Conjugated linoleic acid, unlike other unsaturated fatty acids, strongly induces glutathione synthesis without any lipoperoxidation

Khelifa Arab1, Adrien Rossary2,3, Laurent Soulère4 and Jean-Paul Steghens2,3*

1Division of Toxicology and Cancer Risk Factors, German Cancer Research Center (DKFZ), Im Neuenheimer Feld 280, 69120 Heidelberg, Germany
2UF 21455, Oxidative Stress and Vitamins, Biochemistry Federation, E. Herriot Hospital, Lyon, France
3EA 3090, Claude Bernard University Lyon 1, Lyon, France
4Laboratoire de Chime Organique, UMR CNRS-UCBL 5181, Institut National des sciences appliquées, 20 avenue A. Einstein, 69621 Villeurbanne, France

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Enhancement of the redox status of cells is a cytoprotective strategy against oxidative damage. We recently showed that DHA upregulates glutathione (GSH) content via an induction of its related enzymes γ-glutamylcysteine ligase and glutathione reductase. In the present study, we investigated the effects of eight other fatty acids on the redox status and lipid peroxidation of human fibroblasts. After 48 h, only arachidonic acid and conjugated linoleic acid (CLA) enhanced GSH content through an induction of γ-glutamylcysteine ligase. CLA was more potent than arachidonic acid in inducing GSH synthesis. For all the fatty acids tested, lipoperoxidation, estimated by cell malondialdehyde measurement, did not differ from that of controls at 48 h but dramatically increased at 7 d, except for CLA. Lipoperoxidation is associated at 7 d with a high level of reactive oxygen species and with increased haemoxygenase-1 and cyclooxygenase-2 mRNA expression. As demonstrated by a tert-butyldihydroperoxide cytotoxicity test, the GSH synthesis obtained with arachidonic acid is not sufficient to protect the cells, whereas this protective effect was obvious with CLA at 48 h as well as at 7 d. The present results show that CLA is the only PUFA able to induce GSH synthesis without any change in oxidative balance, whereas an upregulation of cyclooxygenase-2 by other PUFA is concomitant with an overproduction of malondialdehyde and reactive oxygen species. The particular hairpin conformation obtained for CLA by molecular modelling could account for this specific biological effect.


An increased dietary consumption of n-3 PUFA elevates their incorporation into membrane phospholipids of cells or tissues. Thus, n-3 PUFA are claimed to exert protection against CVD and cancer. The molecular mechanisms of these effects are, however, still under study (Jump, 2002).

The potential for preventive actions in CVD was recently bolstered by the finding that PUFA, especially n-3 PUFA, have a protective effect on ischaemia–reperfusion in neuroprotection and an anti-inflammatory action (Pepe & McLennan, 2002; Mukherjee et al. 2004). EPA (C20:5n-3) and DHA (C22:6n-3) are usually consumed in small quantities and are therefore found in relatively low proportions in plasma and tissue lipids. An increased consumption of these fatty acids is, however, associated with their increased proportions in various blood and tissue lipid pools (Chan et al. 2003; Laidlaw & Holub, 2003). Paradoxically, enrichment with n-3 PUFA is thought to enhance the susceptibility of tissues to oxidation by free radicals. The susceptibility of a particular tissue to oxidation is influenced by its PUFA and antioxidant content. Some studies have investigated the effects of dietary or supplemented PUFA on the susceptibility of lipoproteins or cells to oxidation, and have demonstrated a marked reduction in the lag phase for oxidation by PUFA (Oostenbrug et al. 1994; Song et al. 2000). Not all data, however, agree that n-3 PUFA increase overall susceptibility to oxidation, and it is not clear whether the two major n-3 PUFA in fish oil, EPA and DHA, are equally potent in decreasing oxidation (Higdon et al. 2000; Mori et al. 2003).

Lipid peroxidation generates highly reactive aldehydes, such as 4-hydroxynonenal and malondialdehyde (MDA), which are involved in some pathophysiological processes. 4-Hydroxynonenal could modulate protein activity by addition reactions to cysteinyl residues, a mechanism that may explain some gene regulations via lipid peroxidation (Levonen et al. 2004). PUFA serve as substrates for several enzymes in eicosanoid metabolism, and their products play huge roles in the homeostasis of peroxide. Three isosforms of cyclooxygenase (COX), also named prostaglandin endoperoxide H synthase, exist in mammalian tissues, and these enzymes oxygenate arachidonic acid (AA; 20:4n-6) to form a substrate required for prostaglandin synthesis. COX-1 is constitutive and generates...

