Effects of simvastatin on the metabolism of polyunsaturated fatty acids and on glycerolipid, cholesterol, and de novo lipid synthesis in THP-1 cells

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Abstract In the monocytic THP-1 cells, the 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase inhibitor simvastatin (5 μM) enhances the conversion of exogenous linoleic (18:2 n–6) and eicosapentaenoic (20:5 n–3) acids to their long-chain polyunsaturated fatty acid (LC-PUFA) derivatives, and this effect is associated with changes in the desaturation steps. In addition, formation of monounsaturated fatty acids from endogenously synthesized precursors is increased. These metabolic changes lead to elevated LC-PUFA and fatty acid (FA) unsaturation in cells. The effects of simvastatin on FA metabolism are associated with increased synthesis of triglycerides from glycerol. The dose–effect relationships for the activity of simvastatin on total linoleic acid (LA) conversion and cholesterol synthesis reveal that enhancement of PUFA metabolism is already maximal at 0.5 μM simvastatin, whereas cholesterol synthesis is further inhibited by concentrations of simvastatin up to 5 μM. The effects of 5 μM simvastatin on PUFA metabolism are partially prevented by mevalonate (1 mM) and geranylgeraniol (5 μM) but not by farnesol (10 μM). These data indicate that HMG-CoA inhibitors have profound effects on PUFA metabolism, and that the pathways for cholesterol and PUFA synthesis are mutually modulated.—**Risé, P., C. Colombo, and C. Galli.** Effects of simvastatin on the metabolism of polyunsaturated fatty acids and on glycerolipid, cholesterol, and de novo lipid synthesis in THP-1 cells. *J. Lipid Res.* 1997. **38:** 1299–1307.

Supplementary key words THP-1 cells • fatty acid desaturation and elongation • simvastatin • mevalonate • isoprenoids • lipid synthesis from glycerol and acetate

Competitive inhibitors of 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase (1) are widely used in the treatment of hypercholesterolemia. Although their action is considered rather specific for the inhibition of cholesterol synthesis, and fatty acid metabolism has been generally reported to be unaffected (2, 3), recent studies have shown that HMG-CoA reductase inhibitors have additional effects. They enhance fatty acid synthesis and peroxisomal activity (4) and induce elevation of arachidonic acid (AA) and thromboxane production (5) in cultured cells. Furthermore, plasma and erythrocyte long-chain polyunsaturated fatty acids (LC-PUFA) are modified by the use of HMG-CoA reductase inhibitors, in clinical studies (6, 7). As it has been shown that the microsomal cholesterol content may influence the acyl-CoA desaturases (8), the rate-limiting enzymes of PUFA synthesis, this may be relevant to the above findings. In addition, changes in membrane lipids, resulting from complex modifications of lipid metabolism, may be partly involved in the reported effects of lovastatin, a potent HMG-CoA reductase inhibitor, on membrane fluidity and aggregation of platelets in hypercholesterolemic patients (9). It appears, therefore, that inhibition of HMG-CoA reductase results in a wide spectrum of effects on lipid metabolism, but their relationship to their primary mechanism of action is still uncertain.

Although HMG-CoA reductase inhibitors affect the formation of the LC-PUFA AA from its precursor linoleic acid (LA), in the n–6 series (5), it is not clear whether this effect applies generally to the desaturation of FA in other metabolic series, and of endogenously synthesized FA. In addition, the possible effects of HMG-CoA reductase inhibitors on the formation of 22-carbon PUFA, a pathway involving a peroxisomal β-oxidation (10) and the concentration–effect relationships

Abbreviations: AA, arachidonic acid; CE, cholesteryl esters; DAG, diacylglycerol; FA, fatty acids; FFA, free fatty acids; LA, linoleic acid; LC-PUFA, long-chain polyunsaturated fatty acids; MAG, monoacylglycerol; MUFA, monounsaturated fatty acids; MVA, mevalonic acid; PBS, phosphate-buffered saline; PL, phospholipids; PUFA, polyunsaturated fatty acids; SAT, saturated fatty acids; TG, triglycerides; TL, total lipids. HMG-CoA, 3-hydroxy-3-methylglutaryl coenzyme A.

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for the activity on FA and cholesterol, as well as on other metabolic pathways, have not been explored in detail.

We have therefore investigated the activity of the potent HMG-CoA reductase inhibitor simvastatin on the above aspects in the human monocytic cell line THP-1. These cells are widely used in studies on lipid/lipoprotein metabolism (11) and are able to actively desaturate and elongate exogenous n–6 fatty acids to long-chain derivatives, including the synthesis of 22 carbon PUFA (12). Simvastatin is a chemical derivative of lovastatin (13) and both are among the most potent inhibitors of cholesterol synthesis in cultured cells (14). Our study confirms that simvastatin enhances the conversion of LA to AA, and shows also that the synthesis of 22:6 (docosahexaenoic acid, DHA) from 20:5 n–3 (eicosapentaenoic acid, EPA) and the production of oleic acid (18:1) from endogenously synthesized FA, using acetate as substrate, are enhanced. Changes in FA metabolism are associated with enhanced incorporation of glycerol in triglycerides. Finally, maximal effects of simvastatin on PUFA metabolism are attained at concentrations lower than those required for maximal effects on cholesterol synthesis.

