DOI 10.1002/mnfr.200500090 Mol. Nutr. Food Res. 2005, *49*, 1044 – 1049

Review

4-Hydroxynonenal and cholesterol oxidation products in atherosclerosis

Gabriella Leonarduzzi[1], Elena Chiarpotto[1], Fiorella Biasi[2] and Giuseppe Poli[1]

[1]Department of Clinical and Biological Sciences, University of Turin, Turin, Italy
[2]National Research Centre, Turin, Italy

4-Hydroxynonenal (HNE) is by far the most investigated aldehydic end-product of oxidative breakdown of membrane n-6 polyunsaturated fatty acids. Its potential involvement in the pathogenesis of atherosclerosis has been corroborated by its consistent detection in both oxidized LDL and fibrotic plaque in humans. HNE has been shown to activate both macrophage and smooth muscle cells, *i.e.* the two key cell types in chronic inflammatory processes characterized by excessive fibrogenesis. By signalling to the nucleus, the aldehyde may up-regulate in these cells both expression and synthesis of monocyte chemotactic protein 1 (MCP-1) and transforming growth factor β1 (TGFβ1). Oxysterols, namely 27 carbon atoms oxidation products of cholesterol, are found in relatively high amount in LDL from hypercholesterolemic individuals and are consistently detectable in foam cells and necrotic core of human atherosclerotic lesion. As for HNE, the challenge of cells of the macrophage lineage with a mixture of oxysterols like that detectable in hypercholesterolemic individuals led to a marked overexpression of TGFβ1 and MCP-1. Both HNE and oxysterols then appear to be candidates for a primary role in the progression of the atherosclerotic process.

Keywords: Atherosclerosis / Cholesterol / 4-Hydroxynonenal / Inflammation / Oxysterols

Received: June 8, 2005; revised: August 16, 2005; accepted: August 16, 2005

1 Introduction

The oxidative theory of atherosclerosis, first proposed by Steinberg *et al.* about 25 years ago [1], postulates that oxidized or otherwise modified low density lipoproteins (oxLDL) play a major role in the initiation and promotion of fatty streaks and fibrotic plaques. An appropriate update of this concept was recently supported by a number of scientists including Steinberg himself, who underscored the pivotal role made by inflammation in the progression of this globally spread disease [2]. Among oxidative agents present in oxLDL that may be responsible for its proatherogenic effect are aldehydic end-products of lipid peroxidation and cholesterol oxidation products. The potential contribution of these two classes of oxidized lipids to atherosclerosis progression is based on their marked profibrogenic, proapoptotic and proinflammatory effects.

Correspondence: Prof. Giuseppe Poli, Department of Clinical and Biological Sciences, University of Turin, S. Luigi Hospital, 10043 Orbassano (Turin), Italy
E-mail: giuseppe.poli@unito.it
Fax: +39 011-670-5413

Abbreviations: COX-2, cyclooxygenase; **HNE**, 4-hydroxynonenal, **JNKs**, c-Jun amino terminal kinases; **7K**, 7-ketocholesterol; **MAPKs**, mitogen activated protein kinases; **MCP-1**, monocyte chemotactic protein 1; **oxLDL**, oxidize low density lipoproteins; **PDGF-AA**, platelet-derived growth factor-AA; **PKC**, protein kinase C; **ROS**, reactive oxygen species; **TGFβ1**, transforming growth factor β1

2 Chemistry and biochemistry of 4-hydroxynonenal (HNE)

HNE is by far the most investigated aldehydic end-product of oxidative breakdown of membrane n-6 polyunsaturated fatty acids, among which are the two most represented fatty acids in biomembranes, namely arachidonic and linoleic acids. HNE is an unusual compound containing three functional groups that in many cases act in concert and help to explain its high reactivity. There is, first of all, a conjugated system consisting of a C=C double bond and a C=O carbonyl group in HNE. The hydroxy group at carbon 4 contributes to the reactivity both by polarizing the C=C bond and by facilitating internal cyclisation reactions such as thioacetal formation.

