LIPID PEROXIDATION OF MICROSOMES OBTAINED FROM RAT LIVER AND KIDNEY: EFFECT OF *CIS*-9, *TRANS*-11-CONJUGATED LINOLEIC ACID ISOMER

Peroxidación lipídica de microsomas obtenidos del hígado y riñón de rata: efecto del isómero CIS-9, TRANS-11 del ácido linoléico.

Alejandro Palacios[1*], Viviana Angelica Piergiacomi[2]

[1]Cátedra de Bioquímica, Facultad de Ciencias Veterinarias, Universidad Nacional de La Plata, CC 296, B1900AVW, La Plata, Argentina. [2]Comisión de Investigaciones Científicas y Técnicas de la Provincia de Buenos Aires (CIC), Argentina.

*Corresponding author: Alejandro Palacios. Fax: +54-221-425-7980 E-mail address: apalacios@fcv.unlp.edu.ar

ABSTRACT

The polyunsaturated fatty acid composition, chemiluminescence and peroxidizability index of microsomes obtained from rat liver and kidney were studied after oral administration of *cis*-9, *trans*-11-conjugated linoleic acid isomer (c9-t11-*CLA*). After incubation of microsomes in an ascorbate Fe^{++} system (120 min at 37°C) it was observed that the total counts per min/mg protein originated from light emission: chemiluminescence was lower in liver and kidney microsomes in the c9-t11-*CLA* group than in the microsomes obtained from control group. The effect of c9-t11-*CLA* on the polyunsaturated fatty acid composition of native liver microsomes was evidenced by a statistically significant $p<0.007$ decrease of linoleic acid C18:2 n6. When peroxidized microsomes obtained from liver and kidney of both groups (control and c9-t11-*CLA*) were compared with respective natives, it was observed that C18:2 n6, C20:4 n6 decreased in all membranes used in this work, whereas in microsomes obtained from liver c9-t11-*CLA* and control groups C22:6 n3 also decreased. As a consequence, the peroxidizability index – a parameter based on the maximal rate of oxidation of fatty acids – showed significant changes in liver and kidney microsomes. These changes were less pronounced in membranes derived from rats receiving c9-t11-*CLA per os*. Our results would confirm and extend previous observations which indicated that *CLA* could act as an antioxidant, protecting membranes from deleterious effects.

Key words: conjugated linoleic acid, microsomes, peroxidation, chemiluminescence.

RESUMEN

La composición de ácidos grasos, la quimioluminescencia y el índice de peroxidabilidad de microsomas obtenidos de hígado y riñón de rata fueron estudiados después de la administración oral del isómero ácido linoleico conjugado-9-*cis,* 11-trans (c9-t11-*ALC*). Mediante la incubación de microsomas en un sistema ascorbato-Fe^{++} (120 minutos en 37°C), se observó que las cuentas totales por minuto/mg de proteína, originadas por la quimioluminiscencia, eran más bajas en microsomas de hígado y riñón pertenecientes al grupo c9-t11-*ALC* que en los microsomas obtenidos de grupo control. El efecto de c9-t11-*ALC* sobre la composición de ácidos grasos poli-insaturados de microsomas hepáticos nativos fue evidenciado por una disminución estadísticamente significativa $p<0.007$ del ácido linoleico C18:2 n6. Cuando los microsomas peroxidados obtenidos de hígado y riñón, de ambos grupos (control y c9-t11-*ALC*), fueron comparados con sus respectivos nativos, se observó que C18:2 n6, C20: 4 n6 disminuyeron en todas las membranas microsomales usadas en este trabajo, mientras que en los microsomas obtenidos de hígado del grupo c9-t11-*ALC* y grupo control también disminuyó C22: 6 n3. Por consiguiente, el índice del peroxidabilidad – parámetro basado en el índice máximo de oxidación de ácidos grasos – mostró cambios significativos en microsomas de hígado y riñón. Estos cambios fueron menos pronunciados en las membranas microsomales obtenidas de las ratas que recibieron c9-t11-*ALC* por vía oral. Nuestros resultados confirmarían y ampliarían observaciones anteriores que indicaron que *ALC* podría actuar como antioxidante, protegiendo las membranas contra efectos dañinos.

