Strong Increase of 9-Hydroxy-10,12-octadecadienoic Acid in Low Density Lipoprotein after a Hemorrhagic Shock

P. Kreil[a], G. Spiteller[a,*], G. Johannes[b] and W. Wagner[b]

[a] Lehrstuhl Organische Chemie I, Universität Bayreuth, Universitätsstr. 30, D-95440 Bayreuth
[b] Klinikum Bayreuth, Preuschwitzer Straße 101, D-95445 Bayreuth

9-hydroxy-10,12-octadecadienoic acid (9-HODE) is generated by lipid peroxidation (LPO) processes in comparison to other marker compounds in at least 10 fold amount. A 10–25 fold increase of this new marker compound in relation to age matched healthy individuals was observed in the *low density lipoprotein* (LDL) fraction of ten patients suffering from a hemorrhagic shock. The 9-HODE values dropped to normal levels after recovery.

Similarly the 9-HODE content in LDL of patients which had to undergo orthopedic surgery – replacement of their arthritic hip joints by endoprosthesis – were investigated. The rather high HODE values dropped also after recovery reflecting obviously the disappearance of inflammatory processes associated with arthritis.

Introduction

Cell damage induces the activation of enzymes, e.g. phospholipase A_2 which attack phospholipids to produce free fatty acids (Parthasarathy *et al.*, 1985). In addition cell injury activates lipoxygenases (Halliwell, 1993; Herold and Spiteller, 1996). These react with the liberated polyunsaturated fatty acids (PUFAs) in a highly stereospecific reaction to generate lipidhydroperoxides (LOOH) (Glickman and Klinman, 1996; Gardner, 1991). Additionally cell damage activates proteases (Vine and Powell, 1991) which liberate iron ions from iron containing enzymes, e.g. hemoglobin (Gutteridge, 1986; Kanner and Harel, 1985).

Thus during cell damage LOOH **and** iron ions are generated, the prerequesites to induce a nonenzymatic chain reaction: LOOHs are cleaved by iron ions to LO$^{\bullet}$ resp. LOO$^{\bullet}$ radicals (Gardner, 1989; Koppenol, 1994).

In contrast to enzymatic processes targets for radical attack are all activated C-H bonds. Especially prone to radical attack are CH_2 groups located between two double bonds, e.g. those in arachidonic and linoleic acid. Again in contrast to most enzymatic reactions (Folcik *et al.*, 1995), radicals react also with PUFAs in conjugated form, e.g. with PUFAs esterified with cholesterol or glycerol.

The main PUFA in these conjugates is linoleic acid. Consequently most LOOH molecules are derived from linoleic acid conjugates. Hydroperoxyl groups suffer in biological surroundings easily reduction to corresponding hydroxy functions (Wang and Powell, 1991). Such molecules are obviously recognized by enzymes which cleave the ester bond, generating free hydroxy acids (LOHs). Thus hydroxylated linoleic acid conjugates are converted to 9-hydroxy-10,12-octadecadienoic acid (9-HODE) and 13-hydroxy-9,11-octadecadienoic acid (13-HODE). These acids are rather stable to further oxidative attack and therefore enriched. They represent the most abundant lipidperoxidation products, exceeding all other LPO products for a factor of 10 and even more. As a consequence 9- and 13-HODE are excellent markers to recognize LPO processes (Spiteller and Spiteller, 1998).

The main targets of lipid peroxidation in plasma lipoproteins are low density lipoproteins (LDL) (Uemura, 1990). The involvement of oxidative modified LDL in the pathogenesis of vascular diseases, e.g. atherosclerosis, has been shown (Berliner and Heinecke, 1996; Steinbrecher *et al.*, 1990). Oxidized LDL is a potent cytotoxin (Esterbauer *et al.*, 1990; Kuzuya *et al.*, 1991). LDL transports mainly cholesterol linoleate. As a consequence its oxidation results in the generation of HODEs. Analyzing the content of HODEs we have been able to demonstrate an up to fifty fold increase of HODEs in the LDL fraction of patients suffering from atherosclerosis (Jira *et al.*, 1998) and rheumatic arthritis (Jira *et al.*, 1997) compared to the HODE content in LDL of young volunteers.