Virtually all of the biochemical effects of HNE can be explained by its high reactivity towards thiol and amino groups. Primary reactants for HNE are the amino acids

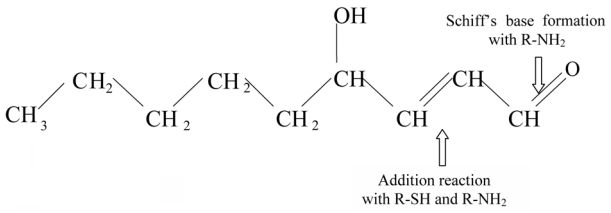

Figure 1. Structure and main reactions of HNE.

cysteine, histidine and lysine, which–either free or protein-bound-undergo readily Michael addition reactions to the C=C double bond. Besides this type of reaction, which confers rotational freedom to the C2–C3 bond, secondary reactions may occur involving the carbonyl and the hydroxy group. Amino groups may alternatively react with the carbonyl group to form Schiff bases [3] (Fig. 1).

An increasing number of reports provide evidence of a causative involvement of this biogenic aldehyde in the pathogenesis of a great number of inflammatory and degenerative processes, including atherosclerosis, Alzheimer's and Parkinson's diseases, liver fibrosis, glomerusclerosis, chronic obstructive bronco-pulmonary diseases and, last but not the least, atherosclerosis (see for a review [4]). With special regard to HNE potential involvement in the pathogenesis of atherosclerosis, this has been corroborated by consistent detection of the aldehyde in both oxLDL [3] and fibrotic plaque in humans [5]. Indeed, the *in vitro* use of HNE in the low μM range appears compatible with the actual concentration the aldehyde may reach in the experimental animal and in human lipoprotein particles [3]. Moreover, the fact that HNE leads to the formation of stable, but reversible [3], lipid and protein adducts points to the ability of this peculiar compound to sustain in the long run biochemical and possibly biological effects generated by the molecule itself.

3 HNE signalling to the nucleus

The availability of fluorescent mAbs raised against histidine-HNE adducts, allowed us to semiquantitatively follow the intracellular fate of micromolar amounts of the aldehyde, added to the incubation medium of a large variety of cells. Cell uptake and intracellular tropism of HNE has so far been successfully monitored by using fluorescent antibodies against HNE-histidine adducts in hepatic stellate cells [6], cells of the macrophage lineage [7], hepatocytes in suspension or in primary culture, neuroblastoma cells, colon carcinoma cells (Poli *et al.*, unpublished data). In all these cells, after 5–10 min of prevailing aldehyde consumption, one can consistently recover HNE in the cytoplasmic space. Further, of great interest, a nuclear tropism of the aldehyde appears more evident with time [6].

In all cell types where HNE has shown intracellular migration, a consistent modulation of protein kinase C (PKC) superfamily has been demonstrated. The trend observed in hepatocytes, neuronal cells and macrophages was the following: in the high nanomolar range (10^{-7} M), the incubation with the aldehyde led to strong activation of classic isoforms β_1 and β_2 [8, 9]. On the contrary, HNE in the low micromolar range (10^{-5}–10^{-6} M) was able to markedly activate hepatocyte novel isoforms of nPKCs, in particular the δ isoform. The latter type of PKC modulation appears related to the well recognized proapoptotic effect of HNE, as proved by the full prevention of apoptosis in macrophages pretreated with rottlerin, a selective inhibitor of nPKCs (Chiarpotto *et al.*, manuscript submitted for publication).

Whether directly interacting with PKC or through the activation of EGF receptor, HNE signalling has been demonstrated by a number of laboratories to consistently involve the mitogen activated protein kinase (MAPK) pathway.

The first demonstration of a net activation of c-Jun amino terminal kinases (JNKs) by HNE, in a concentration (1 μM) definitely compatible with those found *in vivo*, was provided by Parola and colleagues on primary cultures of human hepatic stellate cells. By using mAbs specific for HNE-histidine adducts, authors demonstrated that externally added HNE was able to reach the nucleus of these cells and colocalise with translocated JNK, pointing to the involvement of the aldehyde in the nuclear translocation of the kinase [6]. Several other reports then followed which definitely proved the ability of this aldehyde to markedly up-regulate JNK in a variety of cell types. The group of Uchida also reported on the strong activation of p38 MAPK besides JNKs in rat liver epithelial RL34 cancer cell line challenged with HNE (25 μM final concentration). In the same model system, activity of extracellular signal-regulated kinases (ERKs) was on the contrary scarcely affected [10]. Indeed, most of the so far performed *in vitro* studies did not provide evidence of modulation of ERKs by HNE.