Palabras clave: ácido linoleico conjugado, microsomas, peroxidación, quimioluminiscencia

INTRODUCTION

Conjugated linoleic acid (CLA) is a collective term generally referred to a mixture of positional and geometric conjugated dienoic isomers of linoleic acid, Kramer et al. (1997). Numerous CLA isomers are found in milk-fat, cheese and beef, (Parodi 1997; Sehat et al. 1998). The cis-9, trans-11 isomer of CLA (the principal dietary form) is produced in the rumen of cattle and other ruminants during the microbial biohydrogenation of linoleic and linolenic acids (Kepler et al. 1966; Sehat et al. 1999). CLA is an *in vitro* antioxidant, and it protects membranes from oxidative attack within cells, Sies (1997). It is evident that CLA reduces tumor incidence as well as tumor multiplicity, (Ha et al. 1999; Sebedio et al. 1999).

The mechanism through which CLA inhibits tumor genesis is proposed, O´shea et al. (2000). In relation to other important dietary antioxidants, it quenches singlet oxygen more effectively than α-tocopherol. It appears to act as a chain-breaking antioxidant, by trapping chain-propagating free radicals. Pariza and Ha (1990a) have demonstrated that CLA is a potent antioxidant and that cis-9, trans-11 isomer is selectively incorporated into cellular phospholipids, which may explain, at least in part, the anticarcinogenic activity of CLA as antioxidant. Other reports indicated that CLA exhibited antioxidant properties *in vivo,* Risérius et al. (2004) and *in vitro,* Ha et al. (1999).

The relationship between antioxidant-enzyme defense responses and cellular growth suppression in human cancer cells exposed to CLA in cultures, has also been studied. The activities of superoxide dismutase (SOD), catalase and glutathione peroxidase (Gpx) were induced in cell lines exposed to CLA. The data indicate that CLA-induced cytotoxicity against cancer lines is related to the extent of lipid peroxidation of CLA treated cells, and affirm that the CLA-induced antioxidant enzymes failed to protect these cells from cytotoxic lipid peroxidation products, (O´shea et al. 1999; Richter 1987).

One might speculate that the inhibition of carcinogenesis by CLA could result from the combined effects of a number of CLA activities, possibly including direct effects of

one or more CLA isomers/metabolites on cell differentiation and the effects of one or more CLA isomers on prostaglandin metabolism, which may also influence cancer development at some sites, (Corl et al. 2003; Marnett 1992). Animal fat, which has been maligned for so long, may actually contribute with a potent therapeutic component to our diet, Kritchevsky (2000).

In previous works, we have demonstrated that rat liver microsomal and mitochondrial membranes are protected by vitamin A and CLA (mixture of positional and geometric conjugated dienoic isomers of linoleic acid) when subjected to non enzymatic lipoperoxidation, Palacios et al. (1996). The present study aims to compare the polyunsaturated fatty acid composition and non enzymatic lipid peroxidation of rat liver and kidney microsomes obtained from animals supplemented with conjugated linoleic acid cis-9, trans-11 isomer, (Paradies et al. 1999; Cai et al. 2003).

MATERIAL AND METHODS

Chemicals

Conjugated octadecadienoic acid (c9-t11-CLA) was generously supplied by Natural Inc. (Vernon Hills, IL 60061, USA). Butylated hydroxytoluene (BHT) was purchased from Sigma Chemical Co. (St. Louis, MO). BSA (fraction V) was obtained from Wako Pure Chemicals Industries Ltd; Japan. Standards of fatty acids methyl esters were kindly supplied by NU Check Prep, Inc, Elysian, MN, USA. L[+] ascorbic acid and boron trifluoride-methanol complex were obtained from Merck Laboratories. All other reagents and chemicals were of analytical grade from Sigma.

