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Differential modulation by simvastatin of the metabolic pathways in the *n*-9, *n*-6 and *n*-3 fatty acid series, in human monocytic and hepatocytic cell lines

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Abstract

Statins affect the production of long chain polyunsaturated fatty acids (PUFA), both in vitro and in vivo. Various studies have shown the effects of statins on the pattern of *n*-6 fatty acids (FA), but limited attention has been paid to the *n*-3 FA.

We investigated, in THP-1 and in HepG2 cells, the effects of simvastatin on the conversion of the 18C FA precursors in the *n*-3 and *n*-6 series, [1-^{14}C] α-linolenic acid (α-LNA) and [1-^{14}C] linoleic acid (LA) respectively, and on the metabolism of [1-^{14}C] stearic acid (SA).

THP-1 cells, as in the case of LA, actively converted α-LNA to its products, and after simvastatin treatment, the total conversion was significantly increased (from 57.2 ± 7.2 to $74.3 \pm 8.5\%$, $p < 0.05$). HepG2 cells also converted LA and α-LNA, but simvastatin increased significantly only the conversion of LA ($9.5 \pm 1.9\%$ versus $23.8 \pm 5.1\%$, $p < 0.02$). SA conversion was similar in untreated cells (about 50%), while statin increased the production of oleic acid in HepG2, but in THP-1 cells there was a decrease.

In conclusion, LA, α-LNA and SA are differentially metabolized in THP-1 and in HepG2 cells and their increased conversion by simvastatin is lower in HepG2 than in THP-1. These differences may reflect the distinct features of the two cell lines: monocytes, precursors of phagocytic cells, versus hepatocytes with mainly metabolic functions. Substantial differences concern also cellular FA pools: structural in THP-1 cells, and also depot, resulting in sequestering of the substrates, in HepG2. The greater *n*-3 FA metabolism in THP-1 cells may have favourable functional effects.

Keywords: Simvastatin; Linoleic acid; α-Linolenic acid; Stearic acid; Fatty acid metabolism; Cultured cells

1. Introduction

Statins, the most widely used drugs for the treatment of hypercholesterolemia, acting through the inhibition of the HMGCoA reductase, are endowed of additional anti-atherogenic effects such as an inhibition of cell proliferation in the vascular walls, of tissue factor and of metalloprotease production in macrophages, of lipoprotein oxidation and of platelet aggregation, etc. [1–4]. At higher concentrations, statins induce apoptosis in smooth muscle cells and other cell types [5–7].

Statins affect also the metabolism of polyunsaturated fatty acids in vitro, by increasing the conversion of LA to its derivatives, i.e. AA, through enhanced desaturase activities [8,9].

As a consequence of an increased total LA conversion, patients on statin therapy have enhanced plasma AA levels [10]. Higher cellular levels of AA may explain the greater release of eicosanoids such as thromboxane or prostaglandin I_2 induced by statins in various types of cultured cells [9,11]. However, statin treatment normalizes the excretion of thromboxane metabolites, suggesting that in vivo the modulation of cellular (platelets) functions, rather than the levels of the eicosanoid precursor AA, is the major determinant of eicosanoid production [12].

Within the factors that favourably affect the lipid profile in the hyperlipidemic and hypercholesterolemic patients, contributing to reduce the CHD risk, of special relevance are the polyunsaturated fatty acids and in particular those

Abbreviations: α-LNA, α-linolenic acid; AA, arachidonic acid; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; FA, fatty acids; LA, linoleic acid; OA, oleic acid; PL, phospholipids; PUFA, polyunsaturated fatty acids; SA, stearic acid; TL, total lipids; TG, triglycerides

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of the *n*-3 series. In contrast, there are strong and positive correlations between the intakes of saturated fatty acids and CHD [13].

The protective effects of *n*-3 FA, i.e. α-LNA and the fish oil FA (EPA + DHA), in secondary prevention of CHD have been extensively demonstrated [13]. In addition, a high incorporation of *n*-3 FA in various types of cells results in a shift of the production of the eicosanoids from AA series towards those derived from EPA, much less prothrombotic [14].

