Inhibition of glutathione synthesis increases the toxicity of oxidized low-density lipoprotein to human monocytes and macrophages

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Macrophages are thought to play an important role in the pathogenesis of atherosclerosis by mediating the oxidation of low-density lipoprotein (LDL). However, it is known that these cells show elevated glutathione levels after exposure to oxidized LDL. Here we demonstrate that this increase in the level of intracellular glutathione is due to synthesis *de novo* stimulated by oxidized LDL. Furthermore, inhibition of glutathione synthesis renders oxidized LDL cytotoxic to both monocytes and macrophages at a concentration well tolerated by untreated cells. The stimulation of cholesterol esterification in macrophages by low, non-toxic, concentrations of oxidized LDL is enhanced under conditions where glutathione synthesis is inhibited. These results suggest that the glutathione status of macrophages in the artery wall could be important in both controlling foam-cell formation and the detoxification of oxidized LDL.

INTRODUCTION

Lipid peroxidation may occur in any lipid environment which contains polyunsaturated fatty acids exposed to oxidants capable of abstracting a bis-allylic hydrogen atom. The subsequent reaction of the pentadienyl radical with oxygen to form the peroxyl radical results in a chain reaction which, in effect, greatly amplifies the severity of the initial oxidative insult. The biological membranes which surround cells and form the boundaries of intracellular organelles are protected from the consequences of this reaction by the presence of antioxidants which scavenge peroxyl radicals, enzymes which detoxify peroxides, and other enzymes designed to prevent the formation of oxidants capable of initiating lipid peroxidation. In contrast, the lipoproteins present extracellulary, which function to transport lipids in the body, are protected from oxidation only by antioxidants present in the lipoprotein itself or in the plasma or artery wall. It is now clear that these protective mechanisms are not always adequate to protect low-density lipoprotein (LDL) from oxidation in the artery wall and that extensive lipid peroxidation can occur [1–6]. The consequences of LDL oxidation in this situation are not fully understood, but it is thought that it leads to development of atherosclerosis [1,7–9]. In this extracellular environment it is likely that the peroxidation reaction can proceed unchecked, resulting in the formation of toxic lipid decomposition products such as aldehydes [3,6,7]. In support of this hypothesis, aldehydes such as 4-hydroxynonenal and malondialdehyde have been detected in atherosclerotic lesions, but not in normal tissue [4]. Oxidized LDL has been shown to be cytotoxic and capable of inducing smooth-muscle cell proliferation, and is chemotactic for monocytes (for a review see [8]). A consistent feature of the atherosclerotic lesion is the presence of macrophage-derived foam cells, and this had led to the hypothesis that these cells actively contribute to the development of the disease [9]. Interestingly, these cells are able to bind and take up modified forms of LDL, including oxidized LDL [10,11].

Previously we have shown that macrophages exposed to oxidized LDL for a period of 24 h have greatly elevated glutathione levels when compared with cells treated with either native or acetylated LDL [12]. This effect most likely occurs in response to aldehydes present in the LDL particle, since 4-hydroxynonenal elicited a similar response. This behaviour has also been shown in fibroblasts and is distinct from the response of cells to lipid-derived peroxides, where oxidized glutathione is exported from the cell without a 'rebound synthesis' of glutathione [13,14]. It is known that glutathione and glutathione transferases play a central role in the detoxification of lipid-derived aldehydes and that impairment of this pathway results in an increase in the toxicity of these compounds [15–17]. In the present study we have investigated the role of glutathione in protecting macrophages and monocytes from the toxicity of oxidized LDL.

