HMG-CoA reductase inhibitors perturb fatty acid metabolism and induce peroxisomes in keratinocytes

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Abstract Topical lovastatin stimulates epidermal fatty acid synthesis in vivo; therefore, studies were undertaken to examine the effects of HMG-CoA reductase inhibitors on fatty acid metabolism in cultured keratinocytes. When exposed to fluindostatin or lovastatin for ≥ 24 h, keratinocytes in serum-free media accumulated nile red-fluorescent lipid droplets. By 72 h, the triacylglycerol and phospholipid content were increased 2.5- and 1.3-fold, respectively. Reductase inhibitors (1–10 μM) increased fatty acid synthesis ≈ 1.5-fold; increased synthesis was noted only after > 15 h exposure and was distributed among phospholipids and triacylglycerols. Oxidation of [^{14}C]palmitate to CO_2 was decreased > 50% in inhibitor-treated cultures, and label accumulated in triacylglycerols. Inhibitor-treated keratinocytes exhibited increased numbers of peroxisomes, using diaminobenzidene ultracytochemistry. Peroxisomal hyperplasia was also demonstrated by increased catalase activity (1.5- to 2.5-fold), increased dihydroxyacetone phosphate acyltransferase activity (1.4-fold) and increased peroxisomal (KCN-insensitive) fatty acid oxidation (1.4-fold) in inhibitor-treated cultures. Thus HMG-CoA reductase inhibitors increase fatty acid synthesis, induce triacylglycol and phospholipid accumulation, and induce peroxisomes in cultured keratinocytes. Coincubations with either low density lipoproteins or 25-hydroxycholesterol prevented both the peroxisomal hyperplasia and increased fatty acid synthesis, suggesting that these effects of reductase inhibitors may be linked to their effects on the cholesterol biosynthetic pathway.—**Williams, M. L., G. K. Menon, and K. P. Hanley.** HMG-CoA reductase inhibitors perturb fatty acid metabolism and induce peroxisomes in keratinocytes. *J. Lipid Res.* 1992. **33:** 193–208.

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Compactin, lovastatin, and fluindostatin are competitive inhibitors of 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase, the rate-limiting enzyme in cholesterol biosynthesis (1, 2). Fatty acid metabolism is generally considered to be unaffected by lovastatin and related compounds (2–5); however, modest increases in fatty acid synthesis have been observed in mouse L cells (3), rat lens (6), and in normal and LDL receptor-negative human fibroblasts (7) when incubated with HMG-CoA reductase inhibitors, as well as in rat liver following oral administration (1).

Prolonged exposure to these inhibitors results in a marked increase in reductase content, due both to increased gene transcription and to decreased degradation of message and enzyme (8), and is associated with a striking proliferation of smooth endoplasmic reticulum in liver (9) and UT-1 cells (10). Recently, Mazzocchi et al. (11) have demonstrated that lovastatin in combination with another potent hypocholesterolemic drug, 4-aminopyrazolo-pyrimidine, induces hyperplasia of both smooth endoplasmic reticulum and peroxisomes in rat adrenals. Although HMG-CoA reductase resides predominantly in the endoplasmic reticulum, peroxisomes also have been shown to contain HMG-CoA reductase (12, 13) and other enzymes in the cholesterol biosynthetic pathway (14, 15). Nonetheless, while hepatic peroxisomal hyperplasia is a well-recognized phenomenon in rodents fed clofibrate and related hypocholesterolemic drugs (reviewed in 16, 17), this has not been reported to occur with lovastatin. Moreover, the HMG-CoA reductase content of hepatic peroxisomes is not increased by lovastatin (9), as it is by cholestyramine treatment (13).

In epidermis, cholesterol synthesis is regulated by permeability barrier function (18–21), and may be largely independent of regulation by circulating low density lipoproteins (LDL) (22, 23). Recently, Feingold et al. (24) observed that repeated epicutaneous applications of lovastatin to hairless mice increased transepidermal water loss. While lovastatin initially

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Abbreviations: HMG-CoA, 3-hydroxy-3-methylglutaryl coenzyme A; LDL, low density lipoproteins; PBS, phosphate-buffered saline; DAB, diaminobenzidine; DHAP-AT, dihydroxyacetone phosphate acyltransferase; TLC, thin-layer chromatography.

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inhibited epidermal cholesterol synthesis, by the time a defective barrier developed (i.e., after 5 days), HMG-CoA reductase activity, when dialyzed free of inhibitors, was increased ≈ 2-fold and epidermal cholesterol synthesis and stratum corneum cholesterol content were normal. However, epidermal fatty acid synthesis and context were increased ≈ 2- to 3-fold at this time. Because barrier disfunction itself may stimulate epidermal fatty acid synthesis (22, 25), the lovastatin effect on fatty acid metabolism could have resulted from its effect on barrier function. These studies were undertaken, therefore, to determine whether HMG-CoA reductase inhibitors directly affect fatty acid metabolism in cultured keratinocytes.

Herein we report that the reductase inhibitors, lovastatin and fluindostatin, increase acetate incorporation into fatty acids and inhibit mitochondrial fatty acid oxidation, resulting in accumulation of cytoplasmic triacylglycerol droplets in cultured human keratinocytes. Moreover, increased numbers of peroxisomes are seen ultrastructurally, and both catalase and dihydroxyacetone phosphate acyltransferase (DHAP-AT) activities and peroxisomal fatty acid oxidation are significantly increased in inhibitor-treated keratinocytes. Because neither increased fatty acid synthesis nor peroxisomal hyperplasia were observed during coincubations of reductase inhibitors with either LDL or 25-hydroxycholesterol, these phenomena may be linked to the effects of these inhibitors on the cholesterol biosynthetic pathway.

METHODS

Cell culture

Neonatal foreskin keratinocytes (first to fourth passage) were grown submerged in serum-free medium (KGM, Clonetics) containing 0.07 mM Ca^{2+} ("low" calcium) or 1.2 mM Ca^{2+} ("high" calcium) to retard or enhance differentiation, respectively (26, 27). Neonatal foreskin fibroblasts (passage ≤ 15) were grown in Dulbecco's modified Eagle's medium supplemented with 10% fetal calf serum. Lipoprotein-depleted serum was prepared by ultracentrifugation (28). Low density lipoproteins (LDL) were similarly prepared from human serum and dialyzed against Dulbecco's phosphate-buffered saline (PBS) for 4 h at 4°C. The protein content of the isolated LDL was determined using bicinchonic acid (29). Lovastatin (provided by Dr. A. Alberts, Merck, Sharpe and Dohme, Inc., Rahway, NJ) and fluindostatin (provided by Dr. A. Stutz;, Sandoz Forschungsinstitut, Vienna, Austria) were solubilized in ethanol; the final concentration of vehicle in medium did not exceed 0.1%. Mevalonolacetone (Sigma) was acidified to pH 2.8, then solubilized in medium and added to cultures at a final concentration of 10 mM. This concentration of mevalonic acid was required to inhibit acetate incorporation into cholesterol > 95%.

Lipid metabolism

To assess lipid synthesis, cultures were incubated for 3 h with [2-^{14}C]acetic acid, sodium salt (sp act 50 mCi/mmol; ICN Radiochemicals), supplemented with additional cold acetate, and taken up in PBS to achieve a final concentration of 500 μM (25 μCi/dish). At this concentration, acetate is not limiting to lipid synthesis in keratinocytes (30) and acetate incorporation into fatty acids is linear over the first 6 h. Cultures were harvested by manual scraping with a rubber policeman in 1 ml of buffer (2 M NaCl; 0.05 M H_2NaPO_4; 2 mM EDTA; pH 7.4). Cells were homogenized by brief sonication on ice using a sonic dismembranator (Fisher Scientific Model 300) at a relative output of 35%. Aliquots were taken for DNA content (31) and lipid extraction (32). Polar and neutral lipid fractions were separated by thin-layer chromatography (TLC) in three sequential solvent systems; step 1: develop in chloroform–acetone–methanol 90:5:5 to 8 cm; step 2: double-develop in chloroform–ethyl acetate–methanol–2-propanol–triethylamine–water 65:15:17:0.5:3:2 to 13 cm, then to 8 cm; step 3: develop in hexane–diethylether 90:10 to the top. Lipid fractions were visualized under ultraviolet light after spraying with 0.2% 8-anilino-1-naphthalene sulfonic acid and identified by co-chromatography with known standards. Appropriate zones were scraped directly into scintillation cocktail (Scintiverse II, Fisher Scientific) and radioactivity was determined using a Beckman LS 1800 scintillation counter. Each experiment was conducted in triplicate, and data were expressed as dpm incorporated into lipid fraction/μg DNA (mean ± SE). In other experiments, the lipid extract was saponified overnight at room temperature by incubation of the homogenate in excess 45% KOH–water–70% ethanol 2:1:5. Nonsaponifiable lipids were extracted three times with petroleum ether. The extract was dried, solubilized in chloroform, and chromatographed in ethyl acetate–benzene 1:5, lipid fractions were identified, and radioactivity was quantitated as described above. The saponified lipids were recovered by acidification to pH 2, followed by extraction three times with petroleum ether, dried, resuspended in scintillation fluid, and counted.