Animals

Female Wistar AH/HOK rats (7 weeks-old, 120-137 g) were used. The rats were obtained from Laboratory Animal Facility, Facultad de Ciencias Veterinarias, Universidad Nacional de La Plata. Two groups of ten rats were considered, group A: received conjugated *cis*-9, *trans*-11-conjugated linoleic acid isomer (c9-t11-*CLA*), group B: controls. Each group (control and c9-t11-CLA) have two types of microsomes: a) native (without peroxidation) and b) peroxidized (obtained after incubation in ascorbate Fe^{++} system). All rats were fed with commercial rat chow and water was administered *ad libitum*. Group A received daily 12.5 mg oral administration of c9-t11-*CLA* for 10 days. On day 11, all the rats were sacrificed by cervical dislocation and their liver and kidneys were rapidly removed.

Preparation of microsomes

Liver and kidney were cut into small pieces and washed extensively with 0.15 M NaCl. A homogenate 30% (w/v) was prepared in a solution (0.25 M sucrose, 10 mM Tris-HCl pH 7.4) using the Potter-Elvejhem homogenizer. The homogenate was spun at 10,000 g for 10 min. The supernatant (3 ml) obtained was applied to a Sepharose 4B column (1.6 x 12 cm) equilibrated and eluted with 10 mM Tris-HCl (pH 7.4), 0.01 % NaN_3. The microsomal fraction appearing in the void volume (8-12 ml) was brought to 0.25 M sucrose by addition of solid sucrose. Regarding the concentrations and activities of certain microsomal enzymes, the quality of this microsomal preparation has a similar composition to that obtained by ultracentrifugation, Tangen et al. (1973).

Lipid peroxidation of rat liver and kidney microsomes and chemiluminescence assay

Chemiluminescence and lipid peroxidation were initiated by adding ascorbate to microsomes, Wright et al. (1979). Microsomes (1 mg protein) were incubated at 37 °C with 0.01M phosphate buffer pH 7.4, 0.4 mM

ascorbate; final volume 2 ml. Phosphate buffer was contaminated with enough iron to provide the necessary ferrous or ferric iron for lipid peroxidation (final concentration in the incubation mixture was 2.15 μM), Tadolini et al. (1997). Organelle preparations, which lacked ascorbate, were carried out simultaneously. Membrane light emission was determined over a 120 min period, chemiluminescence was recorded as cpm every 10 min and the sum of the total chemiluminescence was used to calculate cpm/mg protein. Chemiluminescence was measured as counts per min in liquid scintillation analyzer Packard 1900 TR equipment with software for chemiluminescence measurement.

Lipid peroxidation is a branching chain reaction which can be considered as taking place in four main stages: (1) chain initiation, (2) chain propagation, (3) chain branching and (4) chain termination. At least three reactions are known to break the chains: (a) interaction of two radicals leading the chains, (b) interaction of one radical with changing valency metal, and (c) reaction between such a radical and a molecule of "antioxidant".

$LO_2^* + LO_2^*$ $(k) \rightarrow P^* \rightarrow P + \varphi h\nu$ (chemiluminescence)

Reaction (a) is particularly interesting since it is accompanied by chemiluminescence whose intensity (*I)* may serve as a measure of peroxide free radical (LO_2^*) concentration according to the following equation:

$$I = K \varphi k [LO_2^*]^2$$

Where φ represents the chemiluminescence quantum yield and *k* the coefficient depending on the net sensitivity of the instrument. LO_2^* is a free radical produced from lipids molecules, Vladimirov et al. (1980).

Fatty acid analysis

Microsomal lipids were extracted with chloroform/methanol (2:1 v/v), Folch et al. (1957), from both native and peroxidized membranes. Fatty acids were transmethylated with F_3B in methanol at 60 °C for 3 h. Fatty acids methyl esters were analyzed with a GC-14 A gas chromatograph (Shimadzu, Kyoto, Japan) equipped with a packed column (1.80 m x 4 mm i.d.) GP 10 % DEGS-PS on 80/100 Supelcoport. Nitrogen was used as the carrier gas. The injector and detector temperatures were maintained at 250°C, column temperature was held at 200°C. Fatty acid methyl ester peaks were identified by comparison of the retention times with those of standards.

Peroxidizability Index

Peroxidizability Index (PI) was calculated according to the formula PI= (percent of monoenoic acids x 0.025) + (percent of dienoic acids x 1) + (percent of trienoic acids x 2) + (percent of tetraenoic acids x 4) + (percent of pentaenoic acids x 6) + (percent of hexaenoic acids x 8), Pirozhkov et al. (1992).