MATERIALS AND METHODS

Isolation, modification and characterization of LDL

Human LDL was isolated from plasma by differential centrifugation by the method described previously [18]. After dialysis against Ca^{2+}-free phosphate buffered saline (PBS) containing 10 μM EDTA, the LDL was sterilized by filtration through a 0.2 μm-pore-size filter and stored at 4 °C before use [12]. LDL concentration was determined in units of mg of protein by using BSA as standard and the BCA protein assay supplied by Pierce. Oxidation was promoted in 1 mg/ml solutions of LDL by addition of 100 μM $CuSO_4$ followed by overnight incubation at 37 °C. The oxidized LDL was characterized by measurement of its electrophoretic mobility relative to that of native LDL (REM) on agarose gels by using the Beckman Paragon electrophoresis system [12].

Cells and cultures

Cells from the human monocyte cell line THP-1 were grown in Roswell Park Memorial Institute (RPMI) 1640 medium (GIBCO) supplemented with 10% foetal-calf serum, 4 mM glutamine and penicillin/streptomycin (10 i.u./ml). Macrophages were derived by resuspension in medium containing 25–100 ng of phorbol 12-

Abbreviations used: LDL, low-density lipoprotein; BSO, buthionine sulphoximine; PBS, Ca^{2+}/Mg^{2+}-free phosphate-buffered saline; PMA, phorbol 12-myristate 13-acetate; LDH, lactate dehydrogenase; ACAT, acyl-CoA:cholesterol acyltransferase; REM, relative electrophoretic mobility.

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myristate 13-acetate (PMA)/ml, seeded into 12-well plates at a concentration of 1×10^6 cells/ml and incubated for 48 h at 37 °C under air/CO_2 (19:1). The medium containing non-adherent cells was then removed and the cell layer of differentiated macrophages washed with sterile PBS before addition of fresh medium. THP-1 monocytes were similarly incubated for 48 h but in the absence of PMA. Buthionine sulphoximine (BSO), when present, was used at a concentration of 100 μM [16].

Oxidized LDL was prepared as described above at a concentration of 1–4 mg/ml and diluted in medium to give a final concentration of 100–400 μg/ml before addition to macrophages. With this protocol, the accumulation of non-adherent cells after PMA treatment resulted in an apparent decrease in viability of the cultures with time. Additions of oxidized LDL to monocytes were made directly into the cell suspensions to give the desired final concentration. Control medium contained PBS and, where appropriate, Cu^{2+} at the same concentration as that used to oxidize LDL. Incubation with oxidized LDL continued for 24 h, after which the medium was separated from the cells. For macrophages, the cell layer was washed before lysis with 200 μl of ice-cold 0.1 % Triton X-100/PBS. Monocyte suspensions were centrifuged at 2000 *g* for 4 mins at 4 °C. The pellet was washed by resuspension in 1 ml of PBS followed by a second centrifugation, then lysed in 200 μl of ice-cold 0.1 % Triton X-100/PBS. All lysate fractions were stored on ice until assay. Total glutathione was measured spectrophotometrically as the sum of both its oxidized and reduced forms as described by Tietze [19], and results were expressed as nmol of glutathione/mg of cell protein. Lactate dehydrogenase (LDH) activity was determined in both the medium and lysate fractions by measuring the rate of NADH oxidation at 340 nm [20]. Viability was expressed as the percentage of LDH activity in the medium relative to the sum of total LDH activity in both the cell lysate and medium.