To assess incorporation of exogenous fatty acids into lipid, 1.35 μCi of [^{14}C]palmitate (sp act 9.8 mCi/mmol) bound to fatty acid-free bovine serum albumin (Sigma) and taken up in PBS was added to each dish. Cultures were incubated for 2 h, harvested, and lipids were then extracted, fractionated by TLC, and radioactivity was quantitated as described above.

Lipid content

Cultures were harvested and lipid extracted as described above. Lipid fractions were quantitated by quartz rod microchromatography coupled with a flame ionization detector (Iatroscan^R), as previously described (33, 34). Data represent the mean of separations on three or more chromarods each.

Electron microscopy

Keratinocytes were seeded on plastic Petri dishes and exposed to the HMG-CoA reductase inhibitor or vehicle alone for 12, 48, 72 h, or 1 week prior to harvesting. Dishes were rinsed in phosphate buffer, and cultures were fixed by immersion for 1 h at 4°C in 1% paraformaldehyde, 2% glutaraldehyde, and 0.01% $CaCl_2$ in 0.1 M cacodylate buffer (pH 7.4). Cultures were then immersed in 0.1 M cacodylate buffer with 5% sucrose (pH 7.4) for 24 h at 4°C. Catalase activity was then demonstrated using diaminobenzidine (DAB) cytochemistry, as described by Beard et al. (35) using H_2O_2 as the substrate. Controls included incubation medium without H_2O_2, and incubation medium containing the catalase inhibitor, 3-amino-1,2,4-triazole (Sigma). After 2-h incubations, cultures were postfixed in 2% osmium tetroxide, dehydrated, and embedded in an Epon-epoxy mixture (36). Thin sections were reviewed unstained or double-stained with uranyl acetate and lead citrate.

Flourescence microscopy

Keratinocytes were grown on glass coverslips in plastic Petri dishes. At the time of harvest, cultures were rinsed with PBS and the coverslips were mounted on slides under a drop of nile red, sealed with nail polish, and examined by fluorescence microscopy, as previously described (37).

Fatty acid oxidation

Fatty acid oxidation was assessed by the generation of [^{14}C]CO_2 from [1-^{14}C]palmitic acid (38), as modified (34). [^{14}C]CO_2 production was measured during a 1-h incubation at 37°C with 0.5 μCi [1-^{14}C]palmitic acid (57.1 mCi/mmol; New England Nuclear) conjugated to fatty acid-free bovine serum albumin. Data were normalized to DNA content.

Peroxisomal fatty acid oxidation was determined by the oxidation of [1-^{14}C]palmitoyl-CoA to water-soluble products in cell homogenates in the presence of cyanide to prevent mitochondrial fatty acid oxidation, as described by Wanders et al. (39), except that palmitoyl-CoA was not prepared as an α-cyclodextran solution and we used a low-speed spin to sediment unbroken cells. Briefly, cultures were rinsed with PBS, manually harvested in 300 mM sucrose, 2 mM MOPS-NaOH (pH 7.4), and sonicated on ice as described above. After centrifuging at 1500 *g* (10 min; 5°C), 20 μl of supernatant (2–4 mg protein/ml) was incubated with 180 μl of a reaction mixture containing 5 mM KCN, 180 mM sucrose, 75 mM MOPS-NaOH (pH 7.5), 5 mM $MgCl_2$, 10 mM ATP, 3 mM NAD^+, 50 μM FAD, 100 μM CoA, and 10 μM [^{14}C]palmitoyl CoA. The reaction was stopped by transferring a 100-μl aliquot to a glass tube containing 3.25 ml of methanol–chloroform–heptane 1.41:1.25:1. The phases were mixed by shaking (10 min; Burrell wrist action shaker) then separated by centrifugation at 700 *g* (10 min; 4°C). NaOH (2 N, 0.3 ml) was added to the upper phase (1.5 ml) and incubated for 45 min at 50°C to hydrolyze the CoA ester; then 60 μl of concentrated H_2SO_4 was added. The [^{14}C]palmitate was removed by extraction twice through the addition of the lower phase of methanol–chloroform–heptane–0.1 M sodium acetate (pH 4) 1.41:1.25:1:1.18. Radioactivity in the aqueous phase was quantified by scintillation counting in Ecolume (ICN).

Peroxisomal enzyme assays

Catalase was assayed in cellular homogenates by the peroxidation of titanium sulfate oxide (Strem Chemicals, Inc., Newburyport, MA) as described by Leighton et al. (40) and as modified here. Cultures were harvested, homogenized by brief sonication (2 – 15 sec bursts) on ice in 0.25 M sucrose buffer (0.1% Triton X-100 by volume, 1.0 mM Na_4 EDTA, 1.1 mM $NaHCO_3$, 0.1% ethanol), then centrifuged at 1500 *g* for 10 min. The supernatant, diluted with additional buffer as needed to achieve a protein concentration of 100–200 μg in 100 μl, was incubated for 10 min at 0°C with 1.13 ml of imidazole buffer (20 mM, pH 7) containing bovine serum albumin (1 g/l), and 1.5 mM H_2O_2. The reaction was stopped by the addition of a saturated solution of $TiSO_4$ in 2 N H_2SO_4. After 20 min, the yellow titanium peroxysulfate complex was quantitated photometrically by absorption at 410 nm. One unit of enzyme activity (B.U.) is defined as that quantity destroying 50% of the H_2O_2 present in a 50-ml reaction volume in 1 min (41). AcylCoA:dihydroxyacetone phosphate acyltransferase (EC 2.3.1.42) (DHAP-AT) was assayed in cellular homogenates as described by Schutgens et al. (42).

Statistical methods

Significance was determined using the two-tailed Student's *t*-test.

RESULTS

Nile red histochemistry

Keratinocytes, grown on glass coverslips in medium containing 1.2 mM Ca^{2+} to permit differentiation (27),

were exposed to 10–25 μM lovastatin or fluindostatin for 5 days and then stained with the hydrophobic fluorescent probe, nile red. Control keratinocytes form a monolayer at confluence (**Fig. 1** inset), while inhibitor-treated keratinocytes formed clusters of stratifying cells (Fig. 1). Whereas confluent keratinocyte cultures commonly showed small fluorescent cytoplasmic droplets (Fig. 1, inset), treatment with either HMG-CoA reductase inhibitor resulted in more numerous and larger, brightly flourescent droplets (Fig. 1, shown here after exposure for 5 days to 10 μM lovastatin). Increased numbers of lipid droplets appeared as early as 24 h after inhibitor treatment (data not shown). Inhibitor-treated cultures were also sparser than controls. Thus, keratinocytes cultured in serum-free medium in the presence of HMG-CoA reductase inhibitors are growth-inhibited and accumulate lipid.

Lipid content

To determine the composition of accumulated lipids, cultures were exposed to either 10 μM lovastatin or vehicle alone for 72 h, then harvested, and lipids were extracted and fractionated by quartz-rod microchromatography (**Table 1**). Using DNA content as a measure of cell number, the density of cells in lovastatin-treated cultures was significantly reduced. When normalized to DNA, the lipid content of lovastatin-treated keratinocytes was 140% of control cultures (Table 1). Both neutral and polar acyl-lipids were increased, i.e., triacylglycerols and phospholipids were 250% and 130% of control, respectively. These data demonstrate that the increased lipid content of inhibitor-treated cultures is due to accumulation of triacylglycerol and phospholipids.