Cell degenerating processes are also observed after a hemorrhagic shock: A shock induces hypoperfusion of vital organs and thus causes physiological disorders including tissue hypoxia (Schlag and Redl, 1993; Schuster, 1992). This might be regarded as whole body ischemia (McCord *et al.*, 1985). Ischemic processes are connected with cell damage, and consequently LPO processes should be induced, like after a myocardial infarction (Dudda, 1995). Therefore we expected in the LDL fraction of patients suffering from a hemorrhagic shock a substantial increase of HODEs.

During surgery also cells are injured. Thus also in these cases an increase of HODEs is predictable. Therefore we investigated if the HODE level is also changed in patients who had to undergo surgery. The results of these investigations are presented in this paper.

Experimental Procedures / Materials and Methods

N-Methyl-N-trimethylsilyltrifluoroacetamide (MSTFA) was obtained from Macherey and Nagel (Düren, Germany). All other chemicals were purchased from Fluka (Neu Ulm, Germany). Solvents, obtained from Merck (Darmstadt, Germany), were distilled before use.

Blood samples of eleven shock patients were provided by the Klinikum Bayreuth. The samples were withdrawn from patients who were hospitalized after a severe accident following accomplishment of life supporting steps. Reference samples were obtained from the same patients after recovery. In these cases blood was removed in the morning after fasting overnight.

Blood samples from patients whom were inserted hip prosthesis were taken just before the surgery, right after surgery and after recovery (usually two weeks later). Agreement of all patients was obtained for investigation of their blood samples. Blood samples were centrifuged at $1850 \times g/4\ °C$. Serum was stored at $-20\ °C$.

The different kinds of lipoproteins (VLDL, LDL and HDL) were precipitated from the serum (3 ml) following a method of Leiss (1979). Oxidation of the samples was prevented by processing all steps under an atmosphere of argon. The lipoproteins were lyophilized, weighed and dissolved in 8 ml H_2O. Samples were further protected against oxidation by the addition of 10 µl of a BHT-solution (2% in methanol) and 180 µl of a EDTA-Na_2-solution (1% in bidistilled water). 8.27 µg 6-Hydroxyheptadecanoic acid in methanol as a standard compound was added, then the mixture was exposed to a supersonic bath for 1 min. The required amounts of chloroform and methanol for Bligh and Dyer (Bligh and Dyer, 1959) extraction of lipids were added to the solution. After extraction solvent was removed under reduced pressure. The lipid extract was redissolved in 4 ml methanol, PtO_2 was added and the flask was then kept under a H_2 atmosphere of 1 bar for half an hour to transform hydroperoxy groups to corresponding hydroxy functions.

After addition of 10 ml chloroform hydrogen was bubbled through the suspension for 5 min to hydrogenate the double bonds. The catalyst was removed by filtration through silicagel. Following steps were carried out in air since saturated fatty acids are stable against further oxidation.

Saponification of esters was achieved by treatment of the samples with 10 ml of a 1 N methanolic potassium hydroxide solution at 50 °C for 45 min. The solution containing the deprotonated free fatty acids was acidified with hydrochloric acid (pH 4) and extracted three times with 3 ml chloroform. After removing the solvent the residue was dissolved in 0.2 ml of $CHCl_3$ and reacted for 15 min at room temperature with a 2% ethereal solution of diazomethane to convert the acids to their methylates. Separation of the methylates of not oxidized fatty acids from those of the hydroxy fatty acids was achieved by column-chromatography on silicagel (5 g). Fatty acid methylesters were eluted first with 80 ml of cyclohexane/ethylacetate

98:2 (v:v) (lit.), then hydroxy fatty acids methylates were eluted using 80 ml ethylacetate. The solvents were removed at reduced pressure.

