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Biochemistry

Squalene synthetase activity in human fibroblasts: Regulation via the low density lipoprotein receptor

(cholesterol biosynthesis/3-hydroxy-3-methylglutaryl coenzyme A reductase/farnesyl pyrophosphate/cell surface receptors/familial hypercholesterolemia)

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ABSTRACT Squalene synthetase (farnesyltransferase; farnesyl diphosphate:farnesyl-diphosphate farnesyltransferase, EC 2.5.1.21), the enzyme in the cholesterol biosynthetic pathway that converts farnesyl pyrophosphate into squalene, is subject to regulation in cultured human fibroblasts. When cholesterol-carrying low density lipoprotein (LDL) was removed from the serum of the culture medium, squalene synthetase activity increased 8-fold over 24 hr. When LDL was added back to the medium, squalene synthetase was slowly suppressed, 50% and 90% reduction occurring in 15 and 48 hr, respectively. Suppression of squalene synthetase required uptake of LDL via the LDL receptor; hence, it did not occur in mutant fibroblasts from a patient with homozygous familial hypercholesterolemia that lack receptors. The addition of a mixture of 25-hydroxycholesterol and cholesterol suppressed squalene synthetase equally well in normal and mutant fibroblasts. Coupled with previous data, the current findings indicate that cholesterol derived from LDL regulates at least two enzymes in the cholesterol synthetic pathway in fibroblasts: (*i*) its primary action is to rapidly suppress 3-hydroxy-3-methylglutaryl coenzyme A reductase [mevalonate:NADP$^+$, oxidoreductase (CoA-acylating), EC 1.1.1.34], which reduces mevalonate production by 95% within 8 hr, and (*ii*) its secondary action is to slowly suppress squalene synthetase. The LDL-mediated suppression of squalene synthetase does not regulate *de novo* cholesterol synthesis; it occurs after 3-hydroxy-3-methylglutaryl coenzyme A reductase is already suppressed. Rather, we hypothesize that it may function to allow the pool size of farnesyl pyrophosphate to be maintained in the presence of LDL so that low levels of mevalonate can be shunted preferentially into nonsterol products, such as ubiquinone-10 and dolichol. This mechanism may explain the earlier observation that the synthesis of ubiquinone-10 in fibroblasts proceeds at a normal rate in the presence of LDL despite a 95% decrease in mevalonate production.

The synthesis of cholesterol in cultured human fibroblasts and other cell types is regulated primarily by alterations in the activity of 3-hydroxy-3-methylglutaryl coenzyme A reductase (HMG CoA reductase) [mevalonate:NADP$^+$ oxidoreductase (CoA-acylating), EC 1.1.1.34] (1). This microsomal enzyme catalyzes the formation of mevalonate, which is a precursor not only of cholesterol, but also of nonsterol polyisoprenoid compounds, such as ubiquinone-10 and dolichol (2). When fibroblasts are grown in the presence of a usable exogenous source of cholesterol, such as that contained in plasma low density lipoprotein (LDL), HMG CoA reductase is reduced by more than 95% (3). The consequent reduction in mevalonate production leads to a parallel drop in the rate of cholesterol synthesis. Yet, the rate of synthesis of another product derived from mevalonate, ubiquinone-10, continues unchanged in the presence of exogenous LDL (4).

Recent studies suggest that maintenance of ubiquinone-10 synthesis in fibroblasts is made possible by a regulatory mechanism in which LDL-derived cholesterol inhibits the cholesterol biosynthetic pathway at two points: (*i*) a primary inhibition at the level of HMG CoA reductase, which inhibits mevalonate synthesis, and (*ii*) a secondary inhibition at one or more points distal to the last intermediate common to the cholesterol and ubiquinone-10 biosynthetic pathways, which allows the residual mevalonate to be shunted preferentially into ubiquinone-10 (4).

A probable site for this distal enzymatic regulation is squalene synthetase (farnesyltransferase; farnesyl diphosphate:farnesyl-diphosphate farnesyltransferase, EC 2.5.1.21), the enzyme that couples two molecules of farnesyl pyrophosphate to form squalene. Whereas farnesyl pyrophosphate can be converted into nonsterol polyisoprenoid products, such as ubiquinone-10, squalene is committed solely to the cholesterol pathway (2). Squalene synthetase has been studied extensively in mammalian liver and yeast (2, 5–10). However, measurements of its activity in cultured cells and analysis of its regulation have not hitherto been reported.

