Lipid peroxidation in liver microsomes of rats fed soybean, olive, and coconut oil

Mirella Nardini, Cristina Scaccini, Massimo D'Aquino, Paola Corcos Benedetti, Maurizio Di Felice, and Gianni Tomassi*

*Istituto Nazionale della Nutrizione, Roma; and *Universitá della Tuscia, Dipartimento di Protezione delle Piante, Viterbo, Italia*

The effect of varying unsaturation degree of dietary lipid on the oxidative response of rat liver microsomes was studied. Three groups of growing male rats were maintained for 6 weeks on 15% fat diets containing either soybean oil, olive oil, or coconut oil, with the same level of vitamin E. After 6 weeks, microsomal malondialdehyde, vitamin E, and fatty acid composition were measured in liver microsomes. The relative abundance of saturated and unsaturated fatty acids in the microsomes reflected the composition of the dietary lipid. When dietary requirement for vitamin E was satisfied, the increased polyunsaturated fatty acid intake from vegetable oils did not enhance lipid peroxidation in physiological conditions, as demonstrated by similar malondialdehyde concentrations found in the three groups. However, the somewhat lower vitamin E content measured in soybean oil-fed rats confirms an enhanced requirement for dietary antioxidant caused by the increased intake of polyunsaturated fatty acids. The susceptibility of liver microsomes to lipid peroxidation stimulated by the ADP/iron/ascorbate system was also studied. Membranes of soybean oil-fed rats exhibited the highest peroxidation rate, as shown by oxygen consumption and malondialdehyde and 4-hydroxy-2,3-trans-nonenal production, because of the lower concentration of vitamin E and of the higher content of polyunsaturated fatty acids. Microsomes of olive oil- and coconut oil-fed rats showed highest protection against lipid peroxidation.

Keywords: lipid peroxidation; vegetable oils; triolein; tocopherol; malondialdehyde; 4-hydroxy-2,3-trans-nonenal; rat liver microsomes

Introduction

The use of vegetable oils has been recommended as a part of dietary programs to increase the ratio of polyunsaturated to saturated fatty acids to lower serum cholesterol and, indirectly, to prevent the development of atherosclerosis.[1-3]

Nevertheless, because polyunsaturated fatty acids (PUFA) are substrates for free-radical reactions leading to lipid peroxidation, a high dietary intake of PUFA might overwhelm the normal antioxidant defenses of the organism, and increase the need for dietary antioxidants.[4-6]

Lipid peroxidation is thought to play a key role in many pathological processes,[7,8] and evidence is increasing that lipoprotein peroxidation may be involved in the pathology of atherosclerosis.[9,10]

Among vegetable oils, olive oil may be considered to offer a good protection against lipid peroxidation because of the optimal balance between the degree of fatty acid unsaturation and vitamin E content.[11] Oleic acid, the most abundant fatty acid present in olive oil (63–83% of total fatty acids), is resistant to peroxidation.[12] Moreover, oleic acid is known to inhibit lipid peroxidation in in vivo[13] and in vitro[14] experiments, perhaps by chelating available free iron.[15,16] Olive oil is also known to lower serum cholesterol levels and to exert a protective effect against atherosclerosis development.[17,18] Recently, Parthasarathy et al.[19,20] demonstrated that the use of monounsaturated, oleate-rich

Research supported by National Research Council of Italy, Special Project RAISA, Subproject 4, Paper n. 460.
Address reprint requests to Dr. Mirella Nardini at the Istituto Nazionale della Nutrizione, Unitá di Nutrizione Sperimentale, Via Ardeatina 546-00178, Roma, Italia.
Received February 4, 1992; accepted June 12, 1992.

fats rather than polyunsaturated fats generates lipoprotein particles markedly resistant to transition-metal–induced oxidative modifications.

The oxidative metabolism of drugs and carcinogens in the rat liver microsomal fraction is markedly dependent on the nature of the dietary lipid,[21–23] and it is known that the dietary lipid influences the rate of oxidative metabolism by modification of the fatty acids composition of the endoplasmic reticulum.[24–26] It is therefore apparent that the nature and relative quantities of dietary unsaturated fatty acids may have an important role in the regulation of the rate and extent of lipid peroxidation.[4,5,24,27]

In the present investigation the effect of varying the unsaturation degree of dietary lipid on the oxidative response of rat liver microsomes was studied. Rats were fed three different diets contained soybean oil (SO), olive oil (OO), or coconut oil (CO), with the same level of vitamin E in the diets. Oxidative status and susceptibility of liver microsomes to lipid peroxidation stimulated by the ADP/iron/ascorbate system were studied.