For the measurement of cholesterol esterification in THP-1 macrophages, cells were cultured in 6-well plates in RPMI medium containing 10 % foetal-calf serum and 100 ng/ml PMA. After 48 h, the cells were changed to RPMI medium containing 0.2 % BSA and 100 ng/ml PMA, in the presence or absence of BSO. After incubation for a further 24 h, the cells were changed to fresh medium containing 0.2 % BSA, in the presence or absence of BSO, and the incorporation of radiolabelled oleate into cholesteryl oleate was measured. This measurement was performed essentially as described in [21], except that [9,10(n)-^{3}H]oleate/BSA complex (79 Ci/mol) was used. A stock solution of [^{3}H]oleate/BSA complex was prepared, consisting of 10 mM [^{3}H]oleate complexed to 1.2 mg/ml BSA. A sample (1 μl) of this complex was then added to 1 ml of RPMI medium containing 0.2 % BSA, as described above, giving a final concentration of 10 μM [^{3}H]oleate. After incubation for 24 h, the cells were washed three times with PBS and the lipids were extracted from the cell homogenates [22]. Cholesteryl [1-^{14}C]oleate was added as the internal standard (0.45 nCi/ml; 8 nM). The neutral lipids were then separated by t.l.c. in light petroleum (60–80 °C)/diethyl ether/acetic acid (90:30:1, by vol.). The cholesteryl oleate band was identified by co-chromatography with standard, scraped from the plate into Picofluor scintillant (Packard, Groningen, The Netherlands), and the radioactivity was determined in a Beckman LS5801 scintillation counter. Results are expressed as nmoles of [^{3}H]oleate incorporated/mg of cell protein.

RESULTS AND DISCUSSION

In the present study we have used the human monocytic cell line THP-1 as representative of the monocytes, which, after differentiation to macrophages, are known to form 'foam' or fat-filled cells in the artery wall [9]. On treatment with PMA the monocytes adopt the characteristics of macrophages, including the expression of the macrophage scavenger receptor [23,24]. This protein is able to bind modified forms of LDL, including oxidized LDL, and it is this process which is thought to lead to the formation of foam cells in the artery wall [1,9,10].

We have previously shown that exposure of THP-1-derived macrophages to LDL oxidized with Cu^{2+} resulted in a small but significant decrease in glutathione levels, followed by a 2-fold increase after 24 h incubation [12]. This latter result is confirmed in Table 1 and compared with the results found with oxidized LDL prepared by treatment with metmyoglobin. Both forms of oxidized LDL are capable of increasing intracellular glutathione levels, demonstrating that this effect is not specific to Cu^{2+}-modified LDL.

Treatment of THP-1 monocytes, which do not possess the scavenger receptor, with PMA causes the expression of these receptors on the cells, accompanied by a marked decrease in the number of native LDL receptors [23,24]. In order to determine whether uptake of oxidized LDL by the scavenger receptor was essential to elicit an increase in glutathione levels, we incubated monocytes with oxidized LDL and found that, although the basal level of glutathione in these cells was much lower than that found in the macrophages, an increase also occurred after 24 h exposure to oxidized LDL (Table 1). Under the conditions of this experiment we found no evidence that the monocytes had differentiated to macrophages (for example, the cells remained non-adherent), and we conclude therefore that expression of the scavenger receptor is not required for the increase in glutathione levels induced by oxidized LDL. Consistent with this hypothesis, we found that lipid extracted from oxidized LDL could also elicit an increase in glutathione levels (Table 1). In order to confirm that the elevation in glutathione levels was due to synthesis *de novo*, we exposed both macrophages and monocytes to oxidized LDL in the presence of the inhibitor of glutathione synthesis, BSO [16]. The results of this experiment are also reported in Table 1. In the control cells, not treated with oxidized LDL, glutathione levels were depressed by treatment with BSO, indicating a significant turnover of this intracellular antioxidant in these cells. The finding that the glutathione levels of the macro-

Table 1 Effect of oxidized LDL on the glutathione content of monocytes and macrophages in the presence of BSO

Adherent THP-1-derived macrophages or monocytes in suspension (at 10^6 cells/ml) were incubated for 24 h at 37 °C with 10 μM $CuSO_4$ (control) or 100 μg/ml oxidized LDL or the equivalent concentration of lipid extracted from oxidized LDL. Oxidized LDL was prepared by treatment of native LDL with Cu^{2+} (100 μM) or metmyoglobin (Mb^{3+}; 50 μM) and had an REM of 3.5–5.0. When present, BSO was added 24 h before the addition of LDL and was present throughout the experiment. Cells were then washed and subsequently lysed in ice-cold 0.1 % Triton X-100. Results are the means ± S.D. of a minimum of three independent experiments; n.d., not determined.