Hydroxy groups of hydroxy fatty acid methylesters were trimethylsilylated by treatment with MSTFA for 12 h at room temperature. Identification and quantification of derivatized monohydroxy acids was done by mass spectrometry ion tracing and measuring the intensity of the α-cleavage fragment ions. Their ion currents were compared with those of the α-cleavage products of the internal standard. The mass spectrometric determination of derivatized hydroxy acids was repeated three times for each sample. The variation from the average value was less than 10%.

Results

In biological surroundings LOOHs – produced during LPO processes – are reduced rapidly by glutathione peroxidase (Wang and Powell, 1991) to corresponding LOHs. Since the latter are rather stable to further oxidation, they accumulate. Lipidhydroperoxides which had not been reduced enzymatically were subjected to chemical reduction in order to get information about the full extent of LPO. The obtained LOHs contain conjugated double bonds. These disturb the final mass spectrometric determination since mass spectra of unsaturated LOHs are less informative than those of corresponding saturated acids due to complex mass spectrometric degradation reaction caused by the conjugated system. Therefore the mixture of LOHs was subjected to catalytic hydrogenation to remove also double bonds (Nikkari *et al.*, 1995).

Since radicals attack – in contrast to enzymatic LPO – not only free but also conjugated PUFAs, it was of interest to determine the content of free and of esterified acids separately. This was achieved by different workup of a divided sample. One part was hydrolyzed, then the obtained acids were transformed to their methylesters and separated from accompanying fatty acids by column chromatography. After derivatization with MSTFA, the trimethylsilylated monohydroxy fatty esters were analyzed by GC and GC/MS. Mass spectra of each GC peak were registered and the ion currents of the two α-cleavage products were quantified by comparing the area of the α-cleavage fragment ions with those of the standard com-

pound added to the sample at the beginning of sample processing.

The second half of the sample was exactly processed in the same manner but without saponification. Thus the difference between conjugated and free hydroxy fatty acids was determined to be approxinately 50%. This indicates a considerable action of phospholipases which attack obviously preferentially oxidized phospholipids.

A typical distribution pattern of the C-18 hydroxy acids derived from LDL of a shock patient aged 16 years is represented in Fig. 1.
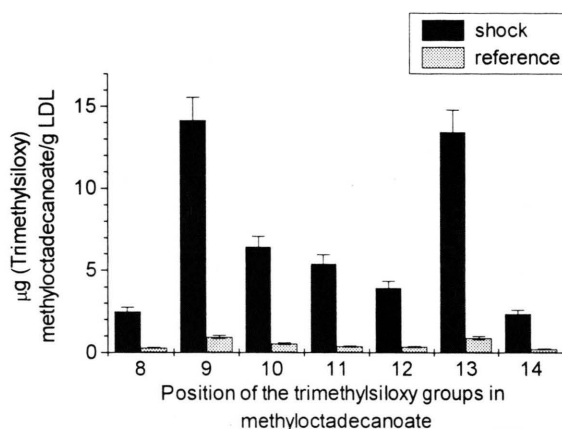

Fig. 1. Concentrations of differently localized hydroxy stearates derived by hydrogenation of hydroxy fatty acids obtained from a patient aged sixteen years during shock (black columns) and after recovery seventeen days later (grey columns).

The values during shock are substantially higher than those after recovery. The 9-HODE content during shock of the patients whose values are represented in Fig. 1 surmounted those after recovery for a factor of about 15.

The distribution of hydroxy groups in the chain is typically for a nonenzymatic LPO process (Lehmann, 1994; Mlakar and Spiteller, 1996). In such reactions 9- and 13-HODE are the most prominant isomers. Consequently we used one of these isomers – 9-HODE – for further investigations.

The investigations were performed with eleven patients suffering from hemorrhagic shock. The increase of the concentration of 9-HODE is observed with all seven patients who survived the shock (Table I).