Accordingly, in the current studies we have established a method for determination of squalene synthetase in cultured human fibroblasts. The results indicate that the activity of this enzyme is suppressed by the receptor-mediated uptake of LDL.

MATERIALS AND METHODS

Isotopes. DL-3-Hydroxy-3-methyl-[3-^{14}C]glutaryl coenzyme A (26.2 Ci/mol; 1 Ci = 3.7 × 10^{10} becquerels), *RS*-[5-^{3}H]-mevalonolactone (7.8 Ci/mmol), [4-^{14}C]cholesterol (54 Ci/mol), sodium [2-^{14}C]acetate (51.9 Ci/mol), and [2-^{14}C]mevalonolactone (27.5 Ci/mol) were obtained from New England Nuclear.

Lipoproteins. Human LDL (density, 1.019–1.063 g/ml), high density lipoprotein (HDL) (density, 1.125–1.215 g/ml), and lipoprotein-deficient serum (LPDS) (density, >1.215 g/ml) were obtained from the plasma of individual healthy subjects and prepared by ultracentrifugation (3). Fetal calf LPDS was prepared as described (11). For LDL and HDL, the mass ratios of total cholesterol to protein were 1.5:1 and 1:4, respectively. The concentrations of LDL and HDL are expressed in terms of their protein content.

Cells. Normal and mutant human fibroblasts were derived from skin biopsies as described (3). Cells were grown in monolayer and used between the 5th and 20th passage. Stock cultures were maintained in a humidified incubator (5% CO_2) at 37°C in 250-ml flasks containing 10 ml of medium A [Eagle's minimum essential medium supplemented with penicillin (100

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Abbreviations: HDL, high density lipoprotein; HMG CoA reductase, 3-hydroxy-3-methylglutaryl coenzyme A reductase; LDL, low density lipoprotein; LPDS, lipoprotein-deficient serum.

units/ml), streptomycin (100 μg/ml), 20 mM Tricine (pH 7.4), 24 mM $NaHCO_3$, and 1% nonessential amino acids] and 10% (vol/vol) fetal calf serum (3). Unless otherwise noted, all experiments were done in a similar format. On day 0, confluent monolayers of cells from stock flasks were dissociated with 0.05% trypsin/0.02% EDTA solution, and 7.5×10^4 cells were seeded into each petri dish (60 × 15 mm) containing 3 ml of medium A with 10% fetal calf serum. On day 3, the medium was replaced with 3 ml of fresh medium A containing 10% fetal calf serum. On day 5, when the cells were in late logarithmic growth, each monolayer was washed with 3 ml of phosphate-buffered saline, after which was added 2 ml of fresh medium A containing 10% (vol/vol) human LPDS (final protein concentration, 5 mg/ml). All experiments were begun on either day 6 or day 7 after incubation for 24 or 48 hr in LPDS.

Preparation of [^{3}H]Farnesyl Pyrophosphate. [^{3}H]Farnesyl pyrophosphate was prepared biosynthetically from [^{3}H]mevalonolactone by a modification of the method of Popjak (12). Five millicuries of *RS*-[5-^{3}H]mevalonolactone (7.8 Ci/mmol) were incubated for 2 hr at 37°C in a 2-ml reaction volume containing 0.1 M Tris·HCl (pH 7.5), 5 mM $MgCl_2$, 2 mM $MnCl_2$, 7 mM ATP, 0.3 mM EDTA, and 26 mg of protein of a rat liver extract prepared as the 30–60% ammonium sulfate fraction of the 100,000 × *g* supernatant (12). The resulting [^{3}H]farnesyl pyrophosphate was purified as described (12) except that Bio-Beads SM-2 were used instead of Amberlite XAD-2, and Cellex D was used for the ion-exchange chromatography. The purified [^{3}H]farnesyl pyrophosphate was passed through a Millipore filter (0.45 μm) and stored at 4°C in 0.2 M Tris·HCl (pH 7.9). The purity of the [^{3}H]farnesyl pyrophosphate was assessed in two ways: (*i*) >90% of the ^{3}H radioactivity cochromatographed with authentic farnesyl pyrophosphate (a gift of Dale Poulter) on silica gel developed in 1-propanol/concentrated ammonium hydroxide/water, 6:3:1 (vol/vol), and (*ii*) treatment of the [^{3}H]farnesyl pyrophosphate with alkaline phosphatase (Worthington, cat. no. 3346) yielded ^{3}H-labeled material that was extracted into petroleum ether and that cochromatographed with authentic farnesol on silica gel developed in chloroform. The specific radioactivity of the [^{3}H]farnesyl pyrophosphate (11,000 cpm/pmol at 22% counting efficiency) was estimated assuming that each mole of the biosynthesized product was formed from 3 moles of *RS*-[5-^{3}H]mevalonolactone without dilution by unlabeled material in the synthetic reaction. Immediately prior to use in the squalene synthetase assay, the [^{3}H]farnesyl pyrophosphate was diluted with water to the desired concentration.