Table 1 Fatty acid composition (%) of experimental diets

Fatty acid	SO	OO	CO
10:0	—	—	0.2
12:0	—	0.2	32.6
14:0	0.2	0.2	20.5
16:0	10.9	12.0	15.5
18:0	3.7	1.9	17.9
18:1 n-9	21.2	75.2	9.0
18:2 n-6	54.1	7.6	2.2
18:3 n-3	7.2	0.5	—
20:0	0.3	0.4	0.4
20:1 n-9	0.4	0.2	0.2
20:4 n-6	0.6	0.6	0.2
22:0	0.4	0.1	1.1
24:0	0.3	0.1	0.4
PUFA	61.9	8.7	2.4
MUFA	21.6	75.4	9.2
SFA	15.8	14.9	88.6
UI[a]	153.8	94.5	14.4

[a]Unsaturation Index (UI) = sum of percentages of individual fatty acids × number of double bonds.

Materials and methods

Diets and animals

Semisynthetic diets containing 15% soybean oil, olive oil, and coconut oil were prepared. The composition of the experimental diets was: 20% casein, 0.3% dl-methionine, 40% rice starch, 17% sucrose, 15% oil, 3% fiber, 3.5% salt mixture (AIN 76), 1% vitamin mixture (AIN 76), 0.2% choline chloride. The diets were prepared weekly and stored at 4° C under nitrogen. Male albino rats weighing 68 ± 6 g were individually housed in wire bottom stainless cages. Lighting was regulated to provide equal hours of light and dark. The animals were randomly divided in three groups of 10 and fed the experimental diets for 6 weeks. Food and water were provided ad libitum. At the end of this period, after an overnight fasting, rats were sacrified.

Diet analysis

Oils were analyzed for tocopherol content according to Carpenter[28], α and γ tocopherols were converted in vitamin E equivalents (UI), and, finally, the content of vitamin E was equalized to 82 IU/Kg diet. The total dietary tocopherol content was measured according to Mc Murray[29] on the same day of preparation of the diet. Samples of diets were analyzed for fatty acid composition by gas-liquid chromatography (GLC)[30] *(Table 1)*.

Microsome preparation

The liver was removed immediately after the rat was killed, weighed, and microsomes prepared by homogenization in 0.25 M sucrose/20 mmol/L TrisHCl pH 7.4 medium and ultracentrifugation at 105,000*g* (ultracentrifuge Beckman Instrument model L5-50B, Palo Alto, CA USA) according to the method described by Slater.[31]

Analytical methods

Protein concentration in microsomal suspensions was estimated by the Biuret method,[32] using bovine serum albumin as standard.

Vitamin E in microsomal samples was measured by high performance liquid chromatography (HPLC) after saponification, as described by Buttris and Diplock.[33]

Malondialdehyde (MDA) in microsomal suspension (containing 0.1 mg/mL butylhydroxytoluene) was determined by HPLC according to the method of Esterbauer et al.[34]

4-Hydroxy-2,3-trans-nonenal (HNE) was prepared according to the chemical synthesis described by Esterbauer and Weger.[35] HNE in microsomal suspensions was determined by HPLC according to Lang et al.[36]

Fatty acid composition of microsomes was determined by GLC[30] after extraction with chloroform/methanol (2:1 vol/vol) in the presence of butylhydroxytoluene according to Folch.[37]

Liver microsome peroxidation

Microsomes (1 mg protein/mL) were preincubated for 2 min at 30° C in 50 mmol/L Tris-HCl/150 mmol/L KCl pH 7.4, containing 500 μmol/L ADP, followed by the addition of 333 μmol/L ascorbate and 8 μmol/L ferric chloride.[38] At different time aliquots were withdrawn and processed for MDA and HNE determination with HPLC.[34,36]

Measurement of oxygen uptake during lipid peroxidation

Microsomal suspensions (1 mg protein/mL) were preincubated for 2 min at 30° C in 50 mmol/L TrisHCl/150 mmol/L KCl pH 7.4, containing 500 μmol/L ADP, followed by the addition of 333 μmol/L ascorbate and 8 μmol/L ferric chloride.[38] O_2 uptake was measured polarographically employing a Clark electrode (Yellow Spring Instruments Co. Inc., Yellow Spring, OH USA) with a Gilson 5/6H oxygraph (Middleton, WI USA). Ambient O_2 concentration in the buffer (30° C) was 270 μmol/L.