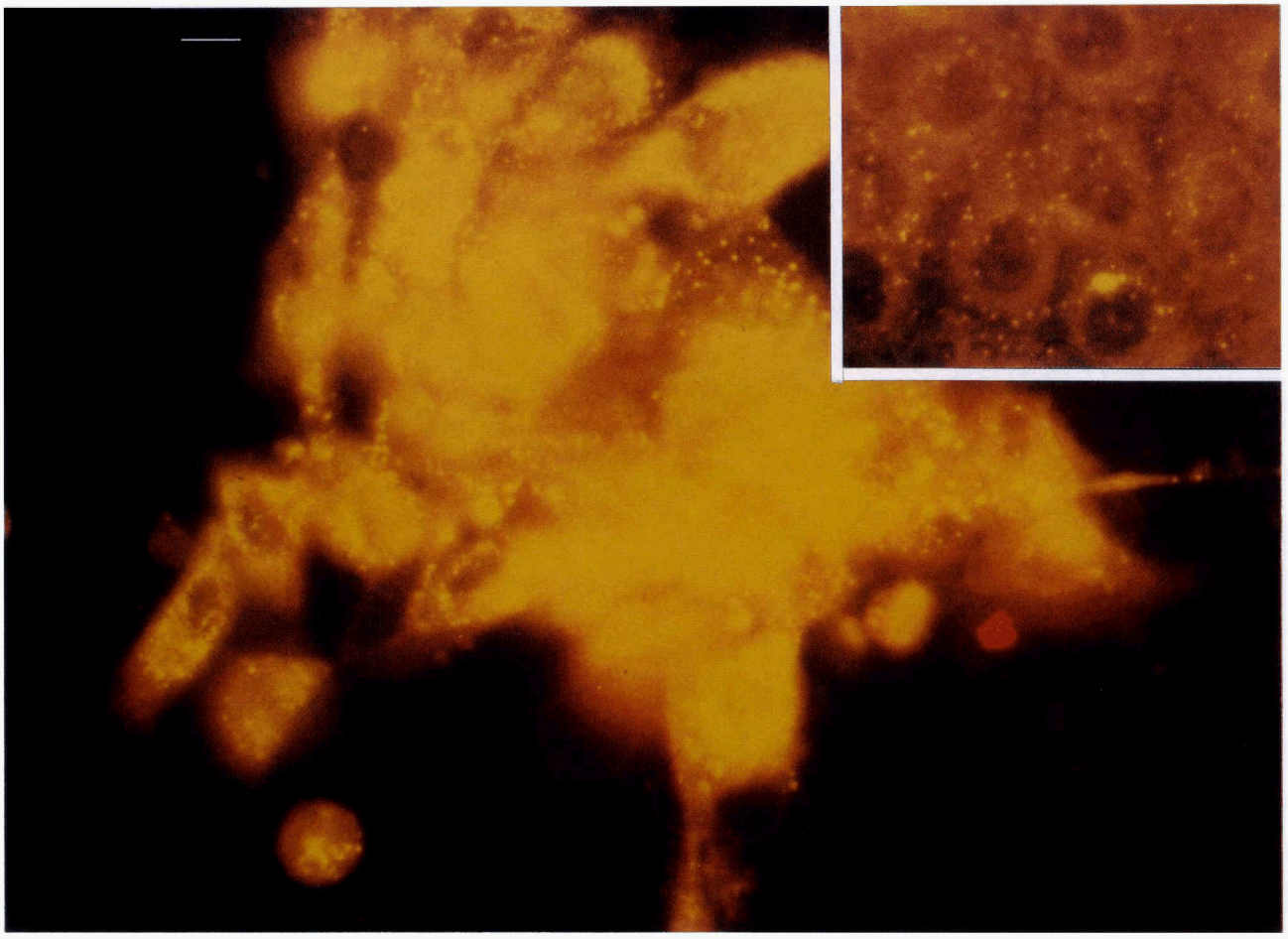

Fig. 1. Nile red histochemistry of keratinocytes incubated with vehicle versus HMG-CoA reductase inhibitors. Keratinocytes cultured on glass coverslips in serum-free media containing 1.2 mM Ca^{2+} were exposed to lovastatin (10 mM) or to vehicle (0.05% ethanol) alone (inset) for 5 days. Coverslips were stained with nile red, mounted, and examined by fluorescent microscopy. While small cytoplasmic lipid droplets are evident in control keratinocyte cultures (inset), lipid droplets are coarser and more numerous in lovastatin-treated cultures. Bar = 10 μm for figure and 20 μm for inset.

TABLE 1. Lipid content of keratinocyte cultures exposed to lovastatin for 72 h

	Control	Lovastatin -Treated	% Control	Significance
	μg	μg		
DNA/dish	152 ± 5	112 ± 4	73	$P < 0.01$
Lipid/dish	295 ± 10	308 ± 14	104	NS
Lipid/μg DNA	1.94	2.76	142	
Lipid fractions	*μg lipid/μg DNA*			
Phospholipids	1.183 ± 0.07	1.579 ± 0.079	133	$P < 0.02$
Sterol esters	ND	ND		
Triacylglycerols	0.256 ± 0.034	0.659 ± 0.082	257	$P < 0.01$
Free fatty acids	0.034 ± 0.002	0.060 ± 0.020	176	NS
Free sterols	0.459 ± 0.055	0.417 ± 0.038	91	NS

Preconfluent keratinocytes in serum-free medium (1.2 mM Ca^{2+}) were cultured for 72 h in the presence of 10 μM lovastatin solubilized in ethanol versus vehicle alone. Cultures were harvested and homogenized, and an aliquot was taken for determination of DNA content. Lipids were then extracted, weighed, and fractionated by quartz rod microchromatography (see Methods). Data represent the mean of triplicate determinations ± SE; ND, none detected; NS, not significant.

Lipid synthesis in keratinocytes and fibroblasts

To determine whether increased fatty acid synthesis contributed to the accumulation of acyl-lipids, preconfluent cultures (in 1.2 mM Ca^{2+}) were exposed for 24 h to varying concentrations of one of the HMG-CoA reductase inhibitors or to vehicle alone, and pulsed during the final 3 h with [^{14}C]acetate, and the incorporation of radiolabel into cholesterol and saponifiable lipids (fatty acids) was determined. Both lovastatin and fluindostatin profoundly suppressed cholesterol synthesis, and with comparable activity (**Table 2**). In addition, both produced a dose-dependent, 50–70% increase in the incorporation of acetate into fatty acids, maximal at 1–10 μM. For the remainder of these studies, inhibitors were used in concentrations of 10–25 μM.

To determine whether HMG-CoA reductase inhibitors stimulate fatty acid synthesis in other cell types, human skin fibroblasts were also examined. To maintain fibroblast growth it was necessary to use a serum-containing medium. When cultures were ≈ 60% confluent, they were placed in medium containing 10% lipoprotein-depleted fetal calf serum and 24 h later media were renewed and either lovastatin (25 μM), fluindostatin (25 μM), or vehicle alone was added. After an additional 21 h, [^{14}C]acetate was added and 3 h later cultures were harvested, and the

TABLE 2. [^{14}C]Acetate incorporation into lipids: dose–response to HMG-CoA reductase inhibitors

Experiment	[^{14}C]Acetate Incorporation into Lipid			
	Fatty Acids		Cholesterol	
	dpm/μg DNA	% Control	dpm/μg DNA	% Control
Exp.1				
Control	1470 ± 80		187.8 ± 16.7	
Lovastatin				
0.1 μM	1850 ± 170	125	33.7 ± 8.5***	18
0.5 μm	1870 ± 180	126	10.3 ± 0.09***	5
1.0 μM	1980 ± 320	134	5.5 ± 0.32***	3
10.0 μM	2550 ± 80***	173	5.6 ± 0.15***	3
50.0 μM	2500 ± 390*	169	19.6 ± 0.44***	10
Exp.2				
Control	1910 ± 40		150.4 ± 10.0	
Fluindostatin				
0.001 μM	2230 ± 50**	117	179.7 ± 8.3	120
0.01 μM	2890 ± 140**	151	84.4 ± 13.2***	56
0.1 μM	2720 ± 260*	142	46.1 ± 6.6****	31
1.0 μM	3060 ± 30****	160	18.2 ± 2.7****	12
10.0 μM	2930 ± 60****	153	6.6 ± 1.6****	4

Preconfluent keratinocytes in serum-free media (1.2 mM Ca^{2+}) were exposed for 24 h to varying concentrations of lovastatin or fluindostatin solubilized in ethanol versus control cultures exposed to vehicle alone. [1-^{14}C]Acetate (25 μCi/dish) was added for the final 3 h. Cells were harvested and homogenized, and an aliquot was taken for DNA content; the remainder was saponified and the nonsaponifiable fraction was chromatographed for determination of cholesterol (see Methods). Data represent the mean ± SE of three dishes each. Significance in comparison to control: *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.02$; ****, $P < 0.001$.

radioactivity in saponifiable lipids was determined. Both inhibitors produced a greater than 3-fold increase in acetate incorporation into fatty acids (experiment #1: control 598 ± 92; lovastatin 2110 ± 244 dpm/μg DNA ($P < 0.01$); experiment #2: control 374 ± 17; fluindostatin 1021 ± 113 dpm/μg DNA; $P < 0.01$)). Thus under these conditions, HMG-CoA reductase inhibitors also increase fatty acid synthesis in fibroblasts. Furthermore, fibroblasts may be even more sensitive to this effect of HMG-CoA reductase inhibitors than keratinocytes.