Assay of Squalene Synthetase. The cells from each fibroblast monolayer were harvested by scraping and were frozen as a pellet at −190°C as described for measurement of HMG CoA reductase activity (3). Extracts for measurement of squalene synthetase activity were prepared by resuspending each cell pellet in 100 μl of 20 mM Tris·HCl (pH 7.5) and incubating at 4°C for 30 min. The resuspended cells were then subjected to two cycles of freeze-thawing, in which the cell suspension was submerged in liquid nitrogen for 1 min followed by incubation for 2 min at 37°C. Aliquots of the whole cell extract (10 μl) were assayed for squalene synthetase activity by measuring the rate of formation of [^{3}H]squalene from [^{3}H]farnesyl pyrophosphate. In the standard assay, each tube contained the following concentrations of reagents in a total volume of 200 μl: 0.1 M Tris·HCl (pH 7.3), 10 mM $MgCl_2$, 0.25 mM NADPH, 1 mM EDTA, and 7–12 μg of extract protein. The mixture was preincubated for 1 min at 37°C, and the reaction was started by addition of 20 μl of [^{3}H]farnesyl pyrophosphate (44,000–67,000 cpm representing approximately 4–6 pmol). (The use of a subsaturating concentration of [^{3}H]farnesyl pyrophosphate was necessitated because of limited availability of the substrate.) After incubation for 1.5 min at 37°C, the reaction was stopped by the addition of 0.5 ml of ethanol. To each tube was added 5 μl of chloroform/methanol, 2:1 (vol/vol), containing 125 μg of [^{14}C]cholesterol (1000 cpm), 15 μg of squalene, 25 μg of lanosterol, and 15 μg of farnesol. Next, 0.3 ml of 5 M NaOH was added, and the mixture was extracted twice with 3.5 ml of petroleum ether. The combined organic extracts were then washed with 2 ml of water. The resulting organic phase was evaporated to dryness, resuspended in 30 μl of chloroform, and spotted onto plastic-backed, silica gel thin-layer chromatography sheets. The chromatograms were developed in chloroform, and the lipids were visualized with iodine vapor. The iodine-sensitive spots corresponding to squalene (R_F = 0.66) and cholesterol (R_F = 0.12) were cut out and radioactivity was determined in 10 ml of Aquasol (New England Nuclear). The recovery of the added [^{14}C]cholesterol was used to correct for procedural losses, which averaged 27%. Squalene synthetase activity is expressed as cpm of [^{3}H]squalene formed per min per mg of cell protein.

Other Assays. HMG CoA reductase activity was assayed by measuring the rate of conversion of 3-hydroxy-3-methyl-[3-^{14}C]glutaryl CoA (10 cpm/pmol) to [^{14}C]mevalonate in detergent-solubilized extracts of fibroblasts as described (3). Reductase activity is expressed as pmol of [^{14}C]mevalonate formed per min per mg of detergent-solubilized protein.

The synthesis of [^{14}C]cholesterol by intact fibroblasts was measured by incubating cell monolayers at 37°C in 2 ml of medium A containing 10% LPDS and either 0.5 mM [2-^{14}C]-acetate or 0.5 mM [2-^{14}C]mevalonolactone. The amount of [^{14}C]cholesterol formed was determined by saponification of the cells and medium- and thin-layer chromatography of the nonsaponifiable lipids (13). The data are expressed as pmol of [^{14}C]acetate or [^{14}C]mevalonolactone incorporated into [^{14}C]cholesterol per hr per mg of total cell protein.

Protein content of cell extracts and lipoproteins was measured by the method of Lowry *et al.* (14), with bovine serum albumin as a standard. The efficiencies for measurement of ^{14}C and ^{3}H radioactivity were 53% and 22%, respectively.