An asterisk indicates the death of the patient as a consequence of the shock. Therefore "recovery values" of these patients (patients 7 and 9 to 11)

Patient	Age	Sex	Days	9-HODE (µg/g LDL) shock / reference / increasing factor
1	16	f	17	14.1 (±1.4) / 0.9 (±0.1) / (16)
2	22	m	14	24.7 (±2.5) / 1.3 (±0.1) / (19)
3	23	f	13	21.4 (±2.1) / 1.0 (±0.1) / (21)
4	32	m	22	33.3 (±3.3) / 1.8 (±0.2) / (19)
5	35	m	34	27.0 (±2.7) / 1.1 (±0.1) / (25)
6	47	f	14	26.3 (±2.6) / 3.4 (±0.3) / (8)
7*	74	m	19	57.1 (±5.7) / 77.6 (±7.8)
8	87	m	16	36.4 (±3.6) / 7.9 (±0.8) / (6)
9*	17	f		29.9 (±3.0)
10*	42	m		43.1 (±4.3)
11*	54	f		42.8 (±4.3)

Table I. Concentration of 9-HODE during shock and recovery. Deceased patients are marked by an asterisk. The column "days" signifies the interval in days between the first blood removal (during shock) and the reference removal (after recovery). In the last column the numbers in brackets specify the factor by which the HODE values after recovery (second number) have to be multiplied to give the value in the shock state (first number).

were not available. A reference sample of patient 7 was taken by chance within a multiple organ failure, one day before death. In this pre-fatal state a very high level of 9-HODE was observed.

The 9-HODE concentrations of all patients during shock arranged against the age are shown in Fig. 2. This figure demonstrates an age related increase of 9-HODE values – in agreement with recent results on the 9-HODE content in LDL of atherosclerotic patients (Jira *et al.* 1998). It also indicates that those samples of patients who had not recovered were characterized by higher 9-HODE values in relation to age matched patients who recovered.

To ascertain whether 9-HODE is increased in other injuries too, for example after surgical operations, altogether twelve patients have been examined in the same way as the accidental victims.

In the course of the surgery hip prosthesis were planted. The blood samples were taken right before surgical intervention, only minutes afterwards

and after recovery as indicated (usually about two weeks later). The distribution pattern of the hydroxy octadecanoic acids was the same as that of samples from shock patients (not shown). 9-HODE values are represented in Table II and 9-HODE concentrations are also given in Fig. 3 in form of a graphical chart.
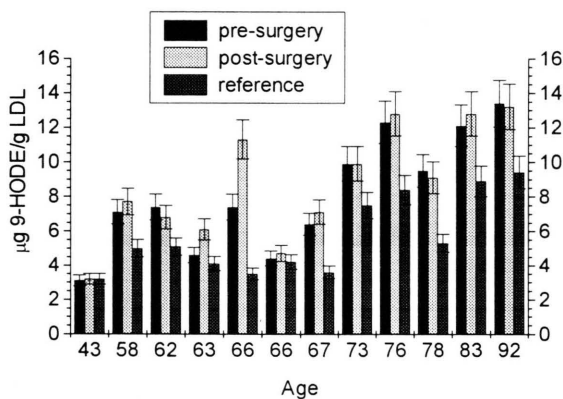

Fig. 3. The concentration of 9-HODE in LDL of twelve patients before and after surgical operation and after recovery. All patients were inserted hip prosthesis. The data are arranged with increasing age.

The values before and after surgery differ not very much but decrease after recovery. The concentrations of HODEs in LDL arranged by increasing age of the patients reveal again a considerable rise with the age – in agreement with previous results (Jira *et al.*, 1996).