Abbreviations: AA, arachidonic acid; CLA, conjugated linoleic acid; COX, cyclooxygenase; γ-GCL, γ-glutamylcysteine ligase; GSH, glutathione; H2DCF-D diamidino fluorescein diacetate; HO-1, haemoxygenase-1; LA, linoleic acid; LLO, α-linolenic acid; GLA, γ-linolenic acid; MDA, malondialdehyde; OA, oleic acid; RA, arachidic acid; ROS, reactive oxygen species; t-BOOH, tert-butyldihydroperoxide.

* Corresponding author: Dr Jean-Paul Steghens, fax +33 472 11 06 75, email jean-paul.steghens@chu-lyon.fr
prostaglandins in response to hormone stimulation, whereas COX-2 is an inducible enzyme, expressed in response to stress stimuli such as cytokines and tumour promoters. The induction of COX-2 is consequently implicated in cellular damage and in several inflammatory diseases; another isoform, COX-3, which was recently discovered, is still under study (Smith et al. 2000; Ringborn et al. 2001).

Glutathione (GSH) is the main cellular antioxidant. As such, GSH is critical for maintaining cellular functions; it provides protection against increases in the level of reactive oxygen species (ROS; Meister, 1983). GSH is involved in detoxification processes via the conjugation of xenobiotics and lipid peroxidation products with γ-glutamyl S transferase. Its level is maintained by both a recycling of oxidised GSH by glutathione reductase and a de novo synthesis by γ-glutamylcysteine ligase (γ-GCL), the rate-limiting enzyme in this synthesis. The transcription of the two subunits of γ-GCL seems to be regulated by dimeric transcription factors such as small MaNF proteins and other bZIP family members, including Nrf1 and 2. In addition, these transcription factors upregulate cytoprotective genes such as haemoxigenase-1 (HO-1; Wild et al. 1999; Leung et al. 2003). Another regulation by NFκB is involved in the transactivation of γ-GCL during induced stress (Urata et al. 1996). The involvement of these transcription factors in GSH homeostasis suggests that they are self-sensitive to the cell redox environment (Wu et al. 1996).

In the present report, we have investigated, on the one hand, the effects of fatty acids on the thiol redox status of cells and their protective effects against oxidative damage, and on the other hand, the involvement of lipid peroxidation in enhancing this damage.

Materials and methods

Chemicals

Chemicals were obtained as follows: arachidonic acid (AA; 20:0), oleic acid (OA; 18:1n-9), linoleic acid (LA; 18:2n-6), α-linolenic acid (LNA; 18:3n-3), γ-linolenic acid (GLA; 18:3n-6), AA (20:4n-6), conjugated LA (CLA; 18:2 [9Z, 11E]), EPA (20:5n-3) and 4-hydroxynonenal were purchased from Cayman Chemical (Interchin, Montluc¸on, France); PBS, 2,3-diaminobutyrate and L-glutamate from Sigma-Aldrich (Saint Quentin Fallavier, France); reduced GSH, ATP, phosphoenolpyruvate and pyruvate kinase from Roche (Meylan, France); enolpyruvate and pyruvate kinase from Roche (Meylan, France); and aminobutyrate from Sigma-Aldrich. GSH was measured by bichromatic spectrophotometry (570 and 700nm) with bicinchoninic acid (Pierce, Interchin) at 37°C on a Kone analyser (Thermo Electron, La Garenne-Colombes, France) with human albumin as standard.

Fatty acid analysis

Lipids were extracted from 200 μl cell extract (approximately 10⁶ cells) with chloroform–methanol (2:1 v/v), with di-palmitoyl-d31-phosphatidylcholine as an internal standard. Fatty acid methyl esters were formed after hydrolysis by transesterification of the lipids with Meth-Prep II. Analyses were carried out on an HP 5980 gas chromatograph (Agilent, Massy, France) equipped with a flame ionisation detector maintained at 220°C, an HP 6980 auto injector (Agilent), a 1 m silanised precolumn and a 30 m × 0.32 mm × 0.2 μm SP-2340 fused silica capillary column (Supelco, Saint Quentin Fallavier, France). Results are expressed as μmol/million cells.