Condition	Glutathione content (nmol/mg of protein)	
	Monocytes	Macrophages
Control	28 ± 5.0	61 ± 9.0
Oxidized LDL (Cu^{2+})	100 ± 16	126 ± 16.0
Oxidized LDL (Lipid fraction)	n.d.	116 ± 4.1
BSO	1 ± 1.7	16 ± 11.0
BSO + oxidized LDL	12.4 ± 5.0	8.0 ± 5.0
Oxidized LDL (Mb^{3+})	n.d.	225 ± 22

Table 2 Effect on cell viability of THP-1 monocytes and macrophages on exposure to oxidized LDL in the presence or absence of BSO

THP-1 monocyte cell suspension or adherent macrophages, both seeded at 10^6 cells/well, were incubated for 24 h at 37 °C with 10 μM $CuSO_4$ (control) or 100 μg/ml oxidized LDL (REM = 4). Similarly prepared wells which had been pretreated with BSO (100 μM) were also incubated with $CuSO_4$ or oxidized LDL. Cell viability was assessed by LDH leakage, and values given represent the means ± S.D. from three experiments performed in duplicate. Those values significantly different from control as assessed by Student's t test at confidence limits of $^{**}P < 0.02$ and $^{*}P < 0.005$ respectively are shown.

	Viability (%)	
Condition	Monocytes	Macrophages
Control	84 ± 2.09	74 ± 2.3
+ BSO	84 ± 1.33	70 ± 2.89
Oxidized LDL	80 ± 1.76	68 ± 3.87
Oxidized LDL + BSO	37 ± 6.4**	24 ± 11*

Table 3 Viability of THP-1 macrophages on exposure to a cytotoxic concentration of oxidized LDL after pre-treatment with a non-cytotoxic concentration of oxidized LDL

Adherent THP-1 macrophages (10^6 cells/ml) were incubated with 10 μM $CuSO_4$ alone (a, b) or 100 μg of oxidized LDL for 24 h (c). After this period the medium was changed and the cells were incubated with (1) 400 μg/ml oxidized LDL (b, c) or (2) $CuSO_4$ alone (a), and viability was assessed after 24 h. Viability is shown relative to the control samples. The viability of the controls fell from 72 ± 0.6% to 51 ± 0.7% (means ± S.E.M., $n = 4$) over the course of the experiment. The data shown represent means ± S.E.M. for four independent determinations. Those values significantly different from the sample pre-treated with oxidized LDL as assessed by Student's t test at a confidence limit of $P < 0.001$ are shown (*).

Treatment	Viability (%)
(a) Control	100 ± 1.35*
(b) No pre-treatment with (100 μg/ml) oxidized LDL, plus 400 μg/ml oxidized LDL	45 ± 3.2*
(c) Pre-treatment with oxidized LDL (100 μg/ml), plus 400 μg/ml oxidized LDL	76 ± 1.3

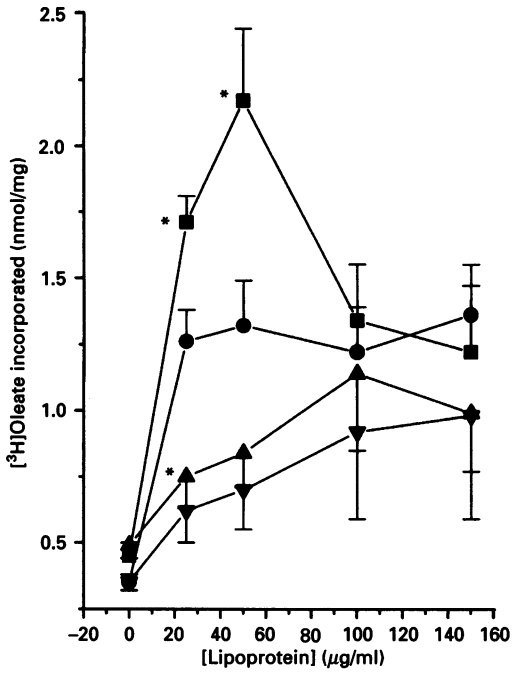

Figure 1 Effect of treatment with BSO on incorporation of [^{3}H]oleate into cholesteryl ester by THP-1 macrophages

