The regulation of 3-hydroxy-3-methylglutaryl-CoA reductase activity, cholesterol esterification and the expression of low-density lipoprotein receptors in cultured monocyte-derived macrophages

Brian L. KNIGHT, Dilip D. PATEL and Anne K. SOUTAR

Medical Research Council Lipid Metabolism Unit, Hammersmith Hospital, London W12 0HS, U.K.

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Human blood monocytes cultured in medium containing 20% whole serum showed the greatest activity of 3-hydroxy-3-methylglutaryl-CoA (HMG-CoA) reductase and [^{14}C]acetate incorporation into non-saponifiable lipids around the 7th day after seeding, the period of greatest growth. Although there was enough low-density lipoprotein (LDL) in the medium to saturate the LDL receptors that were expressed by normal cells at that time, HMG-CoA reductase activity and acetate incorporation were as high in normal cells as in cells from familial-hypercholesterolaemic (FH) patients. Both the addition of extra LDL, which interacted with the cells by non-saturable processes, and receptor-mediated uptake of acetylated LDL significantly reduced reductase activity and increased incorporation of [^{14}C]oleate into cholesteryl esters in normal cells and cells from FH patients ('FH cells'), and reduced the expression of LDL receptors in normal cells. Pre-incubation for 20 h in lipoprotein-deficient medium apparently increased the number of LDL receptors expressed by normal cells but reduced the activity of HMG-CoA reductase in both normal and FH cells. During subsequent incubations the same rate of degradation of acetylated LDL and of non-saturable degradation of LDL by FH cells was associated with the same reduction in HMG-CoA reductase activity, although LDL produced a much smaller stimulation of oleate incorporation into cholesteryl esters. In normal cells pre-incubated without lipoproteins, receptor-mediated uptake of LDL could abolish reductase activity and the expression of LDL receptors. The results suggested that in these cells, receptor-mediated uptake of LDL might have a greater effect on reductase activity and LDL receptors than the equivalent uptake of acetylated LDL. It is proposed that endogenous synthesis is an important source of cholesterol for growth of normal cells, and that the site at which cholesterol is deposited in the cells may determine the nature and extent of the metabolic events that follow.

There is now evidence to suggest that many of the foam cells found in atherosclerotic lesions in arterial walls are macrophages that have taken up large quantities of cholesteryl ester-rich lipoproteins (Fowler *et al.*, 1979). This, coupled with the proposal that the reticuloendothelial system is responsible for a significant proportion of LDL catabolism in man (Goldstein & Brown, 1978), has stimulated interest in the metabolism of lipoproteins by phagocytic cells. Blood monocytes are the natural precursors of tissue macrophages (Gordon & Cohn, 1973) and

can increase in size and develop many of the properties that are characteristic of mature macrophages if maintained in culture in the presence of whole serum (Johnson *et al.*, 1977; Musson *et al.*, 1980). During the period of greatest growth, monocytes from normal human subjects express specific receptors that enable them to take up and degrade LDL present in the medium and to use the cholesterol released for membrane synthesis (Fogelman *et al.*, 1981a; Knight & Soutar, 1982b). However, production of these receptors is not essential for maturation since cells from FH subjects, in whom a genetic defect prevents the expression of active LDL receptors (Goldstein & Brown, 1977), can still grow and develop apparently

Abbreviations used: LDL, low-density lipoprotein; FH, familial hypercholesterolaemia; HMG-CoA, 3-hydroxy-3-methylglutaryl-CoA.

normally (Knight & Soutar, 1982b). Thus a supply of cholesterol that is adequate to support growth can be provided from other sources and these could be important in normal cells as well as those from FH subjects. One probable source is endogenous synthesis and in the experiments reported here we have followed the activity of HMG-CoA reductase, normally the rate-limiting enzyme for cholesterol synthesis, and the incorporation of radioactive acetate into cholesterol during the maturation of both types of cell *in vitro*.

