Down-regulation of hormone-sensitive lipase in sterol ester-laden J774.2 macrophages

Catherine A. JEPSON*, Jillian A. HARRISON*, Fredric B. KRAEMER† and Stephen J. YEAMAN*‡

*Department of Biochemistry and Genetics, Medical School, University of Newcastle upon Tyne, Newcastle upon Tyne NE2 4HH, U.K., and †Division of Endocrinology, Gerontology and Metabolism, Department of Medicine, Stanford University School of Medicine, Stanford, CA 94305, U.S.A.

The development of atherosclerotic plaques in arteries is a key step in atherogenesis, with cholesterol ester accumulation in macrophage-derived foam cells being recognized as a major pathogenic event in this process. In this study, the mouse macrophage cell line J774.2 was induced to accumulate intracellular sterol esters by incubation with 25-hydroxycholesterol in the presence of oleic acid. The accumulation of sterol esters in these cells was found to be accompanied by a marked decrease in

the activity of the enzyme responsible for their hydrolysis, namely hormone-sensitive lipase (HSL); Western blotting studies revealed a corresponding decrease in the levels of the HSL polypeptide. Similar findings were obtained after incubation with oxidized low-density lipoprotein or very-low-density lipoprotein. These findings suggest that down-regulation of the expression of HSL is important in cholesterol ester accumulation in macrophages.

INTRODUCTION

Atherosclerosis is the commonest form of arterial disease, characterized by the thickening, loss of elasticity and obstruction of arteries after the formation of fatty plaques in the vessel walls. The subsequent impaired blood supply to the tissues can result in thrombosis, coronary heart disease and stroke, and contributes to many other diseases of middle and old age [1].

The fatty plaques of atheroma contain foam cells, which are derived at least in part from macrophages that have become overloaded with cholesterol esters [1]. Initially these macrophages either reside in the arterial wall or are circulating monocytes that adhere to the endothelium and penetrate into the subendothelial space at sites of damage [1]. Here they subsequently differentiate and become cholesterol ester-laden, initiated by the uptake of atherogenic lipoproteins [e.g. oxidized low-density lipoprotein (LDL) or β -very-low-density lipoprotein (β -VLDL)]. Under normal conditions the lipoprotein-derived cholesterol esters taken up by macrophages undergo a constant cycle of hydrolysis and re-esterification, catalysed by a cytoplasmic cholesterol ester hydrolase and microsomal acylCoA:cholesterol acyl transferase (ACAT) respectively [2]. If the rate of esterification exceeds that of hydrolysis, cholesterol esters accumulate in the cell. The regulation of the enzymes involved in this macrophage 'cholesterol ester cycle' is therefore important in the pathogenesis of atherosclerosis. The cholesterol ester cycle has been demonstrated not only in foam cells experimentally generated from mouse peritoneal macrophages [2], but evidence suggests that it also occurs, *in vivo*, in foam cells of atherosclerotic lesions [3].

We have previously demonstrated that the enzyme responsible for the hydrolysis of cholesterol esters in mouse peritoneal macrophages and the mouse macrophage cell line WEHI-3b is hormone-sensitive lipase (HSL) [4]. HSL is phosphorylated and activated under conditions where macrophage cAMP levels are raised [5], providing a possible mechanism for acute regulation of

the cholesterol ester cycle. More recently, Khoo et al. [6] demonstrated the expression of HSL mRNA in mouse peritoneal macrophages and the P388D1 mouse macrophage cell line.

In this study we investigate the effect of sterol ester accumulation on the levels of macrophage HSL activity. We demonstrate that the activity of HSL in the J774.2 mouse macrophage cell line is greatly decreased (at most to approx. 20 % of control values) after sterol ester loading of these cells by incubation with 25-hydroxycholesterol. This loss of HSL activity on sterol ester loading is accompanied by a marked decrease in the expression of the HSL protein, as judged by Western blotting of cell extracts with anti-HSL antibodies, thus implicating control of HSL expression as a contributing factor in foam cell development.

MATERIALS AND METHODS

Materials

The J774.2 mouse macrophage cell line was obtained from the European Collection of Animal Cell Cultures (Porton Down, Salisbury, Wilts., U.K.). Cholesterol oxidase (from *Nocardia erythropolis*) and cholesterol esterase (from *Pseudomonas fluorescens*) were from Sigma. Cholesterol [3 H]oleate was from Servicon (Lund, Sweden). Antiserum against a rat HSL/bacterial fusion protein was raised in New Zealand White rabbits as described in Kraemer et al. [7].