Statistical analysis

Data presented are mean ± standard deviation. Data were analyzed by one-factor analysis of variance and Scheffe's method for multiple comparisons; linear regression analysis

was performed using a least squares method. Differences with $P < 0.05$ were considered statistically significant.

Results

Fatty acid composition of liver microsomes is shown in *Table 2*. The fatty acid composition of liver microsomes reflected the dietary lipid composition. Microsomes from SO-fed rats contained more PUFA (47.9% of total fatty acids) in comparison with OO-fed rats (40.4%) and CO-fed rats (29.5%). Microsomes from CO-fed rats had the highest content of SFA (54.9% of total fatty acids). Membranes from OO-fed rats contained middle quantities of PUFA in comparison to SO- and CO-fed rats and high quantities of monounsaturated fatty acids (MUFA). The high content of oleic acid in microsomes of CO-fed animals is possibly due to the quick conversion of stearic acid (18% in coconut diet) to oleic acid through a rapid desaturation.[39,40]

The liver microsomes content of vitamin E and MDA is shown in *Table 3*. Significant differences ($P = 0.018$) in vitamin E content were observed among the three groups, with the lower level in SO-fed rats (in ng/mg protein: SO, 95.6±18.2; OO, 109.6±27.8; CO, 127.7±20.9). The difference between SO-fed rats and CO-fed rats was significant ($P \leq 0.005$), whereas the differences between SO-fed rats and OO-fed rats and between OO-fed rats and CO-fed rats were not.

A different behavior was observed for MDA content. No significative difference ($P = 0.23$) was found among the three groups (in pmol/mg protein: SO, 19.3±5.7; OO, 18.0±5.5; CO, 15.2±4.5). Although SO-fed rats presented the highest value, differences between SO-fed rats and CO-fed rats were not significant ($P \geq 0.05$).

Any attempt to quantitatively determine 4-hydroxy-2,3-trans nonenal, a lipid soluble peroxidation product, failed because its concentration in microsomal suspensions (about 5–8 pmol/mg protein) was near the detection limit of the method.

Table 4 shows the aldehyde production during microsomal peroxidation induced by ADP/iron/ascorbate at different times of incubation. Microsomes from SO-fed rats showed the highest rate of aldehyde production in respect to OO- and CO-fed rats. MDA produced by microsomes of OO-fed rats was not significantly

Table 2 Fatty acid composition (%) of liver microsomes[a]

Fatty acid	SO	OO	CO
12:0	0.2 ± 0.1	0.2 ± 0.1	0.2 ± 0.1
14:0	0.3 ± 0.2	0.2 ± 0.1	0.7 ± 0.2
16:0	18.2 ± 0.2	19.2 ± 0.4	17.4 ± 1.1
18:0	26.1 ± 0.4	24.0 ± 1.0	30.3 ± 1.2
18:1 n-9	3.9 ± 0.2	10.1 ± 1.7	9.9 ± 0.9
18:2 n-6	11.9 ± 0.2	3.6 ± 0.3	5.0 ± 0.6
18:3 n-3	0.4 ± 0.1	—	—
20:0	0.3 ± 0.1	0.1 ± 0.1	0.3 ± 0.1
20:1 n-9	0.9 ± 0.4	0.3 ± 0.1	0.3 ± 0.2
20:4 n-6	27.3 ± 0.4	30.0 ± 1.3	19.2 ± 2.2
22:0	0.2 ± 0.1	1.3 ± 0.1	5.8 ± 0.8
24:0	0.3 ± 0.1	0.2 ± 0.1	0.3 ± 0.1
20:5 n-3	0.6 ± 0.1	0.4 ± 0.2	0.8 ± 0.3
22:4 n-6	0.4 ± 0.1	0.4 ± 0.1	0.4 ± 0.1
22:5 n-3	1.1 ± 0.1	0.4 ± 0.2	0.8 ± 0.3
22:6 n-3	6.4 ± 0.2	5.7 ± 0.4	3.3 ± 0.4
PUFA	47.9 ± 1.3	40.5 ± 2.4	29.5 ± 4.0
MUFA	4.7 ± 0.7	10.4 ± 1.8	10.2 ± 1.1
SFA	45.7 ± 1.0	45.3 ± 1.8	55.0 ± 3.5
UI[b]	186.9 ± 5.8	177.1 ± 11.9	126.6 ± 17.4