While the effects of statins on *n*-6 FA has been studied in vitro and in vivo, little attention has been paid to FA of the *n*-3 series, present in cells and plasma in relative low concentrations. In order to study the effects of treatment on *n*-3 FA in humans, longer periods and higher number of subjects would be required to detect significant differences.

Even in vitro studies on the effects of statins on *n*-3 PUFA metabolism are limited, since only two publications have been devoted to this issue. In addition, these studies were carried out on special types of tumor cell lines using for the two FA series substrates that are not comparable in the metabolic cascade (e.g. 20:3 for the *n*-6 and 18:3 for the *n*-3 series) [15,16].

Therefore, purposes of our study were: first to comparatively investigate, in vitro, the effects of simvastatin on the conversion of 18C FA precursors in the *n*-3 and *n*-6 series, i.e. α-LNA and LA, respectively, and in addition, to evaluate the conversion of the saturated SA of the *n*-9 series, to the monounsaturated OA; secondly to compare the effects of treatment in two different types of cells, a monocytoid (THP-1 cells) and hepatoma (HepG2 cells) cell lines, the first as a relevant type of circulating cells and the second as a typical cellular model for studies of lipid and xenobiotic metabolism.

The results obtained in two cell models with different metabolic features would help in understanding the possible differential effects of simvastatin in vivo in distinct physiological compartments.

2. Materials and methods

2.1. Chemicals

RPMI 1640 and minimum essential medium (MEM), penicillin, streptomycin, glutamine and fetal calf serum (FCS) were from Sigma–Aldrich. All solvents were from E. Merck; [1-^{14}C] 18:2 *n*-6 (LA) (specific activity 55 mCi/mmol) and [1-^{14}C] 18:0 (SA) (specific activity 58 mCi/mmol) were from Amersham whereas [1-^{14}C] 18:3 *n*-3 (α-LNA) (specific activity 53.7 mCi/mmol) was from Perkin-Elmer Life Sciences.

Simvastatin in lactone form (Merck, Sharp & Dohme Research Laboratories) was dissolved in 0.1 M NaOH to obtain the active, open β-hydroxy acid form.

2.2. Cell cultures

THP-1 and HepG2 cells were incubated in the presence of simvastatin at the concentrations that gave the maximal effect on FA conversion as specified below.

THP-1 cells, a human monocytoid cell line obtained from the ATCC, were grown in RPMI medium with 10% FCS, 100 μg/ml penicillin, 100 IU/ml streptomycin and 2 mM glutamine.

Cells were centrifuged at 200 × *g* for 10 min, the pellet was resuspended in RPMI without FCS and cell concentration adjusted to 10^6 cells/ml. THP-1 cells were incubated with 5 μM simvastatin for 48 h and with [1-^{14}C] LA (0.1 μCi/ml), [1-^{14}C] α-LNA (0.1 μCi/ml) or [1-^{14}C] SA (0.1 μCi/ml) for the last 24 h.

HepG2 cells, a human hepatoblastoma cell line obtained from ATCC, were grown in MEM with 10% FCS; at the time of the experiments, when cells were at 70–80% of confluence, the medium was replaced with fresh medium containing 2% FCS; HepG2 cells were incubated with 2 μM simvastatin for 48 h and with [1-^{14}C] LA (1 μCi/dish), [1-^{14}C] α-LNA (1 μCi/dish) or [1-^{14}C] SA (1 μCi/dish) for the last 24 h. At confluence each dish contained about 10 × 10^6 cells.

2.3. Total lipid extraction and fatty acid methylation

After treatment, THP-1 cells were centrifuged (see above) and the pellet was used for lipid extraction; HepG2 cells were scraped off in PBS. Cell lipid extraction was carried out according to Folch et al. [17]; the lipid concentration in the extracts, redissolved in a given volume of chloroform/methanol (2:1, v/v) with 5 μg/ml of butylated hydroxytoluene as antioxidant, was evaluated by microgravimetry, whereas the total radioactivity recovered in the samples was measured with a β-counter.