RESULTS

Extracts prepared from human fibroblasts grown in LPDS rapidly converted [^{3}H]farnesyl pyrophosphate to [^{3}H]squalene (Table 1). The reaction was dependent on the presence of magnesium and NADPH. In experiments not shown, we demonstrated that the synthesized [^{3}H]squalene migrated identically with authentic squalene when subjected to thin-layer chromatography in two systems: (*i*) aluminum oxide developed in methylene dichloride (R_F = 0.88), and (*ii*) silver nitrate-impregnated silica gel developed in chloroform/ethyl acetate, 1:1 (vol/vol) (R_F = 0.31). The radioactive product also cocrystallized with authentic squalene in the presence of thiourea (12). The squalene synthetase reaction was linear with time for 3 min. The pH optimum was 7–7.5 in Tris maleate buffers. The reaction was linear with respect to cell extract up to at least 17 μg of protein per assay tube. More than 98% of the total squalene synthetase activity of fibroblast homogenates was present in the 100,000 × *g* pellet.

Fig. 1 shows saturation curves for the squalene synthetase activity with respect to the concentration of farnesyl pyrophosphate. In extracts of cells grown in LPDS containing no LDL, maximal velocity calculated from Lineweaver–Burk plots was 191 pmol·min^{-1}·mg^{-1}, and half-maximal velocity was attained at a farnesyl pyrophosphate concentration of 0.23 μM. When the cells had been grown in the presence of LDL, maximal velocity of the squalene synthetase reaction was reduced

Table 1. Squalene synthetase activity in extracts of human fibroblasts: Dependence on Mg^{2+}, NADPH, and cell extract

Components	[^{3}H]Squalene formed, cpm/min
Complete system	3830
$-MgCl_2$, -NADPH	11
$-MgCl_2$	14
-NADPH	23
-Cell extract	17

On day 7 of cell growth after incubation for 48 hr in human LPDS, normal fibroblasts were harvested and cell-free extracts were prepared. The complete system contained the following components: 0.1 M Tris·HCl (pH 7.3), 1 mM EDTA, 10 mM $MgCl_2$, 0.25 mM NADPH, 6.6 μg of extract protein, and 53,000 cpm of [^{3}H]farnesyl pyrophosphate. After incubation for 1.5 min at 37°C, the amount of [^{3}H]-squalene formed was determined.

by about 90% to 20 pmol·min^{-1}·mg^{-1}. However, the half-maximal substrate concentration was unchanged at 0.22 μM. In mixing experiments, the activities from the two extracts were additive, indicating that the difference was not due to the presence of soluble activators or inhibitors.

The data of Fig. 1 suggested that squalene synthetase was suppressed by the presence of LDL in the culture medium in a manner similar to the previously reported LDL-mediated suppression of HMG CoA reductase activity (3). Fig. 2 shows that the activities of both of these enzymes increased when LDL was removed from the culture medium. As previously reported (3), HMG CoA reductase increased rapidly, the activity of the enzyme rising nearly 6-fold within 8 hr after removal of LDL. The peak activity of HMG CoA reductase was 40-fold higher than basal activity, and this peak was reached at about 30 hr. On the other hand, the activity of squalene synthetase showed little change up to 8 hr after removal of LDL. Thereafter, however, its activity rose sharply and reached a maximal induction of 8.5-fold at 48 hr (Fig. 2).

When fibroblasts had been subjected to prior growth in the absence of LDL, the subsequent addition of LDL led to a rapid

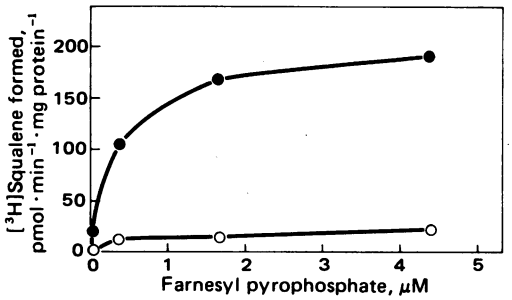

FIG. 1. Squalene synthetase activity in extracts prepared from normal fibroblasts cultured in the absence (●) and presence (○) of LDL plotted as a function of the concentration of farnesyl pyrophosphate in the enzyme assay. On day 5 of cell growth, the medium was replaced with 2 ml of medium A containing 10% human LPDS in the absence (●) or presence (○) of 50 μg of LDL per ml. After incubation for 48 hr, the cells were harvested and cell-free extracts were prepared. Squalene synthetase activity was assayed under standard conditions except that the total concentration of farnesyl pyrophosphate was adjusted to the indicated value by addition of unlabeled farnesyl pyrophosphate. The amount of [^{3}H]farnesyl pyrophosphate was kept constant at 63,000 cpm per assay tube, and the amount of [^{3}H]squalene synthesized was calculated from the final specific radioactivity of the [^{3}H]farnesyl pyrophosphate in each tube. The data were corrected for the known loss of one of the 12 ^{3}H atoms from the two farnesyl pyrophosphate molecules during their coupling to form squalene (15). Each value represents the average of duplicate assays.