MATERIAL AND METHODS

Chemicals

Linoleic acid, essentially fatty acid-free bovine serum albumin, RPMI 1640 medium, penicillin/streptomycin, mevalonic acid, all-*trans* geranylgeraniol, and farnesol were from Sigma (St. Louis, MO); fetal calf serum (FCS), β-mercaptoethanol were from Mascia Brunelli S.p.A. (V.le Monza 272, Milano, Italy); solvents and silica-gel 60R were from E. Merck (D-6100 Darmstadt, Germany). [1-^{14}C]LA (specific activity 53 μCi/μmol), [1(3)-^{3}H]glycerol (specific activity 3.1 Ci/mmol) and [1-^{14}C]acetic acid sodium salt (specific activity 60 mCi/mmol) were purchased from Amersham; [1-^{14}C]EPA (specific activity 52 mCi/mmol) from DuPont-NEN. Simvastatin in lactone form was from Merck, Sharp & Dohme Research Laboratories (Woodbridge, NJ).

Cell culture

THP-1 cells were grown in RPMI 1640 medium with 10% FCS, 100 μg/ml penicillin, 100 IU/ml streptomycin, and 1% β-mercaptoethanol at 37°C in a humidified atmosphere of 5% CO_2 and 95% air. Cells that were not induced by incubation with phorbol esters were used in the various experiments.

Experimental design

The content of several flasks of THP-1 cells was centrifuged at 200 *g* for 10 min; the pellet was resuspended in RPMI medium without FCS and the cells were counted in a Cell Counter R Model 2 M (Coulter Instrument, Milan, Italy). The concentration of cells was adjusted to 10^6 cells/ml. Simvastatin, used in opened form at different (0.5–5 μM) concentrations (15), was added at this time. After 24 h, radioactive substrates, [1-^{14}C]LA 0.1 μCi/ml or [^{14}C]EPA 0.08 μCi/ml or [^{3}H]glycerol 0.5 μCi/ml or [^{14}C]acetic acid sodium salt 1 μCi/ml, were added for additional 24 h. Time course experiments for the conversion of LA were carried out by incubating THP-1 cells with [1-^{14}C]LA 0.1 μCi/ml, for 4, 8, 16, and 24 h. In all experiments FA and glycerol were added dissolved in 0.5% ethanol (final volume). At the end of the incubation period cells were centrifuged and resuspended in PBS; before the second and last washing the pellet was resuspended in a given volume of PBS and cells were counted.

Cell treatment with cholesterol precursors

Mevalonic acid (100–1000 μM), all-*trans* geranylgeraniol (5 μM), and farnesol (10 μM) were added, at the indicated concentrations, at beginning of the experiment, without or with simvastatin.

Lipid extraction

Lipid extraction was carried out according to the method of Folch, Lees, and Sloane Stanley (16). The lipid concentration of the samples, dissolved in chloroform–methanol 2:1, was determined by using a microbalance (C-31, Cahn Instruments, Cerritos, CA) (17).

Determination of cell TG content

Levels of TG in THP-1 cells were determined on lipid extracts by the use of a commercial kit (F. Hoffmann-La Roche Ltd., Basel) and values were expressed as percent of total lipids.

Analysis of FA-associated radioactivity

The determination of the radioactivity incorporated in individual FA was carried out by HPLC. The FA methyl esters, prepared by transesterification with 3 N methanolic-HCl (Supelco, Bellefonte, PA), were separated by a two-solvent system at a flow rate of 1 ml/min: solvent A acetonitrile and solvent B water, in accord with Moore, Yoder, and Spector (18). The column used was a LiChrospher 100, RP-18 (5 μm) (E. Merck, D-6100 Darmstadt, Germany); the HPLC used was a Jasco Model 880-PU (Japan Spectroscopic, Tokyo 192, Japan). The detection of radioactivity associated to FA methyl esters was achieved by using an on-line radiodetector (Flo-one beta A 200, Radiomatic Instruments and Chemicals, A Camberra Company, Tampa, FL) equipped with a 500 μl flow cell, using Flo-Scint A as scintillation fluid.

Separation of lipid classes

The lipid classes (PL, TG, CE, DAG, FFA) were separated by monodimensional TLC using silica-gel 60R plates and as mobile phase hexane–diethyl ether–acetic acid 70:30:1.5; DAG, free cholesterol, and MAG were separated by monodimensional TLC using silica-gel 60R plates and chloroform–methanol 98:2. Lipids were detected on plates dried under N_2 by exposure to iodine vapors; the spots were scraped from plates and the radioactivity was detected after addition of 1 ml methanol–water 1:1 and 10 ml of scintillation fluid.