Discussion

Lipid peroxidation is commonly detected by measuring the amount of malondialdehyde (MDA) (Esterbauer *et al.*, 1991), a degradation
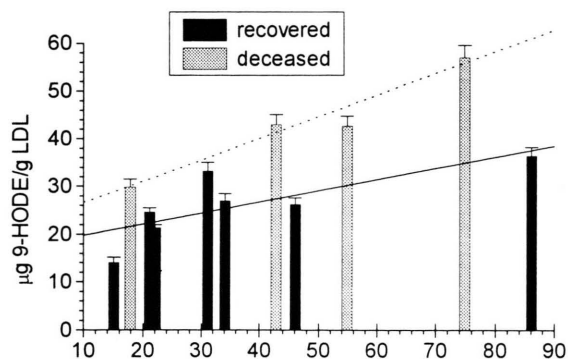

Fig. 2. The 9-HODE values of all eleven patients during shock state arranged against their age (values are recorded in Table I).

Patient	Age	Sex	Days	9-HODE (µg/g LDL) pre / post / reference / increase
1	43	m	11	3.1(±0.3) / 3.2 (±0.3) / 3.2 (±0.3) / +5%
2	58	m	14	7.1(±0.7) / 7.7 (±0.8) / 5.0 (±0.5) / -32%
3	62	f	14	7.4(±0.7) / 6.8 (±0.7) / 5.1 (±0.5) / -28%
4	63	f	13	4.6(±0.5) / 6.1 (±0.6) / 4.1 (±0.4) / -23%
5	66	f	17	7.4(±0.7) / 11.3 (±1.1) / 3.5 (±0.4) / -63%
6	66	m	14	4.4(±0.4) / 4.7 (±0.5) / 4.2 (±0.4) / -8%
7	67	f	11	6.4(±0.6) / 7.1 (±0.7) / 3.6 (±0.4) / -47%
8	73	f	14	9.9(±1.0) / 9.9 (±1.0) / 7.5 (±0.8) / -24%
9	76	f	14	12.3(±1.2) / 2.8 (±1.3) / 8.4 (±0.8) / -33%
10	78	m	14	9.5(±1.0) / 9.1 (±0.9) / 5.3 (±0.5) / -43%
11	83	f	14	12.1(±1.2) / 12.8 (±1.3) / 8.9 (±0.9) / -29%
12	92	f	14	13.4(±1.3) / 13.2 (±1.3) / 9.4 (±0.9) / -29%

Table II. Concentration of 9-HODE pre- and post-surgery and after recovery. The column "days" signifies the interval of days between the day of surgical operation and the day when a reference sample was investigated after recovery. The changes in the contents of 9-HODE are given in the last column.

product of arachidonic acid and other polyunsaturated fatty acids (PUFAs). MDA is mainly generated by thermal decomposition of precursor molecules containing a peroxy group. The yield is very low and the method is unspecific (Janero, 1990). Nevertheless it is widely applied due to its simplicity.

The most serious disadvantage of MDA measurement is the fact that linoleic acid hydroperoxides decompose to MDA only in minute amounts (Esterbauer and Cheeseman, 1990). As pointed out above and deducable from Fig. 1 the main amount of LPO products is generated by nonenzymatically induced LPO reactions. Since in a nonenzymatic LPO process the radical attacks any double allylically activated CH_2 group with about equal probability independant of its location in linoleic or arachidonic acid or other PUFAs and since linoleic acid is the most abundant acid in tissue and blood, most of LPO products escape detection if MDA measurement is applied.

Therefore as outlined recently we use for determination of lipidperoxidation the secondary degradation product of linoleic acid 9-HODE which suffer only slow further degradation (Spiteller and Spiteller, 1998) and hence are enriched by LPO processes in the body. This enhances the sensitivity of the LPO determination for at least one order of magnitude compared to MDA determinations.

9-HODE is especially enriched in the LDL fraction of blood serum of patients suffering from diseases which are connected with inflammation or other processes involved in cell degradation or cell injury (Jira *et al.,* 1997). Cells are injured during a shock situation, transplantation, myocardial infarction or just by surgery. Having this in mind we investigated if increased amounts of LPO might be recognized after a hemorrhagic shock or after surgery and compared the obtained HODE levels in LDL of patients with HODE values after recovery. In the case of surgery we were also able to compare the determined values with those of the same person before surgical intervention.

