Oxidative Stability of Conjugated Linoleic Acids Relative to Other Polyunsaturated Fatty Acids

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ABSTRACT: Contrary to current opinion, conjugated linoleic acids (CLA) as a mixture of several isomers have been previously shown to function as prooxidants in the form of free fatty acids and methyl esters in heated canola oil. Furthermore, CLA oxidizes considerably faster than linoleic acid. However, stability of CLA relative to other polyunsaturated fatty acids remains undetermined. The present study was therefore undertaken to examine the relative oxidation rate of CLA compared with that of linolenic acid (LNA), arachidonic acid (AA), and docosahexaenoic acid (DHA) in air at 90°C. CLA, both in the form of free fatty acids and triacylglycerols, were extremely unstable to the same extent as DHA, but they oxidized considerably faster than LNA and AA. The mechanism by which CLA were readily decomposed was probably due to formation of the unstable free-radical intermediate.

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KEY WORDS: Arachidonic acid, conjugated linoleic acids, docosahexaenoic acid, linoleic acid, linolenic acid, oxidation, prooxidant.

The anticarcinogenic effect of conjugated linoleic acids (CLA) remains poorly understood, but it might be attributed to their antioxidant activity (1). However, strong evidence is lacking to substantiate that CLA is an antioxidant. Recently, van den Berg *et al.* (2) demonstrated that CLA did not act as an antioxidant in a membrane consisting of 1-palmitoyl 2-linoleoyl phosphatidylcholine. We have previously examined the effect of CLA on lipid oxidation in heated canola oil. Contrary to current thought, CLA in the form of free fatty acids or methyl esters functioned as a prooxidant, whereas in the form of triacylglycerols it acted as neither an antioxidant nor a prooxidant (3). It has been known that CLA oxidizes considerably faster than linoleic acid (18:2n-6, LA), either separately or together, when exposed to air (2,3). However, the oxidative stability of CLA relative to other polyunsaturated fatty acids remains unknown. The objective of the present study was therefore to study further the oxidation of CLA relative to more polyunsaturated fatty acids, including linolenic acid (18:3n-3, LNA), arachidonic acid (20:4n-6, AA), and docosahexaenoic acid (22:6n-3, DHA), in the form of free fatty acids or triacylglycerols.

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MATERIALS AND METHODS

Chemicals. CLA, LA, LNA, AA, and DHA in the form of free fatty acids were obtained from Sigma Chemical Company (St. Louis, MO). CLA was purified and analyzed as previously described (3) on a flexible silica capillary column (SP 2560, 100 m × 0.25 mm, i.d.; Supelco, Inc., Bellefonte, PA) in an HP 5890 Series II gas–liquid chromatograph, equipped with a flame-ionization detector (Palo Alto, CA). CLA was found to consist of the following isomers: *c*-9,*t*-11/*t*-9,*c*11, 40.3%; *t*-10,*c*-12/*c*-10,*t*-12, 43.9%; *c*-10,*c*12, 12.4%; *c*-9,*c*-11, 1.8%; *c*-9,*c*-12, 0.3%; others, 1.0%. Lipozyme IM20 [25 units/mg corresponding to 25 μmol of palmitic acid incorporated into triolein per min] was a gift from Novo Nordisk A.S. (Hong Kong).