Separation of FA according to unsaturation levels

Separation of saturated, mono, and polyunsaturated FA methyl esters was carried out by monodimensional TLC on silica-gel 60R plates impregnated with 15% $AgNO_3$, using hexane–diethyl ether 95:5 as developing solvent. Spots, localized using a radio scanner LB 2722-2 Dunnschicht-scanner 2 (Berthold, D 7547 Wildbad 1, Germany), were scraped into vials and the radioactivity was detected after addition of 1 ml methanol–water 1:1 and 10 ml of scintillation fluid. Samples used for further HPLC separations were extracted from silica-gel (14). After solvent evaporation, samples were redissolved in solvents for chromatographic analysis, and aliquots were injected in HPLC.

FA separation in GC

FA methyl esters, prepared from TL extracts, were analyzed on GC (Dani 8610, Monza, Italy) using the capillary column Supelco Omegawax TM 320 (Supelco, Bellefonte, PA), 30 m, 0.32 I.D., 0.25 μm film, and the temperature was programmed from 120°C to 220°C. Peaks were identified by using pure reference compounds and quantified by the use of an internal standard (19:0), added to the samples.

Statistical analysis

Significance of differences, when comparing values in control and simvastatin-treated cells, was assessed by the use of Student's *t*-test.

RESULTS

Effects of simvastatin treatment on the FA composition of THP-1 cell lipids

Cells incubated in the presence of 5 μM simvastatin showed significant, although quantitatively modest, changes in the FA profiles of TL (**Table 1**). In general, saturated FA tended to be reduced by simvastatin, but the difference was significant only for the total SAT. PUFA of the n–6 and n–3 series were all rather low in these cells, when compared to blood monocytes (12); neverless, in the treated cells there was a marked increment of the major products in both series, namely 20:4 n–6 and 22:6 n–3. Elevation of n–9 fatty acids, in addition to 18:1, included 20:3, a product of desaturation and elongation of oleic acid and a marker of essential fatty acid deficiency in vivo. As a consequence of the elevation of most PUFA, the unsaturation index (UI) of cell lipids was significantly increased.

TABLE 1. Major polyunsaturated fatty acids and ratios in control and simvastatin-treated THP-1 cells

Fatty Acids	Control	Simvastatin (5 μM)
	% weight	
18:1	28.29 ± 2.12	33.45 ± 0.98[a]
20:3 n–9	3.02 ± 0.27	4.14 ± 0.28[a]
20:3 n–6	0.64 ± 0.03	0.74 ± 0.43
20:4 n–6	2.73 ± 0.19	3.34 ± 0.24[a]
22:4 n–6	0.27 ± 0.03	0.37 ± 0.03[a]
22:5 n–6	0.06 ± 0.01	0.09 ± 0.01
20:5 n–3	1.12 ± 0.07	1.69 ± 0.22[a]
22:5 n–3	1.20 ± 0.04	1.55 ± 0.11[a]
22:6 n–3	1.72 ± 0.12	2.40 ± 0.20[a]
SAT	51.52 ± 2.51	42.67 ± 1.43[a]
MUFA	36.24 ± 1.97	41.48 ± 0.43[a]
PUFA	12.23 ± 0.72	15.85 ± 1.05[a]
n–6	5.09 ± 0.31	6.00 ± 0.40
n–3	4.12 ± 0.16	5.74 ± 0.53[a]
UI	85.50 ± 4.27	105.21 ± 4.83[a]

SAT, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; UI, unsaturation index = sum of percentage levels × number of double bonds. Values are weight percentage levels and represent the average ± SE of four independent determinations.

[a]Significantly different from controls at $P < 0.05$.

Effects of different concentrations of LA on its conversion and esterification

In order to explore the metabolic changes responsible for the observed modifications in FA composition, we have investigated the synthesis of LC-PUFA from labeled precursors. We have first studied the conversion of LA to AA and the FA esterification into lipid classes at two very different substrate concentrations: 2 and 50 μM. We found that while the proportion of the substrate being converted, at 24 h incubation, was not affected by its concentration (16.2 ± 0.4% with 2 μM vs. 17.1 ± 0.6% with 50 μM LA), the relative esterification into different lipid classes was markedly modified. At 2 μM LA, the relative proportions of incorporation were

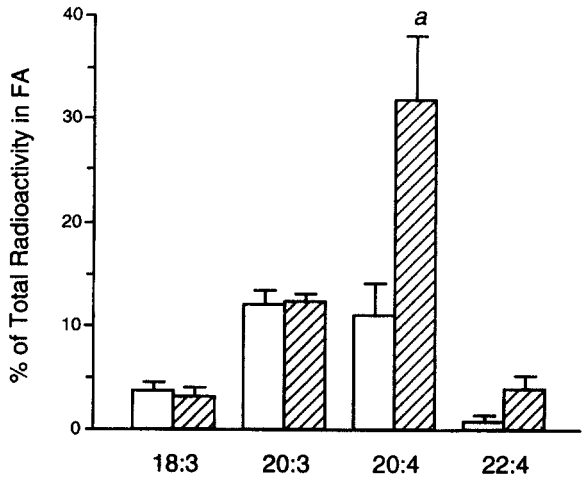

Fig. 1. Radioactivity recovered in individual fatty acids of n–6 series as percentage of total radioactivity, in cells incubated with [^{14}C]LA. Values are the average ± SE of data from four different experiments; ☐ control; ▨ simvastatin, 5 μM; *a*, significantly different from control at $P > 0.05$.