Differences amounting up to a factor of 25 between the HODE content in LDL of shock patients and after their recovery were observed. Interestingly the differences dropped with increasing age of the patients: As already observed earlier, the 9-HODE content increases dramatically with age (Jira *et al.,* 1996). Thus a 60 years old person has on average a 5 fold 9-HODE level compared to a 20–30 years old individual, and starting with 60 the HODE levels increase even much further with age. Obviously the already high individual levels in older patients are not extended in the same degree as in the youth: Older shock patients after recovery had a decrease in the 9-HODE content in LDL only for a factor of about 8.

Highest HODE values in relation to age have been observed in those patients who did not survive the shock. Especially interesting is the case of patient 8: A blood sample was obtained from this patient immediately after the shock and one day before death. The HODE values on the day before death were even increased compared to the first value – a hint that his oxidative status had collapsed.

The difference between patients whom were implanted artificial hip joints (surgery lasted about 1 hour) was much less striking: Nearly no difference was observed between the HODE content in LDL immediately before and immediately after surgery,

somewhat expected, since there was not yet enough time for the circulation to distribute the LPO degradation products among the blood stream. Blood removal at a later point (several hours after surgery when higher values due to the surgical operation might be possible) was prohibited for ethic reasons. The values of samples taken after recovery were all lower with one exception (patient 1) than before surgical operation, reflecting the removal of inflamed sites by the surgery which cause the increase in HODE.

In conclusion the study on the HODE content in LDL of shock patients revealed clearly that the shock situation is connected with a massive increase in LPO processes. The investigation further resulted in the finding that surgery does not influence the HODE content very much, the observed reduction in HODE values after recovery is mainly a consequence of the removal of the source of cell destructions. In addition the investigation confirmed, that LPO increases substantially by aging.

Aust S. D., Svingen B. A. (1982), The role of iron in enzymic lipid peroxidation. In: Free Radicals Biol. (Pryor W. A. ed.), Vol. **5**, Academic Press, New York, pp. 1-28.

Berliner J. A. and Heinecke J. W. (1996), The role of oxidized lipoproteins in atherogenesis. Free Radicals Biol. Med. **20**, 707–27.

Bligh E. G. and Dyer W. J. (1959), A rapid method of total lipid extraction and purification. Can. J. Biochem. Physiol. **37**, 911–917.

Christophersen B. O. (1968), Formation of monohydroxy-polyenic fatty acids from lipid peroxides by a glutathione peroxidase. Biochim. Biophys. Acta **164**, 35–46.

Croft K. P. C., Voisey C. R. and Slusarenko A. J. (1990), Mechanism of hypersensitive cell collapse: Correlation of increased lipoxygenase activity with membrane damage in leaves of Phaseolus vulgaris (L.) inoculated with an avirulent race of *Pseudomonas syringae pv. phaseolicola.* Physiol. Mol. Plant Pathol. **36**, 49–62.

Dudda A., Spiteller G. and Kobelt F. (1996), Lipid oxidation products in ischemic porcine heart tissue. Chem. Phys. Lipids **82**, 39–51.

Eglinton G., Hunneman D. H. and McCormick A. (1968), Gas-chromatographic-mass spectrometric studies of long chain hydroxy acids. III. The mass spectra of the methyl esters trimethylsilyl ethers of aliphatic hydroxy acids. A facile method of double bond location. Org. Mass Spectrom. **1**, 593–611.

Egmond M. R., Vliegenthart J. F. G. and Boldingh J. (1972), Stereospecificity of the hydrogen abstraction at carbon atom n-8 in the oxygenation of linoleic acid by lipoxygenases from corn germs and soybeans. Biochem. Biophys. Res. Commun. **48**, 1055–60.