As monocytes from both normal and FH subjects mature into macrophages they develop distinct receptors that recognize and mediate the uptake and degradation of LDL that has been modified by acetylation (Fogelman *et al.*, 1981a; Knight & Soutar, 1982b). There is a period during the maturation of normal monocytes when the cells express receptors for acetylated LDL as well as those for LDL and can take up similar amounts of each lipoprotein. This offers a good opportunity to compare directly the changes in cellular metabolism that result from the uptake of modified and unmodified LDL. In this paper we report the effects of acetylated LDL and of LDL on the activity of HMG-CoA reductase, cholesterol esterification and expression of LDL receptors in human monocyte-derived macrophages.

Methods and materials

Isolation and maintenance of cells

Culture medium was adjusted to pH 7.4 and contained 100 units of penicillin/ml and 100 µg of streptomycin/ml. All incubations were performed at 37°C in an atmosphere of CO₂/air (1:19) in a humidified incubator.

Mononuclear cells were isolated from freshly drawn blood of normal donors and from the buffy coat obtained during plasma exchange (Thompson *et al.*, 1975) of FH patients (homozygous, type IIa, receptor-defective) by the methods described previously (Knight & Soutar, 1982a; Soutar & Knight, 1982). Cells were seeded at a density of approx. 3×10^6 cells/well in 24 mm Multi-dish wells containing 1 ml of RPMI-1640 medium (Gibco Europe) with 10% (v/v) fetal-calf serum, or at a density of approx. 2×10^6 cells/well in 16 mm Multi-dish wells containing 0.7 ml of the same medium. After incubation at 37°C for 1 h, the lymphocytes and non-adherent cells were removed by washing twice with RPMI-1640 medium. Adherent cells (approx. 20% of those seeded) were then maintained at 37°C in RPMI-1640 medium containing 20% (v/v) fresh serum from normal donors. Normal cells always received autologous serum. On the 3rd day after seeding, cells were washed and fresh medium containing 20% (v/v) of the appropriate serum was added.

Lipoproteins

LDL (ρ 1.019–1.055 g/ml) and lipoprotein-deficient serum were obtained from fresh plasma from normal healthy donors. The methods used for their isolation, radioiodination of LDL and acetylation of LDL with acetic anhydride (Basu *et al.*, 1976) have been described previously (Soutar & Knight, 1982). Lipoproteins were stored sterile at 4°C and were dialysed for 2 h against 100 vol. of Dulbecco's phosphate-buffered saline (Gibco Europe) immediately before each experiment.

Assays

For the assay of HMG-CoA reductase, cells were washed twice with phosphate-buffered saline and lysed in the wells by the addition of 50 mM-potassium phosphate buffer, pH 7.4, containing 5 mM-dithiothreitol, 1 mM-EDTA, 85 mM-KCl and 0.25% (v/v) Kryo EOB (100 µl in 24 mm wells; 75 µl in 16 mm wells). The lysates were incubated for 15 min at 37°C and then centrifuged in a Beckman Microfuge for 1 min. Duplicate samples of the supernatant were assayed for protein by the method of Schaffner & Weissmann (1973) and for reductase activity essentially as described by Gianturco *et al.* (1978). Under these conditions the rate of reaction was constant for at least 2 h at 37°C and was directly related to the amount of extract added. The apparent K_m for DL-3-hydroxy-3-methyl[3-¹⁴C]-glutaryl-CoA was 13 µM. The standard assay contained 20 µl of cell extract and 43 µM-radioactive substrate (approx. 15 d.p.m./pmol) in a final volume of 35 µl and was incubated for 2 h at 37°C.

To measure the incorporation of [¹⁴C]acetate into non-saponifiable lipids, cells were washed twice with RPMI-1640 medium and incubated at 37°C in RPMI-1640 medium (0.5 ml/24 mm well) containing lipoprotein-deficient serum (2.5 mg of protein/ml) and 1.2 mM-[2-¹⁴C]acetate (sodium salt; 7.5 d.p.m./pmol). After 4 h the cells were washed twice with phosphate-buffered saline and lysed in 0.5 ml of 0.05% (w/v) Triton X-100. [1 α ,2 α (n-³H)]Cholesterol was added as an internal standard to assess recovery, followed by 2.5 ml of ethanol and 0.33 ml of 50% (w/v) KOH. After incubation for 1 h at 70°C an equal volume of water was added and the non-saponifiable lipids were extracted into hexane (three extractions, 20 ml each). The pooled extracts were washed twice with 25 ml of 1 M-NaOH and once with 25 ml of water. Hexane was removed in a stream of N₂ and the radioactivity in the remaining lipids was assayed by scintillation spectrometry.