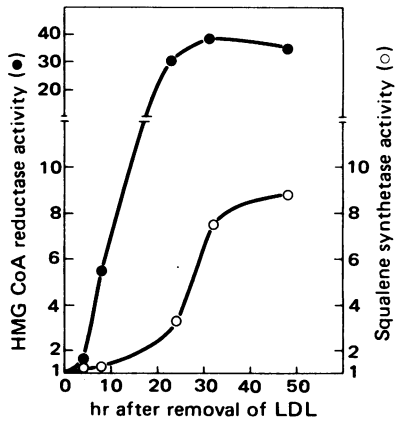

FIG. 2. Increase in HMG CoA reductase (●) and squalene synthetase (○) after removal of LDL from the culture medium. On day 3 of cell growth, the medium was replaced with 3 ml of medium A containing 10% fetal calf LPDS and 50 μg of LDL per ml. On day 4 (zero time), the medium was replaced with 2 ml of medium A containing 10% fetal calf LPDS without LDL. After incubation at 37°C for the indicated time, cells were harvested for measurement of HMG CoA reductase activity (●) and squalene synthetase activity (○). Each value represents the average of duplicate incubations. The results are expressed as the -fold increase above the specific activities for each enzyme at zero time. These "zero time values" were 5.4 pmol·min^{-1}·mg of protein^{-1} for HMG CoA reductase and 57,000 cpm·min^{-1}·mg of protein^{-1} for squalene synthetase.

suppression of HMG CoA reductase, enzymatic activity declining by more than 90% within 8 hr (Fig. 3A). Squalene synthetase was also suppressed by LDL, but the time course was much less rapid (Fig. 3A). The reductions in HMG CoA reductase and squalene synthetase were accompanied by similar reductions in the rates of cholesterol synthesis from [^{14}C]acetate and [^{14}C]mevalonate, respectively (Fig. 3B). Thus, the rate of [^{14}C]acetate incorporation into cholesterol declined by 90% within 8 hr after the addition of LDL, whereas [^{14}C]mevalonate incorporation was unchanged at this time point. Thereafter, however, [^{14}C]mevalonate incorporation into cholesterol declined with a time course that resembled the decline in squalene synthetase (Fig. 3B).

Previous studies have shown that in order to suppress HMG CoA reductase in fibroblasts LDL must bind to a high-affinity cell surface receptor that facilitates its uptake into the cell (1, 16). Cholesterol liberated from LDL within cellular lysosomes then suppresses the reductase (1, 16). In the current experiments, LDL suppressed HMG CoA reductase and squalene synthetase at similarly low concentrations, a finding consistent with an action that involves binding to the high-affinity LDL receptor (Fig. 4). To test this conclusion further, we compared the suppression of both enzymes in normal cells and mutant cells from a patient with homozygous familial hypercholesterolemia that lack functional LDL receptors (Table 2). Whereas LDL suppressed both HMG CoA reductase and squalene synthetase by more than 90% in the normal cells, such suppression did not occur in the mutant cells despite a 48-hr incubation with high levels of LDL. HDL, a cholesterol-carrying lipoprotein that does not bind to the LDL receptor (16), did not suppress either enzymatic activity in either cell strain. The combination of 25-hydroxycholesterol and cholesterol, which enters cells and suppresses HMG CoA reductase activity by a mechanism independent of the LDL receptor (16), also suppressed squalene synthetase (Table 2). This sterol-mediated suppression was similar in the normal and mutant cells.