Finally, in relation to HNE-dependent modulation of MAPKs, it appears important to draw the attention on the observation of JNK activation in primary cultured astrocytes by hydrogen peroxide through a mechanism that apparently implies arachidonic acid metabolism [11]. But arachidonic acid, when undergoes oxidative breakdown, represents a major source of HNE; then, JNK activation by hydrogen peroxide could actually involve HNE as one of the chemical mediators.

4 Up-regulation of redox-sensitive transcription factors by HNE

Among the several transcription factors shown to be modulated by intracellular redox reactions [12], those belonging

to two families of transcriptionally active peptides, called activator protein-1 (AP-1) and nuclear factor kB (NF-kB), have been intensively investigated for potential modulation by HNE.

A marked increase of AP-1 nuclear binding by HNE was among the earlier observations of a cell signalling ability expressed by the aldehyde. Such effect was first demonstrated by Camandola *et al.* in cultivated cells of the macrophage lineage, as exerted by HNE in the low micromolar range (1–10 μM). A significant up-regulation of the transcription factor was already evident 10–15 min after cell challenge with the aldehyde, with a maximum increase between 30 and 60 min incubation, followed by a later slow decrease at least within two hours of observation [13]. The stimulating effect of HNE on the transcription factor was also confirmed by other laboratories, for example, still in the low micromolar concentration range in rat neuronal cells, rat aortic smooth muscle cells and rat epithelial cancer cells [10]. Such strong HNE-dependent activation of AP-1 binding and activity, actually found in all cytotypes so far considered, resulted to be consistently based on a net induction of c-Jun gene expression and synthesis.

Like AP-1, NF-kB is a redox-sensitive transcription factor of primary interest in the pathogenesis of various human diseases. Also, in the case of NF-kB, most of its inducers rely on the production of reactive oxygen species (ROS) and related reactions with cell macromolecules [14].

Of note, NF-kB does not respond to lipid peroxidation-derived aldehydes (HNE) in the way showed by AP-1. In fact, the majority of reports on the possible modulation of NF-kB by HNE showed either inhibition or lack of effect. However, a stimulatory effect was reported as exerted by HNE 1 μM at least on vascular smooth muscle cells [15]. This so far the only evidence of potential up-regulation of NF-kB activity by HNE, still contributes to further outline how different could be the aldehydes action on this factor in the different cell types.

5 HNE-dependent modulation of gene expression

Modulation of AP-1 and maybe NF-kB nuclear binding by HNE should likely interfere with the regulation of expression of a number of genes that have consensus sequences for these peptides in their promoter regions. At present, clear evidence of HNE-dependent overexpression is available for a number of genes whose optimal transcription mainly requires AP-1 activation. Of these genes, some are of primary interest for pathogenetic studies on antioxidants, namely those coding for transforming growth factor β1 (TGFβ1), procollagen type I, platelet-derived growth factor-AA (PDGF-AA), monocyte chemotactic peptide-1 (MCP-1) and cyclooxygenase-2 (COX-2).

The first two reports on the ability of HNE (1–10 μM) to induce gene expression were related to pathophysiology of fibrogenesis. In relation to this, seminal experiments have been those carried out on the profibrogenic effect of the prooxidant hepatotoxin carbon tetrachloride. Following rat chronic intoxication, CCl_4 showed a strong stimulatory effect on the expression of the key-fibrogenic cytokine TGFβ1 and of procollagen type I, which was fully prevented when animals were suitably supplemented with the antioxidant α-tochopherol [16].