Lipoprotein preparation

Lipoproteins were prepared by sequential ultracentrifugation of serum from healthy young individuals, with VLDL being collected between densities 1.006 and 1.019 g/ml and LDL between densities 1.019 and 1.063 g/ml [8].

Abbreviations used: ACAT, acyl CoA:cholesterol acyl transferase; ECL, enhanced chemiluminescence; HDL, high-density lipoprotein; HSL, hormone-sensitive lipase; LDL, low-density lipoprotein; VLDL, very-low-density lipoprotein.

‡ To whom correspondence should be addressed.

LDL (approx. 1 mg/ml) was oxidized by incubation in PBS with 50 μ M CuSO₄ at 37 °C for 24 h, and then desalted before use.

Cell culture

The J774.2 cell line was maintained in RPMI-1640 medium (Gibco/BRL), containing 10 % (v/v) CPSR-1 (a lipid-free serum replacement) (Sigma), 2 mM glutamine, 50 i.u./ml penicillin and 50 μ g/ml streptomycin (Gibco/BRL) (medium A). Macrophages were loaded with sterol esters by incubation for 6–48 h at 37 °C in medium A supplemented with various concentrations of 25-hydroxycholesterol (in 0.15 % DMSO), in the presence or absence of 100 μ g/ml oleic acid (potassium salt) and 2 mg/ml fatty acid-free BSA. Control macrophages were incubated in medium A containing 0.15 % (final volume) DMSO and 2 mg/ml fatty acid-free BSA. Cholesterol ester loading was also achieved by incubation with 50 μ g/ml oxidized LDL or 40 μ g/ml VLDL for 48 h. To confirm that sterol ester loading had taken place, monolayers of macrophages were prepared on glass coverslips in 12-well plates (Costar). Cells were washed three times in PBS and then fixed for 30 min with 3.7 % (v/v) formaldehyde in 2 % (w/v) calcium acetate. Cells were then stained for neutral lipids with Oil Red O, as described by Catalano and Lillie [9].

Quantification of intracellular free sterol and sterol esters

Macrophages were harvested by scraping, followed by centrifugation at 200 *g* for 10 min at room temperature. Cell pellets were washed three times with PBS and then resuspended at a final concentration of 5×10^6 cells/ml in 0.1 % (w/v) Triton X-100. Solubilized cells (0.1 ml, or 0.1 ml of standards containing known concentrations of free cholesterol and cholesterol oleate in 0.1 % Triton X-100) were added to 0.9 ml of 50 mM Tris/HCl, pH 7.4, containing 50 mM MgCl₂, 1 mM dithiothreitol, 0.5 % sodium cholate and either 0.4 i.u./ml cholesterol oxidase (for free sterol determination) or 0.4 i.u./ml cholesterol oxidase supplemented with 0.4 i.u./ml cholesterol esterase (for sterol ester determination). After incubation at 37 °C for 30 min the reaction was terminated by the addition of 0.1 ml of methanol/ethanol (1:1). An aliquot (50 μ l) of reaction mixture was removed and analysed by HPLC on a Vydac C18 column, with isocratic elution with methanol/acetic acid (99:1). Absorbance of the cholest-4-en-3-one generated was monitored at 240 nm [10]. Under the conditions used, the oxidized derivatives of cholesterol and 25-hydroxycholesterol were eluted together. Subsequent modification of the method resolved the two components and indicated that esters of both cholesterol and 25-hydroxycholesterol are present in loaded cells (results not shown). We therefore refer throughout to sterol ester formation after incubation with 25-hydroxycholesterol.

Preparation of cell extracts

Control and sterol ester-laden macrophages were harvested and washed as above, then resuspended at a final concentration of 10^7 cells/ml in 5 mM imidazole/HCl, pH 7.0, containing 0.1 mM benzamidine, 1 mM dithiothreitol, 30 % (w/v) glycerol, 50 mM NaCl and 5 μ g/ml leupeptin. Cells were disrupted by three cycles of freeze–thawing and debris was removed by centrifugation at 13000 *g* for 10 min at 4 °C. The soluble extract was used immediately or stored at –80 °C. Whole-cell extracts for analysis by SDS/PAGE were prepared by resuspending the cell pellet in 0.625 M Tris/HCl, pH 6.8, containing 50 % (w/v) glycerol, 10 % (w/v) SDS, 0.005 % Bromophenol Blue and 12.5 % (w/v) 2-mercaptoethanol, and boiling for 10 min. Insoluble material was

removed by centrifugation at 13000 *g* for 10 min at room temperature.