[a]Values are means ± SD for six rats.
[b]Unsaturation Index (UI) = sum of percentages of individual fatty acid × number of double bonds.

Table 3 Vitamin E and malondialdehyde content of liver microsomes

	SO	OO	CO	*P*
Vitamin E (ng/mg protein)	95.6 ± 18.2[a]	109.6 ± 27.8[a,b]	127.7 ± 20.9[b]	0.018
Malondialdehyde (pmol/mg protein)	19.3 ± 5.7	18.0 ± 5.5	15.2 ± 4.5	ns

Values are means ± SD for 10 rats. Values with different superscript are significantly different by ANOVA (Scheffe *F* test).

Table 4 Aldehydes production during lipid peroxidation of liver microsomes

	SO	OO	CO	*P*
	nmoles/mg protein			
MDA at 5 min	23.3 ± 2.9[a]	15.1 ± 0.3[b]	12.7 ± 0.4[b]	0.01
MDA at 15 min	30.5 ± 1.1[a]	20.8 ± 1.8[b]	15.1 ± 0.6[c]	0.002
MDA at 30 min	36.3 ± 2.5[a]	24.5 ± 0.7[b]	19.3 ± 1.4[b]	0.005
HNE at 30 min	1.0 ± 0.1	0.5 ± 0.1	nd	0.02

Values are means ± SD for six rats. Values with different superscripts are significantly different by ANOVA (Scheffe *F* test). nd, not detectable.

different from that of CO-fed rats, except than at time 15 min. Membranes from SO-fed rats produced twice as much HNE as OO-fed rats. HNE in microsomes of CO-fed rats was undetectable also after 30 min of incubation.

The polarographic traces obtained from peroxidizing microsomes are shown in *Figure 1*. Probably due to vitamin E consumption, the length of the induction period was different in the three groups (curves A, B, C), resulting in significantly different initial rates of O_2 consumption (in nmoles O_2/mg protein: SO, 42.0±1.0; OO, 21.7±1.0; CO, 2.7±0.2; $P=0.0001$) (*Table 5*, first column). Moreover, rates of oxygen uptake in the second, linear part of polarographic traces are also significantly different ($P = 0.0001$), with the highest rate of oxidation observed for SO-fed rats (in nmoles/min/mg protein: SO, 133.2±14.7; OO, 111.4±10.3; CO, 74.4±14.0) (*Table 5*, second column).

To clarify the effect of vitamin E content and fatty acid composition on the course of polarographic traces, we studied the peroxidation of microsomes from rats fed a diet supplemented with 15% (wt/wt) triolein, a synthetic mixture of triglycerides (75% oleic acid) mimicking olive oil in fatty acid composition. Microsomes from triolein-fed rats had a fatty acid composition quite similar to that of OO-fed rats (C16:0, 18.0±1.5%; C18:0, 24.8±2.0%; C18:1 n-9, 9.5±1.4%; C18:2 n-6, 4.1±0.2% C20:4 n-6, 29.7±2.9%) with 40.8% PUFA, 14.2% MUFA, 46.0% saturated fatty acid (SFA), and a vitamin E content (74.4±9.4 ng/mg protein) significantly lower in respect to the other three groups, particularly the OO-fed group ($P = 0.0001$). As shown in *Table 5*, the rate of oxygen consumption in the second linear part of polarographic traces was similar to that of microsomes from OO-fed rats (113.5±12.5 nmoles O_2/min/mg protein for triolein-fed group, 111.4±10.3 nmoles O_2/min/mg protein for OO-fed group), but the length of the induction period (*Figure 1*, curve D, dotted line) and the initial rate (55.0±7.5 nmoles O_2/mg protein for triolein fed-group, 21.7±1.0 nmoles O_2/mg protein for OO-fed rats) (*Table 5*) were markedly different from those of OO-fed rat microsomes.