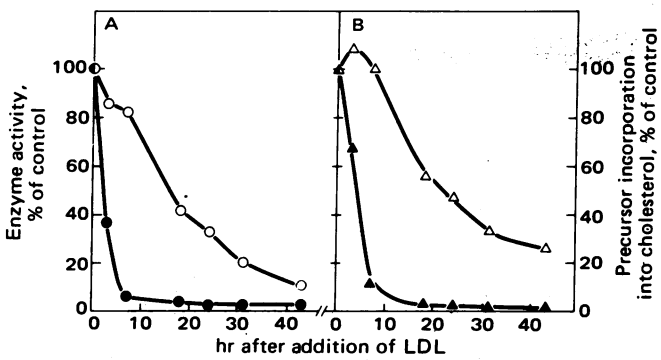

FIG. 3. Time course of LDL-mediated suppression of cholesterol synthesis in normal fibroblasts. On day 5 of cell growth, the medium was replaced with 2 ml of medium A containing 10% human LPDS. On day 6 the medium was replaced with 2 ml of medium A containing 10% human LPDS. On day 6, 7, or 8, 100 μg of LDL was added to the appropriate dish, and the cells were incubated at 37°C. The time of addition of LDL was arranged so that all dishes were harvested at the same time on day 8. After incubation with LDL for the indicated time, duplicate dishes of cells were harvested for measurement of HMG CoA reductase activity (●) and squalene synthetase activity (○) (*A*), and duplicate dishes of cells were pulse-labeled for 2 hr with either 0.5 mM [^{14}C]acetate (60 cpm/pmol) (▲) or 0.5 mM [^{14}C]mevalonolactone (32 cpm/pmol) (△), after which the cellular content of [^{14}C]cholesterol was determined (*B*). The "100% of control values" for HMG CoA reductase and squalene synthetase were 122 pmol·min^{-1}·mg of protein^{-1} and 380,000 cpm·min^{-1}·mg of protein^{-1}, respectively. The "100% of control values" for [^{14}C]acetate and [^{14}C]-mevalonate incorporation were 700 and 170 pmol·hr^{-1}·mg of protein^{-1}, respectively.

DISCUSSION

The current results disclose an additional level of complexity in the regulation of cholesterol metabolism in cultured human fibroblasts. Uptake of LDL-cholesterol via the LDL receptor affects the cholesterol biosynthetic pathway in at least two ways: (*i*) a rapid suppression of HMG CoA reductase, which immediately reduces mevalonate production, and (*ii*) a more gradual suppression of squalene synthetase.

The LDL-mediated suppression of squalene synthetase does not function primarily to suppress cholesterol synthesis because it occurs only after HMG CoA reductase activity (and consequently the rate of cholesterol synthesis from the physiologic precursor acetyl CoA) has been reduced by more than 95%. Moreover, under all conditions the maximal rate of the squalene synthetase reaction is at least 10-fold greater than the rate of the HMG CoA reductase reaction when expressed in equivalent terms. For example, when cells are grown in LPDS, the specific activity of HMG CoA reductase at saturating substrate levels is about 120 pmol/min per mg of detergent-solubilized protein (equivalent to 100 pmol/min per mg of total cell protein). Under the same conditions, the maximal velocity of squalene synthetase is about 200 pmol/min per mg of total cell protein. Inasmuch as each molecule of squalene is derived from six molecules of mevalonate, this reaction velocity is equivalent to 1200 pmol of mevalonate incorporated into squalene per min per mg of cell protein, a value that is 12-fold higher than the rate of mevalonate production from HMG CoA reductase. When LDL is added, HMG CoA reductase is suppressed to a greater degree than squalene synthetase (Figs. 3 and 4; Table 2), and so the relative excess of squalene synthetase becomes even greater. Thus, under all conditions so far studied, HMG CoA reductase, and not squalene synthetase, is the primary rate-limiting enzyme in cholesterol biosynthesis in fibroblasts.

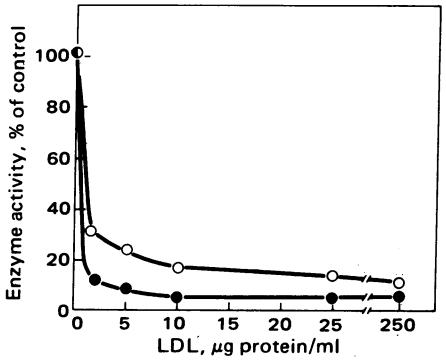

FIG. 4. Suppression of HMG CoA reductase (●) and squalene synthetase (○) by LDL. On day 5 of cell growth, the medium was replaced with 2 ml of medium A containing 10% human LPDS. On day 6 the medium was replaced with 2 ml of medium A containing 10% human LPDS and the indicated concentration of LDL. After incubation for 48 hr in the presence of LDL, cells were harvested for measurement of HMG CoA reductase (●) and squalene synthetase (○) activity. Each value represents the average of duplicate incubations. Enzyme specific activities determined in extracts of cells receiving no LDL ("100% of control values") were: 155 pmol·min^{-1}·mg of protein^{-1} for HMG CoA reductase and 342,000 cpm·min^{-1}·mg of protein^{-1} for squalene synthetase.