Cholesterol ester hydrolase activity

Macrophage extract was assayed for hydrolase activity against cholesterol [³H]oleate essentially as described by Tornqvist et al. [11]; the final substrate concentration was 0.6 mM, with a specific radioactivity of 6.25 μ Ci/nmol. Under optimal loading conditions, the non-radioactive sterol esters in the extract would decrease the specific activity of the substrate by a maximum of 25 % if all the cellular sterol esters were present in the soluble extract. The calculated enzyme activities have not been adjusted for this possible effect. One unit of enzyme activity catalyses the release of 1 μ mol of fatty acid per min. Protein was determined by the method of Bradford [12].

Western blotting

Cell-free extracts of J774.2 macrophages were subjected to SDS/PAGE, on 8 % or 10 % (w/v) polyacrylamide resolving gels [13]. Gels were stained with Coomassie Blue R or proteins were transferred to Fluorotrans transfer membrane (Pall) with an electroblotting buffer consisting of 10 mM 3-cyclohexylamino-1-propane sulphonic acid in 10 % (v/v) methanol. Western blotting was performed with anti-HSL antiserum [7] as primary antibody and anti-rabbit IgG (peroxidase-conjugated) as secondary antibody. The blots were developed with the enhanced chemiluminescence (ECL) system (Amersham), and visualized by exposure to Hyperfilm-ECL film (Amersham). The developed films were analysed by densitometry with a Hoefer GS300 scanning densitometer linked to 'hsi gel scan' software (Hoefer Scientific Instruments).

RESULTS

Incubation of J774.2 macrophages for 24 h with 7.5 μ g/ml 25-hydroxycholesterol in the presence of 100 μ g/ml oleic acid resulted in accumulation of intracellular neutral lipid, as indicated by staining with Oil Red O (results not shown). Quantification of the sterol ester content of these macrophages demonstrated that this treatment resulted in an approx. 30-fold increase in intracellular sterol esters (means $\pm$ S.D. from 5.6 ± 6 to 161 ± 23 μ g/mg cellular protein), whereas levels of free sterol increased approx. 9-fold (means $\pm$ S.D. from 2.6 ± 2.5 to 24 ± 11 μ g/mg cellular protein). This 25-hydroxycholesterol-induced accumulation of sterol esters in J774.2 macrophages was found to be both dose-dependent (Figure 1a) and time-dependent (Figure 1b). In all subsequent experiments the concentration of 25-hydroxycholesterol used was 2.5 μ g/ml. 25-Hydroxycholesterol was used in these studies because it has previously been shown to stimulate rapidly the incorporation of oleic acid into the macrophage cholesterol ester pool by increasing the amount of cholesterol available for esterification, rather than by directly increasing the activity of ACAT [14].

To investigate the effect of this treatment on HSL activity, control and 25-hydroxycholesterol-treated macrophage extracts were assayed with cholesterol [³H]oleate as substrate. Treatment of the cells with 25-hydroxycholesterol was found to be associated with decreased HSL activity (Figures 1a and 1b). As with cholesterol ester accumulation, loss of activity in J774.2 macrophages was found to be dependent on the amount of 25-hydroxycholesterol in the incubation medium (Figure 1a). At the highest concentration tested (7.5 μ g/ml), 25-hydroxycholesterol induced a 65 ± 5 % decrease in cholesterol ester hydrolase activity after 24 h of incubation (Figure 1a). As expected, loss of activity

Figure 1 The effect of 25-hydroxycholesterol on sterol ester accumulation and HSL activity in J774.2 macrophages

J774.2 macrophages were incubated in medium A containing 2 mg/ml fatty acid-free BSA (a) for 24 h at 37 °C in the presence of the indicated amounts of 25-hydroxycholesterol and 100 μg/ml oleic acid, and (b) in the presence of 2.5 μg/ml 25-hydroxycholesterol and 100 μg/ml oleic acid at 37 °C for the times indicated. After extensive washing with PBS, cell-free extracts were prepared and assayed for HSL activity (■) or cells were solubilized in 0.1% Triton X-100 and the intracellular concentration of sterol ester was determined (●), as described in the Materials and methods section. Results are shown as means ± S.D. ($n = 4$ independent experiments). In (a) the activity in the absence of 25-hydroxycholesterol was 165 ± 13 μ-units/mg of protein, whereas in (b) the activity at zero time was 172 ± 21 μ-units/mg.

in J774.2 macrophages was also found to be time-dependent (Figure 1b), with incubation of cells in the presence of 2.5 μg/ml 25-hydroxycholesterol for 48 h resulting in a $79 \pm 8\%$ reduction in cholesterol ester hydrolase activity.