Other biochemical methods

Proteins were determined by the Method of Lowry et al. (1951).

Statistical analysis

Results are expressed as mean ($\overline{\chi}$) ± standard deviation (SD) of three independent determinations. Results were statistically evaluated by one-way analysis of variance (ANOVA) and Tukey test. The statistical criteria for significance were selected at different *p* values, which were indicated in each case.

RESULTS

Polyunsaturated fatty acid composition of total lipids from liver and kidney microsomes (native vs. peroxidized)

The polyunsaturated fatty acid composition of total lipids isolated from rat liver and kidney microsomes obtained from c9-t11-*CLA* and control groups is shown in Tables 1 and 2. The changes in fatty acids composition of organelles from control group were more significant than those from c9-t11-*CLA* group. The effect of c9-t11-*CLA* on the polyunsaturated fatty acid composition of native liver microsomes was evidenced by a statistically significant ($p<0.007$) decrease of linoleic acid C18:2 n6. When peroxidized microsomes obtained from liver and kidney of both groups (control and c9-t11-*CLA*) were compared with their respective natives, it was observed that C18:2 n6, C20:4 n6 and C22:6 n3 decreased in all membranes used in this work.

After lipid peroxidation using ascorbate-Fe^{++} system, the content of C20:4 n6 and C22:6 n3 in liver microsomes obtained from c9-t11-*CLA* group was less affected than that one of control group. In kidney microsomes obtained from the *CLA* group C22:6 n3 was less affected in comparison with control group.

Light emission of microsomes obtained from liver and kidney during lipid peroxidation

The incubation of microsomes in the presence of ascorbate-Fe^{++} system resulted in the peroxidation of membranes, as evidenced by emission of light (chemiluminescence). It was observed that the total cpm/mg of protein originated from light emission, was significantly lower in liver and kidney microsomes from c9-t11-*CLA* treated animals compared to the control group (Table 3).

Peroxidizability Index

There were marked differences when peroxidizability index (PI) of peroxidized liver and kidney microsomes from control and c9-t11-*CLA* groups were compared; in this case, the PI of c9-t11-*CLA* group was higher, this meaning more protection against lipid peroxidation (Table

Table 1. **Polyunsaturated fatty acid composition (area %) of native and peroxidized microsomes from control group ($\bar{\chi}$± SD of three experiments).**

fatty acid	liver		kidney	
	native	peroxidized	native	peroxidized
C18:2 (n-6)	$11.621\pm1.678^{a,1}$	3.255 ± 1.761^{a}	22.612 ± 2.984^{b}	4.563 ± 2.850^{b}
C20:4 (n-6)	21.622 ± 2.057^{c}	$2.831\pm0.408^{c,3}$	12.921 ± 2.525^{e}	2.624 ± 0.637^{e}
C22:6 (n-3)	2.531 ± 0.834^{d}	$0^{d,2}$	0.979 ± 0.096^{f}	$0^{f,4}$

Native: without incubation in ascorbate Fe^{++} system; peroxidized: with incubation in the presence of 0.4 mM ascorbate. Statistically differences between native vs. peroxidized microsomes of liver and kidney are indicated by $p^{a}<0.006$, $p^{b}<0.003$, $p^{c}<0.002$, $p^{d}<0.04$, $p^{e}<0.0001$, $p^{f}<0.009$. Statistically differences between fatty acids of Table 1 and 2 are indicated by $p^{1}<0.007$, $p^{2}<0.003$, $p^{3}<0.02$ and $p^{4}<0.001$.

Table 2. **Polyunsaturated fatty acid composition (area %) of native and peroxidized microsomes from c9-t11-CLA group ($\bar{\chi}$± SD of three experiments).**

fatty acid	liver		kidney	
	native	peroxidized	native	peroxidized
C18:2 (n-6)	8.032±1.632 [a,1]	3.122±0.732[a]	22.144±1.727[d]	7.601±1.272[d]
C20:4 (n-6)	20.498±2.035[b]	6.028±1.036 [b,3]	13.006±2.325[e]	3.144±0.638[e]
C22:6 (n-3)	2.842± 0.655[c]	0.978± 0.123[c,2]	1.035±0.379	0.405±0.096[4]

Native: no incubation in ascorbate Fe^{++} system; peroxidized: incubation in the presence of 0.4 mM ascorbate. Statistically differences between native vs. peroxidized microsomes of liver and kidney are indicated by $p^a < 0.01$, $p^b < 0.002$, $p^c < 0.02$, $p^d < 0.001$, $p^e < 0.001$. Statistically differences between fatty acids of Table 1 and 2 are indicated by $p^1 < 0.007$, $p^2 < 0.003$, $p^3 < 0.02$ and $p^4 < 0.001$.