THP-1 macrophages were preincubated for 24 h in serum-free medium, in the presence (■, ▲) or absence (●, ▼) of BSO. The cells were then incubated for a further 24 h in serum-free medium containing 10 μM [^{3}H]oleate, native LDL (▼), native LDL with BSO (▲), Cu^{2+}-oxidized LDL (●) or Cu^{2+}-oxidized LDL with BSO (■). Results are expressed as nmol of [^{3}H]oleate incorporated/mg of cell protein, and are means ± S.E.M. of four independent experiments. Values significantly different from the condition in the absence of BSO are denoted by $^{*}P < 0.05$.

phages are some 3-fold higher than those in the monocytes is interesting, and appears to be a consequence of PMA treatment (result not shown). The increase in glutathione content stimulated by oxidized LDL was inhibited by BSO, consistent with the hypothesis that the lipid fraction of oxidized LDL induces synthesis *de novo* of this intracellular antioxidant.

We have previously reported that macrophages exposed to oxidized LDL under these conditions show no apparent loss of viability, and we proposed that the increase in glutathione synthesis could be important in preventing cell death [12]. In order to test this hypothesis, we measured the viability of cells treated with oxidized LDL after treatment with BSO. The results of this series of experiments are shown in Table 2. Incubations with either BSO or oxidized LDL alone did not adversely affect the viability of macrophages or monocytes (remaining above 70–80% throughout the experiment). In contrast, when cells were treated with oxidized LDL in combination with BSO, a dramatic loss in viability occurred.

The previous experiments show that depletion of intracellular glutathione enhances the cytotoxicity of oxidized LDL. The next series of experiments were designed to determine whether the induction of glutathione synthesis initiated by a non-cytotoxic level of oxidized LDL offered any protection when cells were subsequently challenged with a cytotoxic concentration of oxidized LDL. In the first part of the experiment THP-1 macrophages were exposed to 100 μg/ml oxidized LDL and the glutathione content was measured. Under these conditions, with this sample of oxidized LDL, glutathione was increased from 117 ± 4.8 nmol/mg in the control sample to 134 ± 3.1 nmol/mg (both means ± S.D., $n = 4$) for the cells treated with oxidized LDL. Viability, at approx. 70%, remained unchanged after this treatment. An identical series of cells was then challenged with a cytotoxic concentration of oxidized LDL (400 μg/ml) and the results are shown in Table 3. Under these conditions the viability of the cells pre-treated with oxidized LDL was approx. 1.7-fold greater than that of those receiving no treatment after exposure to a cytotoxic level of oxidized LDL. This result supports our hypothesis that the induction of glutathione synthesis by macrophages when challenged with oxidized LDL increases their resistance to the cytotoxicity of this form of modified LDL.

The formation of foam cells by macrophages requires that cholesterol be esterified by the enzyme acyl-CoA:cholesterol acyl transferase (ACAT). It has been reported that, although oxidized LDL stimulates ACAT activity, it does so less effectively than do other, non-oxidized, forms of modified LDL [25]. We have therefore examined the effect of glutathione depletion upon cholesterol esterification in THP-1 macrophages. In this series of experiments, the levels of glutathione in the cells after the various treatments were similar to those reported in Table 1. Treatment