To determine whether the loss of HSL activity in sterol ester-laden macrophages could be accounted for by a corresponding reduction in the level of the HSL polypeptide, or whether other regulatory mechanisms are responsible, Western blotting studies were performed on soluble extracts of the sterol ester-laden cells with polyclonal antiserum raised against rat HSL [7]. As seen in Figure 2a, the level of the HSL protein is clearly decreased in sterol ester-laden cells in a time-dependent manner. The possibility that the decrease in HSL enzyme protein observed in the cell-free extracts of the sterol ester-laden macrophages could be due to HSL's associating with fractions of the cell removed during the extract preparation was discounted by performing Western blots on whole-cell homogenates of sterol ester-laden macrophages (Figure 2b); the levels of HSL protein were found to decrease by similar amounts in the whole-cell and soluble extracts, confirming that there is a decrease in the cellular content of HSL enzyme.

Densitometric scans of the ECL-stained Western blots allowed the amounts of HSL protein in control and sterol ester-laden cells to be quantitated. As seen in Figure 2c, the loss of HSL enzyme protein correlates closely with the time-dependent re-

Figure 2 Loss of the HSL enzyme protein in sterol ester-laden macrophages

J774.2 macrophages were incubated for the indicated times at 37 °C in the presence of 2.5 μg/ml 25-hydroxycholesterol, 100 μg/ml oleic acid and 2 mg/ml fatty acid-free BSA. (a) Cell-free extracts or (b) whole-cell lysates prepared from washed cells were subjected to SDS/PAGE, electrotransfer on to membrane and Western blotting with anti-HSL antiserum as described in the Materials and methods section. Incubation times: (a) lane 1, 0 h; lane 2, 6 h; lane 3, 12 h; lane 4, 24 h; lane 5, 48 h; (b) lane 1, 0 h; lane 2, 48 h. (c) Cell-free extracts were assayed for HSL activity (■) and for the presence of HSL polypeptide by Western blot analysis and densitometric scanning (●), as described in the Materials and methods section. Results are shown as means ± S.D. ($n = 4$ independent experiments). The activity at zero time was 157 ± 17 μ-units/mg.

duction in HSL activity in the 25-hydroxycholesterol-treated cells (being maximally decreased by $87 \pm 5\%$). The decrease in HSL enzyme protein also correlates with the loss of HSL activity in a dose-dependent manner (results not shown), being maximally decreased by $71 \pm 7\%$ in the presence of 7.5 mg/ml 25-hydroxycholesterol for 24 h. In both cases similarity between the decrease in HSL protein and the loss of HSL activity suggests that it is unlikely that other mechanisms are involved in the observed loss of HSL activity in the sterol ester-laden cells.

The observed loss of HSL enzyme protein in sterol ester-laden cells is not exclusively associated with 25-hydroxycholesterol treatment. As seen in Figure 3(a), treatment with oleic acid alone induced a smaller but significant decrease in HSL enzyme protein, as occurred when cells were treated with 25-hydroxycholesterol alone. However, treatment with both oleic acid and 25-hydroxycholesterol induced a much greater loss of HSL enzyme protein, which was associated with a more extensive accumulation of intracellular sterol ester (Figure 3b). As expected, the changes in the levels of the HSL protein again correlated with changes in HSL activity (Figure 3b). After a 24 h preincubation in the presence of 25-hydroxycholesterol and oleic acid, removal of lipid for 24 h failed to alter further either the level of HSL enzyme protein or the levels of intracellular sterol esters (Figure 3b). These observations suggest that the level of HSL protein is directly related to the amount of intracellular sterol ester and that the decreased levels are not dependent on the continued presence of exogenous lipid.