Esterbauer H. and Cheeseman K. H. (1990), Determination of aldehydic lipid peroxidation products: Malonaldehyde and 4-hydroxynonenal. Methods Enzymol. **186**, 407–21.

Esterbauer H., Dieber-Rotheneder M., Waeg G., Striegl G. and Jürgens G. (1990), Biochemical, structural, and functional properties of oxidized low-density lipoprotein. Chem. Res. Toxicol. **3**, 77–92.

Esterbauer H., Schaur R. J. and Zollner H. (1991), Chemistry and biochemistry of 4-hydroxynonenal, malonaldehyde and related aldehydes. Free Radicals Biol. Med. **11**, 81–128.

Folcik V. A., Nivar-Aristy R. A., Krajewski L. P. and Cathcart M. K. (1995), Lipoxygenase contributes to the oxidation of lipids in human atherosclerotic plaques. J. Clin. Invest. **96**, 504–510.

Gardner H. W. (1989), Oxygen radical chemistry of polyunsaturated fatty acids. Free Radicals Biol. Med. **7**, 65–86.

Gardner H. W. (1991) Recent investigations into the lipoxygenase pathway of plants. Biochim. Biophys. Acta **1084**, 221–39.

Glickman M. H. and Klinman J. P. (1996), Lipoxygenase reaction mechanism: demonstration that hydrogen abstraction from substrate precedes dioxygen binding during catalytic turnover. Biochemistry **35**, 12882–92.

Gutteridge J. M. C. (1986), Iron promoters of the Fenton reaction and lipid peroxidation can be released from hemoglobin by peroxides. FEBS Lett. **201**, 291–5.

Halliwell B. (1993), The role of oxygen radicals in human disease, with particular reference to the vascular system. Haemostasis **23** (Suppl. 1), 118–26.

Herold M. and Spiteller G. (1996), Enzymatic Production of hydroperoxides of unsaturated fatty acids by injury of mammalians cells. Chem. Phys. Lipids **79**, 113–21.

Holman R. T. and Burr G. O. (1945), Spectrophotometric studies of the oxidation of fats. IV. UV-Absorption spectra of lipoxidase-oxidized fats. Arch. Biochem. **7**, 47–54.

Janero D. R. (1990), Malondialdeyhde and thiobarbituric acid-reactivity as diagnostic indices of lipid peroxidation and peroxidative tissue injury. Free Radicals Biol. Med. **9**, 515–40.

Jira W., Spiteller G. and Schramm A (1996), Increase in hydroxy fatty acids in human low density lipoproteins with age. Chem. Phys. Lipids **84**, 165–73.

Jira W., Spiteller G. and Richter A. (1997), Increased levels of lipid oxidation products in low density lipoproteins of patients suffering from rheumatoid arthritis. Chem. Phys. Lipids **87**, 81–89.

Jira W., Spiteller G. and Carson W. (1998), Strong increase in hydroxy fatty acids derived from linoleic acid in human low density lipoproteins of atherosclerotic patients. Chem. Phys. Lipids **91**, 1–11.

Kanner J. and Harel S. (1985), Initiation of membranal lipid peroxidation by activated metmyoglobin and methemoglobin. Arch. Biochem. Biophys. **237**, 314–21.

Koppenol W. H. (1994), Chemistry of iron and copper in radical reactions. New Compr. Biochem. **28**, 3–24.

Kosugi H., Kojima T. and Kikugawa K. (1989), Thiobarbituric Acid-Reactive Substances from Peroxidized Lipids. Lipids **24**, 873–881.

Kuehn H., Belkner J., Wiesner R., Lankin V. Z., Tikchase A. K. and Schewe T. (1992), Structure elucidation of oxygenated lipids in human atherosclerotic lesions. Eicosanoids **5**, 17–22.

Kuzuya M., Naito M., Funaki C., Hayashi T., Asai K. and Kuzuya F. (1991), Lipid peroxide and transition metals are required for the toxicity of oxidized low density lipoprotein to cultured endothelial cells. Biochim. Biophys. Acta **1096**, 155–161.