92.5% in PL, 6.6% in TG, with less than 0.5% in DAG, FFA and CE, whereas at 50 μM the proportions were 36.3% in PL, 55% in TG, 4.3 in DAG, 2.8% in FFA, and 1.4% in CE. These data prompted us to use the 2 μM LA concentration for studies on the effects of simvastatin as the major esterification occurred in PL and a small incorporation occurred in the most labile lipid pools (DAG and FFA).

Effect of simvastatin on the conversion of LA

The concentration of the drug and the time period of incubation were chosen on the basis of available data (15), and the initial set of experiments was carried out with 5 μM simvastatin. The incubation of cells with labeled LA, in the presence of this concentration of the drug did not modify the total incorporation of the substrate in cell lipids (2,854 ± 117 × 10^3 cpm/mg TL in control vs. 2,773 ± 177 × 10^3 cmp/mg TL in simvastatin-treated cells), but tended to shift the relative incorporation of LA and metabolites in TG versus PL from a TG/PL ratio of 0.132 ± 0.02 to a value of 0.185 ± 0.026 (nonsignificant difference). Measurements of the conversion of LA to its metabolites showed that treatment with simvastatin, while it did not enhance the total radioactivity incorporated into the cells, resulted in significant increment of the total conversion of LA to its derivatives, from 29.5 ± 1.5% to 53.1 ± 3.2%, and in markedly increased incorporation of radioactivity in 20:4 (**Fig. 1**). Measurements of product/precursor ratios for the desaturation and elongation reactions (**Table 2**) indicate that simvastatin enhanced exclusively the Δ5 desaturation step, with no involvement of the other steps. The calculated net AA synthesis in control and simvastatin treated cells was 3.3 and 9.2 nmol/mg cell TL, respectively. The relative labeling of individual FA in cell PL and TG (**Table 3**) indicates that greater increments of AA, in treated cells, occurred in PL rather than in TG. Labeling of 22:4 was also markedly increased in PL, by simvastatin, whereas labeling of this FA was not detectable in TG in both control and treated cells.

In order to explore whether the changes of labeling of LA-derived products were related to possible changes in their turnover rates rather than in their biosynthesis, we have also carried out a time course experiment in which the formation of products was monitored between 4 and 24 h incubation of the substrate, in control and simvastatin-treated cells. **Figure 2** reports the values only for the incorporation of radioactivity in 20:3 and 20:4 as these FA underwent the most prominent modifications in control and treated cells. In controls maximal total conversion of LA occurred already at 4 h (37.2% conversion in simvastatin vs. 20.5% in controls), with limited changes later on. In addition, in the simvastatin cells, the labeling of 20:4 exceeded that of 20:3 already at 4 h, and there was an additional increment of 20:4 up to 16 h, without significant changes in 20:3 labeling. In the simvastatin-treated cells there was also

TABLE 2. Product–precursor ratios at the desaturation and elongation steps in the conversion of LA to AA

	Control	Simvastatin 5 μM
18:3/18:2 (Δ6 desaturase)	0.06 ± 0.03	0.10 ± 0.06
20:4/20:3 (Δ5 desaturase)	0.86 ± 0.37	2.46 ± 1.27[a]
20:3/18:3 (C18 elongase)	3.57 ± 0.43	3.64 ± 0.65
22:4/20:4 (C20 elongase)	0.24 ± 0.14	0.11 ± 0.02

LA, linoleic acid; AA, arachidonic acid. Values are the average ± SE of 4 experiments.
[a]Significantly different from controls at $P < 0.05$.

TABLE 3. Percentage of the total radioactivity incorporated in fatty acids derived from linoleic acid

Fatty Acids	Control	Simvastatin 5 μM
In phospholipid		
18:3	4.13 ± 0.21	4.20 ± 0.21
20:4	15.24 ± 2.27	36.11 ± 5.38[a]
20:3	11.05 ± 2.79	10.95 ± 1.87
22:4	1.22 ± 0.99	5.66 ± 0.12[a]
20:2	1.81 ± 0.38	1.11 ± 0.34
In triglyceride		
18:3	8.49 ± 0.01	11.11 ± 0.83
20:4	9.16 ± 3.01	14.97 ± 2.73
20:3	7.16 ± 3.73	5.92 ± 2.09
20:2	5.55 ± 1.18	3.11 ± 1.97

Values are the average ± SE of 4 experiments.
[a]Significantly different from control at $P < 0.05$.