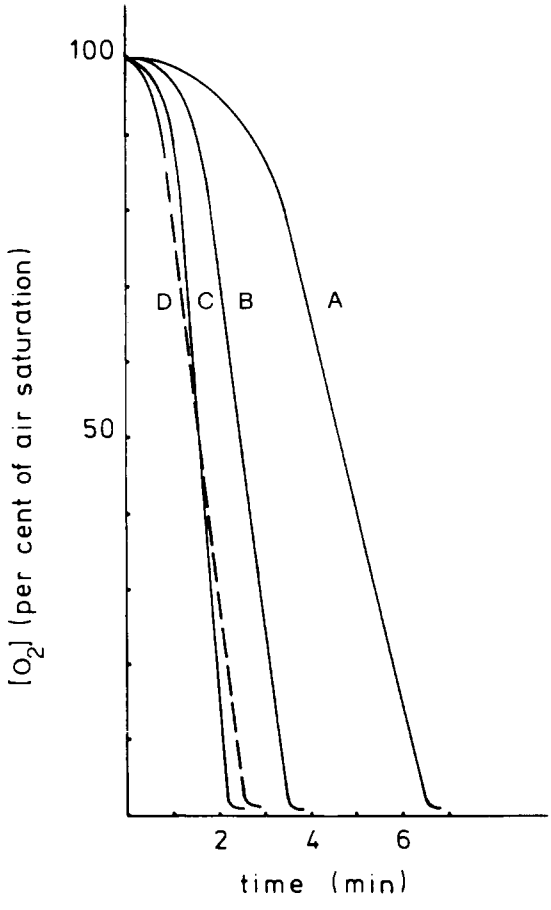

Figure 1 Polarographic traces obtained by inducing lipid peroxidation in liver microsomes. Microsomes (1 mg protein/mL) were preincubated for 1 min at 30° C in 50 mmol/L Tris-HCl/150 mmol/L KCl pH 7.4 containing 500 μmol/L ADP, 333 μmol/L ascorbate followed by the addition of $FeCl_3$ (8 μmol/L). Sample from: A, CO-fed rats; B, OO-fed rats; C, SO-fed rats; D (dotted line), triolein-fed rats.

Table 5 Oxygen consumption during lipid peroxidation of liver microsomes[a]

Diet	Phase I[b]	Phase II[c]
	nmoles/mg protein	nmoles/min/mg protein
SO	42.0 ± 1.0	133.2 ± 14.7
OO	21.7 ± 1.0	111.4 ± 10.3
CO	2.7 ± 0.2	74.4 ± 14.0
Triolein	55.0 ± 7.5	113.5 ± 12.5

[a]Values are means ± SD for five rats.
[b]Values referred to oxygen consumption in the first minute of reaction.
[c]Values referred to oxygen consumption in the linear phase of polarographic trace.

Linear regression analysis showed a statistically significant negative correlation between the initial rates of oxygen consumption and vitamin E content ($r = 0.987$, $P = 0.013$) and a highly significant positive correlation between rate of O_2 consumption in the second linear phase and PUFA content of microsomes ($r = 0.998$, $P = 0.002$). No correlation whatsoever was found between initial rates and PUFA content or between vitamin E content and rates of oxygen consumption in the second linear phase. These results confirm that the induction period was really due to consumption of vitamin E, and that peroxidation rates in the linear phase were only determined by the fatty acids unsaturation degree of microsomes.

Discussion

The relative abundance of saturated and unsaturated fatty acids in the microsomal membranes reflects the consumption of the fatty acids present in the diet. However a certain aliquot of MUFA is converted to PUFA.[41] This phenomenon is particularly evident in the olive oil diet.

All diets used in our experiment were equalized for

vitamin E content to 82 IU/Kg diet, a quantity that fully satisfies the requirement of rats (about 50 IU/Kg according to National Research Council),[42] but vitamin E levels in SO-fed rats were somewhat lower than those of OO-fed rats and CO-fed rats, confirming an enhanced requirement for dietary antioxidants caused by high intake of PUFA.