In primary cultures of rat aortic smooth muscle cells, three hours incubation with 1 μM HNE led to strongly up-regulate the synthesis of PDGF-AA. Simultaneous treatment with *N*-acetyl cysteine was able to prevent the overexpression of this cytokine, indirectly indicating that this other evidence relevant to atherosclerosis was likely oxidant-mediated [17]. Still related to inflammation is the reported effect of HNE on the expression and synthesis of MCP-1 and of COX-2. Parola and colleagues [18] have found this aldehyde able to up-regulate the expression of the key C-C chemokine MCP-1 in primary cultures of human liver stellate cells, at very low steady-state concentration (1 μM). Increased synthesis and activated secretion of MCP-1 by murine macrophages (J774A.1) challenged with 1–10 μM HNE was then reported by Domenicotti *et al.* [19]. Certainly, the demonstration that HNE is able to markedly up-regulate MCP-1 levels in tissues like liver and arterial wall, where phagocytes and extracellular matrix cells are accumulating, provides important proofs in favour of this aldehyde as mediator molecule primarily involved in inflammatory processes, especially those with chronic trend like liver fibrosis and atherosclerosis. In the latter disease process, HNE is likely transported by oxLDL within the core of fibrotic plaque where it may accumulate and chemically attract cells of the macrophage and smooth muscle lineages. For this reason, *in vitro* studies on the potential involvement of HNE in atherosclerosis have essentially been focused on these two types of cells, paying much less attention to another important type of vascular cell, *i.e.* the endothelial cell. In addition, HNE was shown to bind to lysine residues of LDL apolipoproteins [3], thus it is not apparently free to react with molecular targets of the endothelial lining barrier while lipoprotein micelles are taken up through a receptor-mediated mechanism.

Taking all available data together, it seems reasonable to consider HNE as one of the molecules consistently involved in the atherosclerotic process, both in the formation of the atheroma and in the fibrotic transformation of the arterial wall. But rather involved in the progression of the atherosclerotic lesion than in its initiation.

6 Oxysterols: Biochemical background and occurrence *in vivo*

Among the oxidation products of the lipid moiety of LDL, cholesterol oxidation products called oxysterols are of great interest as possible reactive mediators of structural and functional changes of the vascular wall, which are affected by the atherosclerotic process (for a review see [20]). Oxysterols are well known for their toxicity. Indeed, the notion that pure cholesterol is reactive *per se* appears remote, whereas oxysterols should be viewed as toxic because they cause dysfunction of vascular endothelial cells [21] and play an active role in atherosclerotic lesion development [22, 23]. Oxysterols are 27-carbon products of cholesterol oxidation. Besides being intermediates or end products in cholesterol degradation, oxysterols have been implicated in many cellular processes. They have a broad spectrum of biological effects [24–27]. Notably, one of the critical properties of oxysterols is their ability to pass lipophilic membranes rapidly due to the additional oxygen function [28, 29].

Oxysterols may enter the blood circulation through the diet; they are also generated endogenously through oxidation of the lipoprotein lipid moiety and through intracellular metabolism. In the first case, they derive from autoxidation (nonenzymatic oxidation) of cholesterol present in various foodstuffs. In the second case, plasma oxysterols may arise either from autoxidation of LDL cholesterol, which is mainly mediated by reactive prooxidant species, or specific enzymatic reactions [20]. The vast majority of the oxysterols of interest in pathophysiology are found in food and food derivatives: in particular, 7-ketocholesterol (7K), 7α-hydroxycholesterol, 7β-hydroxycholesterol, 5α,6α-epoxycholesterol, 5β,6β-epoxycholesterol, cholestan-3β,5α,6β-triol and 25-hydroxycholesterol. They may be present in fresh or raw foodstuffs containing cholesterol and also in seasonings. However, more important, several factors are known to accelerate the oxidation of food cholesterol: in particular, γ- and UV-radiation, photo-oxidation, heat, presence of oxygen, presence of prooxidant agents and storage conditions [30].

7 The cytotoxicity of oxysterols is quenched when they are in a mixture

Oxysterols, as with the other oxidation products of the lipid moiety of plasma LDL, are consistently found within the walls of major arteries, mainly in the characteristic lesions of atherosclerosis. Over the last decade, reliable *in vitro* studies have characterized the potential proapoptotic effect of the major oxysterols with regard to vascular cells, namely smooth muscle cells, endothelial cells, fibroblasts and monocyte-macrophages (see [20] for a comprehensive review).