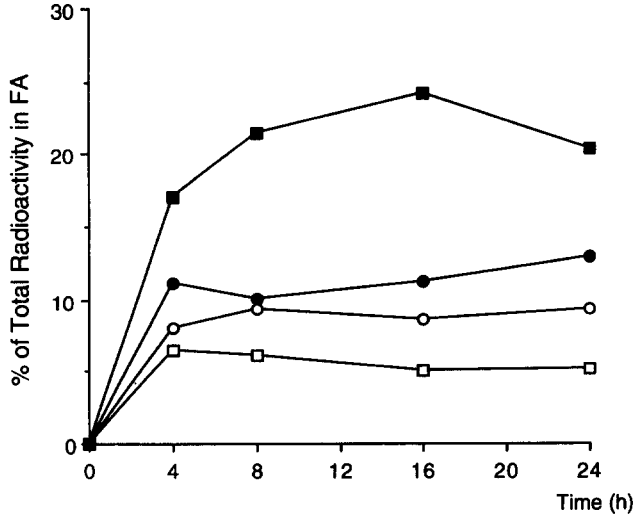

Fig. 2. Time-course of the labeling of 20:3 and 20:4 after incubation with [^{14}C]LA, in control and simvastatin-treated cells. Values are the average of duplicate analyses carried out on samples made up by least three pooled cell preparations for each time point; (○) 20:3 control; (□) 20:4 control; (●) 20:3 simvastatin 5 μM; (■) 20:4 simvastatin 5 μM.

formation of 22:4, amounting to about 1.5% of the radioactivity, versus undetectable levels in controls. In controls, labeling of 20:4 remained somewhat lower than that of 20:3 throughout the whole time period.

Subsequent experiments were devoted to investigate the effects of simvastatin on the conversion of labeled 20:5 n–3 to its derivatives (**Table 4**). Total conversion was enhanced of about 30% and this was reflected in enhanced labeling of both 22:5 and 22:6. We could not detect, in our conditions, formation of peaks corresponding to the intermediate products 24:5 and 24:6 (10), possibly because of the relatively low rates of conversion of EPA to DHA in THP-1 cells, resulting in labeling of intermediates below the detection limit. The product/precursor ratios for the elongation and the desaturation at position 4 (Table 4), were both enhanced by simvastatin.

TABLE 4. Conversion of 20:5 n–3 (EPA)

	Control	Simvastatin 5 μM
% of Total conversion	28.68 ± 0.43	37.61 ± 1.36[a]
% Radioactivity		
22:5 n–3	24.63 ± 0.32	32.00 ± 0.95[a]
22:6 n–3	0.93 ± 0.13	2.53 ± 0.35[b]
Product–precursor ratio		
22:5/20:5 (C20 elongase)	0.33 ± 0.006	0.49 ± 0.02[a]
22:6/22:5 (desaturase)	0.04 ± 0.006	0.08 ± 0.01[b]

Values are the average ± SE of 3 experiments.
[a]Significantly different from controls at $P < 0.005$.
[b]Significantly different from controls at $P < 0.05$.

TABLE 5. Incorporation of [^{14}C]acetate in total lipids and lipid classes

Lipid Classes	Control	Simvastatin 5 μM
cpm/mgTL × 100	17100 ± 9.41	15578 ± 184[b]
% of Incorporated radioactivity		
CE	2.66 ± 1.42	2.59 ± 1.06
TG	20.73 ± 4.79	24.25 ± 6.01
FFA	0.38 ± 0.01	0.33 ± 0.04
MAG	2.08 ± 0.44	0.91 ± 0.23[b]
Cho	9.27 ± 0.60	0.38 ± 0.01[a]
DAG	2.71 ± 0.04	1.36 ± 0.20[b]
PL	62.10 ± 4.54	70.17 ± 3.66

CE, cholesteryl esters; TG, triglycerides; FFA, free fatty acids; MAG, monoacylglycerol; Cho, cholesterol; DAG, diacylglycerol, PL, phospholipids. Values are the average ± SE of 4 experiments.
[a]Significantly different from controls at $P < 0.01$.
[b]Significantly different from controls at $P < 0.05$.

Lipid synthesis from acetate

Acetate incorporation into TL was substantially the same in both control and treated cells. The major difference, concerning the incorporation of acetate into individual lipid classes (**Table 5**), was the almost complete inhibition of cholesterol synthesis, as expected, in simvastatin-treated cells, and this was associated with significant reduction also of MAG and DAG labeling. Labeling of CE was not affected, in spite of the drastic reduction of cholesterol synthesis. Alkaline hydrolysis of TL and preparation of methyl esters revealed that over 90% of the radioactivity in control cells and more than 98% in simvastatin-treated cells was associated with the FA moiety. This prompted us to analyze the distribution of the radioactivity in the FA, separated as methyl esters by $AgNO_3$-impregnated TLC and by HPLC coupled with a radiodetector. In simvastatin treated cells (**Fig. 3**), there was a markedly greater incorporation into MUFA, and a correspondingly lower incorporation into SAT (panel A), when compared to control cells, with an increment of the MUFA/SAT ratio from 0.42 to 0.99. The incorporation into individual FA (panel B) showed reduction of 16:0 and increment of 18:1 in simvastatin-treated versus control cells.