Thiol redox status

Oxidized and reduced glutathione. GSH was measured by LC–MS as previously described (Stephens et al. 2003). The processed sample was diluted 1:5 (v/v) in distilled water and subjected to LC–MS analysis.

γ-Glutamyl-cysteine ligase activity. The activity of γ-GCL was determined by a new kinetic LC–MS method (Chik et al. 2005). Briefly, after incubating the cell extract (three aliquots of 10 μl each) at 37°C in 70 μl of the first reagent (Tris 25 mmol/l, KCl 150 mmol/l, MgCl2 20 mmol/l, EDTA 2 mmol/l, L-glutamate 40 mmol/l, Na2ATP 5·6 mmol/l, phosphoenolpyruvate 2·2 mmol/l, pyruvate kinase 3·4 IU/ml, pH 8.6), the reaction was triggered by 20 μl 2·α-aminoaminobutyrate 20 mmol/l and then stopped after 2, 4 or 6 min (depending on the aliquot) by adding sulfo-5-salicylic acid. The slope of production of γ-glutamyl aminoaminobutyrate, measured by LC–MS, corresponded to the catalytic activity, and the results were expressed as IU/mg protein.

Real-time PCR mRNA quantification

Total RNA was isolated from fibroblasts by the total RNA isolation kit (Rneasy mini kit; Qiagen, Courtaboeuf, France). Reverse transcription was performed with 1 μg total RNA for each condition with a first-strand cDNA synthesis kit (Amersham, Orsay, France). Human-specific primers were used for β-actin, the catalytic subunit of γ-GCL, HO-1 and COX-2. Primer sequences, gene references and fragment sizes are shown in Table 1. The specificity of the products for different periods of time, up to 7 d, and the culture medium was changed every day. At 48 h or 7 d, cells were harvested by trypsinisation and washed twice with PBS 1X. Cells were collected in 500 μl Tris-HCl 50 mmol/l, pH 7.4 and Tween20 0.2%, and 50 μl were processed immediately to measure oxidised and reduced GSH, as previously described (Stephens et al. 2003). For other tests, the cells were lysed with two successive freezing and thawing operations with 15 s sonication. The cell lysates were stored at −20°C until analysis.

For fatty acid analysis, cells were collected after washing and lysed by repeated freezing and thawing in 500 μl Tris-HCl 50 mmol/l, pH 7-4. Cell lysates were stored at −80°C until analysis.

Cell lysate proteins were measured by bichromatic spectrophotometry (570 and 700nm) with bicinchoninic acid (Pierce, Interchin) at 37°C on a Kone analyser (Thermo Electron, La Garenne-Colombes, France) with human albumin as standard.

Cell culture

Human skin fibroblasts were a gift from Dr Damour of the Tissue and Cell Bank HCL, Hopital Edouard Herriot, Lyon, France. The human fibroblasts were grown in DMEM containing 10% FCS (Gibco; Pontoise, France) and 2·5 ml, 98 IU penicillin/ml and 98 μg streptomycin/ml (Cambrex, Emerainville, France). Cultures were maintained at 37°C in a humidified atmosphere with 5% CO2.

The culture medium was supplemented with each fatty acid at final concentrations in incubation or culture medium.
was demonstrated for each fragment by melting curve analysis and gel electrophoresis. The following thermocycler program was used for quantitative PCR: 10 min preincubation at 95 °C, followed by 55 cycles of 95 °C for 15 s, and then 53 °C for HO-1, 56 °C for γ-GCL, 61 °C for COX-2 for 5 s, and 72 °C for 10 s. β-Actin was used to standardise the total amount of cDNA. qPCR was carried out on a Light Cycler (Roche) by normalising all cDNA to β-actin.

**Flow cytometry analysis of reactive oxygen species production**

H₂DCF-DA, after deacetylation inside the cell, becomes a probe sensitive to ROS such as H₂O₂, the superoxide anion (O₂⁻) and hydroxyl radicals (OH). The procedure for its use was adapted from that of Carter et al. (1994). After 48 h and 7 d of culture, cells were washed twice with PBS, incubated with 2 µmol H₂DCF-DA/l in PBS at 37 °C for 30 min, and subjected to fluorescent flow cytometry analysis (Vantage; Becton Dickinson, Le Pont-de-Claix, France); the oxidation of H₂DCF was measured, with the excitation set at 488 nm, as an increase in fluorescence at 530 nm on a log scale for 10 000 events (cell counts).