In addition, it is now possible to conclude that various oxysterols of pathophysiological interest appear to up-regulate intracellular steady-state levels of ROS, essentially through enhancement of NADPH oxidase activity [31, 32]. Solid proof of a causative role for oxidative stress in apoptosis provoked by 7K was recently provided. Indeed, a marked or almost complete inhibition of 7K-dependent ROS increase by two selective inhibitors of NADPH oxidase, namely diphenyleneiodonium chloride (DPI) and 4-(2-aminoethyl)benzenesulfanyl fluoride (AEBSF) and by the antioxidant epicatechin allowed to significantly protect macrophage cells against 7K-induced apoptotic death, as shown both in morphological and biochemical terms [32].

A very interesting point has recently been addressed, namely the relatively lower cytotoxicity of oxysterols when given to cell models as a mixture rather than as individual compounds. Indeed, oxysterols are always present as a mixture in foods, oxLDL or in the core region of atherosclerotic plaque and molecular interactions often occur among mixed compounds. Using cells of the macrophage lineage, Leonarduzzi and colleagues analysed the profibrogenic effect both of 7K and of a biologically representative mixture of oxysterols in a percentage composition consistent with that found in the plasma LDL of hypercholesterolemic patients. Within a concentration range of pathophysiological interest (10–30 μM), the oxysterol mixture was markedly profibrogenic, while an equimolar amount of 7K was not [33]. Starting from this evidence, we analysed the effect of 7K on murine J774A.1 macrophages, in the presence or in the absence of equimolar concentrations of 7β-hydroxycholesterol, in terms of ROS generation, cytochrome c release from mitochondria, caspase-3 activation, p21 up-regulation and morphological appearance of condensed nuclei and apoptotic bodies. All events along the mitochondrial apoptotic pathway triggered by 7K were significantly quenched when cells were cotreated with identical amounts of the second sterol oxide [34].

8 Proinflammatory effect of oxysterols

During progression of atherosclerosis, inflammation certainly plays a pivotal role [2]. Oxysterols likely contribute to the proinflammatory effect of oxLDL because of both their quantitative relevance and their biochemical activity. Consistently, an increasing number of reports point to the modulation of proinflammatory molecules by cholesterol oxidation products accumulating within human fibrotic plaques. To date enough literature is available which supports an inflammatory action of oxysterols exerted on vascular cells (for a review see [20]).

Also, our laboratory has been recently involved in investigating the potential proinflammatory action of oxysterols found in human oxLDL. In fact, we observed that an oxysterol mixture compatible with that detectable in human hypercholesterolemic plasma, unlike equimolar concentrations of 7K, markedly up-regulated TGFβ1 expression and synthesis in cells of the macrophage lineage [33]. Subsequently, using the same human promonocytic cell line (U937), we observed that the model oxysterol mixture employed was also able to up-regulate the steady-state levels of messenger RNA specific for a small number of chemokines, in particular that of MCP-1 [35]. Of note, knocking out the MCP-1 gene reduces lesion size in murine models of atherosclerosis [36], pointing to a leading role for monocyte recruitment in relatively early phases of atherosclerosis. We then performed immunoenzymatic analysis to confirm the gene expression data regarding MCP-1 up-regulation by the oxysterol mixture. The amount of chemokine actually synthesized by macrophages *in vitro* challenged with the mixture was indeed found to be significantly higher than in control cells. On the contrary, MCP-1 levels in the cell samples treated with equimolar concentrations of 7K or nonoxidized cholesterol did not show any variation compared to untreated cells [35].

Thus, the apparent ability of oxysterols to favour the migration of monocytic cells points to a probably significant contribution of these products to the promotion and progression of atherosclerotic lesions within arteries.

9 Mechanisms underlying the proinflammatory effect of oxysterols

Very little evidence is available thus far to elucidate the mechanisms by which cholesterol oxides contribute to the progression of atherosclerotic lesions. The only established step is the ability of various oxysterols to up-regulate steady-state levels of ROS in target cells by stimulating NADPH oxidase activity [31, 32].