To determine the net incorporation of [¹⁴C]oleate into cholesteryl esters an appropriate volume of a solution of phosphate-buffered saline containing 2% (w/v) bovine serum albumin and 2 mM-[1-¹⁴C]oleate (sodium salt, approx. 5 d.p.m./pmole) was added to

the culture medium to give a final concentration of 0.1 mM- $[^{14}\text{C}]$ oleate. After the required incubation at 37°C the cells were washed twice with phosphate-buffered saline and the cholesteryl esters were extracted directly from the wells into hexane/propan-2-ol (3:2, v/v) and then separated, as described by Brown *et al.* (1980).

For the measurement of lipoprotein degradation, cells were washed twice with RPMI-1640 medium and incubated for 4 h at 37°C in RPMI-1640 medium (0.5 ml for 24 mm wells or 0.3 ml for 16 mm wells) containing lipoprotein-deficient serum (2.5 mg of protein/ml) and either 50 μg of protein/ml of radioactive LDL or 15 μg of protein/ml of radioactive acetylated LDL. At the end of the incubation samples of medium were assayed for radioactive acid-soluble non-iodide products of lipoprotein catabolism as described previously (Soutar & Knight, 1982).

For measurement of protein or DNA, cells were washed twice with phosphate-buffered saline and dissolved in 0.1 M-NaOH or 0.3 M-KOH respectively. Protein was then assayed by the method of Lowry *et al.* (1951) and DNA by the method of Donkersloot *et al.* (1972).

Materials

Constituents of tissue culture medium were obtained from Gibco Europe, Paisley, Scotland, U.K., and Linbro Multi-Dishes (12 $\times$ 24 mm and 24 $\times$ 16 mm) from Flow Laboratories, Irvine, Scotland, U.K. Na^{125}I (carrier-free), $[1-^{14}\text{C}]$ oleic acid (sp. radioactivity > 50 Ci/mol), $[2-^{14}\text{C}]$ acetic acid (sodium salt, sp. radioactivity 40–60 Ci/mol) and 3-hydroxy-3-methyl $[3-^{14}\text{C}]$ glutaryl-coenzyme A (sp. radioactivity 40–60 Ci/mol) were obtained from Amersham International, Amersham, Bucks., U.K.

Results

Blood monocytes from normal and FH subjects were assayed for HMG-CoA reductase activity and incorporation of $[^{14}\text{C}]$ acetate into non-saponifiable lipids at various times during culture in medium containing normal human serum (Fig. 1). Under these conditions the cells rapidly increased in size, as indicated by the increase in the ratio of protein to DNA (Fig. 1c), although the development of cells from FH subjects ('FH cells') lagged slightly behind that of the normals. In this experiment the ability of both types of cell to degrade acetylated LDL increased markedly between days 2 and 7 and remained high for the next 5 days. Also the normal cells, but not the FH cells, exhibited saturable degradation of unmodified LDL, which was highest between the 5th and 12th days after seeding. These results, which are not shown, generally agree with those that we have presented before (Knight &

Fig. 1. Changes in HMG-CoA reductase activity (a), incorporation of acetate into non-saponifiable lipids (b) and cellular protein content (c) of human monocyte-derived macrophages during maturation *in vitro*

Cells were seeded into 24 mm Multi-Dish wells and maintained in 1 ml of RPMI-1640 medium containing 20% normal human serum. At various times after seeding duplicate dishes were taken for assay of protein, DNA, HMG-CoA reductase and incorporation of $[^{14}\text{C}]$ acetate (see the Methods and materials section for further details). The points shown are averages of values obtained with cells from two normal (●) and two FH (○) subjects, all of which were seeded at the same time. Individual values did not vary by more than 15% from the average. To give a direct comparison between the two types of cell, values for the ratio of protein to DNA are given as a percentage of the values obtained for each cell line on day 1, which were 11.4 and 7.2 mg of protein/mg of DNA for the normal cells and 7.4 and 8.7 mg of protein/mg of DNA for the cells from FH subjects.