Effect of mevalonate and isoprenoids on LA conversion

In order to assess whether the effects of simvastatin on PUFA metabolism were related to inhibition of the formation of intermediates in cholesterol synthesis, we have supplemented mevalonate at the concentrations of 100, 500, and 1000 μM (5, 15), farnesol (10 μM) and geranylgeraniol (5 μM) (15) together with the administration of simvastatin. There was a dose-dependent reduction (**Table 6**) of the effects of simvastatin on LA conversion and AA synthesis by mevalonate (MVA), al-

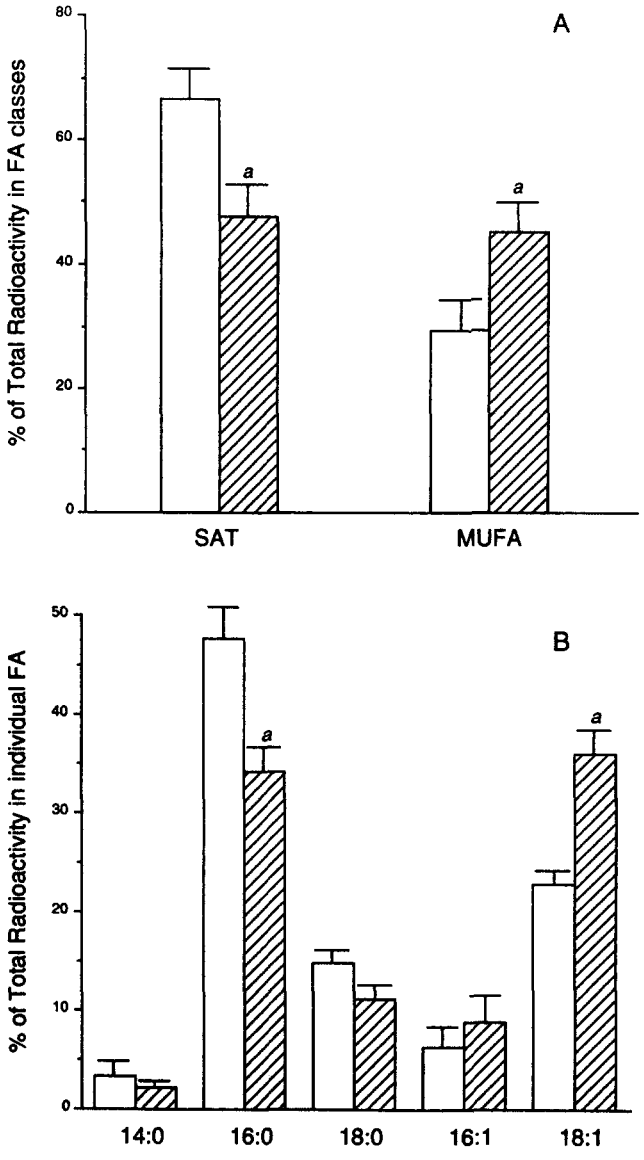

Fig. 3. FA labeling in cells incubated with [^{14}C]acetate. Panel A: percentage of radioactivity incorporated in saturated and monounsaturated FA in control and simvastatin-treated cells. SAT, saturated; MUFA, monounsaturated fatty acids. Panel B: percentage of radioactivity incorporated in individual fatty acids in control and simvastatin-treated cells. Values are the average ±SE of data from three experiments; ☐ control; ▨ simvastatin 5 μM. *a*, significantly different from control at $P < 0.05$.

though even at 1000 μM the effects were reduced only about 50%. Administration of geranylgeraniol, but not of farnesol, was also able to markedly reduce the effects of simvastatin on PUFA metabolism.

Lipid synthesis from glycerol

As FA metabolism may be coupled to lipid synthesis, we then explored the effects of simvastatin on the production of glycerolipids from labeled glycerol. More than 95% of the radioactivity was incorporated into TG and PL, in both control and simvastatin treated cells. The percentage of incorporation of glycerol in TG was enhanced, whereas that in PL was reduced by the drug (**Fig. 4**) with a significantly greater relative incorporation into TG.