Very recent results from our laboratory demonstrated the inhibition of oxysterol-induced MCP-1 overexpression when macrophages were pretreated with inhibitors of classic PKC isoform (Leonarduzzi et al, unpublished data). These isoenzymes are recognized to enhance cellular ROS production through NADPH oxidase up-regulation. However, there are as yet no data to explain how oxysterol-dependent ROS production may induce cells to express proinflammatory rather than proapoptotic stimuli. In our opinion, the natural occurrence of oxysterols as a mixture would first favour an overall proinflammatory action, partly because of the quenching of the toxicity of specific components, as reported above. In the long run, the concentration threshold for toxic compounds such as 7K might be overcome, probably through selective metabolism and then suicide or necrogenic signals would prevail.

Investigation of the transcription pathways effectively stimulated by oxysterol-induced ROS increase will undoubtedly be a major research target in the near future with regard to the pathogenetic role of cholesterol in atherosclerosis.

10 Concluding remarks

The *in vivo* generation of aldehydic end-products of lipid peroxidation and cholesterol oxidation products has been confirmed by several laboratories. Further, increasing experimental evidence points to a primary contribution of oxysterols and, in the second instance, of biogenic unsaturated aldehydes in the development of atherosclerosis. In contrast to the so-called stable atherosclerotic plaque, the unstable variety may become enlarged and lead to vascular complications such as further stenosis of the lumen or plaque rupture and thrombosis. Unstable plaque is characterized by the presence of a sustained inflammatory stimulus, which in turn is maintained by sustained partial cell death. Oxysterols and HNE, but even other lipid oxidation products, consistently present in significant amounts in unstable atherosclerotic plaque, most likely provide a primary contribution to the whole process through their pronounced proinflammatory, profibrogenic and proapoptotic effects.

Further molecular research has to focus on the mechanisms through which oxysterols and HNE signal to the nucleus of vascular cells in a way to exert proatherogenic stimuli. Cell signalling by both oxidized cholesterol and HNE, as reported in a recent review dedicated to oxidative stress-mediated cell response, may also include modulation of antioxidant pathways [10], a fact that further complicates the overall picture. In addition, of great potential interest appears the common ability of different molecules like oxysterols and HNE to up-regulate expression and synthesis of TGFβ1 and MCP-1. Even if a clear explanation of this finding is not available yet, we tend to consider a fine tuning of ROS production as the common and initial pathway through which oxidized cholesterol and HNE up-regulate the two considered cytokines. While activation of NADPH-oxidase has already been confirmed in vascular cells treated with oxysterols, similar effect still has to be proven for HNE. But, the observed ability of this aldehyde to enhance PKC activity [10, this paper], allows to consider a prooxidant effect of HNE as a very likely phenomenon in the vasculature undergoing atherosclerotic challenge.

Original data reported in this review have been obtained with the support of Italian Ministry of Education, PRIN 2002 and 2004, Region of Piedmont and University of Turin, Italy.

11 References

[1] Steinberg, D., Parthasarathy, S., Carew, T. E., Khoo, J. C., Witztum, J. L., *N. Engl. J. Med*. 1989, *320*, 915 – 924.

[2] Steinberg, D., *Nat. Med.* 2002, *8*, 1211 – 1217.

[3] Esterbauer, H., Schaur, R. J., Zollner, H., *Free Radic. Biol. Med.* 1999, *11*, 81 – 128.

[4] Poli, G., Schaur, R. J., *IUBMB Life* 2000, *50*, 315 – 321.

[5] Napoli, C., D'Armiento, F. P., Mancini, F. P., Postiglione, A. *et al.*, *J. Clin. Invest.* 1997, *100*, 2680 – 2690.

[6] Parola, M., Robino, G., Marra, F., Pinzani, M. *et al.*, *J. Clin. Invest.* 1998, *102*, 1942 – 1950.

[7] Leonarduzzi, G., Arkan, M. C., Basaga, H., Chiarpotto, E. *et al*., *Free Radic. Biol. Med.* 2000, *28*, 1370 – 1378.

[8] Chiarpotto, E., Domenicotti, C., Paola, D., Vitali, A. *et al.*, *Hepatology* 1999, *29*, 1565 – 1572.

[9] Paola, D., Domenicotti, C., Nitti, M., Vitali, A. *et al*., *Biochem. Biophys. Res. Commun.* 2000, *268,* 642 – 646.

[10] Poli, G., Leonarduzzi, G., Biasi, F., Chiarpotto, E., *Curr. Med. Chem.* 2004, *11*, 1023 – 1042.