Table 1. *Effect of lipoproteins on HMG-CoA reductase activity and the degradation of LDL by monocyte-derived macrophages*

Cells were cultured for 7–9 days in medium containing 20% normal human serum. On the day before the experiment medium in some wells was changed to RPMI-1640 containing lipoprotein-deficient serum (5 mg of protein/ml). Other wells were left unchanged or received non-radioactive lipoproteins to give an additional concentration of 200 μ g of protein/ml of LDL or 40 μ g of protein/ml of acetylated LDL. After incubation for 20 h the cells were lysed and assayed for HMG-CoA reductase activity or washed and incubated for a further 4 h with 125 I-labelled LDL (50 μ g of protein/ml; approx. 60 c.p.m./ng of protein) to assay LDL degradation (see the Methods and materials section for further details). Values are means $\pm$ S.E.M., for the numbers of different experiments indicated in parentheses. Data were obtained from several different experiments with cells from four normal and two FH subjects. Values with LDL, acetylated LDL and lipoprotein-deficient serum were significantly different from controls in normal serum ($P < 0.05$; Student's t test for HMG-CoA reductase activity, paired t test for LDL degradation).

Incubation conditions	HMG-CoA reductase (nmol/2 h per mg of protein)		LDL degradation in normal cells (ng/mg of protein)
	Normal cells	FH cells	
Normal serum (20%)	20.2 $\pm$ 2.0 (7)	21.2 $\pm$ 3.0 (4)	339 $\pm$ 79 (4)
Normal serum (20%) + LDL (200 μ g of protein/ml)	7.6 $\pm$ 1.1 (5)	10.3 $\pm$ 2.8 (3)	228 $\pm$ 61 (4)
Normal serum (20%) + acetylated LDL (40 μ g of protein/ml)	3.5 $\pm$ 2.0 (4)	3.1 $\pm$ 1.1 (3)	171 $\pm$ 82 (4)
Lipoprotein-deficient serum (5 mg of protein/ml)	13.9 $\pm$ 1.6 (5)	12.6 $\pm$ 1.9 (3)	790 $\pm$ 181 (4)

Soutar, 1982b). HMG-CoA reductase activity (Fig. 1a) and acetate incorporation (Fig. 1b) were relatively low for the first 2 days after seeding, then rose, remained high between the 5th and 9th day and fell again as growth slowed. There were no obvious differences between the normal and FH cells in either the pattern of changes or the activity of the reductase and the amount of acetate incorporated.

In subsequent experiments we employed monocyte-derived macrophages that had been cultured for 7–9 days. At this stage both reductase activity and the expression of LDL receptors were highest and the cells had developed the receptors that recognize acetylated LDL. Table 1 summarizes results from a number of experiments and shows the effects of LDL, acetylated LDL and lipoprotein-deficient serum on HMG-CoA reductase activity in normal and FH cells and the ability of normal cells to degrade LDL. Degradation of LDL was assayed at a concentration of 50 μ g of LDL protein/ml and in normal cells represents approx. 80% of the saturable degradation, mediated by the LDL receptors, with a low rate of degradation by non-saturable processes (Soutar & Knight, 1982). At this concentration, degradation of LDL by FH cells, which was non-saturable, was approx. 80 ng/mg of cell protein and was not affected by the addition or removal of lipoproteins (results not shown). Under each set of incubation conditions the mean activity of HMG-CoA reductase in FH cells was the same as that in normal cells. Although the standard culture medium already contained approx. 130 μ g of LDL protein/ml, which was enough to saturate the LDL receptors (apparent K_m of 30 μ g of LDL protein/ml), incubation for 20 h with an additional 200 μ g of

LDL protein/ml produced a fall in both HMG-CoA reductase activity and in the ability of normal cells to degrade LDL. Incubation with a saturating concentration of acetylated LDL (apparent K_m of approx. 2 μ g of protein/ml) produced a greater fall in both activities. After 20 h in lipoprotein-deficient serum, LDL degradation in normal cells had greatly increased, but the activity of HMG-CoA reductase had unexpectedly fallen (Table 1).