Table 3. **Total chemiluminescence (cpm) of rat liver and kidney microsomes oxidized by ascorbate-Fe^{++} ($\bar{\chi}$± SD of three experiments).**

	liver		kidney	
	control	c9-t11 CLA	control	C9-t11-CLA
light emission	1432 ± 107 [a]	1126 ± 102 [a]	1234 ± 098 [b]	936 ± 59 [b]
inhibition (%)	0	27,1	0	24,1

Statistically differences between CLA vs. control group are indicated by $p^a < 0.005$, $p^b < 0.008$.

Table 4. **Peroxidizability Index ($\bar{\chi}$± SD of three experiments).**

		liver	kidney
control group	native	157.235± 12.932[a]	121.057± 6.025
	perox.	19.047± 1.215[b]	43.975± 7.163[c]
c9-t11-CLA group	native	127.456± 6.784[a]	120.672± 5.759
	perox.	57.962± 5.864[b]	64.096± 4.374[c]

Perox.: peroxidized. Statistically significant differences are indicated by $p^a < 0.02$, $p^b < 0.001$ and $p^c < 0.02$.

4). Significant changes in the peroxidizability index were also observed in microsomes from liver and kidney. These changes were less pronounced in those membranes derived from rat receiving c9-t11-*CLA*. The PI of native liver microsomes obtained from c9-t11-*CLA* group was lower than the PI of those ones obtained from liver microsomes of control group, but higher in peroxidized organelles of c9-t11-CLA group. These results are in agreement with the results shown in Table 3 obtained by chemiluminescence. This suggests that conjugated linoleic acid acts by decreasing polyunsaturated fatty acids content in native membranes, thus protecting them against lipid peroxidation. The changes observed in the fatty acid composition of membranes subjected to lipid peroxidation in the presence of ascorbate -Fe^{++}, also revealed an important decrease in the relative content of those fatty acids with higher level of unsaturation. As a result, the peroxidizability index of the peroxidized membranes obtained from liver and kidney in the c9-t11-*CLA* group was significantly higher than the one in the control group.

DISCUSSION

Although considerable research has already been performed to characterize the changes in structure, composition and physical properties of membranes subjected to oxidation, (Udilova et al. 2003; Satoru et al. 2001), it is important to know how biological compounds with antioxidant properties contribute to the protection of specialized membranes against deleterious effects produced by reactive oxygen species and other free radicals. Evidence leading to the recognition of the anticarcinogenic activity of the conjugated dienoic derivatives of linoleic acid has been reviewed. Recent results indicated that CLA has potent antioxidant activity, Liangli et al. (2002). Because the c9-t11-*CLA* isomer is present in its esterified form, it may represent a heretofore unrecognized in situ defense mechanism against membrane attack by oxygen radicals, Pariza and Ha (1999b).

In relation to other important dietary antioxidants, it quenches less singlet oxygen, being therefore more effective than α-tocopherol, it appears to act as a chain-breaking antioxidant, by trapping chain propagating free radicals, McGuire and McGuire (2000). One might speculate that the inhibition of carcinogenesis by CLA could result from the combined effects of a number of CLA activities, possibly including direct effects of one or more CLA isomers/metabolites on cell differentiation, Kang et al. (2003). Effects of CLA on vitamin A metabolism that would also influence cell differentiation, and effects of one or more CLA isomers on prostaglandin metabolism, Risérius et al. (2004), which may also influence cancer development at some sites, Corl et al. (2003).