**Lipid peroxidation**

After 48 h and 7 d of cell culture, intracellular MDA was measured by LC–MS using a method based on diaminonaphthalene derivatisation, as previously described (Peiro et al. 2005). The quantification was carried out with a deuterated MDA internal standard, and the derivatizations of MDA and d2-MDA were detected at m/z 195-2 and 197-2, respectively. The chromatographic mobile phase (ammonium acetate 5 mmol/l, adjusted at pH 1.8 with formic acid containing 15 % (v/v) of a methanol–acetonitrile (1:1) mix) was adjusted to enable full separation of acetaldehyde (m/z = 183.2) and MDA derivatives.

In order to exclude the effect of contaminating lipoperoxides in fatty acid preparations, we measured lipoperoxides in cell culture media, alone as a control or supplemented with 30 µmol/l fatty acids, as previously described (Arab & Stephens, 2004).

**Cell viability**

Fibroblasts were grown with different fatty acids for 48 h and 7 d, and cells were harvested in DMEM-based incubation media at a density of 1 x 10⁶ cells/well in a standard 96-well plate. The cells were then incubated at 37 °C for 4 h with different concentrations of tert-butylhydroperoxide (t-BOOH), followed by incubation at 37 °C for 3 h with the tetrazolium salt (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl tetrazolium bromide). Cell viability was measured as a whole by the tetrazolium salt assay (Mosmann, 1983), according to the protocol proposed by Thermo Electron with its kit assay. Metabolically active cells reduced the dye to purple formazan. Formazan crystals were dissolved in isopropanol with 0.4 M HCl. Absorbance was measured bichromatically at 570 and 600 nm using a multiwell plate reader (SpectraMax Model 190; Molecular Devices, Saint Grégoire, France).

**Molecular modelling of fatty acids**

Conformational studies were achieved on a PC workstation using the software package Sybyl 7.0 for Linux (Tripos Associates Inc., St Louis, MO, USA). A conformational grid search was performed on each PUFA by varying key torsion angles adjacent to double bonds. All the resulting conformations were then classified according to the increasing order of energy values. Conformational analysis of PUFA was achieved by calculating the energy of conformations obtained by relative variation of key torsion angles ω₁ and ω₂ for CLA (1296 conformations), ω₁–ω₃ for LA (1728 conformations), ω₁–ω₄ for LLA and GLA (1296 conformations), ω₁–ω₄ for AA and EPA (7776 conformations) and ω₁–ω₆ for DHA (46 656 conformations). Conformations with the lowest energy were finally minimised using the Tripos force field to give the representative preferential conformation for each PUFA.

**Statistical analysis**

Statistical analysis was carried out with GraphPad software (version 2.1; GraphPad, Deltapoint, Monterey, CA, USA). All data were expressed as means and their standard errors. Comparisons between groups were performed by one-way ANOVA with 95 % CI, followed by Dunnett’s multiple range tests.

**Results**

**Incorporation of fatty acids in cell lipids**

To assess the role of fatty acids in the modulation of thiol redox status in fibroblasts, eight fatty acids representative of the main classes were used: RA, a saturated fatty acid (20:0), OA, a MUFA (18:1n-9), three n-6 PUFA (LA (18:2n-6), GLA (18:3n-6) and AA (20:4n-6)), two n-3 PUFA (LLA (18:3n-3) and EPA (20:5n-3)) and CLA (18:2Z9, 11E)). Total fatty acids were analysed after 48 h culture in order to check that added fatty acids had been incorporated into cell lipids (Table 2). All fatty acids were highly incorporated, with an increase of at least 100 %, except for RA. OA and n-6 PUFA displayed a systematic increase in

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**Table 1. DNA sequences of sense and antisense primers used for quantitative PCR analysis**