To examine these effects in more detail we first monitored reductase activity and LDL degradation in normal cells at various times after the changes in composition of the medium (Fig. 2). There was a lag of 2 h after the addition of LDL before HMG-CoA reductase activity fell. Maximal reduction by all concentrations of LDL tested was achieved within 16 h of the addition of the lipoprotein. Lipoprotein-deficient serum produced an early rise in reductase activity, which was followed by a gradual decline to values below the controls (Fig. 2a). The changes in LDL degradation occurred appreciably later than the changes in reductase activity, although the effects had again reached their maximum within 16 h. There was a lag of almost 5 h before lipoprotein-deficient serum produced a rise in LDL degradation and of 8 h before the addition of LDL resulted in a fall (Fig. 2b). A separate experiment using different cells showed that the decline in HMG-CoA reductase activity that resulted from the addition of acetylated LDL followed a similar course to that produced by LDL, and that the uptake of both lipoproteins led to an increase in the net incorporation of [14 C]oleate into cholesteryl esters by the cells (Fig. 3). An increase in incorporation could be observed within 2 h of the additions and the

Fig. 2. Time course of the effects of LDL and lipoprotein-deficient serum on HMG-CoA reductase activity (a) and LDL degradation (b) by normal monocyte-derived macrophages

Cells from a normal subject were seeded into 16 mm Multi-Dish wells and cultured for 8 days in RPMI-1640 medium containing 20% autologous serum. At time zero the medium in some wells was changed to RPMI-1640 containing 5 mg of protein/ml of lipoprotein-deficient serum (●). Other wells were left unchanged (○) or received an additional 200 µg of LDL protein/ml (■). After incubation at 37°C for the times indicated cells were assayed immediately for HMG-CoA reductase activity or were washed and incubated for a further 4 h with ^{125}I -labelled LDL (53 µg of protein/ml; 70 c.p.m./ng protein) to assay LDL degradation (see the Methods and materials section for further details). The values given are means of results from duplicate wells.

Fig. 3. Time course of effects of LDL and acetylated LDL on HMG-CoA reductase activity (a) and net incorporation of oleate into cholesteryl esters (b) by normal monocyte-derived macrophages

Monocytes from a normal subject were seeded into 16 mm Multi-Dish wells and cultured for 8 days in RPMI-1640 medium containing 20% autologous serum. At time zero some wells received an additional 200 µg of LDL protein/ml (■), some received 40 µg of acetylated LDL protein/ml (Δ) and others were left unchanged (○). Wells to be used for the assay of oleate incorporation also received a solution of [^{14}C]oleate to give a final concentration of 0.1 mM (see the Methods and materials section). After incubation at 37°C for the time indicated cells were assayed for HMG-CoA reductase activity and net incorporation of [^{14}C]oleate into cholesteryl esters as described in the Methods and materials section. Points are means of results from duplicate wells.

amount of radioactivity present in the cholesteryl esters reached a maximum about 14 h later. Further studies indicated that the values for HMG-CoA

reductase activity, degradation of LDL and net incorporation of oleate that were observed after 16 h with LDL, acetylated LDL or lipoprotein-deficient serum remained constant during the next 24 h.

Fig. 4. The effect of LDL and acetylated LDL on monocyte-derived macrophages maintained in medium containing 20% normal human serum

Cells from a normal (filled symbols) and an FH (open symbols) subject were cultured for 8 days in RPMI-1640 medium containing 20% serum from the normal donor. The required concentrations of LDL, acetylated LDL and [14 C]oleate were added as appropriate and the cells were incubated at 37°C for a further 20h. Cells were then assayed for HMG-CoA reductase activity (○ and ●) and net incorporation of oleate into cholesteryl esters (△ and ▲) or were washed and incubated for 4h with [125 I]-labelled LDL (53 µg of protein/ml; 65 c.p.m./ng of protein) to determine their ability to degrade LDL (□ and ■) (see the Methods and materials section for further details). Points are averages of values from duplicate wells.