TL were transmethylated (with CH_3OH/HCl 3 N, for 1 h at 90 °C) and the radioactivity associated to the individual fatty acids was determined after separation by HPLC equipped with a radiodetector (Flow Scintillation Analyzer 500TR, Perkin-Elmer), as reported [8].

2.4. Lipid classes analysis

The lipid classes (PL, TG, diglycerides, cholesteryl esters and free fatty acids) were separated by TLC using as mobile phase exane–diethyl ether–acetic acid (70:30:1.5).

Lipids were detected by exposure to iodine vapors; the spots were scraped from plates and the radioactivity was detected, after addition of 1 ml water–methanol (1:1) and 10 ml of scintillation fluid, in a β-counter.

2.5. Statistical analysis

Student's *t*-test was applied to evaluate the significance of differences between control and treated cells. All the

values are the means ± standard error of different experiments.

3. Results

3.1. Effect of simvastatin treatment on n-6 fatty acid conversion

In control conditions, THP-1 cells metabolise [1-^{14}C] LA more actively than HepG2 cells, as shown by a percentage of total LA conversion, expressed as the sum of all *n*-6 FA derived from LA, of 40.99 ± 3.21% versus 9.52 ± 1.9%, respectively. The treatments with simvastatin (5 μM for THP-1 and 2 μM for HepG2 cells) greatly affect LA total conversion in both cell lines (Fig. 1). Simvastatin significantly increases not only LA total conversion but also Δ6 and Δ5 desaturase activities (expressed as product/precursor ratios, 18:3/18:2 and 20:4/20:3) both in THP-1 and HepG2 cells (Table 1). In THP-1 cells, Δ5 desaturase is more affected than Δ6 desaturase with an increase of 52% for Δ6 and of 198% for Δ5 desaturase. In HepG2 cells, in contrast, Δ6 increases of 117% and Δ5 desaturase of 44% in treated cells versus controls.

When the specific substrate of the Δ5 desaturase, [1-^{14}C] di-homo-γ-linolenic acid (20:3 *n*-6), is incubated similar results are obtained with an increment of FA conversion from 31.7 ± 0.3 to 58.1 ± 1.9 (+87%) in THP-1 and from 13.8 ± 0.1 to 16.5 ± 0.5 (+20%) in HepG2 cells, after simvastatin treatment. The specific increments in Δ5 desaturase activity are of about 225 and 25% (not shown).

3.2. Effect of simvastatin treatment on n-3 fatty acid conversion

When the radiolabelled FA substrate is the initial precursor in the *n*-3 series, in control conditions the conversion of α-LNA is similar in the two types of cells, 57.16 ± 7.16% in THP-1 and 50.69 ± 4.86% in HepG2 (Fig. 2).

After treatment with simvastatin, total α-LNA conversion (Fig. 2) and Δ6 and Δ5 desaturase activities (Table 2) significantly increase in THP-1 cells, whereas in HepG2 the trend observed is the same, without however reaching significance.

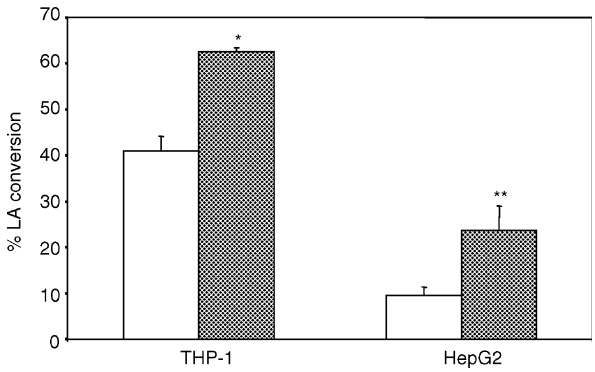

Fig. 1. Total conversion of [1-^{14}C] linoleic acid (LA) in THP-1 and HepG2 cells, in control conditions and after simvastatin treatment 5 and 2 μM respectively, for 48 h. Open bars represent control cells, full bars simvastatin treated cells. Values are the mean ± S.E. of five different experiments; significantly different from control at $^{*}p < 0.0004$; $^{**}p < 0.02$.