Nevertheless, plasma ascorbate concentration was not significantly different among the three groups of rats (data not shown). Malondialdehyde concentration was quite similar in all groups of animals, suggesting that when dietary requirement for vitamin E is satisfied, increased PUFA intake from vegetable oils does not enhance lipid peroxidation in physiological conditions. We cannot exclude that malondialdehyde, a hydrophilic compound, once formed would diffuse partially from the place of its origin in lipid phase into the surrounding aqueous phase, as shown for peroxiding liver microsomes.[43]

In the presence of an induced oxidative stress, microsomes from SO-fed rats were the most susceptible to lipid peroxidation, as indicated by oxygen consumption and MDA and HNE production, both for the lower concentration of vitamin E, SFA, and MUFA and for the increased levels of PUFA. OO-fed rats showed a better protection toward induced lipid peroxidation in comparison with SO-fed rats, due to the lower content of PUFA and to the higher content of vitamin E, SFA and MUFA. HNE produced during lipid peroxidation of microsomes from OO-fed rats was half of the amount produced by membranes of SO-fed rats. Moreover, the amount of MDA produced during peroxidation was not significantly different from that produced by microsomes of CO-fed rats. CO-fed rats were the most resistent to lipid peroxidation both for the higher levels of vitamin E and SFA and for the lower content of PUFA.

To lower serum cholesterol levels, the consumption of oils with high contents of polyunsaturated-monounsaturated fatty acids is recommended instead of highly saturated fats (butter, lard), which are known to raise cholesterol levels. However, polyunsaturated fatty acids are easily susceptible to peroxidation and high dietary intake of PUFA might overwhelm the normal antioxidant defenses of the organism. Our results show that in the absence of induced oxidative insults, diets with high PUFA content do not cause substantial increases in microsomes' oxidative status when dietary requirement for vitamin E is satisfied. Nevertheless, olive oil in the diet seems to assure a better protection against oxidative risk in comparison to rich PUFA vegetable oils.

Abbreviations

PUFA	polyunsaturated fatty acids
MUFA	monounsaturated fatty acids
SFA	saturated fatty acids
SO	soybean oil
OO	olive oil
CO	coconut oil
MDA	malondialehyde
HNE	4-hydroxy-2,3-transnonenal
GLC	gas-liquid chromatography
HPLC	high-performance liquid chromatography

Acknowledgments

The authors acknowledge Mrs. Pierina Rami and Mrs. Rita Rami for their skillful assistance. The authors thank Professor Silvestro Duprè for his valuable collaboration.