with BSO did not significantly affect the rate of cholesterol esterification in the absence of exogenous lipoprotein, suggesting that glutathione levels do not directly affect basal ACAT activity (see data in Figure 1 when no LDL added). The addition of increasing amounts of Cu^{2+}-oxidized LDL to the cells resulted in a 3.6–3.8-fold increase in incorporation of [^{3}H]oleate into cholesteryl ester in the control incubation. The increase appeared to be saturable, with near-maximal rates of esterification at 25 μg/ml oxidized LDL, consistent with stimulation of ACAT activity due to the uptake of oxidized LDL by the macrophage scavenger receptor and a concomitant increase in intracellular cholesterol. Cells which had been pretreated with BSO exhibited significantly higher rates of esterification at concentrations of 25 and 50 μg/ml oxidized LDL. Incubation of THP-1 macrophages with native LDL also stimulated cholesterol esterification in a saturable manner, indicating that these cells do possess functional native LDL receptors. Treatment with BSO did not markedly increase esterification in response to native LDL; the only significant difference was observed at 25 μg/ml. These results probably reflect the combination of two distinct processes which are controlling ACAT activity in macrophages when treated with oxidized LDL. The first process is the well-established stimulation of ACAT activity due to increased intracellular cholesterol, and the second appears to be a suppression of this effect by glutathione, with the net effect, under these conditions, of increased ACAT activity.

Interestingly, it is apparent from these experiments that glutathione depletion results in elevated levels of cholesterol esterification in response to low concentrations of oxidized LDL. If this effect were to occur *in vivo*, it could result in enhanced foam-cell formation under conditions of oxidative stress in the vasculature. That the increased level of esterification is not maintained at higher concentrations of oxidized LDL may be due to the observed loss of viability detected in the presence of BSO and oxidized LDL (Table 2). The increased rate of esterification may be due to increased lipoprotein uptake, as suggested above, or to effects on cholesterol-biosynthesis rates. However, BSO does not appear to affect significantly the down-regulation of cholesterol biosynthesis in response to oxidized LDL, as monitored by incorporation of [^{14}C]acetate into cholesterol (results not shown).

The effects of BSO on ACAT activity stimulated by native LDL and oxidized LDL are strikingly different (Figure 1). We do not know the identity of the components apparently unique to oxidized LDL which mediate these effects, but it is probable that the lipid moiety is responsible, for example a hydroxy (or hydroperoxy) derivative of cholesterol [25]. The activity of the membrane-bound phospholipid glutathione peroxidase, which is responsible for detoxifying such components [26], may be compromised by glutathione depletion. This would generate increased amounts of oxysterols, known to stimulate ACAT [25], within the cell. Since these agents are also cytotoxic, they may also contribute to the loss of cell viability observed in the glutathione-depleted cells.

In summary, our results support the hypothesis that monocytes and macrophages are capable of detoxifying the decomposition products of lipid peroxidation, such as aldehydes, and that they require glutathione for this process. The toxicity of oxidized LDL is evident in both monocytes and macrophages depleted of glutathione, suggesting that uptake of oxidized LDL by the scavenger receptor is not required for its manifestation. This is not unexpected, since aldehydes are able to partition from the lipid phase of the oxidized LDL particle into the plasma membrane of a cell. If this process were to occur in the artery wall, then the macrophage scavenger receptor may provide an important mechanism for the removal of this toxic particle and, as we have shown, the glutathione in the cell would take part in its detoxification. In support of this argument, oxidized LDL has been shown to be cytotoxic to endothelial cells [15]. In addition, it has been shown that the glutathione content of atherosclerotic lesions in fat-fed rabbits are elevated when compared with control non-lesioned areas, suggesting that glutathione-dependent detoxification of LDL may also be important *in vivo* [27].