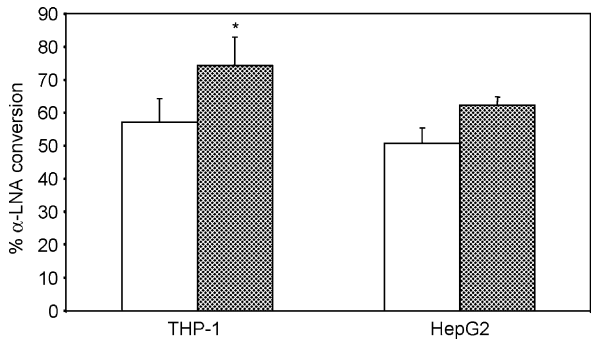

Fig. 2. Total conversion of [1-^{14}C] α-linolenic acid (α-LNA) in THP-1 and HepG2 cells, in control conditions and after simvastatin treatment 5 and 2 μM respectively, for 48 h. Open bars represent control cells, full bars simvastatin treated cells. Values are the mean ± S.E. of four different experiments; significantly different from control at $^{*}p < 0.05$.

Table 3 shows the percentage of the radioactivity associated to different fatty acids, derived from α-LNA. In THP-1 cells the major metabolite is 20:5 *n*-3 (EPA) followed by 20:4 *n*-3 and 22:5 *n*-3, whereas no radioactive 22:6 is detected in control conditions. In HepG2 cells EPA the major metabolite, is followed by 20:4 *n*-3 and by consistent amounts (about 6%) of 20:3 *n*-3 and also 22:6 *n*-3 (DHA). Intermediates in DHA production, 24:5 *n*-3 and 24:6 *n*-3, not present in THP-1 cells, are also detected. After treatment, in THP-1 cells, the increased conversion of α-LNA is accompanied by a significant increase of 22:5 *n*-3 and by the appearance of DHA

Table 1
Product-precursor ratios at the desaturation steps in the conversion of [1-^{14}C] linolenic acid (LA, 18:2 *n*-6)

	THP-1		HepG2	
	Control	Simvastatin	Control	Simvastatin
18:3/18:2 Ratio (Δ6 desaturase)	0.07 ± 0.00	0.10 ± 0.01**	0.02 ± 0.00	0.05 ± 0.02**
20:4/20:3 Ratio (Δ5 desaturase)	1.08 ± 0.09	3.22 ± 0.13*	0.35 ± 0.01	0.50 ± 0.05***

THP-1 and HepG2 cells were treated with 5 and 2 μM simvastatin respectively, for 48 h. Values are the mean ± S.E. of five experiments.
* Significantly different from control at $p < 0.0001$.
** Significantly different from control at $p < 0.005$.
*** Significantly different from control at $p < 0.01$.

Table 2
Product-precursor ratios at the desaturation steps in the conversion of [1-^{14}C] α-linolenic acid (α-LNA, 18:3 *n*-3)

	THP-1		HepG2	
	Control	Simvastatin	Control	Simvastatin
18:4/18:3 Ratio (Δ6 desaturase)	0.08 ± 0.00	0.12 ± 0.01*	0.03 ± 0.01	0.04 ± 0.02
20:5/20:4 Ratio (Δ5 desaturase)	4.78 ± 0.36	10.55 ± 1.85*	2.09 ± 0.11	2.96 ± 0.65
24:6/24:5 Ratio (Δ6 desaturase)	–	–	1.25 ± 0.35	2.02 ± 0.30

THP-1 and HepG2 cells were treated with 5 and 2 μM simvastatin respectively, for 48 h. Values are the mean ± S.E. of four experiments.
* Significantly different from control at $p < 0.05$.

associated with a significant decrease of 20:4 *n*-3. In HepG2 cells, after treatment, all the FA, derived from EPA in the metabolic pathway, increase but the increments are significant only for DHA and 24:6 *n*-3.

3.3. Effect of simvastatin treatment on stearic acid conversion

Fig. 3 shows the conversion of SA (18:0) to OA (18:1 *n*-9); in control conditions the conversion of SA is the same in THP-1 and in HepG2 cells, 52.77 ± 1.1 and 54.11 ± 0.92%, respectively. After treatment, in THP-1 cells SA conversion (panel A) and the activity of Δ9 desaturase (panel B), expressed as 18:1/18:0 ratio, are decreased, whereas in HepG2 cells they are significantly increased versus controls.