Figure 3 Effect of different lipid loading conditions on HSL enzyme protein and intracellular cholesterol ester accumulation

J774.2 macrophages were incubated for 48 h at 37 °C in medium A containing 2 mg/ml fatty acid-free BSA and (1) 0.15% (final concentration) DMSO, (2) 0.15% (final concentration) DMSO and 100 μg/ml oleic acid, (3) 2.5 μg/ml 25-hydroxycholesterol, or (4) 2.5 μg/ml 25-hydroxycholesterol and 100 μg/ml oleic acid. Macrophages were also incubated in medium A containing 2 mg/ml fatty acid-free BSA, 2.5 μg/ml 25-hydroxycholesterol and 100 μg/ml oleic acid for (5) 24 h or (6) 24 h, followed by extensive washing with PBS and incubation for a further 24 h in lipid-free medium. (a) Cell-free extracts were prepared and subjected to Western blotting as in the legend to Figure 2. (b) Western blots were analysed by densitometric scanning (open bars), cell-free extracts were assayed for HSL activity (filled bars) and the intracellular concentration of cholesterol ester determined (hatched bars) as described in the Materials and methods section. Results are shown as the averages of two independent experiments and are the percentages of the control value of 170 μ-units/mg.

Figure 4 Effect of lipoprotein or 25-hydroxycholesterol loading on HSL enzyme protein and cellular proteins

Whole-cell lysates were prepared from washed J774.2 macrophages, incubated for 48 h in medium A (lane 1), medium A supplemented with 2.5 μg/ml 25-hydroxycholesterol, 100 μg/ml oleic acid and 2 mg/ml fatty acid-free BSA (lane 2), 50 μg/ml oxidized LDL (lane 3) or 40 μg/ml VLDL (lane 4), and subjected to SDS/PAGE. (a) Western blotting with anti-HSL antiserum as described in the Materials and methods section. (b) Stained with Coomassie Blue R (15 μg of protein loaded per lane).

The HSL content of macrophages is also significantly decreased after incubation with either oxidized LDL or VLDL (Figure 4a). Furthermore analysis of the protein profile of the cells by

SDS/PAGE and staining indicates that the effect of lipoproteins and 25-hydroxycholesterol on HSL is a specific event in that the relative amounts of the major cellular proteins are unaffected by this treatment (Figure 4b).

DISCUSSION

The levels of cholesterol esters in macrophages depend on the balance between their rate of esterification catalysed by ACAT and their hydrolysis catalysed by HSL. In macrophage foam cells an imbalance clearly exists in favour of cholesterol ester deposition because of increased ACAT activity, decreased HSL activity, or both. Several studies have reported an increase in ACAT activity in lipoprotein-treated macrophages [15–17]. This activation of ACAT seems not to be due to stimulation by the direct influx of cholesterol liberated by hydrolysis of lipoproteins in the lysosomes, but to the expansion of intracellular cholesterol pools to a critical threshold level [17]. Correspondingly ACAT inhibitors have been shown to reduce lipid deposition and fatty streak formation in experimental atherosclerosis in rabbits [18,19].

Until recently, less emphasis was placed on the role of the hydrolytic phase of the cholesterol ester cycle in foam cell formation. However, by a comparison of cholesterol ester metabolism in rat peritoneal and alveolar macrophages, Ishii et al. [20] demonstrated that β -VLDL-induced cholesterol ester deposition in these cells is regulated not only by lipoprotein uptake, but also by neutral cholesterol ester hydrolase activity attributable to HSL. Furthermore Yancey and St. Clair [21] observed that the slow rate of cholesterol ester clearance in macrophages from atherosclerosis-susceptible White Carneau pigeons is partly attributable to a defect in cholesterol ester hydrolysis in these cells. This conclusion was reached when it was observed that this slow rate of cholesterol ester clearance could not be accounted for by reduced desorption of cholesterol from the plasma membrane or an increased rate of cholesterol re-esterification by ACAT. However, cholesterol ester clearance was stimulated by treatment of the macrophages with cAMP, which also increased the rate of cholesterol ester hydrolysis [21].