Next, to determine the time-course for the induction of fatty acid synthesis and the distribution of labeled fatty acids among acyl-lipid classes, preconfluent keratinocyte cultures in 1.2 mM Ca^{2+} were exposed to 10 μM lovastatin for 4, 8, 15, 24, or 72 h and were labeled with [^{14}C]acetate for the final 4 h of each incubation. Thereafter, the lipids were extracted, fractionated by TLC, and the radioactivity in lipid fractions was quantitated (**Fig. 2A–C**). The DNA content of lovastatin-treated cultures did not change over the duration of the experiment; however, the deviation in DNA content from control cultures was significant only at 72 h (data not shown). As expected, cholesterol synthesis was profoundly (> 90%) inhibited by lovastatin at all time points (data not shown). Incorporation of acetate into both free fatty acids (Fig. 2A) and phospholipids (Fig. 2B) was increased at 15 h ($P < 0.05$; $P < 0.02$, respectively), 24 h ($P < 0.01$; $P < 0.001$, respectively), and 72 h (both $P < 0.01$) but not at earlier time points (i.e., 4 and 8 h). In contrast, increased incorporation into triacylglycerols was delayed to 24 and 72 h (both $P < 0.001$) (Fig. 2C). Thus, fatty acid synthesis was increased by HMG-CoA reductase inhibitors only after a considerable delay (i.e., 15 h), while no comparable delay (within the limits of the assay) in the inhibition of cholesterol synthesis was observed. The increased fatty acid synthesis was initially directed primarily to phospholipids and later to both phospholipids and triacylglycerols; these data are consistent with the changes in lipid composition of inhibitor-treated cultures described above.

The ability of LDL, 25-hydroxycholesterol, and mevalonic acid to prevent the increased fatty acid

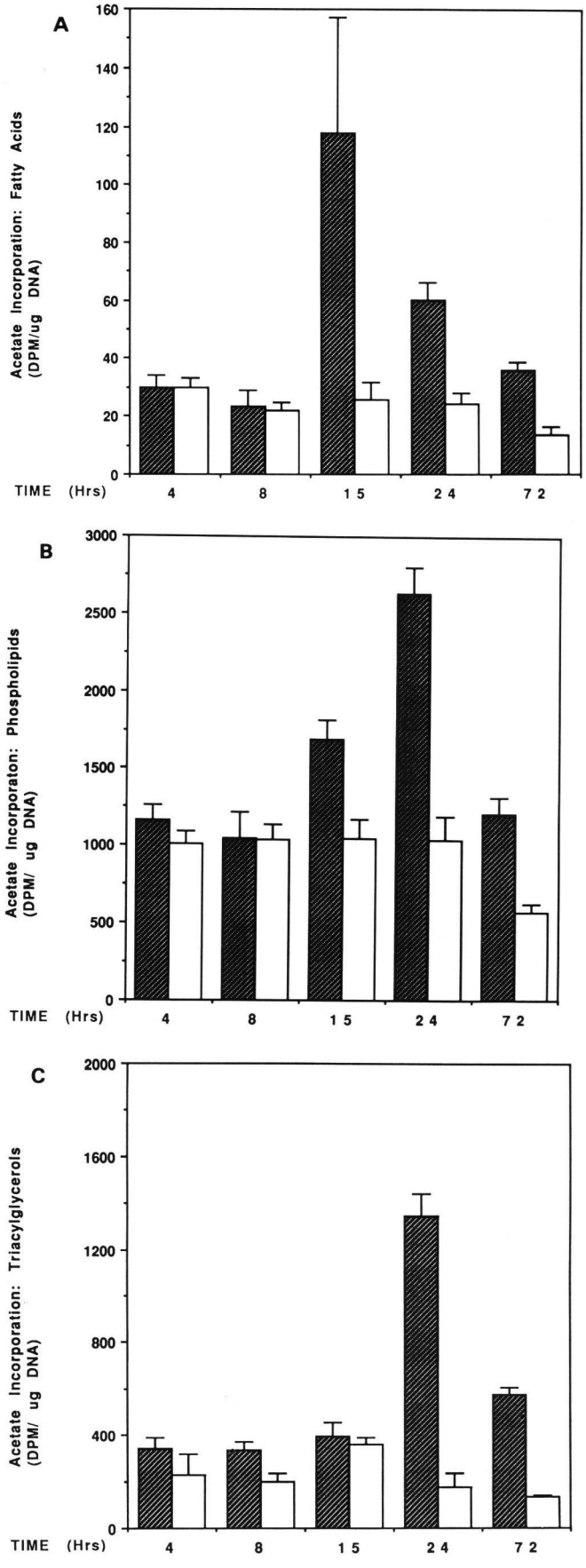

Fig. 2. Lipid synthesis in keratinocytes treated with HMG-CoA reductase inhibitors: time-course. Preconfluent cultures of keratinocytes in serum-free medium (1.2 mM Ca^{2+}) were exposed to 10 μM lovastatin (hatched bars) for 4, 8, 15, 25, or 72 h or vehicle alone (open bars) and labeled with [^{14}C]acetate during the final 4 h. Cultures were harvested for determination of DNA content and the radioactivity in lipids fractionated by TLC and quantitated (panels A–C) (see Methods). Data represent the mean of three dishes each ± SE. Significantly increased incorporation of acetate into free fatty acids (panel A) and phospholipids (panel B) is evident by 15 h, while incorporation into triacylglycerols (panel C) is increased by 24 h.

synthesis in HMG-CoA reductase inhibitor-treated keratinocytes was examined next. Pre-confluent keratinocytes were maintained in low calcium (0.07 mM) medium, because keratinocytes express LDL-receptors and down-regulate their cholesterol biosynthesis in response to serum lipoproteins under these conditions (43–45). As expected, when cultures were exposed to either LDL or 25-hydroxycholesterol for 24 h, cholesterol synthesis was markedly inhibited (≥ 95% inhibition, data not shown). The DNA content of experimental groups did not differ significantly from controls in these studies. Neither 25-hydroxycholesterol nor LDL when given alone significantly altered fatty acid synthesis (**Fig. 3**). Lovastatin significantly increased fatty acid synthesis when used alone, but not when co-administered with either LDL or 25-hydroxycholesterol (Fig. 3). Like the HMG-CoA reductase inhibitors, mevalonate (10 mM) alone also induced a modest but significant increase in fatty acid synthesis; however, there was no further increase in fatty acid synthesis in the presence of both lovastatin and mevalonate (Fig. 3).

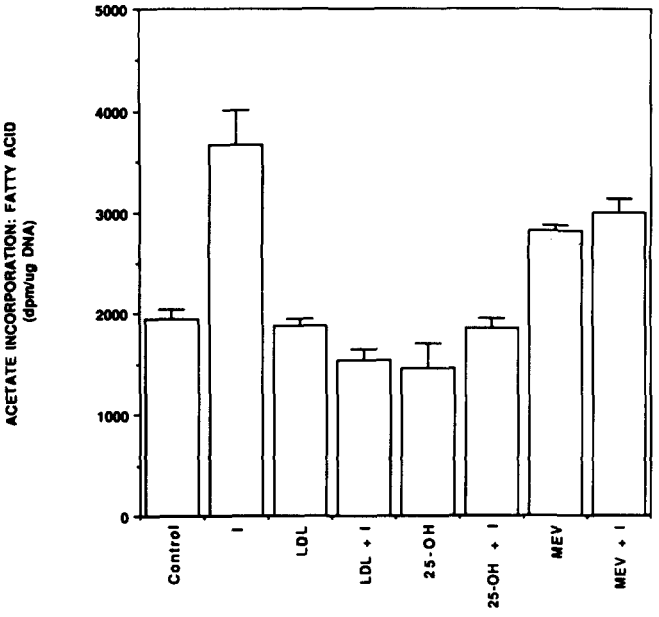

Fig. 3. Fatty acid synthesis: effect of HMG-CoA reductase inhibitor alone or in combination with LDL, 25-hydroxycholesterol or mevalonate. Keratinocytes were cultured in low calcium (0.07 mM) medium. Pre-confluent cultures were exposed LDL (30 μg/ml), 25-hydroxycholesterol (25-OH) (12.5 μM), or mevalonate (MEV) (10 mM), alone or in combination with HMG-CoA reductase inhibitor (I) (lovastatin; 10 μM), or to vehicle alone (Control), for 24 h, and were radiolabeled with [^{14}C]acetate during the final 3 h. Lipids were extracted and saponified as described. Data represent the mean of three dishes each ± SE. Compared to control, fatty acid synthesis was significantly increased in inhibitor-treated cultures ($P < 0.001$). Neither LDL nor 25-hydroxycholesterol alone or in combination with inhibitor significantly increased fatty acid synthesis. In contrast, mevalonate alone or in combination with inhibitor significantly increased fatty acid synthesis over control cultures (Mev alone: $P < 0.05$; Mev + I: $P < 0.02$); the stimulatory effects of mevalonate and inhibitor on fatty acid synthesis were not additive.