The distribution of the radioactivity, after incubation with SA, in different lipid classes of THP-1 and HepG2 cells is reported in Table 4. SA and its metabolite OA are incorporated mainly into PL, about 70%, and in TG (>20%), without any significant difference between the two cell lines. The treatment with simvastatin affects the incorporation of SA in both cell lines, with a decrease in PL (−6.6% in THP-1 and −18.1% in HepG2) and an increase in TG (27.8% in THP-1 and 62% in HepG2).

HPLC analyses of PL and TG fractions in the two types of cells reveals that, in TG of THP-1 cell, OA is present in much lower concentration respect to SA, whereas in TG of untreated HepG2 cells OA is in large excess over the precursor. After simvastatin treatment, opposite changes occur in PL of THP-1 cells (increase of SA and reduction of OA), with respect of the reduction of SA and increment of OA in TG, versus controls ($p < 0.03$), whereas in HepG2 cells SA decreases and OA increases both in PL and TG (Table 4).

Table 3
Percentage of the total radioactivity incorporated in fatty acids derived from [1-^{14}C] α-linolenic acid (α-LNA, 18:3 *n*-3)

	THP-1		HepG2	
	Control (%)	Simvastatin (%)	Control (%)	Simvastatin (%)
18:4	3.30 ± 0.58	2.91 ± 0.69	1.62 ± 0.44	1.44 ± 0.56
20:3	1.48 ± 0.15	0.48 ± 0.47	6.79 ± 0.73	6.70 ± 0.91
20:4	7.81 ± 1.38	3.82 ± 0.45**	9.73 ± 1.07	8.80 ± 0.76
20:5	36.81 ± 3.80	39.50 ± 2.28	20.48±3.29	25.52 ± 4.94
22:5	7.78 ± 2.33	25.08 ± 5.74**	3.34 ± 0.57	5.94 ± 1.03
22:6	–	2.25 ± 1.38	5.70 ± 0.17	8.82 ± 0.83*
24:5	–	–	1.35 ± 0.04	1.66 ± 0.15
24:6	–	–	1.68 ± 0.42	3.32 ± 0.06*

THP-1 and HepG2 cells were treated with 5 and 2 μM simvastatin respectively, for 48 h. Values are the mean ± S.E. of four experiments.
* Significantly different from control at $p < 0.04$.
** Significantly different from control at $p < 0.05$.