In the present study we have directly demonstrated a loss of neutral cholesterol ester hydrolase activity in sterol ester-laden macrophages and have also shown that it is associated with a loss of the HSL enzyme protein. Although expression of HSL is decreased markedly during sterol ester accumulation, sufficient activity must remain to allow the cycling of intracellular cholesterol ester reported previously [2]. The observation that high-density lipoprotein (HDL) alone does not induce cholesterol ester clearance from acetyl-LDL-treated J774 macrophages [22] might be related to the reduction in HSL enzyme protein in cholesterol ester-laden cells. In the presence of HDL alone, the rate of cholesterol ester hydrolysis attainable by the lower levels of HSL is presumably not sufficient to result in loss of cholesterol ester to HDL. However, HDL will promote cholesterol efflux from J774 cells in the presence of 8-(4-chlorophenylthio)-cAMP, which is likely to lead to phosphorylation and activation of HSL. This may be a necessary step to generate sufficient hydrolytic activity for clearance of cholesterol ester from the cell.

It has previously been reported that treating mouse peritoneal macrophages with acetyl-LDL to cause cholesterol ester accumulation results in changes in expression of specific cellular proteins [23]; two-dimensional gel electrophoresis of [35 S]methionine-labelled cellular proteins identified five proteins that decreased in expression, whereas two others increased. One of the proteins that was found to decrease on cholesterol ester loading has a mass of 81 kDa and a pI of 6.75. Mouse HSL has

been reported to have a mass of 82 kDa and pI 6.7–6.8 [24], consistent with the possibility that a decrease in HSL was occurring in these studies. A decrease in cellular content during cholesterol ester accumulation is apparently specific to a small subset of macrophage proteins. In our study, analysis of macrophage extracts by SDS/PAGE and protein staining indicated that no obvious changes had occurred in the range and amounts of the major proteins expressed.

The mechanism known so far by which HSL activity is controlled is reversible phosphorylation of the enzyme protein (reviewed in [25]). The observation that the decrease in HSL activity in cholesterol ester-laden cells correlates closely with a decrease in HSL protein suggests that a second regulatory mechanism for HSL activity other than reversible phosphorylation operates in these cells. This is further supported by the observation that the activity of HSL against the analogue of diacylglycerol, (3)-mono-oleoyl-2-*O*-oleylglycerol, is decreased by proportionately the same amount as that against cholesterol oleate (results not shown). As the activity of HSL against diacylglycerol is apparently unaffected by reversible phosphorylation [26], this suggests that dephosphorylation is not a major contributory factor in the observed loss of HSL activity.

Further studies must be performed to determine the mechanism by which the down-regulation of HSL expression in cholesterol ester-laden macrophages is initiated; it remains to be established whether the 'trigger' for loss of the HSL enzyme protein is increased cholesterol uptake, increased intracellular sterol ester levels or other cellular changes associated with sterol ester accumulation. This is the first report of altered HSL expression in macrophages, although decreased activity has been observed in the human macrophage cell line THP-1 after cholesterol loading [27]. HSL expression in cultured adipocytes has previously been shown to be reduced in response to tumour necrosis factor [28] and increased in response to dexamethasone [29]. The relationship between down-regulation of HSL expression and lipid accumulation is apparently cell-specific; the differentiation of 3T3-L1 fibroblasts to adipocyte-like cells is accompanied by a marked (19-fold) increase in HSL activity, even though differentiation is accompanied by a massive accumulation of stored lipid [30]. Significantly, recent work on isolation of the mouse HSL gene has detected regulatory elements in the 5' flanking region that control expression in specific tissues [31]. Of particular interest is the presence of the sterol regulatory element 1, which regulates transcription of several genes encoding proteins involved in cholesterol metabolism [32]. In conclusion, decreased HSL activity resulting from decreased expression of the enzyme protein might be important in the development of macrophage foam cells. The mechanism underlying this remains to be elucidated.

C.A.J. was supported by a project grant (to C.A.J. and S.J.Y.) and J.A.H. by a studentship from the British Heart Foundation. This work was supported in part by

research grants from the Research Service of the Department of Veterans Affairs and by grant HL 46942 from the National Institutes of Health.