Lehmann W. D. (1994), Regio- and stereochemistry of the dioxygenation reaction catalyzed by (S)-type lipoxygenase or by the cyclooxygenase activity of prostaglandin H synthases. Free Radicals Biol. Med. **16**, 241–53.

Leiss O., Murawski U. and Egge H. (1979), Mikrobestimmung von Lipoproteinlipiden im Serum. J. Clin. Chem. Clin. Biochem. **16**, 619–625.

McCord J. M. (1985), Oxygen-derived free radicals in postischemic tissue injury. New Engl. J. Med. **312**, 159–63.

Mlakar A. and Spiteller G. (1996), Distinction between enzymic and nonenzymic lipid peroxidation. J. Chromatogr. A **743**, 293–300.

Nikkari T., Malo-Ranta U., Hiltunen T., Jaakkola O. and Ylä-Herttuala S. (1995), Monitoring of lipoprotein oxidation by gas chromatographic analysis of hydroxy fatty acids. J. Lipid Res. **36**, 200–7.

Parthasarathy S., Steinbrecher U. P., Barnett J., Witztum J. L. and Steinberg D. (1985), Essential role of phospholipase A_2 activity in endothelial cell-induced modification of low density lipoprotein. Proc. Natl. Acad. Sci. USA **82**, 3000–3004.

Pryor W. A., Stanley J. P. and Blair E. (1976), Autoxidation of polyunsaturated fatty acids: II. A suggested mechanism for the formation of TBA-reactive materials from prostaglandin-like endoperoxides. Lipids **11**, 370–9.

Schlag G. and Redl H. (1993), Organ in shock, early organ failure, late organ failure. In: Pathophysiology of Shock, Sepsis, and Organ Failure (Schlag G., Redl H. eds.). Springer Press, Berlin Heidelberg, pp. 1-3.

Schuster F. P. (1992), Kreislaufschock. In: Innere Medizin in Praxis und Klinik, Band I: Herz, Gefäße, Atmungsorgane, Endokrines System (Hornbostel H., Kaufmann W., Siegenthaler W. eds.). Georg Thieme Verlag, Stuttgart New York, pp. 1.422–1.447.

Spiteller P. and Spiteller G. (1997), 9-Hydroxy-10,12-octadecadienoic acid (9-HODE) and 13-hydroxy-9,11-octadecadienoic acid (13-HODE): Excellent markers for lipid peroxidation. Chem. Phys. Lipids **89**, 131–39.

Spiteller P. and Spiteller G. (1998), Epoxyhydroxy acids, hydroxyoxo acids and aldehydes are main products in Fe^{3+} catalyzed air oxidation of the 13-hydroperoxide of linoleic acid. Biochim. Biophys. Acta **1392**, 23–40.

Steinbrecher U. P., Zhang H. and Lougheed M. (1990), Role of oxidatively modified LDL in atherosclerosis. Free Radicals Biol. Med. **9**, 155–168.

Uemura T. (1990), Biological function and lipid constituents of oxidatively modified lipoproteins. Domyaku Koka **18**, 81–90.

Vine N. and Powell J. T. (1991), Metalloproteinases in degenerative aortic disease. Clin. Sci. **81**, 233–9.

Wang T. and Powell W. S. (1991), Increased levels of monohydroxy metabolites of arachidonic acid and linoleic acid in LDL and aorta from atherosclerotic rabbits. Biochim. Biophys. Acta **1084**, 129–38.

Yagi K. (1976) A simple fluorometric assay for lipoperoxide in blood plasma. Biochem. Med. **15**, 212–16.

Yamamoto Y., Niki E. and Kamiya Y. (1982), Oxidation of lipids. I. Quantitative determination of the oxidation of methyl linoleate and methyl linolenate. Bull. Chem. Soc. Jpn. **55**, 1548–50.