Synthesis of triacylglycerols containing CLA, LA, LNA, AA, and DHA. Incorporation of CLA, LA, LNA, AA, and DHA into triheptadecanoin was accomplished by lipase-catalyzed interesterification (4). In brief, a mixture of 20 mg each of free CLA, LA, LNA, AA, and DHA with 100 mg triheptadecanoin, 40 mg lipozyme, and 4 mL hexane was stirred at 60°C for 6 h. The mixture was then centrifuged, and the supernatant hexane phase was saved, followed by evaporation under a gentle stream of nitrogen. An aliquot of the mixture was then subjected to thin-layer chromatography (TLC) separation (20 mg/plate) by using a solvent system of hexane/diethyl ether/acetic acid (80:20:1, vol/vol/vol). The triacylglycerol band was scratched off the plate and eluted with 60 mL of hexane/diethyl ether (9:1, vol/vol). After evaporation of hexane/diethyl ether under nitrogen, the total triacylglycerols were redissolved in hexane (10 mg/mL). To confirm and quantitate the acyl exchange of CLA, LA, LNA, AA, and DHA with haptadecanoic acid (17:0, HA) in triheptadecanoin, 5 mg of the triacylglycerols, isolated from the TLC plate, were converted to the corresponding fatty acid methyl esters. Gas–liquid chromatography analysis showed that the lipase-catalyzed acyl exchange between the free fatty acids (CLA, LA, LNA, AA, DHA) and triheptadecanoin was efficient in hexane. The resulting triacylglycerols contained 59.0% HA, 12.1% LA, 9.1% LNA, 7.8% CLA, 7.1% AA, and 4.9% DHA.

Thermal stability of CLA relative to other polyunsaturated fatty acids. One mL of hexane with 2 mg each of free CLA, LA, LNA, AA, DHA, and HA or 1 mL of hexane with 10 mg

of the triacylglycerols containing these fatty acids was delivered into each Pyrex tube (13 × 100 mm; Corning, NY). The hexane was removed under a gentle stream of nitrogen. The tube was then flushed with air and heated at 90°C. After heating, the mixture was cooled at room temperature, followed by conversion to the corresponding fatty acid methyl esters as described below. After the gas–liquid chromatographic analysis, the remaining CLA, LA, LNA, AA, and DHA in the mixture of free fatty acids or the triacylglycerols heated at 90°C were quantitated with HA as an internal standard.

Fatty acid analysis. CLA, LA, LNA, AA, DHA, and HA as a mixture of free fatty acids or triacylglycerols were converted to the corresponding fatty acid methyl esters by two methods—with 2 mL of 14% BF_3 in methanol and toluene (1:1, vol/vol) or with 2 mL of methanolic hydrogen chloride under nitrogen at 90°C for 45 min (5,6). Fatty acid methyl esters were analyzed on a flexible silica capillary column (SP 2560; 100 m × 0.25 mm, i.d.; Supelco, Inc.) in an HP 5980 Series II gas–liquid chromatograph, equipped with a flame-ionization detector and an automated injector. Column temperature was programmed from 180 to 220°C at a rate of 1°C/min and then held for 10 min. Injector and detector temperatures were set at 250 and 300°C, respectively. Hydrogen was used as the carrier gas at a head pressure of 15 psi.

Statistics. Data were expressed as mean ± SD and subjected to the analysis of variance. This was done by running data on the PC ANOVA software (PC ANOVA For the IBM Personal Computer, Version 1.1, 1985; IBM, Armonk, NY). Differences were considered to be significant when $P < 0.05$.

RESULTS AND DISCUSSION

To assess thermal stability of CLA relative to other polyunsaturated fatty acids, equal amounts of CLA, LA, LNA, AA, DHA, and HA were exposed as a mixture to air at 90°C for varying times. The stability of each fatty acid was expressed as a percentage of its original amount before oxidation, with HA being the internal standard. The results from the two methylation methods used in the present study were similar. To simplify the presentation, only data from BF_3-methanol method are shown. The oxidative rate of free CLA was similar to that of free DHA, but it was considerably faster than for free AA, LNA, and LA (Fig. 1). When exposed to air under similar conditions, CLA in triacylglycerols was oxidized faster than LA and LNA (Fig. 2). In contrast to the free fatty acids, CLA in triacylglycerols oxidized slightly slower than DHA but slightly faster than AA (Fig. 2). These data were the first to suggest that CLA in either free fatty acids or triacylglycerols is most susceptible to oxidation when compared with other polyunsaturated fatty acids, including LA, LNA, and AA. Furthermore, the oxidative susceptibility of CLA was similar to that of DHA.