In order to explore whether changes in the synthesis of TG from different precursors (glycerol and acetate) were also associated with enhanced TG levels, we have then measured the endogenous concentration of this lipid class in control and treated cells and found significant elevation of TG concentration after simvastatin

TABLE 6. Effect of mevalonate and isoprenoids on LA conversion

	Total LA Conversion	AA Synthesis
	% radioactivity	
Control	23.9 ± 0.5	8.7 ± 0.3
Simvastatin		
5 μM	47.3 ± 0.6	30.2 ± 0.2
5 μM + MVA 100 μM	43.2 ± 0.6[a]	27.4 ± 0.1[b]
5 μM + MVA 500 μM	39.0 ± 0.1[b]	22.8 ± 0.1[c]
5 μM + MVA 1000 μM	32.8 ± 0.1[c]	16.4 ± 0.3[c]
5 μM + farnesol 10 μM	45.5 ± 0.3	30.9 ± 0.3
5 μM + GG 5 μM	36.4 ± 0.4[c]	20.0 ± 0.1[c]

LA, linoleic acid; AA, arachidonic acid; MVA, mevalonic acid; GG, geranylgeraniol. Values are the average ± SE of different experiments.

[a]Significantly different from simvastatin at $P < 0.05$.
[b]Significantly different from simvastatin at $P < 0.01$.
[c]Significantly different from simvastatin at $P < 0.005$.

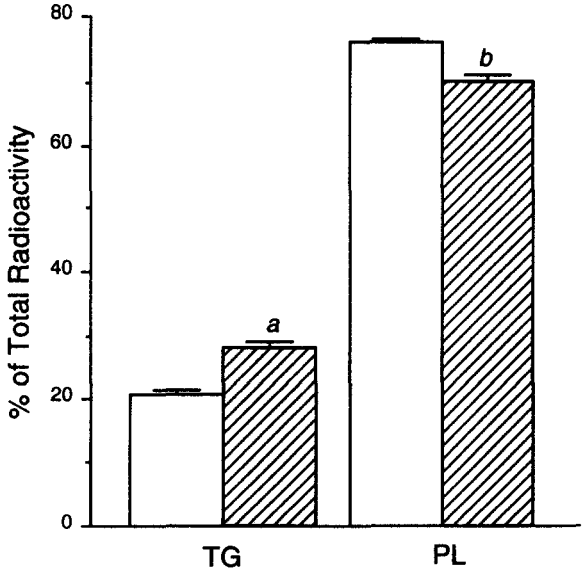

Fig. 4. Incorporation of [^{3}H]glycerol in lipids of control and simvastatin-treated cells. PL, phospholipids; TG, triglycerides. Values are the average ±SE of data from three experiments; ☐ control, ▨ simvastatin 5 μM. *a*, statistically different from control at $P < 0.01$; *b*, statistically different from control at $P < 0.02$.

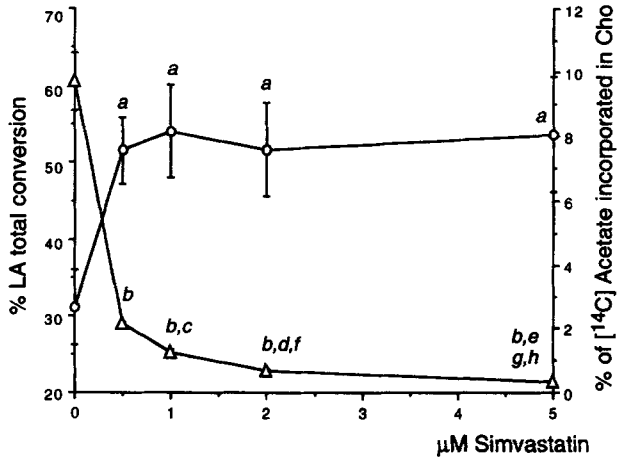

Fig. 5. Dose–response curves for the effects of simvastatin on LA metabolism and cholesterol synthesis. Left axis: total [^{14}C]LA conversion in control and simvastatin-treated cells. Values are the average ±SE of data from three experiments. *a*, Statistically different from control at $P < 0.05$; Right axis: cholesterol labeling from [^{14}C]acetate as percentage of the incorporation in total lipids. Values at each time point are different from each other, at the specified levels: *b*, Statistically different from control at $P < 0.01$; *c*, statistically different from 0.5 µM simvastatin at $P < 0.005$; *d*, statistically different from 0.5 µM simvastatin at $P < 0.01$; *e*, statistically different from 0.5 µM simvastatin at $P < 0.02$; *f*, statistically different from 1 µM simvastatin at $P < 0.01$; *g*, statistically different from 1 µM simvastatin at $P < 0.02$; *h*, statistically different from 2 µM simvastatin at $P < 0.02$. (○) linoleic acid, (△) cholesterol.

(25.2 ± 3.5 SE as % of TL in treated cells vs. 19.6 ± 2.0 SE in controls, $P = 0.046$).

After analyzing the effects of simvastatin on various pathways of lipid metabolism, at the concentration of 5 µM simvastatin, we proceeded to evaluate the comparative dose–response curves for the effects of the drug on cholesterol synthesis and on FA metabolism. We have therefore measured the conversion of labeled LA to LC-PUFA and of acetate to cholesterol in the presence of concentrations from 0.5 to 5 µM simvastatin (**Fig. 5**). Total conversion of LA was maximally elevated already at 0.5 µM simvastatin, with minimal additional changes at greater concentrations, whereas the inhibition of incorporation of acetate into cholesterol was further reduced from 9.3% acetate incorporated in controls to 2.1% at 0.5 µM and 0.35% at 5 µM simvastatin, that is with a further statistically significant reduction between 0.5 and 5 µM simvastatin.