Incorporation into lipid and β-oxidation of exogenously supplied fatty acid

The effect of HMG-CoA reductase inhibitors on fatty acid β-oxidation was determined next. During a 1-h incubation, oxidation of [^{14}C]palmitate to CO_2 was decreased by more than 50% in keratinocytes that had been preincubated for 24 h with either 25 μM lovastatin (control: 6.39 ± 0.87, lovastatin: 2.87 ± 0.10, nmol/min per μg DNA; $P < 0.01$) or 25 μM fluindostatin (control: 6.60 ± 0.19, fluindostatin: 2.81 ± 0.19 nmol/min per μg DNA; $P < 0.001$). Co-incubation with either LDL or 25-hydroxycholesterol did not restore [^{14}C]palmitate oxidation. To determine whether this was a specific effect on fatty acid oxidation or represented a more general repression of cellular respiration, the oxidation of [^{14}C]acetate was similarly assessed. In contrast to the decreased fatty acid oxidation, inhibitor-treated cultures exhibited a 40% increase in the oxidation of [^{14}C]acetate (sp act 59.9 mCi/mmol) to CO_2 (control 40.23 ± 3.57; fluindostatin 57.03 ± 2.16 nmol/min per μg DNA; $P < 0.02$).

Table 3 shows the distribution of [^{14}C]palmitate into cellular lipids following a 2-h incubation. While the overall uptake and incorporation into lipid of [^{14}C]palmitate did not differ in inhibitor-treated cultures, the incorporation of [^{14}C]palmitate into triacylglycerols was increased by 50% ($P < 0.05$), and the incorporation of [^{14}C]palmitate into sterol esters was markedly decreased. Thus, keratinocytes exposed to HMG-CoA reductase inhibitors display significant inhibition of mitochondrial fatty acid β-oxidation, as well as accumulation of exogenously supplied palmitate in triacylglycerols.

TABLE 3. Incorporation of [^{14}C]palmitate into cellular lipids in lovastatin versus control keratinocytes

Lipid	[^{14}C]Palmitate Incorporation		Significance
	Control	Lovastatin	
	dpm/μg DNA		
Total lipid extract	8,313 ± 202	9,424 ± 421	NS
Phospholipids	5,548 ± 230	6,098 ± 110	NS
Free fatty acids	351 ± 31	279 ± 1	NS
Triacylglycerols	1,538 ± 221	2,263 ± 37	$P < 0.05$
Sterol esters	211 ± 26	32 ± 1	$P < 0.001$

Keratinocytes in medium containing 1.2 mM Ca^{2+} were exposed to either 25 μM lovastatin or vehicle alone for 24 h and incubated with [1-^{14}C]palmitate, 1.3 μCi/dish (sp act 9.8 mCi/mmol) during the final 2 h. Cultures were harvested and lipid-associated radioactivity was determined (see Methods). Data represent the mean of three dishes ± SE; NS, not significant.

Ultrastructure and DAB-cytochemistry

The ultrastructures of keratinocytes exposed for varying periods of time to HMG-CoA reductase inhibitors versus vehicle controls are illustrated (see Figs. 4–6). Whereas keratinocytes treated for 12 h with 10 μM lovastatin demonstrated increased numbers of both membrane-bound and non-membrane-bound lipid droplets (**Fig. 4B**), keratinocytes of control cultures contained smaller numbers of lipid droplets that were not membrane-bound (Fig. 4A; see also Fig. 6). In control cultures, lipid droplets were confined predominantly to the more differentiated suprabasilar cells while, in contrast, prominent lipid droplets were apparent in all strata of HMG-CoA reductase inhibitor-treated cells (data not shown).

Small vesicular structures were frequently present within these larger membrane-bound droplets. To determine whether these might represent peroxisomes, diaminobenzidine (DAB) ultracytochemistry was used (35). DAB-reaction products were not apparent in samples incubated without substrate (H_2O_2) (**Fig. 5B**) or in the presence of the catalase inhibitor, aminotriazole (data not shown). Control keratinocytes exhibited occasional peroxisomes (Fig. 4A; **Fig. 6,** inset). Multivescular bodies were also occasionally encountered, but DAB-reaction products usually did not localize to these structures in control keratinocytes (Fig. 6). However, DAB-reaction products were observed primarily within multivesicular bodies in lovastatin-treated cultures (Figs. 4B, 4C; Fig. 5A, inset). Increased quantities of DAB-positive organelles appeared as early as 12 h after addition of the HMG-CoA reductase inhibitor (Fig. 4B). Both the size and number of DAB-positive multivesicular bodies increased with prolongation of exposure to the inhibitor (Fig. 4C and Fig. 5, 48 h and 7 days, respectively). Lipid droplets were also found frequently in close proximity to peroxisomes (Fig. 4B). These studies demonstrate that keratinocytes exposed to HMG-CoA reductase inhibitors for ≥ 12 h exhibit both increased quantities of cytoplasmic lipid vacuoles and increased numbers of peroxisomes, that are primarily found within multivesicular bodies.

Peroxisomal enzyme activities

To confirm the ultrastructural impression of peroxisomal hyperplasia, peroxisomal fatty acid oxidation, as well as the activities of two peroxisomal enzymes, catalase and dihydroxyacetone phosphate acyltransferase (DHAP-AT), were assayed. Because catalase activity increases in relationship to keratinocyte differentiation in vivo (46), the activity of this enzyme in response to lovastatin under culture conditions that favor proliferation (0.07 mM Ca^{2+}) versus differentiation (1.2 mM Ca^{2+}) (26, 27) and at various stages of confluence was examined. Vehicle-treated controls showed considerable variation in catalase activity depending upon culture conditions, with the highest activities found in preconfluent cultures in low calcium medium (**Table 4**). Despite these variations, exposure to 10 μM lovastatin for 48 h induced a 1.5- to 2.5-fold increase in catalase activity that was highly significant ($P < 0.001$) under all culture conditions.

HMG-CoA reductase inhibitors also induced a significant increase in peroxisomal fatty acid oxidation, using an assay based upon the ability of potassium cyanide to inhibit mitochondrial but not peroxisomal fatty acid oxidation (39). Exposure of confluent cultures in 1.2 mM Ca^{2+} to 10 μM lovastatin increased peroxisomal fatty acid oxidation by 40% after 48 h (control 22.1 ± 0.3; lovastatin 30.6 ± 1.7, pmol/min per mg protein; $P < 0.01$). Similarly, the activity of DHAP-AT, an enzyme localized exclusively to peroxisomes, also was increased by 40% in cultures exposed to fluindostatin (25 μM) for 48 h (control 14.59 ± 76; fluindostatin 20.64 ± 31; $P < 0.001$). Thus, using three independent biochemical measures, catalase activity, peroxisomal fatty acid oxidation, and DHAP-AT activity, significant increases in peroxisomal function in keratinocytes exposed to HMG-CoA reductase inhibitors were observed. In conjunction with the ultrastructural cytochemistry (vide supra), we conclude that HMG-CoA reductase inhibitors induce peroxisomal hyperplasia in keratinocytes.

To determine the effect of sterols and mevalonate on the induction of peroxisomes by HMG-CoA reductase inhibitors, keratinocytes were grown in low calcium media (0.07 mM Ca^{+}) to permit LDL-receptor expression (see above) and exposed for 48 h to either LDL, 25-hydroxycholesterol, mevalonate, or vehicle alone (control) in the presence or absence of fluindo-

TABLE 4. *Effect of culture conditions on lovastatin induction of catalase*

Culture	Catalase Activity		
	Control	+ Lovastatin	% Control
	B.U./ml/ μg protein		
Pre-confluent			
0.07 mM Ca^{2+}	5.44 ± 0.01	7.92 ± 0.07	150
1.2 mM Ca^{2+}	2.73 ± 0.01	6.56 ± 0.13	230
Confluent			
0.07 mM Ca^{2+}	3.67 ± 0.05	7.34 ± 0.14	200
1.2 mM Ca^{2+}	2.92 ± 0.05	5.78 ± 0.01	200
Post-confluent			
0.07 mM Ca^{2+}	1.78 ± 0.03	4.29 ± 0.01	240
1.2 mM Ca^{2+}	2.28 ± 0.03	4.06 ± 0.03	180

Keratinocytes were grown in serum-free medium with either 0.07 mM or 1.2 mM extracellular calcium and exposed to 10 μM lovastatin or vehicle alone (control) for 48 h prior to harvesting. Cultures were harvested when estimated visually to be 50% pre-confluent, confluent, or 5 days post-confluent, and catalase activity was determined (Methods). Data represent the mean of triplicate determinations ± SE.