<table>
<thead>
<tr>
<th>Name</th>
<th>Primers sense/antisense</th>
<th>Amplicon size (bp)</th>
<th>GenBank accession number</th>
</tr>
</thead>
<tbody>
<tr>
<td>β-Actin</td>
<td>5′-TCG-TGC-GTG-ACA-TTA-AGG-AG-3′</td>
<td>262</td>
<td>NM:001101</td>
</tr>
<tr>
<td>Catalytic subunit of γ-glutaryl/lysine ligase</td>
<td>5′-AGC-AGG-GAA-ATT-AAC-GAG-AG-3′</td>
<td>204</td>
<td>NM:001498</td>
</tr>
<tr>
<td>Cyclooxygenase-2</td>
<td>5′-TCT-CCT-AGA-AAA-ATT-AAC-GAG-AG-3′</td>
<td>198</td>
<td>NM:000963</td>
</tr>
<tr>
<td>Haemoglobinase-1</td>
<td>5′-ACA-GTT-GCT-GTA-GGG-GTT-AT-3′</td>
<td>247</td>
<td>NM:002133</td>
</tr>
</tbody>
</table>
their content and in that of their elongation products associated with a decrease in n-3 PUFA content.

For n-3 PUFA, LLA supplementation increased EPA content, whereas EPA supplementation had no effect on DHA. Furthermore, these results showed that PUFA n-6 elongation was more efficient than that of n-3 PUFA.

It is noteworthy that CLA supplementation did not give rise to any changes in fatty acid content.

### Fatty acid supplementation and lipoperoxides

The concentrations of lipoperoxides in the cell culture medium alone or supplemented with each fatty acid were (μmol/l): 1.9 (SE 0.15) for controls, 1.9 (SE 0.15) for RA, 2.0 (SE 0.04) for oleic acid, 1.9 (SE 0.15) for LLA, 1.9 (SE 0.10) for LLA, 2.2 (SE 0.15) for GLA, 2.1 (SE 0.12) for AA, 2.0 (SE 0.18) for LLA, 2.2 (SE 0.16) for EPA and 2.1 (SE 0.12) for CLA (n 3). We can therefore exclude the hypothesis that lipoperoxides are responsible for the biological effects we have described.

### Arachidonic acid and conjugated linoleic acid induce glutathione synthesis

Thiol redox status was assessed by measuring cell GSH content and γ-GCL activity. OA, LA and RA were tested and had no effect on thiol redox status.

During the first 48 h, AA and CLA induced a strong antioxidant response by increasing intracellular GSH (more than 100% for CLA, Fig. 1), associated with an activation of γ-GCL (Fig. 2A). The activation and induction of the γ-GCL enzyme suggests a response against oxidative conditions, as confirmed by a 3-fold increase in mRNA expression by AA and CLA. Other fatty acids had no effect on the antioxidant response (Fig. 2B).

After 7 d supplementation, AA and CLA maintained their effects by increasing GSH content and γ-GCL activity, but with a non-significant increase in the GSH level for CLA. GLA, LLA and EPA activated γ-GCL with no increase in intracellular GSH. Analysis of the mRNA expression level for the catalytic subunit of γ-GCL confirmed the induction (3–4-fold) for these three fatty acids (Fig. 2).

### Measurement of intracellular reactive oxygen species

GSH is known as the main intracellular antioxidant, and it is often assumed that its content maintains the redox status of the cell; in this way, it is related to intracellular ROS levels. For this reason, intracellular ROS production was assessed in cells supplemented with fatty acids: OA, LA and RA were tested and had no influence.

![Fig. 1. Glutathione (GSH) content measured in total cell lysate after 48 h (G)](https://doi.org/10.1017/Bnj20061910)
Conjugated linoleic acid and glutathione synthesis

Association of haemoxygenase-1 and cyclooxygenase-2 induction with lipoperoxidation

HO-1, formerly known as heat shock protein 32, is the inducible form of HO, implicated in the cell response against oxidative damage. It is also considered to be a good biomarker of the antioxidant response. Lipid peroxidation is a biomarker of oxidative damage. Thus, the association of this biomarker with HO-1 mRNA induction seems to confirm the increase in oxidative status. In addition, Uchida and colleagues reported that 4-hydroxy-2-nonenal, a lipid peroxidation byproduct, could be responsible for the elevation of COX-2 mRNA (Kumagai et al. 2004), and we could hypothesise that an induction of HO-1 and an increase in MDA are suitable conditions for COX-2 induction.

At 48 h, lipoperoxidation did not increase, whatever the fatty acid used, whereas after 7 d, EPA was the strongest inducer of lipid peroxidation (MDA 233 (SE 4·17) pmol/mg protein), with significant and similar, but lower, lipoperoxidation for AA, GLA and LLA (about 130 pmol/mg protein; Fig. 4). In this context, the quantification of the mRNA level of HO-1 showed a 3-fold induction for EPA and a lower but significant induction for GLA, AA and LLA (Fig. 5).