References

1 Committee on Diet and Health, Food and Nutrition Board, Commission on Life Sciences, National Research Council. (1989). National Academy Press, Washington, DC USA
2 Marr, J.W. (1985). In *The role of fats in human nutrition*, (F.B. Padley and J. Podmore eds.), p.200–205, Ellis Horwood Ltd., Chichester, UK
3 Singman, H.S., Berman, S.N., Cowell, C., Maslansky, E. and Archer, M. (1980). The anti-coronary club. *Am. J. Clin.* **33**, 1183–1191
4 Buckingham, K.W. (1985). Effect of dietary polyunsaturated/saturated fatty acid ratio and dietary vitamin E on lipid peroxidation in the rat. *J. Nutr.* **115**, 1425–1435
5 Leibovitz, B.E., Miao-Lin, H. and Tappel, A.L. (1990). Lipid peroxidation in rat tissue slices: effect of dietary vitamin E, corn oil-lard and menhaden oil. *Lipids* **25**, 125–129
6 D'Aquino, M., Corcos Benedetti, P., Di Felice, M., Gentili, V., Tomassi, G., Maiorino, M. and Ursini, F. (1991). Effect of fish oil and coconut oil on antioxidant defence system and lipid peroxidation in rat liver. *Free Rad. Res. Comms.*, **12–13**, 147–152
7 Mead, J.F. (1976). In *Free Radicals in Biology*, (W.A. Pryor, ed.) Vol. 1, p. 51–68, Academic Press, New York, NY USA
8 Yagi, K. (1987). Lipid peroxides and human diseases. *Chem. Physics Lipids* **45**, 337–351
9 Steinberg, D., Parthasarathy, S., Carew, T.E., Khoo, J.C. and Witztum, J.L. (1989). Modifications of low-density lipoprotein that increase its atherogenicity. *N. Engl. J. Med.* **320**, 915–924
10 Steinbrecher, U.P., Parthasaraty, S., Leake, D.S., Witztum, J.L. and Steinberg, D. (1984). Modification of low density lipoprotein by endothelial cell involves lipid peroxidation and degradation of low density lipoprotein phospholipids. *Proc. Natl. Acad. Sci. USA* **81**, 3883–3887
11 Battistini, N., Bini, A., Bedogni, G., Di Felice, M., Gatti, G., Monzani, N. and Tomassi, G. (1990). Dietary fatty acids composition susceptibility to mitochondrial and microsomal membranes to oxidative stress and cellular injury in rat liver. In *Advances in free radicals in disease II*, (A. Tomasi, F. Ursini, and V. Vannini, eds.), p. 49–57, Cleup Editrice, Padova, Italy
12 Halliwell, B.H. and Gutteridge, J.M.C. (1985). The peroxidation process. In *Free radicals in biology and medicine*, p. 139–189, Clarendon Press, Oxford, UK
13 Diplock, A.T., Balasubramanian, K.A., Manohar, M., Mathan, V.J. and Ashton, D. (1988). Purification and chemical characterization of the inhibitor of lipid peroxidation from intestinal mucosa. *Biochim. Biophys. Acta* **962**, 42–50
14 Balasubramanian, K.A., Nalini, S., Cheeseman, K.H. and Slater, T.F. (1989). Nonesterified fatty acids inhibit iron-dependent lipid peroxidation. *Biochim. Biophys. Acta.* **1003**, 232–237
15 Simpson, R.J. and Peters, T.J. (1987). Iron binding lipids of rabbit duodenal brush-border membrane. *Biochim. Biophys. Acta.* **898**, 181–186
16 Simpson, R.J., Moore, R. and Peters, T.J. (1988). Significance of nonesterified fatty acids in iron uptake by intestinal brush-border membrane vesicles. *Biochim. Biophys. Acta.* **941**, 39–47
17 Keys, A. (1970). Coronary heart disease in seven countries. *Circulation* **41**, 1–211

18 The Expert Panel. (1988) Report of the National Cholesterol Education Program Exper Panel on detection, evaluation and treatment of high blood cholesterol in adults. *Arch. Intern. Med.* **148**, 36–39

19 Parthasarathy, S., Khoo, J.C., Miller, E., Barnett, J., Witzum, J. and Steinberg, D. (1990). Low density lipoprotein rich in oleic acid is protected against oxidative modifications: implications for dietary prevention of atherosclerosis. *Proc. Natl. Acad. Sci. USA* **87**, 3894–3898

20 Reaven, P., Parthasarathy, S., Grasse, B.J., Miller, E., Almazan, F., Mattson, F.H., Khoo, J.C., Steinberg, D. and Witzum, J.L. (1991). Feasibility of using an oleate-rich diet to reduce the susceptibility of low-density lipoprotein to oxidative modification in humans. *Am. J. Clin. Nutr.* **54**, 701–706

21 Marshall, W.J. and Mc Lean, A.E.M. (1971). A requirement for dietary lipids for induction of cytochrome P-450 by phenobarbitone in rat liver microsomal fraction. *Biochem. J.* **122**, 569–573

22 Rowe, L. and Wills, E.D. (1976). The effect of dietary lipids and vitamin E on lipid peroxide formation cytochrome P-450 and oxidative demethylation in the endoplasmic reticulum. *Biochem. Pharmacol.* **25**, 175–179

23 Lambert, L. and Wills, E.D. (1977). The effect of dietary lipid peroxides, sterols and oxidized sterols on cytochrome P-450 and oxidative demethylation in the endoplasmic reticulum. *Biochem. Pharmacol* **26**, 1417–1421

24 Wills, E.D. (1985). The role of dietary components in oxidative stress in tissue. In *Oxidative Stress,* (H. Sies, ed.), p.197–216, Academic Press, London, UK