[11] Tournier, C., Thomas, G., Pierre, J., Jacquemin, C. *et al*., *Eur. J. Biochem.* 1997, *244*, 587 – 595.

[12] Arrigo, A. P., *Free Radic. Biol. Med.* 1999, *27*, 936 – 944.

[13] Camandola, S., Scavazza, A., Leonarduzzi, G., Biasi, F. *et al*., *Biofactors* 1997, *6*, 173 – 179.

[14] Schreck, R., Rieber, P., Baeuerle, P. A., *EMBO J.* 1991, *10*, 2247 – 2258.

[15] Ruef, J., Moser, M., Bode, C., Kubler, W., Runge, M. S., *Basic Res. Cardiol*. 2001, *96*, 143 – 150.

[16] Parola, M., Muraca, R., Dianzani, I., Barrera, G. *et al*., *FEBS Lett.* 1992, *308*, 267 – 270.

[17] Ruef, J., Rao, G. N., Li, F., Bode, C. *et al.*, *Circulation* 1998, *97*, 1071 – 1078.

[18] Marra, F., DeFranco, R., Grappone, C., Parola, M. *et al*., *J. Invest. Med.* 1999, *47*, 66 – 75.

[19] Nitti, M., Domenicotti, C., d'Abramo, C., Assereto, S. *et al*., *Biochem. Biophys. Res. Commun.* 2002, *294*, 547 – 552.

[20] Leonarduzzi, G., Sottero, B., Poli, G., *J. Nutr. Biochem*. 2002, *13*, 700 – 710.

[21] Peng, S. K., Taylor, C. B., Hill, R. J., Morin, R. J., *Atherosclerosis* 1985, *54*, 121 – 133.

[22] Carpenter, K. L., Taylor, S. E., van der Veen, C., Williamson, B. K. *et al*., *Biochim. Biophys. Acta* 1995, *1256*, 141 – 150.

[23] Breuer, O., Dzeletovic, S., Lund, E., Diczfalusy, U., *Biochim. Biophys. Acta* 1996, *1302*, 145 – 152.

[24] Brown, A. J., Jessup, W., *Atherosclerosis* 1999, *142*, 1 – 28.

[25] Schroepfer, G. J. Jr., *Physiol. Rev.* 2000, *80*, 361 – 554.

[26] Björkhem, I., Diczfalusy, U., *Arter. Thromb. Vasc. Biol.* 2002, *22*, 734 – 742.

[27] Guardiola, F., Dutta, P. C., Codony, R., Savaga, G. P. (Eds.), *Cholesterol and Phytosterol Oxidation Products: Analysis, Occurence and Biological Effects*, AOCS, Champaign, IL 2002.

[28] Smondyrev, A. M., Berkowitz, M. L., *Chem. Phys. Lipids* 2002, *112*, 31 – 39.

[29] Meaney, S., Bodin, K., Diczfalusy, U., Björkhem, I., *J. Lipid Res.* 2002, *43*, 2130 – 2135.

[30] Dionisi, F., Golay, P. A., Aeschlimann, J. M., Fay, L. B., *J. Agric. Food Chem.* 1998, *46*, 2227 – 2233.

[31] Rosenblat, M., Belinky, P., Vaya, J., Levy, R. *et al.*, *J. Biol. Chem.* 1999, *274*, 13790 – 13799.

[32] Leonarduzzi, G., Vizio, B., Sottero, B., Verde, V. *et al.*, *Antiox. Redox Signal*. 2005, (in press).

[33] Leonarduzzi, G., Sevanian, A., Sottero, B., Arkan, M. C. *et al*., *FASEB J.* 2001, *15*, 1619 – 1621.

[34] Leonarduzzi, G., Biasi, F., Chiarpotto, E., Poli, G., *Mol. Aspects Med.* 2004, *25*, 155 – 167.

[35] Leonarduzzi, G., Gamba, P., Sottero, S., Kadl, A. *et al., Free Radic. Biol. Med.* 2005, (in press).

[36] Gu, L., Okada, Y., Clinton, S. K., Gerard, C. *et al.*, *Mol. Cell* 1998, *2*, 275 – 281.