At 48 hours as well as at 7 d, CLA did not modify the MDA content or the HO-1 and COX-2 mRNA levels (Fig. 6). It should be noted that OA, LA and RA had no effect on either lipoperoxidation or HO-1 and COX-2 mRNA expression.
Conjugated linoleic acid protects cells from oxidative stress

In order to compare the potential protection of different fatty acids against t-BOOH-induced oxidative stress, we assessed cell viability by the methyl tetrazolium salt test after 48 h and 7 d culture with different fatty acids. As shown in Table 3, CLA was the only PUFA able to enhance cell resistance against oxidative stress at 48 h as well as at 7 d; cell resistance increased to 130%, compared with the control at 48 h and 7 d, and was associated with a high GSH cell content or GCL activity. Conversely, after 7 d, cell resistance was significantly decreased with GLA (20%), AA (15%) and EPA (27%). Cell susceptibility to t-BOOH seems to be related to intracellular MDA cell content.

Discussion

In the present report, we investigated the effect of several fatty acids on the cellular antioxidant response and on lipoperoxidation. Fatty acid supplementation increased their concentration in the cells; the present results showed that the elongation products of fatty acids increased, except for EPA, which did not modify DHA content. Conversely, we have previously shown that DHA supplementation increased EPA cell content (Arab et al. 2006). The results obtained with GLA, LA and AA suggest that n-6 elongation is more efficient than n-3 PUFA elongation in human fibroblasts. It is worth noting that CLA supplementation led to a lack of change in fatty acid content, suggesting that this effect uses a different signalling pathway.

The modulation of redox status of the cells appears to be a cytoprotective strategy to reduce oxidative damage, and we showed that GSH synthesis is upregulated by AA and CLA, mainly through an induction of γ-GCL; an induction of γ-GCL is characteristic of an antioxidant response (Lee et al. 2003; Rahman, 2005), as previously demonstrated with different compounds such as DHA and sulforaphane (Gao et al. 2001; Kim et al. 2003; Arab et al. 2006). So we tested the hypothesis that other PUFA could induce an antioxidantive response and could explain their beneficial effect in preventing oxidative damage (Manna et al. 1999; Suh et al. 2004). It is noteworthy that the results in Table 2 are for total cell fatty acids (i.e. mainly fatty acids in membrane phospholipids) and that GLA, a precursor of AA, unlike AA, is unable to induce GSH content and other protective enzymes. We may thus suppose that the fatty acids which efficiently induce an antioxidant response are in a ‘free form’.

As a consequence of their chemical structure, which is very sensitive to free radicals, it is assumed that the oxidation of n-3 PUFA generates byproducts such as 4-hydroxynonenal and MDA, which seem to play a role in the exacerbation of oxidative damage. Conversely, EPA is often described as a potential cytoprotective agent against oxidative stress by downregulating proinflammatory genes, interleukin-8 and COX-2 (Obata et al. 1999; Storey et al. 2005). For EPA, in our model, the present results enable us to exclude the enhancement of thiol redox status to explain this cytoprotection as well as the downregulation of genes such as COX-2. However, the ability of EPA and other n-3 PUFA to induce apoptosis and tumour suppression through lipid peroxidation is well described (Lynch et al. 2003; Utomo et al. 2004; Ng et al. 2005) and is in excellent agreement with the large production of MDA detected in our human fibroblasts and in an MRC5 cell culture model (data not shown). We suggest that this lipoperoxidation may be a protective mechanism against malignancy and carcinogenesis (Maheo et al. 2005).

The presence of protein-bound lipoperoxidation products has been reported in many chronic diseases in man, including atherosclerosis and neurodegenerative disorders (Terasawa et al. 2000; Liu et al. 2005). On the one hand, lipoperoxidation is associated with an induction of HO-1, an association reported to be a defence mechanism during oxidative injury (Hill-Kapturczak et al. 2003; Kim et al. 2005). On the other hand, the involvement of COX-2 in lipoperoxidation has been demonstrated by several studies, but the effect of lipoperoxidation products on its expression is less known. In addition, some reports have indicated that 4-hydroxynonenal induces COX-2 mRNA and promotes oxidative damage (Nanj et al. 2001; Ishii et al. 2004; Kumagai et al. 2004). COX-2 might then, via lipoperoxidation, mediate the formation of DNA adducts and increase the risk of tumorigenesis (Lee et al. 2005). Taking these relations into account, we tested the hypothesis that lipoperoxidation products might be involved in the upregulation of COX-2 and compared the level of MDA, a terminal lipoperoxidation product, with ROS production and COX-2 and HO-1 induction. Except for CLA, the present results showed that a high production of MDA was associated with a high induction of HO-1, COX-2 and ROS production. The fact that COX-2 is suggested to be