What, then, is the function of the LDL-mediated suppression of squalene synthetase? We hypothesize that this suppression functions to limit the incorporation of farnesyl pyrophosphate into cholesterol under conditions in which only small amounts of farnesyl pyrophosphate are formed by the cells—i.e., when HMG CoA reductase is suppressed by LDL. This suppression allows the pool size of farnesyl pyrophosphate to remain nearly constant despite the reduction in mevalonate synthesis. As a result of the suppression of squalene synthetase, a higher proportion of synthesized farnesyl pyrophosphate would be incorporated into nonsterol polyisoprenoids such as ubiquinone-10 as compared with cholesterol. These data may account for the earlier observation that synthesis of ubiquinone-10 continues in the presence of LDL despite a 95% decrease in mevalonate and farnesyl pyrophosphate production due to the suppression of HMG CoA reductase (4). If this hypothesis is correct, farnesyl pyrophosphate is the postulated last common intermediate in the cholesterol and ubiquinone-10 pathways whose disposition is regulated by LDL-cholesterol (4). Although other enzymes distal to squalene are probably also suppressed by LDL-cholesterol (unpublished observations), their suppression would lead only to the accumulation of squalene or more distal products and would not directly expand the farnesyl pyrophosphate pool.

The squalene synthetase activity in human fibroblast extracts resembles the activity previously described in yeast and mammalian liver (2, 5–10). The farnesyl pyrophosphate concentration giving a half-maximal reaction velocity (0.23 μM) is similar to the apparent K_m (0.4 μM) reported for a partially purified preparation of squalene synthetase from hog liver (9). The specific activity of the enzyme at saturating substrate levels in whole extracts of fibroblasts grown in LPDS was 191 pmol/min per mg of total cell protein. The specific activity for this conversion in hog liver microsomes was reported to be 940 pmol/min per mg of microsomal protein (9). Thus, fibroblasts grown in LPDS have about as much squalene synthetase activity per mg of cell protein as do hog liver cells. Like the mammalian liver enzyme (5, 9) and the yeast enzyme (8), the human fibroblast squalene synthetase occurred primarily in a membrane-bound form and required a divalent cation (Mg^{2+}) and a reduced pyridine nucleotide coenzyme (NADPH).

When fibroblasts were grown for relatively long periods in

Table 2. Regulation of HMG CoA reductase and squalene synthetase in normal and homozygous familial hypercholesterolemia (FH) fibroblasts after prolonged incubation with LDL, HDL, and sterols

Addition to medium	Concentration in medium, μg/ml		HMG CoA reductase activity*		Squalene synthetase activity†	
	Protein	Sterol	Normal	FH homozygote	Normal	FH homozygote
None	0	0	120	130	550	540
LDL	12	18	1.2	89	51	380
	24	36	0.4	92	40	370
	100	150	1.0	120	38	319
HDL	50	13	130	200	470	540
	150	38	110	140	450	530
25-Hydroxycholesterol	—	0.6				
+ cholesterol	—	12	6.3	12	54	73

On day 4 of cell growth, the medium was replaced with 2 ml of medium A containing 10% human LPDS. On day 5, the medium was replaced with 2 ml of medium A containing 10% human LPDS, 5 μl of ethanol, and the indicated addition. After incubation for 48 hr in the presence of the indicated addition, cells were harvested for measurement of HMG CoA reductase activity and squalene synthetase activity. Each value represents the average of duplicate incubations.

* pmol·min^{-1}·mg of protein^{-1}.

† cpm·min^{-1}·μg of protein^{-1}.

the presence of LDL, the maximal assayable squalene synthetase activity was reduced by more than 90%. The shape of the farnesyl pyrophosphate saturation curve was unchanged, suggesting that the maximal velocity of the reaction was reduced without a change in the affinity of the enzyme for this substrate. Inasmuch as the kinetics of the squalene synthetase reaction are complex (8, 9), it is not possible to conclude from kinetic data alone that the LDL-mediated change in maximal velocity is due to a reduction in the amount of enzyme protein. Moreover, it has been reported that cytosolic proteins enhance the rate of the hepatic squalene synthetase reaction (17). Although such stimulation has not been observed by all workers (18), it remains possible that the LDL receptor-mediated changes in squalene synthetase activity in fibroblasts are due not to changes in the amount of enzyme, but rather to changes in the amount of a rate-limiting cofactor.