Having established that the changes were always complete within 16h, we could study the way in which the concentration of the lipoproteins affected the three cellular processes. Fig. 4 shows the values obtained 20h after the addition of different concentrations of LDL and acetylated LDL to medium containing 20% normal human serum. There was an essentially linear relationship with both normal and FH cells between the increase in LDL concentration and the fall in the activity of HMG-CoA reductase (Fig. 4e). This confirms that the LDL receptors on normal cells were fully saturated by the LDL already present in the medium and indicates that the reduction in reductase activity resulted from non-

saturable interactions between the cells and the LDL. The increase in net oleate incorporation into cholesteryl esters by both types of cell (Fig. 4a) and the decrease in LDL degradation by normal cells (Fig. 4c) were also directly related to the amount of LDL added to the incubation medium. In contrast, the relationships between the concentration and the effects of acetylated LDL were saturable (Figs. 4b, 4d and 4f). The increase in net oleate incorporation and the decrease in HMG-CoA reductase activity were the same in FH cells as in normal cells.

To study the effects that resulted from the uptake of LDL through LDL receptors, normal cells were left for 20h in lipoprotein-deficient serum to induce fully the formation of the receptors. They were then incubated for a further 20h with different concentrations of lipoproteins so that the effects of LDL could be compared with those produced by acetylated LDL (Fig. 5). The amount of each lipoprotein that was degraded during the 20h incubation period is shown in Fig. 5(b). At each concentration the rate of degradation of acetylated LDL remained constant throughout the incubations. However, degradation of LDL was reduced by the presence of LDL in the medium (Fig. 5c). Thus the rate of degradation of LDL did not remain constant, and the overall accumulation of degradation products during the incubations with the two lipoproteins should not be directly compared. The increase in net oleate incorporation with LDL followed a saturable curve, and was lower than the increase with acetylated LDL over the range of concentrations tested (Fig. 5a). Cells pre-incubated in lipoprotein-deficient serum (Fig. 5) responded to acetylated LDL in the same way as cells left in 20% normal serum (Fig. 4), but there was a marked difference in their response to LDL. Whereas normal cells in whole serum expressed LDL receptors and a high activity of HMG-CoA reductase in the presence of a saturating concentration of LDL (Table 1), reductase activity in cells pre-incubated with lipoprotein-deficient serum was essentially abolished by concentrations of LDL as low as 20 µg of protein/ml (Fig. 5d). Moreover, LDL produced the same maximal reduction in LDL degradation as acetylated LDL and had a greater effect than acetylated LDL at low concentrations (Fig. 5c).

Pre-incubation with lipoprotein-deficient serum also changed the response of FH cells to LDL, but not to acetylated LDL (Fig. 6). Again, HMG-CoA reductase activity could be abolished by LDL, though a higher concentration was required than with normal cells. The linear relationship between response and concentration that was seen with FH cells kept in whole serum (Fig. 4e) was retained (Fig. 6c). Degradation of LDL by FH cells was constant throughout the incubations (results not shown). Thus the amount degraded, which was directly related to

Fig. 5. The effect of LDL (filled symbols) and acetylated LDL (open symbols) on normal monocyte-derived macrophages pre-incubated for 20 h with lipoprotein-deficient serum

Monocytes from a normal subject were cultured for 7 days in RPMI-1640 medium containing 20% autologous serum. The cells were washed and incubated for 20 h in RPMI-1640 medium containing lipoprotein-deficient serum (5 mg of protein/ml). Further incubations were then performed with different concentrations of lipoproteins as follows. (a) Wells received [^{14}C]oleate and LDL ($\blacktriangle$) or acetylated LDL ($\triangle$). Cells were incubated for a further 20 h at 37°C and then assayed for net incorporation of oleate into cholesteryl esters. (b) Wells received [^{125}I]-labelled LDL ($\blacktriangledown$) or [^{125}I]-labelled acetylated LDL ($\triangledown$), each of which had a specific radioactivity of approx. 20 c.p.m./ng of protein. After 20 h at 37°C , medium was removed and assayed for the radioactive products of lipoprotein catabolism that had accumulated. (c) Wells received LDL ($\blacksquare$) or acetylated LDL ($\square$) and were incubated for 20 h at 37°C . The cells were then washed and incubated for a further 4 h at 37°C with [^{125}I]-labelled LDL (53 μg of protein/ml; 65 c.p.m./ng of protein) to determine their ability to degrade LDL. (d) Wells received LDL ($\bullet$) or acetylated LDL ($\circ$). After 20 h at 37°C the cells were immediately assayed for HMG-CoA reductase activity. All points are means of values from duplicate wells.