It remains uncertain if a conjugated double bond is more susceptible to oxidation than a nonconjugated double bond (2,7). The present results were, however, in agreement with previous observations (2,3) in which CLA with two conjugated double bonds was more susceptible to oxidation than LA with two nonconjugated double bonds when incubated together at room temperature or 90°C. In a separate study, van den Berg (7) has demonstrated that parinaric acid with four conjugated double bonds was more susceptible to oxidation than AA with four nonconjugated double bonds. The present study further suggests that CLA was even less stable than LNA (three nonconjugated double bonds) and AA (four nonconjugated double bonds).

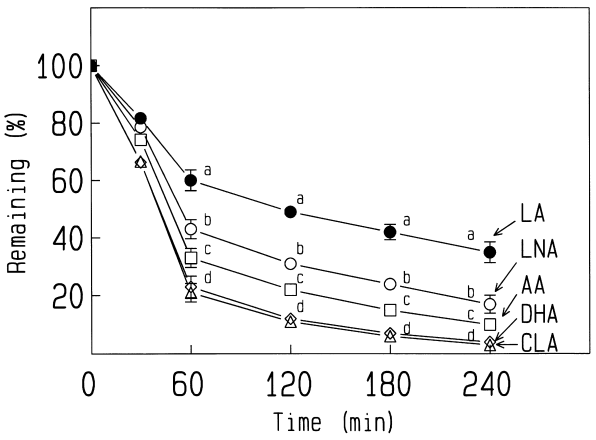

FIG. 1. Time course of the remaining free conjugated linoleic acids (CLA), free linoleic acid (LA), free linolenic acid (LNA), free arachidonic acid (AA), and free docosahexaenoic acid (DHA), heated as a mixture at 90°C. Data are expressed as mean ± SD for $n = 5$ samples. Means at the same time point with different superscript letters (a–d) differ significantly ($P < 0.05$).

An antioxidant, in general, should be an excellent donor of electrons or protons, and the resulting antioxidant free-rad-

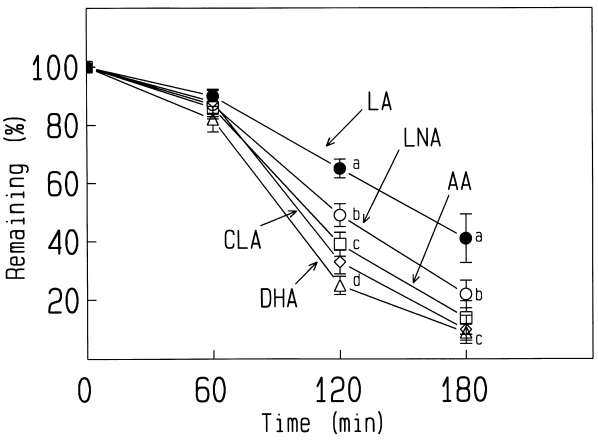

FIG. 2. Time course of the remaining conjugated linoleic acids (CLA), linoleic acid (LA), linolenic acid (LNA), arachidonic acid (AA), and docosahexaenoic acid (DHA), heated as a mixture of triacylglycerols at 90°C. Data are expressed as mean ± SD for $n = 5$ samples. Means at the same time point with different superscript letters (a–d) differ significantly ($P < 0.05$).

ical intermediate should be relatively stable. In this regard, CLA theoretically is unlikely an antioxidant. This is because, like phenolic antioxidants, CLA can readily donate an electron or a hydrogen due to resonance delocalization but, unlike phenolic antioxidants, its free-radical intermediate may not be stable and subjected to further oxidative degradation. In fact, CLA has been shown to be rapidly decomposed to form furan fatty acids (8).

In conclusion, the present study does not support the view that CLA is an antioxidant, at least under the present accelerated experimental conditions. Regardless of the length of carbon chains, CLA in triacylglycerols or free fatty acids behave like DHA and are much less stable, not only in comparison with the nonconjugated isomer, LA, but also more polyunsaturated fatty acids, including LNA and AA, when exposed to air oxidation at 90°C.

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