In vitro lipid peroxidation studies are useful for the elucidation of a possible mechanism of peroxide formation in vivo, Benzi (1988), since the high concentration of polyunsaturated fatty acids in membranes causes susceptibility to lipid peroxidative degradation. Previous investigations from our laboratory have shown that the fatty acid composition of rat liver microsomes is modified after non-enzymatic peroxidation in the presence of ascorbate-Fe^{++}, Piergiacomi et al. (2001). In previous works carried out in our laboratory the antioxidant effect of a CLA isomer mixture was studied (Palacios et al. 2003; Piergiacomi et al. 2006). Due to the wide variety of isomeric forms present in this mixture the study using pure isomers to determine the antioxidant activity of each one of them was chosen. In the study reported here, the effect of conjugated linoleic acid (c9-t11-*CLA*) on the polyunsaturated fatty acid composition, chemiluminescence and peroxidizability index of microsomes isolated from rat liver and kidney were analyzed.

The effect of c9-t11-*CLA* on the polyunsaturated fatty acid composition of native liver microsomes was evidenced by

a statistically significant decrease of linoleic acid C18:2 n6, when compared with control group. Rat liver and kidney or microsomes obtained from c9-t11-*CLA* group were protected against lipid peroxidation when compared to similar organelles obtained from control rats, as shown by the results obtained by chemiluminescence, polyunsaturated fatty acid composition and peroxidizability index. The analysis of PI of peroxidized microsomes and microsomal membranes revealed that c9-t11-*CLA* group was more resistant against lipid peroxidation, compared to the control group. In our experimental conditions, before incubation in an ascorbate Fe^{++} system, we also found changes in the polyunsaturated fatty acid composition of microsomes from liver of rats treated with CLA when compared to control group, being the latter in agreement with previous results derived from other studies, (Belury and Kempa-Steczko 1997; Furr and Olson 1989).

Our results show that c9-t11-*CLA* decrease the content of polyunsaturated fatty acids in microsomes of liver; another studies state that CLA does not appear to act as an antioxidant, but its ability to decrease polyenoic fatty acid concentration could decrease the formation of highly cytotoxic lipid peroxidation products, Belury and Kempa-Steczko (1997). In contrast, other reports suggested an antioxidant mechanism of CLA, (Pariza and Ha 1990b; Pariza et al. 1999). Some authors have also reported a higher antioxidant activity for the isomer trans-10, cis-12-Conjugated Linoleic Acid Isomer compared to the isomer used in this work, Yan and Rui (2000). Nevertheless, the same authors postulate that CLA may induce both non-enzymatic and enzymatic in vivo lipid peroxidation, (Basu et al. 2000; Leung and Liu 2000).

In conclusion, our results are consistent with the hypothesis that c9-t11-*CLA* consumption may provide a previously unrecognized in situ defense mechanism against membrane attack by oxygen radicals in lipid membranes. Further studies are needed for a more adequate evaluation of these observations.

ACKNOWLEDGMENTS

We are grateful to Macarena Nasif and Lola Moya for her excellent technical assistance.

REFERENCES

BASU S., SMEDMAN A., VESSBY B. 2000. Conjugated linoleic acid induces lipid peroxidation in humans. FEBS Lett. 468: 33-36.

BELURY M..A., KEMPA-STECZKO A. 1997. Conjugated linoleic acid modulates hepatic lipid composition in mice. Lipids 32: 199-204.

BENZI G.1988. Peroxidation, Energy Transduction and Microsomes during Aging, Jhon Libery Eurotext, London, 51-117.

CAI Y.J., FANG J.G., MA L.P., YANG L., LIU Z.L. 2003. Inhibition of free radical-induced peroxidation of rat liver microsomes by resveratrol and its analogues. Biochim. Biophys. Acta 1637: 31-38.

CORL B.A., BARBANO D.M., BAUMAN D.E., IP C. 2003. *cis*-9, *trans*-11 CLA Derived Endogenously from *trans*-11 18:1 Reduces Cancer Risk in Rats. J. Nutr. 133: 2893-2900.

FOLCH J., LEES M., SLOANE STANLEY G.H. 1957. A simple method for the isolation and purification of total lipids from animal tissues. J. Biol. Chem. 226: 497-509.

FURR H.C., OLSON J.A.1989. The effect of high vitamin A intake on fatty acid composition of rat liver retinyl esters and total liver fatty acids. Nutr. Res. 9: 395-403.

