, E.A., and R.O. Recknagel, Spectrophotometric Detection of Lipid-Conjugated Dienes, in In Vitro *Toxicity Indicators*, edited by C.A. Tyson and J.M. Frazier, Academic Press, San Diego, 1994, pp. 400–406.
3. Porter, N.A., L.S. Lehman, and D.G. Wujek, Oxidation Mechanisms of Polyunsaturated Fatty Acids, in *Oxygen Radicals in Chemistry and Biology,* Walter De Gruyter & Co. Berlin, 1984, pp. 235–247.
4. Banni, S., R.W. Evans, M.G. Salgo, F.P. Corongiu, and B. Lombardi, Conjugated Diene and *Trans* Fatty Acids in Tissue Lipids of Rats Fed an Hepatocarcinogenic Choline-Devoid Diet, *Carcinogenesis 11*:2053–2057 (1990).
5. Banni, S., B.W. Day, R.W. Evans, F.P. Corongiu, and B. Lombardi, Liquid Chromatography Mass Spectrometric Analysis of Conjugated Diene Fatty Acids in a Partially Hydrogenated Fat, *J. Am. Oil Chem. Soc. 71*:1321–1325 (1994).
6. Kepler, R.C., P.W. Tucker, and S.B. Tove, Biohydrogenation of Unsaturated Fatty Acids. IV. Substrate Specificity and Inhibition of Linoleate 12-*cis*,11-*trans* Isomerase from *Butyrivibrio fibrisolvens, J. Biol. Chem. 245*:3612 (1970).
7. Fogerty, A.C., G.L. Ford, and D. Svoronos, Octadeca-9,11-dienoic Acid in Foodstuffs and in the Lipids of Human Blood and Breast Milk, *Nutr. Rep. Int. 38*:937–944 (1995).
8. Banni, S., G. Carta, M.S. Contini, E. Angioni, M. Deiana, M.A. Dessì, M.P. Melis, and F.P. Corongiu, Characterization of Conjugated Diene Fatty Acids in Milk, Dairy Products, and Lamb Tissues, *J. Nutr. Biochem. 7*:150–155 (1996).
9. Banni, S., B.W. Day, R.W. Evans, F.P. Corongiu, and B. Lombardi, Detection of Conjugated Diene Isomers of Linoleic Acid in Liver Lipids of Rats Fed a Choline-Devoid Diet Indicates That the Diet Does Not Cause Lipoperoxidation, *Ibid. 6*:281–289 (1995).
10. Ha, Y.L., J. Storkson, and M.W. Pariza, Inhibition of Benzo(a)pyrene-Induced Mouse Forestomach Neoplasia by Conjugated Dienoic Derivatives of Linoleic Acid, *Cancer Res. 50*:1097–1101 (1990).
11. Ip, C., M. Singh, H.J. Thompson, and J.A. Scimeca, Conjugated Linoleic Acid Suppresses Mammary Carcinogenesis and Proliferative Activity of the Mammary Gland in the Rat, *Ibid. 54*:1212–1215 (1994).
12. Liew, C., H.A.J. Schut, S.F. Chin, M.W. Pariza, and R.H. Dashwood, Protection of Conjugated Linoleic Acids Against 2-Amino-3-methylimidazo[4,5-f]quinoline-Induced Colon Carcinogenesis in the F344 Rat: A Study of Inhibitory Mechanisms, *Carcinogenesis 16*:3037–3043 (1995).
13. Lee, K.N., D. Kritchevsky, and M.W. Pariza, Conjugated Linoleic Acid and Atherosclerosis in Rabbits, *Atherosclerosis 108*:19–25 (1994).
14. Dormandy, T.L., and D.G. Wickens, The Experimental and Clinical Pathology of Diene Conjugation, *Chem. Phys. Lipids 45*: 353–364 (1987).
15. Banni, S., L. Lucchi, A. Baraldi, B. Botti, G. Cappelli, F.P. Corongiu, M.A. Dessì, A. Tomasi, and E. Lusvarghi, No Direct Evidence of Increased Lipid Peroxidation in Hemodialysed Patients, *Nephron 72*:177–183 (1996).
16. Thompson, S., and M.T. Smith, Measurements of Diene Conjugated from Linoleic Acid in Plasma by High-Performance Liquid Chromatography: A Questionable Noninvasive Assay of Free Radical Activity? *Chem. Biol. Interact. 55*:357–366 (1985).
17. Halliwell, B., and S. Chirico, Lipid Peroxidation: Its Mechanism, Measurement, and Significance, *Am. J. Clin. Nutr. 57*: 715S–725S (1993).
18. Ip, C., S.F. Chin, J.A. Scimeca, and M.W. Pariza, Mammary Cancer Prevention by Conjugated Dienoic Derivative of Linoleic Acid, *Cancer Res. 51*:6118–6124 (1991).
19. Van Den Berg, J.J.M., N.E. Cook, and D.L. Tribble, Reinvestigation of the Antioxidant Properties of Conjugated Linoleic Acid, *Lipids 30*:599–605 (1995).
20. Banni, S., M.S. Contini, E. Angioni, M. Deiana, M.A. Dessì, M.P. Melis, G. Carta, and F.P. Corongiu, A Novel Approach to Study Linoleic Acid Autoxidation: Importance of Simultaneous Detection of the Substrate and Its Derivative Oxidation Products, *Free Radical Res. 25*:43–53 (1996).
21. Banni, S., R.W. Evans, M.G. Salgo, F.P. Corongiu, and B. Lombardi, Conjugated Diene and *Trans* Fatty Acids in a Choline-Devoid Diet Hepatocarcinogenic in the Rat, *Carcinogenesis 11*: 2047–2051 (1990).
22. Folch, J., M. Lees, and G.H. Sloane-Stanley, A Simple Method for the Isolation and Purification of Total Lipids from Animal Tissues, *J. Biol. Chem. 226*:497–509 (1957).
23. Chiang, S.D., C.F. Gessert, and O.H. Lowry, Colorimetric Determination of Extracted Lipids. An Adaptation for Microgram Amounts of Lipids Obtained from Cerumen, *Curr. List Med. Lit. Res. Rep. 33*:56–113 (1957).
24. Halliwell, B., and J.M.C. Gutteridge, *Free Radicals in Biology and Medicine*, 2nd edn., Clarendon Press, Oxford, 1989.
25. Tomasi, A., S. Banni, A. Bini, B. Botti, M.A. Dessì, and A. Ian-

none, A Critical Overview on Spin Trapping, Salicylate Oxidation, and Conjugated Dienes Determination as Probes for the Detection of Free Radical Reactions *In Vivo*, in *Free Radicals in the Environment, Medicine and Toxicology*, edited by H. Nohl, H. Esterbauer, and C. Rice-Evans, Richelieu Press, London, 1994, pp. 265–286.
26. Banni, S., E. Angioni, G. Carta, V. Casu, M. Deiana, M.A. Dessì, L. Lucchi, M.P. Melis, A. Rosa, S. Scrugli, D. Sicbaldi, E. Solla, and F.P. Corongiu, Metabolism of Conjugated Linoleic Acid in Pathological States, 88th AOCS Annual Meeting (Abstract), 1997.
27. Nugteren, D.H., Inhibition of Prostaglandin Biosynthesis by 8*cis*, 12*trans*, 14*cis*-Eicosatrienoic Acid and 5*cis*, 8*cis*, 12*trans*, 14*cis*-Eicosatetraenoic Acid, *Biochim. Biophys. Acta 121*: 171–176 (1970).

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