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REFERENCES

1 Steinberg, D., Parthasarathy, S., Carew, T. E., Khoo, J. C. and Witztum, J. L. (1989) N. Engl. J. Med. **320**, 915–924
2 Steinbrecher, U. P., Zhang, H. and Lougheed, M. (1990) Free Radicals Biol. Med. **9**, 155–168
3 Palinski, W., Rosenfeld, M. E., Yla-Herttuala, S., Gurtner, G. C., Socher, S. S., Butler, S. W., Parthasarathy, S., Carew, T. E., Steinberg, D. and Witztum, J. L. (1989) Proc. Natl. Acad. Sci. U.S.A. **86**, 1372–1376
4 Yla-Herttuala, S., Palinski, W., Rosenfeld, M. E., Parthasarathy, S., Carew, T. E., Butler, S., Witztum, J. L. and Steinberg, D. (1989) J. Clin. Invest. **84**, 1086–1095
5 Mowri, H., Ohkawa, S. and Takano, T. (1988) Biochim. Biophys. Acta **963**, 208–214
6 Salonen, J. T., Yla-Herttuala, S., Yamamoto, R., Butler, S., Korpela, H., Salonen, R., Nyyssonen, K., Palinski, W. and Witztum, J. (1992) Lancet **339**, 883–887
7 Esterbauer, H., Dieber-Rothender, M., Waeg, G., Striegl, G. and Jurgens, G. (1990) Chem. Res. Toxicol. **3**, 77–92
8 Esterbauer, H., Gebicki, J., Puhl, H. and Jurgens, G. (1992) Free Radicals Biol. Med. **13**, 341–390
9 Ross, R. (1986) N. Engl. J. Med. **314**, 488–500
10 Brown, M. S., Basu, S. K., Falck, J. R., Ho, Y. K. and Goldstein, J. L. (1980) J. Supramol. Struct. **13**, 67–81
11 Sparrow, C. P., Parthasarathy, S. and Steinberg, S. (1989) J. Biol. Chem. **264**, 2599–2604
12 Darley-Usmar, V. M., Severn, A., O'Leary, V. J. and Rogers, M., (1991) Biochem. J. **278**, 429–434
13 Poot, M., Verkerk, A., Koster, J. F., Esterbauer, H. and Jongkind, J. F. (1987) Eur. J. Biochem. **162**, 287–291
14 Ishikawa, T. and Sies, H. (1984) J. Biol. Chem. **259**, 3838–3843
15 Kuzuya, M., Naito, M., Funaki, C., Hayashi, T., Asai, K. and Kuzuya, F. (1989) Biochem. Biophys. Res. Commun. **163**, 1466–1472
16 Meister, A. (1992) Biochem. Pharmacol. **44**, 1905–1915
17 Jensson, H., Guthenbera, C., Alin, P. and Mannervik, B. (1986) FEBS Lett. **203**, 207–209
18 Chung, B. H., Wilkindon, T., Geer, J. C. and Segrest, J. P. (1980) J. Lipid Res. **21**, 284–291
19 Tietze, F. (1969) Anal. Biochem. **27**, 502–522
20 Saggerson, E. D. and Greenbaum, A. L. (1969) Biochem. J. **115**, 405–417
21 Goldstein, J. L., Basu, S. K. and Brown, M. S. (1983) Methods Enzymol. **98**, 246–260
22 Bligh, E. G. and Dyer, W. J. (1959) Can. J. Biochem. Physiol. **37**, 911–917
23 Via, D. P., Pons, L., Dennison, D. K., Fanslow, A. E. and Bernini, F. (1989) J. Lipid Res. **30**, 1515–1524
24 Hara, H., Tanishita, H., Yokoyama, S., Tajima, S. and Yamamoto, A. (1987) Biochem. Biophys. Res. Commun. **146**, 802–808
25 Zhang, H., Basra, H. J. and Steinbrecher, U. P. (1990) J. Lipid Res. **31**, 1361–1369
26 Thomas, J. P., Maiorino, M., Ursini, F. and Girotti, A. W. (1990) J. Biol. Chem. **265**, 454–461
27 DelBoccio, G., Lapenna, D., Porreca, E., Pennelli, A., Savini, F., Feliciani, P., Ricci, G. and Cuccurullo, F. (1990) Atherosclerosis **81**, 127–135

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