Squalene synthetase appears to be under remarkably similar regulation in human fibroblasts and in rat liver. In both cases the enzymatic activity declines when exogenous cholesterol enters the cell, and in both cases the changes follow much more rapid and profound declines in HMG CoA reductase activity. For example, when rats are fed cholesterol, [^{14}C]acetate incorporation into cholesterol by liver slices declines by more than 95% within 1 day (19, 20) and this is accompanied by a precipitous fall in HMG CoA reductase activity. On the other hand, [^{14}C]mevalonate incorporation into cholesterol declines much more slowly, the rate falling by about 80% in 2 days (19). This latter fall was postulated to be due primarily to a decrease in the conversion of farnesyl pyrophosphate to squalene, although small decreases in conversion of mevalonate to farnesyl pyrophosphate were also observed (19). Slakey *et al.* (21) demonstrated that squalene synthetase activity in rat liver is reduced by fasting and re-induced by feeding a fat-free diet. Similar changes were seen in the activities of several enzymes in the pathway between mevalonate pyrophosphate and farnesyl pyrophosphate. Whether these latter enzyme activities are reduced in fibroblasts as a result of LDL uptake is not yet known.

Considered together with previous data on the LDL receptor pathway (16), the current findings indicate that at least four cellular metabolic reactions are regulated in fibroblasts by the receptor-mediated endocytosis of LDL: (*i*) suppression of HMG CoA reductase, (*ii*) suppression of squalene synthetase, (*iii*) stimulation of acyl-CoA:cholesterol acyltransferase, the microsomal enzyme that catalyzes the re-esterification of LDL-derived cholesterol, and (*iv*) suppression of the synthesis of LDL receptors. Together, these reactions serve to stabilize the cellular concentration of unesterified cholesterol, while at the same time allowing the continual synthesis of essential nonsterol polyisoprenoid compounds.

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1. Brown, M. S. & Goldstein, J. L. (1976) *Science* **191**, 150–154.
2. Beytia, E. D. & Porter, J. W. (1976) *Annu. Rev. Biochem.* **45**, 113–142.
3. Brown, M. S., Dana, S. E. & Goldstein, J. L. (1974) *J. Biol. Chem.* **249**, 789–796.
4. Faust, J. R., Goldstein, J. L. & Brown, M. S. (1979) *Arch. Biochem. Biophys.* **192**, 86–99.
5. Goodman, DeW. & Popjak, G. (1960) *J. Lipid Res.* **1**, 286–300.
6. Lynen, F., Eggerer, H., Henning, U. & Kessel, I. (1958) *Angew. Chem.* **70**, 738–742.
7. Rilling, H. C. (1966) *J. Biol. Chem.* **241**, 3233–3246.
8. Shechter, I. & Bloch, K. (1971) *J. Biol. Chem.* **246**, 7690–7696.
9. Dugan, R. E. & Porter, J. W. (1972) *Arch. Biochem. Biophys.* **152**, 28–35.
10. Agnew, W. S. & Popjak, G. (1978) *J. Biol. Chem.* **253**, 4574–4583.
11. Brown, M. S. & Goldstein, J. L. (1974) *J. Biol. Chem.* **249**, 7306–7314.
12. Popjak, G. (1969) *Methods Enzymol.* **15**, 303–454.
13. Brown, M.S., Faust, J. R., Goldstein, J. L., Kaneko, I. & Endo, A. (1978) *J. Biol. Chem.* **253**, 1121–1128.
14. Lowry, O. H., Rosebrough, N. J., Farr, A. L. & Randall, R. J. (1951) *J. Biol. Chem.* **193**, 265–275.
15. Popjak, G., Goodman, DeW., Cornforth, J. W., Cornforth, R. H. & Ryhage, R. (1961) *J. Biol. Chem.* **236**, 1934–1947.
16. Goldstein, J. L. & Brown, M. S. (1977) *Annu. Rev. Biochem.* **46**, 897–930.
17. Rilling, H. C. (1972) *Biochem. Biophys. Res. Commun.* **46**, 470–475.
18. Gavey, K. L. & Scallen, T. J. (1978) *J. Biol. Chem.* **253**, 5470–5475.
19. Gould, R. G. & Swyryd, E. A. (1966) *J. Lipid. Res.* **7**, 698–707.
20. Siperstein, M. D. & Guest, M. J. (1960) *J. Clin. Invest.* **39**, 642–652.
21. Slakey, L. L., Craig, M. C., Beytia, E., Briedis, A., Feldbruegge, D. H., Dugan, R. E., Qureshi, A. A., Subbarayan, C. & Porter, J. W. (1972) *J. Biol. Chem.* **247**, 3014–3022.