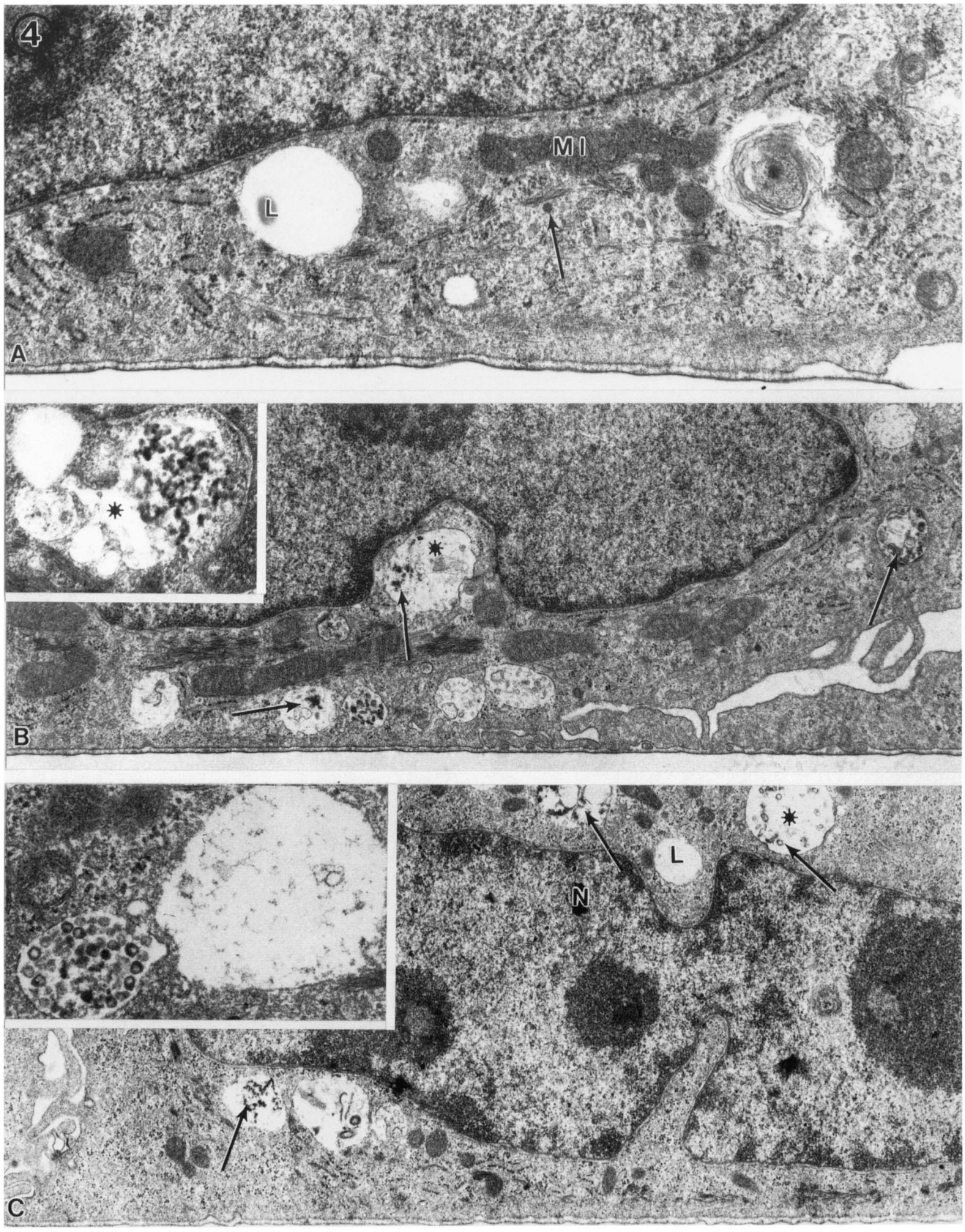

Fig. 4. Electron micrographs of keratinocytes using DAB cytochemistry. Panel A: Keratinocytes prior to addition of HMG-CoA reductase inhibitor, time zero. Keratinocytes exhibit occasional nonmembrane-bound lipid droplets (L) as well as discrete DAB-positive microbodies (↑) (16,000 x). Panel B: Keratinocytes exposed to lovastatin (10 μM) for 12 h. Increased numbers of DAB-positive organelles (↑) are noted by 12 h (15,750 x). Inset: DAB-positive microbodies are frequently concentrated within multivesicular bodies (*), and often in close association to lipid droplets (25,000 x). Panel C: Keratinocytes exposed to lovastatin (10 μM) for 48 h. Similar numbers and distribution of DAB-positive structures are also evident at this time (11,000 x). Mi, mitochondria; N, nucleus.

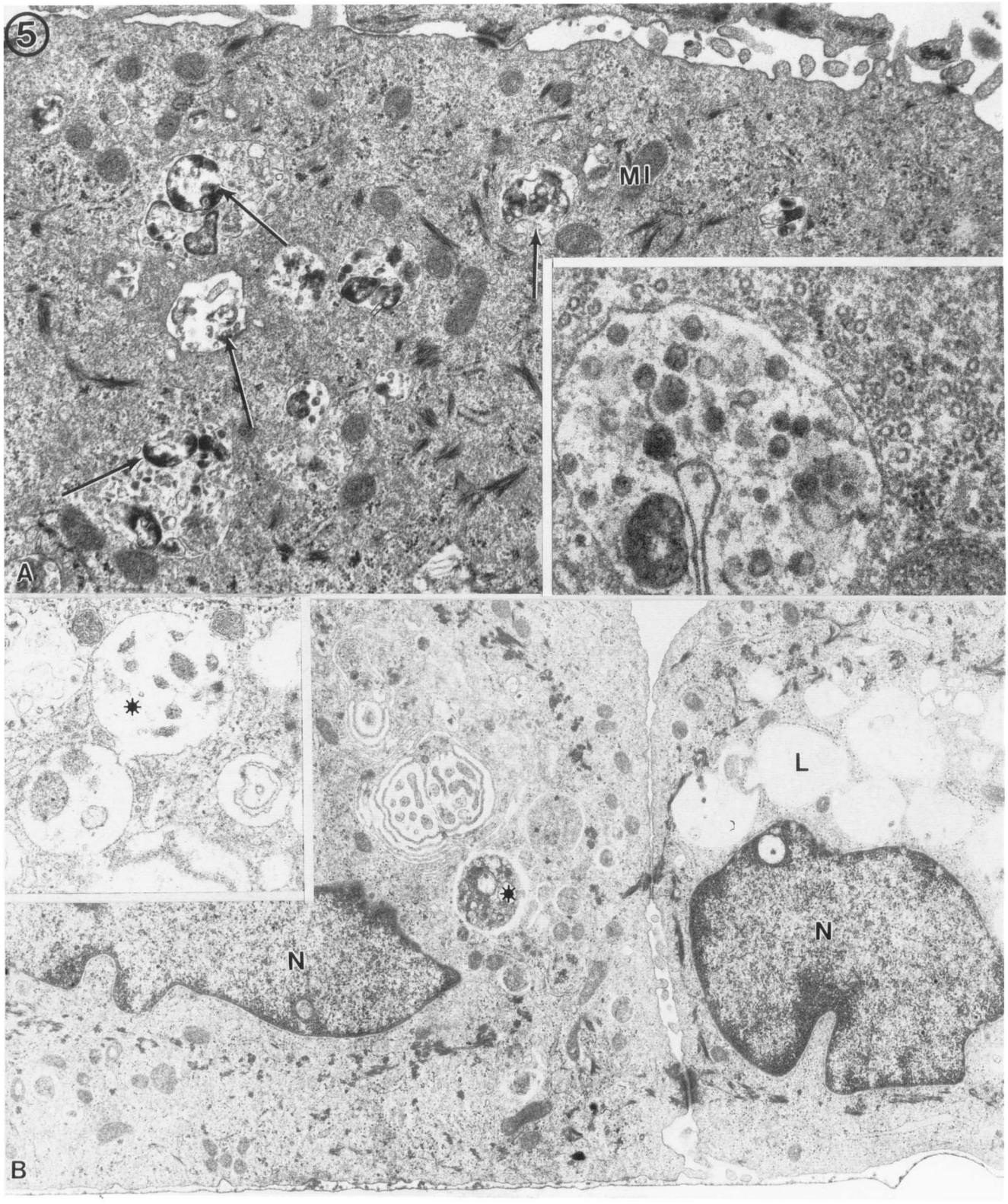

Fig. 5. Electron microscopy of keratinocytes using DAB cytochemistry. Panel A: Keratinocytes after treatment with lovastatin (10 μM) for 1 week. DAB-positive organelles are even more numerous and continue to be found primarily within multivesicular bodies; 24,000 x. Inset: 825,000 x. Panel B: Control for DAB cytochemistry (incubation without H_2O_2) in keratinocytes treated with lovastatin (10 μM) for 1 week. Note that the multivesicular bodies (*) are devoid of DAB reaction products. Numerous lipid droplets (L) are evident after prolonged exposure to the inhibitor (12,000 x). Inset: Higher magnification of multivesicular bodies (32,000 x). Mi, mitochondria; N, nucleus.