the concentration of LDL in the medium (Fig. 6a), could be compared with the degradation of acetylated LDL. It can be seen from Fig. 6 that a 50% reduction in HMG-CoA reductase activity was achieved with a concentration of 85 μg of LDL protein/ml or 6 μg of acetylated LDL protein/ml, concentrations that resulted in the same amount of lipoprotein degradation (2.1 μg /mg of cell protein). In contrast, the relationship between lipoprotein degradation and the stimulation of net oleate incorporation into cholesteryl esters was not the same for LDL as for acetylated LDL. At concentrations that gave the same amount of degradation of each lipoprotein, the increase in net incorporation of oleate was much lower in incubations with LDL than in those with acetylated LDL (Figs. 6a and 6b).

Discussion

In a previous paper we proposed that monocytes from FH patients developed apparently normally *in vitro* because they could compensate for the absence

of cholesterol uptake through LDL receptors by an increase in the rate of cholesterol synthesis within the cells (Knight & Soutar, 1982b). However, the results presented here do not support such a proposal. During the period of greatest growth normal and FH cells exhibited the same activity of HMG-CoA reductase and the same rate of incorporation of [^{14}C]acetate into non-saponifiable lipids. Neither of these activities can be taken as an estimate of the true rate of cholesterol synthesis since they take no account of limitations or changes in the supply of endogenous substrates. Nevertheless, the results suggest that cellular synthesis and non-saturable uptake of cholesterol could be the most important sources of the cholesterol required to support growth, even in normal cells that have the ability to express LDL receptors. There is other circumstantial evidence that points to the same conclusion. Incubation of rapidly growing cells with lipoprotein-deficient serum caused only a slight and transient increase in HMG-CoA reductase activity and had no effect on the incorporation of [^{14}C]-

Fig. 6. The effect of LDL and acetylated LDL after pre-incubation of monocyte-derived macrophages from an FH subject for 20 h with lipoprotein-deficient serum

Cells from an FH subject were seeded into 16mm Multi-Dishes and cultured for 7 days in medium containing 20% normal human serum. Cells were pre-incubated for 20h in medium containing lipoprotein-deficient serum (5 mg of protein/ml) and for a further 20h with additions as given in the legend to Fig. 5. The Figure shows the effects on HMG-CoA reductase activity (○), net incorporation of [14 C]oleate (Δ) and the amount of lipoprotein degraded during the 20h incubation with lipoproteins (▼). Values are means of results from duplicate wells.

acetate in either type of cell (results not shown), suggesting that the rate of cholesterol synthesis was already high in the presence of normal serum and could not increase to compensate for the absence of lipoproteins in the medium. Incorporation of [14 C]-acetate can represent anything between 5 and 60% of the actual rate of cholesterol synthesis, depending on the tissue and conditions employed (Gibbons & Pullinger, 1979; Andersen & Dietschy, 1979). It can be estimated from the rate of [14 C]acetate incorporation (Fig. 1b), without any correction for dilution of the radioactivity, that endogenous synthesis could provide approx. 20% of the increase in cellular cholesterol content between days 7 and 9, calculated from the increase in the ratio of protein to DNA (Fig. 1c) and assuming a ratio of 25 μg of cholesterol per mg of protein (Knight & Soutar, 1982b). Despite the apparently high rate of cholesterol synthesis, normal cells did not express their maximal number of LDL receptors. Incubation with lipoprotein-deficient serum could still increase the capacity of the cells for receptor-mediated degra-

dation of LDL. Without a more accurate measurement of the rate of cholesterol synthesis any conclusions can only be tentative, but taken overall the available evidence indicates that endogenous synthesis provided a significant proportion of the cholesterol required by both normal and FH cells. Together with non-saturable uptake it could satisfy the needs of FH cells to sustain a rapid rate of growth. Uptake of LDL through LDL receptors in normal cells offered only a marginal advantage, if any, and was not the preferred source of cholesterol for growth under normal conditions.