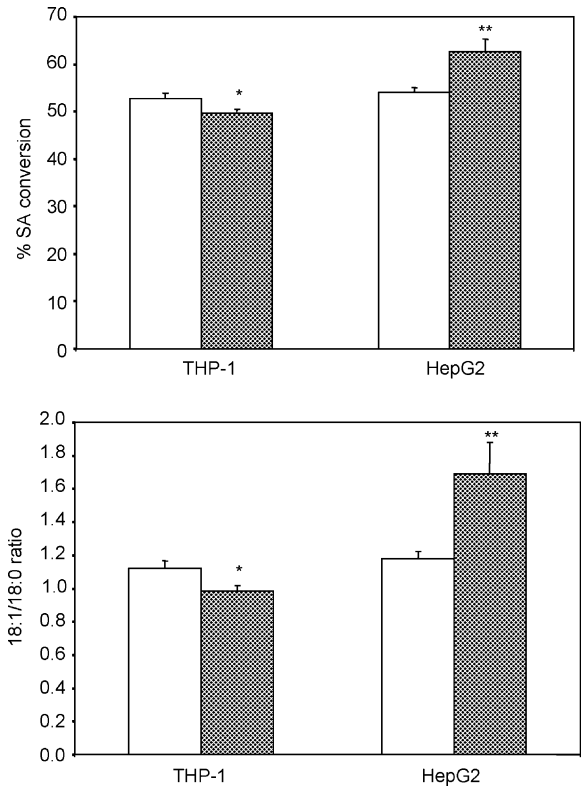

Fig. 3. Panel A: total conversion of [1-^{14}C] stearic acid (SA) in THP-1 and HepG2 cells, in control conditions and after simvastatin treatment 5 and 2 μM, respectively, for 48 h. Open bars represent control cells, full bars simvastatin treated cells. Values are the mean ± S.E. of four different experiments; significantly different from control at $^{*}p < 0.04$; $^{**}p < 0.05$. Panel B: Δ9 desaturase activity, expressed as 18:1/18:0 ratio, in THP-1 and HepG2 cells, in control conditions and after simvastatin treatment 5 and 2 μM, respectively, for 48 h. Open bars represent control cells, full bars simvastatin treated cells. Values are the mean ± S.E. of four different experiments; significantly different from control at $^{*}p < 0.04$; $^{**}p < 0.05$.

4. Discussion

Statins have been reported to increase total conversion of LA to its longer and more unsaturated metabolites and in

Table 4
Percentage of the radioactivity, derived from stearic acid (SA, 18:0) incorporated in lipid classes

		THP-1		HepG2	
		Control (%)	Simvastatin (%)	Control (%)	Simvastatin (%)
PL		71.35 ± 3.30	66.63 ± 1.53	69.10 ± 4.74	56.59 ± 4.70
TG		21.12 ± 2.98	26.99 ± 1.17	24.28 ± 4.28	39.33 ± 4.29
PL	18:0	80.08 ± 11.45	92.19 ± 3.19	69.60 ± 13.40	62.01 ± 9.30
	18:1	19.91 ± 11.45	7.91 ± 3.19	30.40 ± 13.40	37.99 ± 9.30
TG	18:0	78.95 ± 6.64	53.45 ± 2.53*	23.03 ± 1.46	18.39 ± 2.43
	18:1	21.05 ± 6.64	46.55 ± 2.53*	76.97 ± 1.46	81.61 ± 2.43

THP-1 and HepG2 cells were treated with 5 and 2 μM simvastatin respectively, for 48 h. PL, phospholipids; TG, triglycerides. Values are the mean ± S.E. of four experiments.
* Significantly different from control at $p < 0.04$.

particular AA levels are increased both in monocytic and hepatocytic cell lines after treatment [8,9]. In the present work evaluation of the intermediates, in the metabolic pathway of LA, shows that desaturation steps (expressed as product/precursor ratios) are greater in THP-1 than in HepG2 cells, Δ5 desaturation being more active than Δ6. After simvastatin treatment, Δ5 is more affected than Δ6 in THP-1 cells whereas the reverse is true in HepG2 cells. The incubation with di-homo-γ-linolenic acid (20:3 *n*-6), the direct substrate for Δ5 desaturase, confirms this result; in fact, simvastatin markedly affects Δ5 desaturase, with an higher increment of this conversion step in THP-1 cells, with respect to that in HepG2 cells. In accordance, in the human lung adenocarcinoma A549 cells simvastatin increases Δ5 desaturase activity of about 126% when the substrate is di-homo-γ-linolenic acid [15].

While consistent and numerous data are available concerning the effects of statins on LA conversion both in vitro and in vivo [8–10], on α-LNA conversion data are scarce [15,16,18,19]. THP-1 and HepG2 cells actively metabolize α-LNA to its derivatives, mainly EPA; this conversion is increased after simvastatin treatment. In a previous work, we demonstrated that THP-1 cells convert [1-^{14}C] EPA to 22:5 *n*-3 and DHA and that these elongation and desaturation reactions are significantly affected by simvastatin [8]. It has been shown that DHA is not produced through directly desaturation of 22:5 *n*-3, but through a more complex pathway in which 22:5 is elongated to 24:5 and then desaturated to 24:6 by Δ6 desaturase. Finally 24:6 is retroconverted to 22:6 DHA, by peroxisomal β-oxidation [20]. In THP-1 cells the longer and more unsaturated fatty acid derived from labelled α-LNA is 22:5, while DHA is not detectable in control conditions. In contrast, in HepG2 cells DHA and also 24:5 and 24:6 *n*-3 are found. It is conceivable that β-oxidative steps in the conversion of 22:5 to 22:6 *n*-3 are more active in HepG2 than in THP-1 cells, resulting in the detection of the intermediates only in HepG2 cells.