REFERENCES

- Steinberg, D. (1987) in *Hypercholesterolaemia and Atherosclerosis: Pathogenesis and Prevention* (Steinberg, D. and Olefsky, J. M., eds.), pp. 5–23, Churchill Livingstone, New York
- Brown, M. S., Ho, Y. K. and Goldstein, J. L. (1980) *J. Biol. Chem.* **255**, 9344–9352
- St. Clair, R. W. (1976) *Ann. N. Y. Acad. Sci.* **275**, 228–237
- Small, C. A., Goodacre, J. A. and Yeaman, S. J. (1989) *FEBS Lett.* **247**, 205–208
- Small, C. A., Rogers, M. P., Goodacre, J. A. and Yeaman, S. J. (1991) *FEBS Lett.* **279**, 323–326
- Khoo, J. C., Reue, K., Steinberg, D. and Schotz, M. C. (1993) *J. Lipid Res.* **34**, 1969–1973
- Kraemer, F. B., Patel, S., Saedi, M. S. and Sztalryd, C. (1993) *J. Lipid Res.* **34**, 663–671
- Havel, R., Edner, H. and Bragdon, J. (1955) *J. Clin. Invest.* **34**, 1345–1353
- Catalano, R. A. and Lillie, R. D. (1975) *Stain Technology* **50**, 279–300
- Contreras, J. A., Castro, M., Bocos, B., Herrera, E. and Lasuncion, M. A. (1992) *J. Lipid Res.* **33**, 931–936
- Tornqvist, H., Bjorgell, P., Krabisch, L. and Belfrage, P. (1978) *J. Lipid Res.* **19**, 654–656
- Bradford, M. M. (1976) *Anal. Biochem.* **72**, 248–254
- Laemmli, U.K. and Favre, M. (1973) *J. Mol. Biol.* **80**, 575–599
- Miller, S. C. and Melnykovich, G. (1984) *J. Lipid Res.* **25**, 991–999
- Brown, M. S., Goldstein, J. L., Kreiger, M., Ho, Y. K. and Anderson, R. G. W. (1979) *J. Cell Biol.* **82**, 597–613
- Tabas, I., Boykow, G. C. and Tall, A. R. (1987) *J. Clin. Invest.* **79**, 418–426
- Xu, X. X. and Tabas, I. (1991) *J. Biol. Chem.* **266**, 17040–17048
- Bocan, T. M. A., Bak Mueller, S., Uhlendorf, P. D., Newton, R. S. and Krause, B. R. (1991) *Arteriosclerosis Thromb.* **11**, 1830–1841
- Tanaka, H., Ohtsuka, I., Kogushi, M., Kimura, T., Fujimori, T., Saeki, T., Hayashi, K., Kobayashi, H., Yamada, T., Hiyoshi, H. and Saito, I. (1994) *Atherosclerosis* **107**, 187–192
- Ishii, I., Oka, M., Shirai, K., Saito, Y. and Hirose, S. (1992) *Arteriosclerosis Thromb.* **12**, 1139–1145
- Yancey, P. G. and St. Clair, R. W. (1994) *J. Lipid Res.* **35**, 2114–2129
- Bernard, D. W., Rodriguez, A., Rothblat, G. H. and Glick, J. M. (1991) *J. Biol. Chem.* **266**, 710–716
- Bottalico, L. A., Kendrick, N. C., Keller, A., Li, Y. and Tabas, I. (1993) *Arteriosclerosis Thromb.* **13**, 264–275
- Holm, C., Fredrikson, G., Cannon, B. and Belfrage, P. (1987) *Biosci. Rep.* **7**, 897–904
- Yeaman, S. J., Smith, G. M., Jepson, C. A., Wood, S. L. and Emmison, N. (1994) *Adv. Enzyme Regul.* **34**, 355–370
- Fredrikson, G., Stralfors, P., Nilsson, N. O. and Belfrage, P. (1981) *J. Biol. Chem.* **256**, 6311–6320
- Graham, A., Angel, A. D. R., Jepson, C. A., Yeaman, S. J. and Hassall, D. G. (1996) *Atherosclerosis* **120**, 135–145
- Sumisa, M., Sekiya, K., Okuda, H., Tanaka, Y. and Shiosaka, T. (1990) *J. Biochem.* **107**, 1–2
- Slavin, B. G., Ong, J. M. and Kern, P. A. (1994) *J. Lipid Res.* **35**, 1535–1541
- Kawamura, M., Jensen, D. F., Wancewicz, E. V., Joy, L. L., Khoo, J. C. and Steinberg, D. (1981) *Proc. Natl. Acad. Sci. U.S.A.* **78**, 732–736
- Li, Z., Sumida, M., Birchbauer, A., Schotz, M. and Reue, K. (1994) *Genomics* **24**, 259–265
- Goldstein, J. L. and Brown, M. S. (1990) *Nature (London)* **343**, 425–430