DISCUSSION

In the monocytic cell line THP-1, inhibition of cholesterol synthesis, almost complete in the presence of 5 µM simvastatin, was associated with enhanced labeling of n–6 LC PUFA from LA, as previously described (5), and with enhanced conversion of 20:5 n–3 to 22:5 and 22:6. The effects of simvastatin on AA labeling appeared to be mainly the result of enhanced synthesis and to be selective for the Δ5 desaturation, on the basis of calculations of the product/precursor ratios. An initial and selected activation of the Δ5 desaturation step was also supported by time-course studies indicating that in simvastatin-treated cells, labeling of 20:4 was markedly greater than that of 20:3 already at 4 h of incubation with LA, with a further increment exclusively for 20:4, in contrast to the situation in control cells. It is also unlikely that the enhanced AA labeling was the result of reduced degradation, as in control cells, i.e., in the absence of simvastatin, labeling of this fatty acid remained constant between 4 and 24 h of incubation, without significant losses of radioactivity. The elevation of 22:5 and 22:6 labeling from 20:5, and the enhanced formation of MUFA from acetate further support a generalized stimulation of FA desaturation by simvastatin. Total lipid synthesis from acetate, however, with the exception of cholesterol labeling, was not affected by simvastatin. It should be pointed out, however, that uncertainties exist in quantitative interpretations of fatty acid and cholesterol synthesis from radiolabeled acetate due to possible differences in the specific activity of cellular acetyl CoA substrate pools. The use of tritiated water would have allowed a more reliable assessment of lipid synthesis. Formation of CE from acetate was also marginally affected, suggesting that the endogenous cholesterol pool, not modified by treatment (not shown), served as substrate for esterification of the newly synthesized FA. In cells exposed to acetylated LDL, instead, simvastatin was shown to inhibit the incorporation of [1-^{14}C]oleate in cholesteryl esters (19). The enhanced labeling of TG from glycerol in simvastatin-treated cells, associated with the already reported (4) elevation of cellular TG, suggests that the production of the unsaturated FA exceeded the rates of esterification into PL, shifting the process towards TG synthesis.

The effects of simvastatin on FA metabolism were dependent upon inhibition of mevalonate formation, as supplementation of this metabolic intermediate normalized the rates of PUFA formation, enhanced by simvastatin (5). The reversal of the effect of simvastatin on FA metabolism by mevalonate was concentration-dependent, although at high concentrations, and marked reversal was obtained also with geranylgeraniol at much lower concentrations (5 µM), farnesol (10 µM) being, instead, inactive. The above are the highest nontoxic concentrations of the isoprenoids reported in the literature (15, 20). Although the discrepancy between geranylgeraniol and farnesol demands further investigation, it may reflect a different fate of the two isoprenoids.

The C_{15} isoprenoid farnesyl, in its pyrophosphate form functions poorly as a substrate for elongation to geranylgeranyl pyrophosphate (21) and, at least in the liver, a separate enzyme appears to catalyze the synthesis of geranylgeranyl pyrophosphate from geranyl-pyrophosphate (22). Furthermore, in mammalian cells incubated with labeled famesol or geranylgeraniol, completely different sets of protein become labeled (23). In addition, as the major class of labeled prenylated proteins produced in eukaryotic cells is derived from geranylgeranyl-pyrophosphate (20). Inhibition of protein geranylgeranylation may be a major component of the effect of simvastatin on FA metabolism.

Our data confirm the complexity of the interactions between cholesterol and FA metabolism described in studies on somewhat different aspects, pharmacologically and/or nutritionally approached. Enhanced activity of the hepatic mitochondrial carnitine palmitoyltransferase has been observed, for instance, after inhibition of the HMG-CoA reductase (24), whereas the administration of exogenous cholesterol resulted in enhanced TG synthesis while reducing FA oxidation (25). Conversely, the administration of FA stimulated cholesterol synthesis (26, 27).

In summary, simvastatin in the monocytic cell line THP-1 enhances the unsaturation of exogenously supplemented PUFA and of FA formed from endogenous precursors, thus resulting in increased unsaturation of cell lipids; TG synthesis is also enhanced. The machinery for unsaturated FA formation is quite sensitive to the activity of simvastatin, as maximal effects occur already at the lowest concentration tested. The observed changes in FA and lipid metabolism may be responsible for the reported effects of HMG-CoA reductase inhibitors on plasma FA in hypercholesterolemic subjects (6, 7) and possibly for the modifications of platelet function (9). The mechanisms are not clear but it would appear that changes in the lipid environment surrounding the FA metabolizing enzymes, induced by the drug, may play a role in the described processes (28). Inhibition of protein geranylgeranylation may also be involved.

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