Clinical considerations forced us to maintain the FH cells used in this study in 20% normal serum, which contains less LDL than the autologous FH serum used previously (Knight & Soutar, 1982b). It is possible that the slower development of the FH cells seen here resulted from their inability to make up for the lower rate of non-saturable uptake of LDL from the normal serum. However, this did not prevent the cells from producing receptors that recognized acetylated LDL. As well as reducing

HMG-CoA reductase activity and stimulating net incorporation of oleate into cholesteryl esters, uptake of acetylated LDL by normal cells led to a reduction in saturable degradation of LDL, indicating that the two types of receptor were probably expressed together on the same cells. Addition of LDL to normal cells at concentrations above that required to saturate the LDL receptors caused a reduction in reductase activity and LDL degradation, and an increase in oleate incorporation, each of which was directly related to the amount of LDL added. Virtually identical results were obtained with FH cells, which confirms that monocyte-derived macrophages from both normal and FH subjects, unlike the corresponding cultured fibroblasts (Brown *et al.*, 1974), can respond to LDL that interacts with the cells through processes that do not involve the LDL receptor. Fogelman *et al.* (1981b) reached the same conclusion from experiments that were recently reported in abstract form. The effects eventually produced by LDL and acetylated LDL appeared to be limited by the interaction between the lipoproteins and the cells. However, the timing of the changes in the different processes was not the same. For instance, reductase activity began to fall after 2 h with the lipoproteins, whereas LDL degradation was unaltered for at least 8 h. Whether this reflects a difference in the mechanism of regulation of the two activities or merely a difference in the rate of turnover of the components involved remains to be determined.

Incubation of monocyte-derived macrophages from normal and FH subjects with lipoprotein-deficient serum did not alter their metabolism of acetylated LDL. After 20 h the activity of HMG-CoA reductase in both types of cell was significantly reduced, whereas in normal cells saturable degradation of LDL was increased. Moreover, the uptake of LDL through the LDL receptors present on normal cells could now abolish reductase activity and saturable degradation of LDL, and increase net oleate incorporation into cholesteryl esters. Thus the cells more closely resembled fibroblasts that had been cultured in the same artificial conditions (Goldstein & Brown, 1977), and had probably adapted to the deficiency of lipoproteins with a slower rate of growth.

With FH cells that had been pre-incubated with lipoprotein-deficient serum, the same rate of degradation of acetylated LDL and non-saturable degradation of LDL was accompanied by the same reduction in HMG-CoA reductase activity. However, acetylated LDL produced a much greater increase in net oleate incorporation into cholesteryl esters. A similar direct comparison between the effects of the lipoproteins on normal cells pre-incubated without lipoproteins is not possible because receptor-mediated degradation of LDL fell

during the subsequent 20 h incubations as the receptors were repressed and it is difficult to know at what stage the rates of the various processes should be compared. At the end of the incubations the cells degraded much less LDL than acetylated LDL and the amount of [^{14}C]oleate present in cholesteryl esters was correspondingly reduced. In spite of this, saturable uptake of LDL had resulted in the same, or possibly a greater, decrease in HMG-CoA reductase activity and a much greater fall in LDL degradation (Fig. 5). Since maximal saturable degradation of LDL was lower than that of acetylated LDL even at the beginning of the incubations, the results suggest that the LDL taken up through the LDL receptor might produce a greater effect on HMG-CoA reductase activity and the expression of LDL receptors than the same amount of modified LDL taken up through the receptor for acetylated LDL. Thus it cannot be assumed that cholesterol supplied by different pathways, even different receptor-mediated pathways, produces the same effects on cellular metabolism. The site at which it is deposited in the cell may be important in determining the nature and extent of the changes that follow. It is clear that the relationships between lipoprotein uptake, cholesterol synthesis and cholesterol esterification are complex and that careful experimentation will be required to unravel the mechanisms by which these processes are balanced by the cells under different conditions.

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