![Fig. 6. mRNA expression level of cyclooxygenase-2 (COX-2) in cells supplemented with each fatty acid. At 48 h (C) and 7 d (Q). Data are expressed as means with their standard errors indicated by vertical bars (n=3). GLA, γ-linolenic acid; AA, arachidonic acid; LLA, α-linolenic acid; CLA, conjugated linoleic acid. Mean values were significantly different from those of the control group: *P<0.05.](https://www.cambridge.org/core/core/10.1017/BJN20061910)
auto-inducible by its preferential substrate AA could explain the elevation in COX-2 mRNA, detected as soon as 48 h, when cells are supplemented with AA.

The measurement of cell viability in the presence of t-BOOH was used as a further test to check cell protection: only CLA protected cells against oxidative damage induced by t-BOOH (Table 3). For CLA, this protection seems to be mediated by the high level of GSH; the same protective effect was found for DHA (EC₅₀ 0.95 (SE 0.03) mmol/l; data not shown), which also induced a strong antioxidant response associated with a 4-fold increase in GSH at 7 d (Arab et al. 2006). The elevated MDA content and the induction of COX-2, associated with a lower increase in GSH in cells supplemented with AA, could explain the absence of a protective effect of GSH against oxidative damage. Cell viability might be dependent on a balance between thiol redox status and lipoperoxidation. The model with GLA and EPA is in agreement with this hypothesis, and further work is necessary to confirm whether there is a link between lipoperoxidation and COX-2 induction.

CLA (18:2[9Z, 11E]) is a member of the linoleic acid isomers, described in the 1990s as potent bioactive molecules against insulin resistance and recently as agonists of PPAR-γ (Ryder et al. 2001; Kuniyasu et al. 2006). We used CLA (18:2[9Z, 11E]) because it is the most abundant isomer in the diet of man. The study of the dose effect for AA and CLA showed that both were already significantly active at 6 μmol/l (Supplementary Fig.) but with a different slope for each fatty acid. In addition, CLA induced GSH synthesis, without any induction of ROS production (even at 4 or 8 h; data not shown). This demonstrates that GSH synthesis was induced through a mechanism independent of ROS production. As the ability of CLA to enhance GSH content relies on γ-GCL induction, and the main inducers of this activity are ROS and PPAR-γ, our results suggest that the induction of γ-GCL by CLA, unlike other PUFA, could be mediated through PPAR-γ.

To explain this specific behaviour of CLA, a structure–activity relationship was attempted through a conformational study of PUFA. The molecular modelling study was carried out in order to delineate preferential conformations that PUFA would adopt in a biological medium. This conformation analysis of PUFA clearly shows the following:

1. Double bonds induce the presence of curved regions.
2. The packed conformation that allows intramolecular hydrophobic interactions increases with the degree of unsaturation of the PUFA. The position of the double bonds also enhances these interactions in GLA, for example, compared with LLA (two double bonds each).
3. Particular preferential conformations are remarkable for CLA (hairpin shape) and for AA, EPA and DHA (globular shape; Fig. 7).

Except for GLA, whose conformation is difficult to classify, this study suggests that the particular hairpin conformation of CLA (similar to that of the isomer trans-10, cis-12; data not shown) may allow specific hydrophobic interactions with proteins and could explain its specific interaction with PPAR-γ.

In the present report, we showed that, of the eight fatty acids tested, AA and CLA were the most potent inducers of GSH synthesis, and only CLA had a protective action, due to GSH synthesis without lipoperoxidation. The supposed relationships between fatty acid structures and their actions need to be explored to clarify the role of CLA and its isomers in modulating the redox status of the cell. The protective effect of a CLA-rich diet has already been described and could be related to the enhancement of the thiol redox status described here.

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References


Ryder JW, Portocarrero CP, Song XM, et al. (2001) Isoemer-specific antiabetic properties of conjugated linoleic acid. Improved...
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