In a human larynx tumor cell line α-LNA is converted only to EPA, while in human lung adenocarcinoma cells and in HepG2 this substrate is converted to DHA without traces of 24:5 and 24:6 *n*-3 [15,16]. The discrepancy between our results in HepG2 cells and those of Hrboticky et al., is probably due to the different analytical methods (HPLC + radiodetector versus GC with capillary column and FID as detector) and to the different experimental conditions: we have used [1-^{14}C] α-LNA as tracer (final concentration about 2 μM) for 24 h, whereas Hrboticky has used 40 μM cold α-LNA for 72 h [18]. The higher concentration and the longer period of incubation could have resulted in a total conversion of 22:5 to DHA with difficulties in the detection of the "cold" intermediates and possibly with disappearance of 24:5 and 24:6 *n*-3.

After simvastatin treatment, the conversion of α-LNA and the activities of Δ6 (first desaturation step, 18:4/18:3) and Δ5 desaturases increased significantly in THP-1 with production of DHA; in HepG2 cells these increments are not significant, although the 24:6/24:5 ratio (second Δ6 desaturation step), is increased of 61%.

As reported recently by de Antueno et al. [21], the same Δ6 and Δ5 desaturases catalyse both *n*-6 and *n*-3 substrates, and a single Δ6 desaturase is active on α-LNA and 24:5 *n*-3 and on LA and 24:4 *n*-6, confirming previous works [22,23].

Most cancer cells in culture have low levels of Δ6 desaturase [24] and, in general, the activity of FA desaturases correlates with their mRNA abundance in cultured cells [25]. Δ6 desaturase shows a preference for α-LNA versus LA [26,27]. Our data, on the basis of the substrate conversion, indicate that in both cell lines *n*-3 are more actively converted than *n*-6 FA.

The saturated substrate SA is converted through the Δ9 desaturase to OA similarly in THP-1 and in HepG2 control cells (about 52%). After simvastatin treatment, in THP-1 cells total SA conversion (and Δ9 desaturase activity) decreases whereas in HepG2 cells increases, when evaluated in TL. This could be explained by the different incorporation of the substrate SA and of the product OA in different lipid classes in the two cell lines.

As reported by Bruce and Salter [28], SA is preferentially incorporated in PL whereas its metabolite OA is rather incorporated in TG, and our data are in accordance with these findings. After statin treatment there is a further increment in OA incorporation in TG. It could be postu-

lated that in HepG2 cells, a model for hepatic cell metabolism, the TG pool undergoes more extensive remodelling resulting in greater incorporation of OA in this lipid pool. Data in the literature indicate, in fact, that the TG pool in HepG2 cells is about 45 μg/mg cell proteins [29] whereas in the monocytic line THP-1, where cell lipids are mainly structural, the TG content is about 25 μg/mg cell proteins (data not shown).

In conclusion, the comparative incorporation of the three FA (LA, α-LNA and SA) in the two types of cells is similar (not shown) but they are differentially metabolized in cultured cells. In both cell lines, *n*-3 FA are more actively converted than *n*-6 FA, before and after simvastatin treatment. In addition, the effects of simvastatin on *n*-3 and *n*-6 FA conversion are lower in HepG2 versus THP-1 cells. On the other hand the conversion of SA is similar in the two types of cells in control conditions, but after simvastatin treatment the conversion increased in HepG2 and decreased in THP-1.

The major significance of our results can be summarized as follows: (a) the assumption that a type of cell such as HepG2, with major functions in lipid metabolism, with respect to e.g. THP-1 cells, would respond more effectively to simvastatin in terms of PUFA metabolism is not directly appliable to overall PUFA metabolism, due to the different (qualitative and quantitative) contribution of cell lipid pools; (b) the greater increment in *n*-3 FA conversion in THP-1 versus HepG2 cells, after simvastatin treatment, may have some clinical implication such as favourable functional changes in cells involved in inflammatory and immune responses.

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