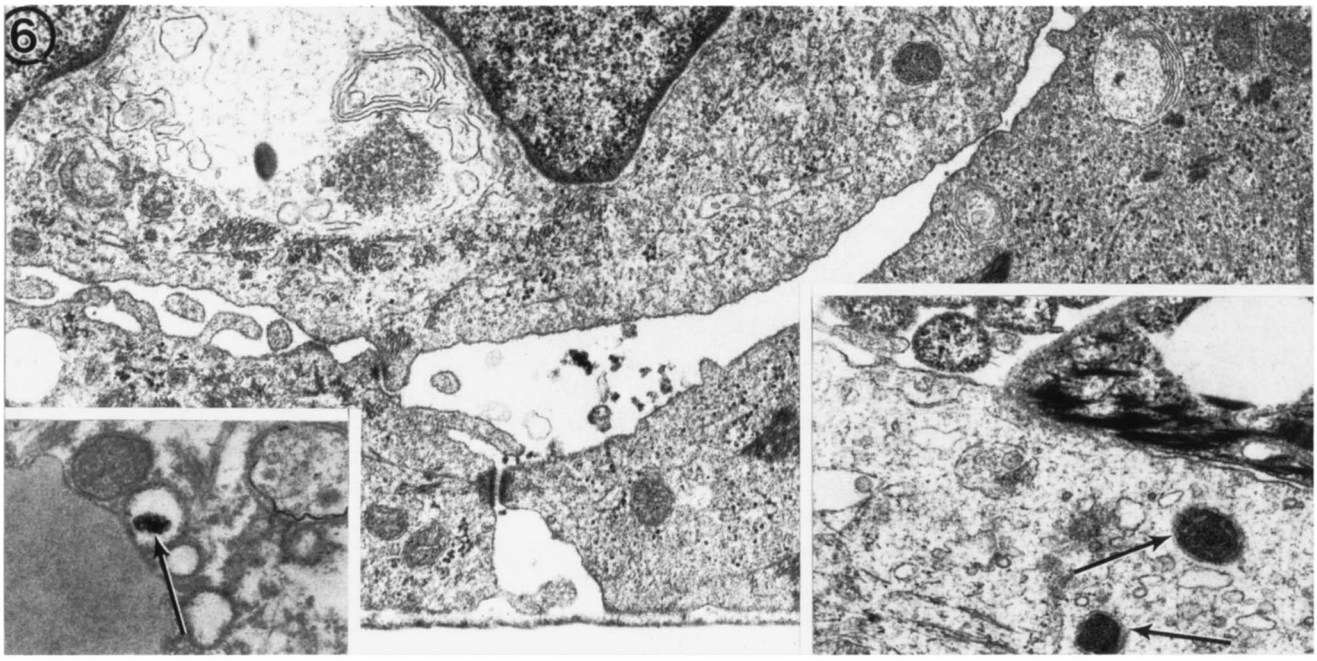

Fig. 6. Electron micrographs of keratinocytes using DAB cytochemistry. Keratinocytes were exposed to vehicle alone for 1 week. In contrast to inhibitor-treated cultures, peroxisomes are not evident within multivesicular bodies (22,000 x), and only occasionally are peroxisomes (↑) encountered (insets: left, 32,000 x; right, 25,000 x).

statin (25 μM), and catalase activity was assayed (**Fig. 7**). As shown in prior experiments, fluindostatin alone increased catalase activity approximately 2-fold (Fig. 7). In contrast, LDL, 25-hydroxycholesterol, or mevalonate alone each produced a modest decrease in catalase activity (18%, 33%, and 19%, respectively; each $P < 0.01$). The addition of fluindostatin to these cultures resulted in either no effect (LDL and mevalonate) or only a modest increase (25-hydroxycholesterol vs. 25-hydroxycholesterol + fluindostatin; 28% increase ($P < 0.02$)) in catalase activity. In no instance did the combination of an HMG-CoA reductase inhibitor and LDL, 25-hydroxycholesterol, or mevalonate increase catalase activity above levels in control cultures.

DISCUSSION

These studies demonstrate that prolonged (i.e., ≥ 12 h) exposure of keratinocytes to competitive inhibitors of HMG-CoA reductase results in increased fatty acid synthesis, phospholipid and triacylglycerol accumulation, and peroxisomal hyperplasia (**Table 5**). These observations imply that the effects of these agents on cellular lipid metabolism may be broader than generally recognized.

While lipid synthesis in response to HMG-CoA reductase inhibitors has been extensively studied, dif-

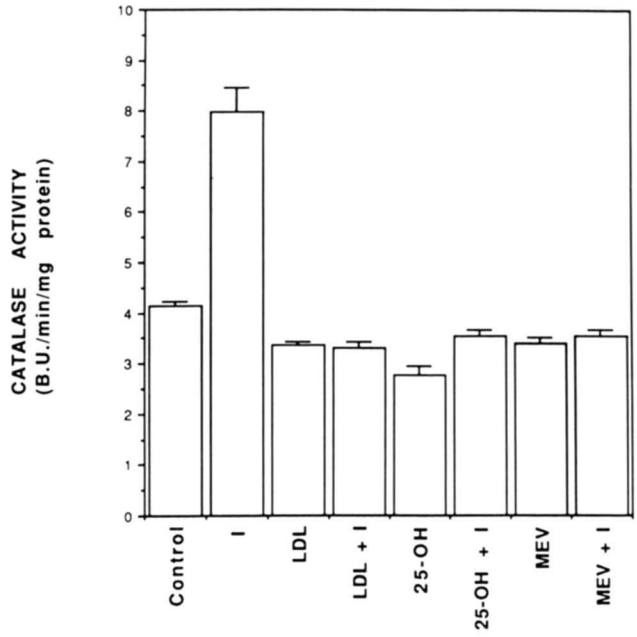

Fig. 7. Catalase activity: effect of HMG-CoA reductase inhibitor alone or in combination with LDL, 25-hydroxycholesterol or mevalonate. Keratinocytes were cultured in serum-free low calcium (0.07 mM) medium. When cultures were 70–80% confluent, HMG-CoA reductase inhibitor (I) (fluindostatin; 25 μM) and/or 25-hydroxycholesterol (25-OH) (12.5 μM), mevalonate (MEV) (10 mM), LDL (30 μg/ml), or vehicle alone (Control) were added (see Methods). Forty-eight hours later cultures were harvested, and catalase activity was assayed (see Methods). Data represent the mean of three dishes each ± SE. Only keratinocytes treated with inhibitor alone exhibit increased catalase activity.

ferences in experimental conditions, e.g., dose and duration of exposure to inhibitor, culture density, or cell type, could account for the infrequency that effects on fatty acid metabolism have been previously noted (3, 6, 7). For example, Kaneko, Hazama-Shimada, and Endo (3) noted no stimulation of fatty acid synthesis in fibroblasts treated with compactin for up to 6 h and in doses of up to 5 μg/ml. However, their data do show a modest increase in fatty acid synthesis at higher concentrations. Lovastatin (0.1 μM) increased fatty acid synthesis in the rat lens during a 4.5-h incubation, while 3 μM pravastatin had no effect (6). Pravastatin, however, was a significantly less potent inhibitor of cholesterol biosynthesis. In our studies, HMG-CoA reductase inhibitors increased fatty acid synthesis only after 15 h and this effect was maximal at 1–10 μM concentrations. Bensch, Ingebritsen, and Diller (7) also observed increased fatty acid synthesis in response to compactin in cultured fibroblasts; however, the time required to produce this effect was not indicated. We also observed increased fatty acid synthesis in fibroblasts after 24 h incubation with HMG-CoA reductase inhibitors.

Lipid synthesis in cultured cells is closely linked to growth (47–50); in the present studies fatty acid synthesis was increased despite growth-arrest of inhibitor-treated keratinocytes. Cholesterol (48) and other post-mevalonate products (51) are required for cell growth; and cell cycle-arrest of HMG-reductase inhibitor-treated cultured cells is a well-recognized phenomenon (52). The increased incorporation of acetate into fatty acids in response to HMG-CoA reductase inhibitors cannot be attributed to shift of acetate pools from an inhibited (cholesterol) to an uninhibited (fatty acid) pathway because: *1)* of differences in time course, i.e., acetate incorporation into cholesterol is inhibited immediately (0–3 h), while increased incorporation into fatty acids is delayed (15 h) (Fig. 2); and *2)* other inhibitors of cholesterol biosynthesis (e.g., 25-hydroxycholesterol) do not increase acetate incorporate into fatty acids (Fig. 3). Moreover, using [^{3}H]water, Feingold et al. (24) also observed increased epidermal fatty acid synthesis in vivo during prolonged topical lovastatin treatment. The interpretation that these data reflect increased fatty acid synthesis is also consistent with the accumulation of acyl-lipids in inhibitor-treated cultures. Although triacylglycerol accumulation is a characteristic feature of keratinocytes in postconfluent, differentiated cultures (50, 53), the triacylglycerol content of lovastatin-treated cultures was 2.5-fold greater than control cultures. Because mitochondrial fatty acid oxidation is also reduced > 50% in inhibitor-treated cultures, and exogenously supplied fatty acids also accumulate under these conditions, the lipid accumulation we observed reflects both increased fatty acid synthesis and decreased oxidation.