25 Hammer, C.I. and Wills, E.D. (1979). The effect of dietary fats on the composition of the liver endoplasmic reticulum and oxidative drug metabolism. *Br. J. Nutr.* **41**, 465–475

26 Saito, M., Kubota-Shirao, M., Kobatake, Y., and Yamaguchi, M. (1990). Influence of different types and levels of dietary lipids on liver microsomal mixed function oxidase system in rats. *Ann. Nutr. Metab.* **34**, 288–296

27 Miao-Lin, Hu, Frankel, E.D., Leibovitz, B.E. and Tappel, A.L. (1989). Effect of dietary lipids and vitamin E on in vitro lipid peroxidation in rat liver and kidney homogenates. *Lipids* **119**, 1574–1582

28 Carpenter, A.P. (1979). Determination of tocopherols in vegetable oils. *J. Am. Oil Chem. Soc.* **56**, 668–671.

29 Mc Murray, C., Blauchflower, W.J. and Rice, D.A. (1980). Influence of extraction techniques on determination of alpha-tocopherol in animal food-stuff. *J. Assoc. Off. Anal. Chem.* **63**, 1258–1261

30 Metcalfe, L.D. and Schmitz, A.A. (1961). Rapid preparation of fatty acid ester for gas-chromatography analysis. *Anal. Chem.* **33**, 363–369

31 Slater, T.F. and Sawyer, B.C. (1971). The stimulatory effect of carbon tetrachloride and other alogenoalkanes on peroxidative reaction in rat liver fractions. *Biochem. J.* **123**, 805–814

32 Layne, E. (1957). Biuret method. In *Methods in Enzymology,* (S.P. Colowick and N.O. Kaplan, eds.), Vol. 3, p. 450–451, Academic Press, New York, NY USA

33 Buttris, J.L. and Diplock, T. (1984). High-performance liquid chromatography methods for vitamin E in tissues. In *Methods in Enzymology,* (L. Packer, ed.) vol. 105, p. 131–147, Academic Press, New York, NY USA

34 Esterbauer, H., Lang, J., Zadravec, S. and Slater, T.F. (1984). Detection of malondialdehyde by high performance liquid chromatography. In *Methods in Enzymology,* (L. Pacher, ed.) vol.105, p. 319–328, Academic Press, New York, NY USA

35 Esterbauer, H. and Weger, W. (1967). Uber die Wirkungen von Aldehyden auf gesunde und maligne Zellen, 3. Mitt: synthese von homologen 4-hydroxy-2-alkenalen, II. *Monatsh. Chem* **98**, 1994–2000

36 Lang, J., Celotto, C. and Esterbauer, H. (1985). Quantitative determination of the lipid peroxidation product 4-hydroxynonenal by high performance liquid chromatography. *Anal. Biochem.* **150**, 369–378

37 Folch, J., Less, M., and Stanley, S. (1957). A single method for the isolation and purification of total lipids from animal tissues. *J. Biol. Chem.* **226**, 497–502

38 Wills, E.D. (1969). Lipid peroxide formation in microsomes. *Biochem. J.* **113**, 315–324

39 Elovson, J. (1965). Immediate fate of albumin bound [I-^{14}C] stearic acid following its intraportal injection into carbohydrate refed rats. Early course of desaturation and esterification. *Biochim. Biophys. Acta* **106**, 480–494

40 Bonanome, A. and Grundy, S.M. (1989). Intestinal absorption of stearic acid after consumption of high fat meals in humans. *J. Nutr.* **119**, 1556–1560

41 Hammer, C.T. and Wills, E.D. (1979). The effect of dietary fats on the composition of the liver endoplasmic reticulum and oxidative drug metabolism. *Br. J. Nutr.* **41**, 465–475

42 National Research Council. (1972). Nutrient Requirement of Laboratory Animals. National Academy of Science N.10 p. 64, NAS, Washington, DC USA

43 Esterbauer, H., Cheeseman, K.H., Dianzani, M., Poli, G. and Slater, T.F. (1982). Separation and characterization of the aldehydic product of lipid peroxidation stimulated by ADP-Fe^{2+} in rat liver microsomes. *Biochem. J.* **208**, 129–140