HA Y.L., STORKSON J., PARIZA M.W. 1999. Inhibition of benzo(a)-pyrene-induced mouse forestomach neoplasia by conjugated dienoic derivatives of linoleic acid. Cancer Res. 50: 1097-1110.

KANG K., LIU W., ALBRIGHT J.K., PARK Y., PARIZA M.W. 2003. *trans*-10, *cis*-12 CLA inhibits differentiation of 3T3-L1 adipocytes and decreases PPARγ expression. Biochem. Biophys. Res. Comm. 303: 795-799.

KEPLER C.R., HIRONS K.P., MCNEILL J.J., TOVE S.B. 1966. Intermediates and products of the biohydrogenation of linoleic acid by Butyrivibrio fibrisolvens. J. Biol. Chem. 241: 1350-1354.

KRAMER J.K., FELLNER V., DUGAN M.E., SAUER F.D., MOSSOBA M.M., YURAWECZ M.P. 1997.Evaluating acid and base catalysts in the methylation of milk and rumen fatty acids with special emphasis on conjugated dienes and total trans fatty acids. Lipids 32: 1219-1228.

KRITCHEVSKY D. 2000. Antimutagenic and some other effects of conjugated linoleic acid. British J. Nutr. 83: 459-465.

LEUNG Y.H., LIU R.H. 2000. Trans-10, cis-12-conjugated linoleic acid isomer exhibits stronger oxyradical scavenging capacity than cis-9, trans11-conjugated linoleic acid isomer. J. Agric. Food Chem. 48: 5469-5475.

LIANGLI Y., DWAYNE A., MICHAEL G. 2002. Conjugated Linoleic Acid Isomers Differ in Their Free Radical Scavenging Properties. J. Agric. Food Chem. 50: 4135 -4140.

LOWRY O.H., ROSEBROUGHT N.J., FARR A.L., RANDALL R.J. 1951. Protein measurement with Folin phenol reagent. J. Biol. Chem. 193: 265-275.

MARNETT L.J.1992. Aspirin and the potential role of prostaglandins in colon cancer. Cancer Res. 52: 5175-5589.

MCGUIRE M.A., MCGUIRE M.K. 2000. Conjugated linoleic acid (CLA): A ruminant fatty acid with beneficial effects on human health. J. Anim. Sc. 77: 1-8.

O´SHEA M., STANTON C., DEVERY R. 1999. Antioxidant enzyme defense responses of human MCF-7 and SW480 cancer cells to conjugated linoleic acid. Anticancer. Res. 19: 1953-1959.

O´SHEA M., DEVERY R., LAWLESS F., MURPHY J., STANTON C. 2000. Milk fat conjugated linoleic acid (CLA) inhibits growth of human mammary MCF-7 cancer cells. Anticancer. Res. 20: 3591-3601.

PALACIOS A., PIERGIACOMI V.A., CATALÁ A. 1996. Vitamin A supplementation inhibits chemiluminescence and lipid peroxidation in isolated rat liver microsomes and microsomes. Mol. Cell. Biochem. 154: 77-82.

PALACIOS A., PIERGIACOMI V.A., CATALÁ A. 2003. Antioxidant effect of conjugated linoleic acid and vitamin A during non enzymatic lipid peroxidation of rat liver microsomes and mitochondria. Mol. Cell. Biochem. 250: 107-113.

PARADIES G., PETROSILLO G., PISTOLESE M., DI VENOSA N., SERENA D., RUGGIERO F.M. 1999. Lipid peroxidation and alterations to oxidative metabolism in microsomes isolated from rat heart subjected to ischemia and reperfusion. Free Radic. Biol. Med. 27: 42-50.

PARIZA M.W., HA Y.L. 1990a. Conjugated dienoic derivatives of linoleic acid: a new class of anticarcinogens. Med. Oncol. Tumor Pharmacother. 7: 169-171.

PARIZA M.W., HA Y.L. 1990b. Newly recognized anticarcinogenic fatty acids. Basic Life Sci. 52: 167-70.

PARIZA M.W., PARK Y., COOK M.E. 1999. Conjugated linoleic acid and the control of the cancer and obesity. Toxicol. Sci. 52: 107-110.