TABLE 5. Summary of effects of HMG-CoA reductase inhibitors on fatty acid synthesis, mitochondrial fatty acid oxidation, and catalase activity: modifications by addition of LDL, 25-hydroxycholesterol, or mevalonate

	HMG-CoA Reductase Inihibitor	Fatty Acid Synthesis	Fatty Acid Oxidation	Catalase Activity
Control	–	N	N	N
Control	+	↑↑	↓	↑↑
LDL	–	N	ND	(↓)
LDL	+	(↓)	↓	(↓)
25-Hydroxy-cholesterol	–	(↓)	ND	↓
25-Hydroxy-cholesterol	+	N	↓	(↓)
Mevalonate	–	↑↑	ND	(↓)
Mevalonate	+	↑↑	ND	(↓)

Abbreviations: N, normal; (↑) or (↓), ↑ or ↓, ↑↑ or ↓↓ indicate a significant change ≤ 20%, 20–50%, or > 50% of control, respectively; ND, not determined.

The ability of either LDL or 25-hydroxycholesterol to prevent the stimulatory effect of HMG-CoA reductase inhibitors (Table 5) on fatty acid synthesis suggests that this effect is linked in some manner to their inhibition of cholesterol biosynthesis. We cannot readily explain our observation that mevalonate alone stimulates fatty acid synthesis, although it may be pertinent to note that it was necessary to use relatively high (i.e., 10 mM) concentrations of this metabolite (see Methods). Moreover, mevalonate is used for the synthesis of other isoprenoids (51, 52); one of these products may stimulate fatty acid synthesis. Because the stimulatory effects on fatty acid synthesis of the HMG-CoA reductase inhibitors and mevalonate were not additive, they may each act through a common mechanism; or, perhaps more likely, the conversion of mevalonate to cholesterol may block the response to the inhibitor, leaving only the baseline mevalonate effect on fatty acid synthesis. The ability of LDL to override the reductase inhibitors' effect on fatty acid synthesis may account for the infrequent reports of increased acyl-lipid synthesis in response to systemic administration of these agents. Hypertriglyceridermia has not been reported in clinical use of lovastatin (2). Moreover, although reductase inhibitors simulate fatty acid synthesis in both fibroblasts and keratinocytes in the absence of LDL, there may be tissue-specificity for this effect, because Middleton, Hatton, and White (54) noted that compactin inhibits fatty acid synthesis in hormonally-stimulated mammary glands in vitro.

There are several mechanisms whereby fatty acid synthesis and cholesterol synthesis may be interrelated. First, the key regulatory enzymes in fatty acid biosynthesis, acetyl CoA carboxylase and fatty acid syn-

thetase, may be regulated coordinately with HMG-CoA reductase at the genomic level. If so, increased transcription of these genes would occur in response to unopposed HMG-CoA reductase inhibitors, while provision of exogenous sterol would repress gene transcription (8, 55–57). However, coordinate genomic regulation of these enzymes with HMG-CoA reductase has not yet been demonstrated. Alternatively, because both HMG-CoA reductase and acetyl CoA carboxylase are reversibly inactivated through phosphorylation by the same protein kinase (58), down-regulation of this protein kinase by sterol deficiency could increase acetyl coenzyme A carboxylase activity when HMG-CoA reductase inhibitors were given in the absence, but not in the presence, of regulatory sterols. Finally, in UT-I cells (10) and liver (9) HMG-CoA reductase inhibitors induce marked proliferation of smooth endoplasmic reticulum as a consequence of a massive increase in reductase content. Similarly, hyperplasia of both smooth endoplasmic reticulum and peroxisomes has been observed in adrenal glands of lovastatin-treated rats (14). And in the present studies we observed hyperplasia of peroxisomes in response to HMG-CoA reductase inhibitors. The requirement for lipid for new organellar membranes may thus be the stimulus for fatty acid synthesis in reductase inhibitor-treated cultures.

Peroxisomal hyperplasia in response to HMG-CoA reductase inhibitors has not been previously reported in cultured cells. Other hypolipidemic agents, particularly clofibrate or related compounds, are well-recognized inducers of hepatic peroxisomes (reviewed in 16, 17, 59). It has been noted that all xenobiotics that induce peroxisomes possess a carboxylic functional group in common (59). Reddy et al. (16) have proposed that these drugs induce transcription of peroxisomal β-oxidation enzymes via a specific nuclear receptor. Yet, while the active metabolites of HMG-CoA reductase inhibitors also possess a carboxylic functional group, it is unlikely that they act directly on this putative nuclear receptor, because co-incubation with either LDL, 25-hydroxycholesterol, or mevalonic acid prevented peroxisomal induction in these studies (Table 5). An alternate theory postulates that peroxisomes are induced in response to substrate overload; i.e., increased flux of fatty acids (reviewed in 17, 59). Although HMG-CoA reductase inhibitors increased fatty acid synthesis in these studies, this alone is not sufficient to induce peroxisomes, because mevalonate alone or in combination with a reductase inhibitor also stimulated fatty acid synthesis without inducing peroxisomes (Table 5). Inhibition of mitochondrial fatty acid oxidation has also been proposed to underlie xenobiotic-induced peroxisomal hyperplasia (17). Yet, while HMG-CoA reductase inhibitors also inhibited mitochondrial fatty acid oxidation in keratinocytes, this is unlikely to be the mechanism underlying the peroxisomal hyperplasia in response to HMG-CoA reductase inhibitors because, in the presence of either LDL or 25-hydroxycholesterol, peroxisomal induction was prevented while inhibition of mitochondrial fatty acid oxidation was not (Table 5).

Alternatively, the peroxisomal induction by HMG-CoA reductase inhibitors may not be directly related to their effects on acyl-lipid synthesis and storage, but instead may occur as a consequence of the marked increase in HMG-CoA reductase content induced by these inhibitors. Keller et al. (12, 13) have demonstrated the localization of HMG-CoA reductase to peroxisomes in liver. Under normal conditions, less than 5% of the enzyme in rat liver is localized to peroxisomes; but, after induction of peroxisomes with cholestyramine, 20–30% of the enzyme becomes localized to peroxisomes. However, hepatic peroxisome reductase content was not increased in rats treated with reductase inhibitors (9). In future studies it will be important to directly assess reductase content and its subcellular distribution in keratinocytes treated with HMG-CoA reductase inhibitors.

While these studies demonstrate that under certain experimental conditions HMG-CoA reductase inhibitors stimulate fatty acid synthesis and induce peroxisomes in cultured cells, one would predict that these phenomena would be unlike to occur in most tissues in vivo. In the first place, because lovastatin is primarily cleared during first pass through the liver (2), most tissues may not be exposed to high enough concentrations during systemic administration of the drug. Moreover, tissues that utilize LDL cholesterol should not be sensitive to these effects, because neither increased fatty acid synthesis nor peroxisomal induction was observed in inhibitor-treated keratinocytes in the presence of LDL (c.f. Figs. 3, 7). However, Feingold et al. (24) have shown that the repeated topical application of HMG-CoA reductase inhibitors to the skin of hairless mice stimulates epidermal fatty acid synthesis and acyl-lipid accumulation. In epidermis, LDL receptors are only expressed on basal cell layers (60), while upper cell strata remain active sites of sterologenesis (18, 61, 62), and overall cutaneous sterologenesis is not regulated by serum cholesterol (22, 23). Thus, topical administration of HMG-CoA reductase inhibitors may achieve high enough local concentrations of inhibitor within a tissue whose cholesterol requirements are dependent upon de novo synthesis.

The studies reported herein describe the conditions under which HMG-CoA reductase inhibitors perturb fatty acid metabolism and induce peroxisomes in vitro. Further studies will be required to delineate the

mechanisms that underlie these effects. For example, delineation of the mechanism(s) whereby HMG-CoA reductase inhibitions stimulate fatty acid synthesis may provide important insights into interrelationships between cholesterol and fatty acid metabolism. It will also be important to determine whether HMG-CoA reductase inhibitors also induce peroxisomal hyperplasia in epidermis in vivo. Delineation of the mechanism whereby HMG-CoA reductase inhibitors induce peroxisomes may yield insights into the manner in which cells regulate their peroxisomal content.

In summary, HMG-CoA reductase inhibitors stimulate fatty acid synthesis and produce accumulation of acyl-lipids in keratinocytes cultured in serum-free media. Addition of either LDL or 25-hydroxycholesterol prevents this response. Under similar conditions, HMG-CoA reductase inhibitors also induce peroxisomal hyperplasia. Delineation of the mechanism(s) whereby HMG-CoA reductase inhibitors induce these responses represents a goal of future studies.

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