PARODI P.W. 1997. Cow's milk fat components as potential anticarcinogenic agents. J. Nutr. 127: 1055-1060.

PIERGIACOMI V.A., PALACIOS A., CATALÁ A. 2001. Comparative studies on lipid peroxidation of microsomes and mitochondria obtained from different rat tissues: effect of retinyl palmitate. Prostaglandins Leukot. Essent. Fatty Acids 64: 75-80.

PIERGIACOMI V.A., PALACIOS A. 2006. Conjugated linoleic acid and fatty acid binding protein as antioxidants. InVet .8: 139-148.

PIROZHKOV S.V., ESKELSON C.D., WATSON R.R., HUNTER G.C., PIOTROWSKI J.J., BERNHARD V. 1992. Effect of chronic consumption of ethanol and vitamin E on fatty acid composition and lipid peroxidation in rat heart tissue Alcohol 9: 329-334.

RICHTER C. 1987. Biophysical consequences of lipid peroxidation in membranes. Chem. Phys. Lipids 44: 175-189.

RISÉRUS U., VESSBY B., ÄRNLÖV J., BASU S. 2004. Effects of *cis*-9, *trans*-11 conjugated linoleic acid supplementation on insulin sensitivity, lipid peroxidation, and proinflammatory markers in obese men. Am. Soc. Clin. Nutr. 80: 279-283.

SATORU G., KENTARO K., KAZUTOYO A., YUKARI K., KATSUHIRO K., EIJI Y., HIROSHI T.2001. Efficient radical trapping at the surface and inside the phospholipid membrane is responsible for highly potent antiperoxidative activity of the carotenoid astaxanthin Biochim. Biophys. Acta 1512: 251-258.

SEBEDIO J.L., GNAEDIG S., CHARDIGNY J.M.1999. Recent advances in conjugated linoleic acid research. Clin. Nutr. Metab. Care 2: 499-506.

SEHAT N., YURAWECZ M.P., ROACH J.A., MOSSOBA M.M., KRAMER J.K., KU Y.1998. Silver-ion high-perfomance liquid chromatography separation and identification of conjugated linoleic acid isomers. Lipids 33: 217-221.

SEHAT N., RICKERT R.R., MOSSOBA M.M., KRAMER J.K., YURAWECZ M.P., ROACH J.A., ADOLF R.O., MOREHOUSE K.M., FRITSCHE J., EULITZ K.D., STENHART H., KU Y.1999. Improved separation of conjugated fatty acid methyl esters by silver ion-high-performance liquid chromatography. Lipids 34: 407-413.

SIES H.1997. Oxidative stress: oxidants and antioxidants. Exp. Physiol. 82: 291-295.

TADOLINI B., CABRINI L., MENNA C., PINNA G.G., HAKIM G.1997. Iron [III] stimulation of lipid hydroperoxide-dependent lipid peroxidation. Free Radical Res. 27: 563-576.

TANGEN O., JONSSON J., ORRENIUS S.1973. Isolation of rat liver microsomes by gel filtration. Anal. Biochem. 54: 597-603.

UDILOVAN., JUREKD., MARIANB., GILLE L., SCHULTE-HERMANN R., NOHL H. 2003. Induction of lipid peroxidation in biomembranes by dietary oil components. Food Chem. Toxicol. 41:1481-1489.

VLADIMIROV Y.A., OLENEV V.I., SUSLOVA T.B., CHEREMISINA Z.P. 1980. Lipid peroxidation in mitochondrial membrane. Adv. Lipid Res. 17: 173-249.

WRIGHT J.R., RUMBAUGTH R.C., COLBY H.D., MILES P.R. 1979. The relationships between chemiluminescence and lipid peroxidation in rat hepatic microsomes. Arch. Biochem. Biophys. 192: 344-351.

YAN H.L., RUI H.L. 2000. *trans*-10,*cis*-12-Conjugated Linoleic Acid Isomer Exhibits Stronger Oxyradical Scavenging Capacity than *cis*-9,*trans*-11-Conjugated Linoleic Acid Isomer. J. Agric. Food Chem. 48: 5469-5475.