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Effects of Membrane Fatty Acids on Thermal and Oxidative Injury in the Human Premonocytic Line U937

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ABSTRACT. Heat shock (HS) proteins (HSP) function as molecular chaperones and protect cells from thermal and oxidative injury. The signals leading to HSP synthesis, i.e. the "cellular thermometer(s)," are still a matter of debate. In the human premonocytic line U937, we investigated the effects of specific modification of membrane fatty acid (FA) composition by incubation with various saturated and unsaturated fatty acids (UFA) on the HS response and on hydrogen peroxide (H_2O_2)-induced cell death. FA readily incorporated into U937 cell membranes. UFA did not modulate the HS response but potentiated H_2O_2-mediated damage, while pre-exposure to HS protected the UFA-treated cells from this increased H_2O_2 toxicity. BIOCHEM PHARMACOL 54;7:773–780, 1997. © 1997 Elsevier Science Inc.

KEY WORDS. dietary fatty acids; heat shock proteins; reactive oxygen species; lipid peroxidation; human cells; cellular thermometer

The heat shock (HS)¶ response is a conserved, physiological, immediate, and transient response to cellular injuries such as elevated temperatures, oxidative injury, or, *in vivo*, ischemia and reperfusion injury. The HS response is associated with the transcriptional activation of a specific set of genes, the HS genes, secondary to the activation and binding of HS factor(s) (HSF) to the HS consensus sequences (*n*-GAA-*nn*-TTC-*n*) (HS element, HSE) [1]. Subsequently, the HS proteins (HSP) are synthesized; they function as molecular chaperones and protect cells from thermal and oxidative injury [2, 3].

The signals leading to HSF activation and HSP synthesis, i.e. the "cellular thermometer(s)," are still a matter of debate. Among the proposed hypotheses are the presence of abnormal, unfolded, or misfolded proteins, alterations in membrane physical state, classical second messengers, or reactive oxygen species (ROS) [4–13]. A relationship between membrane lipids and the HS response was first suggested, based on the observation that poikilotherms adjust their membrane lipids as the temperature increases. Several groups, including our own [5, 6], have investigated the effects of changes in membrane lipids on thermosensitivity, thermotolerance, and HSP synthesis and have proposed that the ratio between saturated vs. unsaturated fatty acids (the SFA/UFA ratio) and subsequent perturbations of membrane lipoprotein complexes could represent the cellular thermometer. These perturbations could be involved in the perception of rapid temperature changes, and, under HS, disturbances of a pre-existing membrane physical state might cause transduction of a signal that induces transcription of HS genes.

Here, we investigated in the human premonocytic line U937 the effects of specific modifications of membrane fatty acid (FA) composition on the HS and oxidative stress response. The more polyunsaturated FA (PUFA) reduced cell proliferation, as previously described [14], without altering cell viability. While PUFA had no effect on HSP synthesis after HS or thermosensitivity, they potentiated oxidative injury. Thus, our results argue against a role for alterations in the SFA/UFA ratio as cellular thermometer in the current model but support the hypothesis that PUFA are preferential targets for ROS-mediated cell lesions.

MATERIALS AND METHODS

Reagents

FA were purchased from the Sigma Chemical Company (St. Louis, MO, USA), diluted in pure ethanol or DMSO

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¶ *Abbreviations:* AA, arachidonic acid; DCHA, docosahexaenoic acid; EPA, eicosapentanoic acid; FA, fatty acids; HS, heat shock; HSE, heat shock element; HSF, heat shock factor; HSP, heat shock proteins; LA, linoleic acid; OA, oleic acid; ROS, reactive oxygen species; SA, stearic acid; SFA, saturated fatty acids; UFA, unsaturated fatty acids.

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and kept in the dark at −70° under nitrogen or argon. FA were freshly diluted in medium for each experiment. The controls contained the carrier alone. Fura-2 AM was obtained from Calbiochem (La Jolla, CA, USA).

Cells and Media

Mycoplasma-free U937 cells were cultured in RPMI 1640 (Gibco Laboratories, Grand Island, NY, USA, or Paisley, Scotland) containing 10% fetal calf serum (Gibco) and maintained at 37° in a humidified atmosphere containing 95% air and 5% CO_2. U937 cells were incubated with FA at concentrations ranging from 10^{-6} to 10^{-4} M, or carrier alone, for 24 to 72 h. In most experiments shown here, we used 10^{-5} M of the various FA. For the experiments involving labeling with [^{35}S]methionine, cells were grown in methionine-free RPMI (Gibco).

Determination of Membrane FA Composition

After incubation with the various FA, the cells were rinsed thoroughly in cold phosphate-buffered saline (PBS, Gibco). Cell phospholipids were extracted in chloroform:methanol (2:1), and the extract was washed twice in 0.02% aqueous calcium chloride. The aqueous phase was removed, and the organic phase was dried under nitrogen. The dried lipid was reconstituted in chloroform, and the phospholipid fraction was separated by silicic acid chromatography and eluted in methanol. FA in the phospholipid fraction were transesterified to their methyl esters using boron trifluoride-methanol.

FA methyl esters were extracted from the reaction mixture in hexane, and the hexane dried over anhydrous sodium sulfate. The hexane extracts were dried under nitrogen and resuspended in chloroform for gas-liquid chromatographic analysis. FA methyl esters were separated and identified quantitatively with a Perkin-Elmer 8500 gas-liquid chromatograph model utilizing a Carbowax 20 capillary column (0.25 mm × 30 cm) (Supelco, Bellafonte, PA, USA), and peaks were detected with a flame ionization detector. Quantitation was achieved with a Perkin-Elmer integrator, and FA composition was expressed as a percent of the total FA identified by comparison of their retention time with standard FA methyl esters (PUFA I and II, Supelco).

Cell Viability, Growth, and DNA Synthesis

Cell viability was estimated using trypan blue exclusion, thereby determining the number of stained cells among 200 cells in two individual microscopic fields as previously described [15], and protein synthesis (see below). Cell growth was assessed by following over time cell numbers using a Coulter Counter (Coulter Electronics Inc., Hialeah, FL, USA) as previously described [15], with both floating and adherent cells being counted. [^{3}H]Thymidine incorporation was used as an index for the cell proliferation assay. 2×10^6 cells in 200 μL of culture medium were added to each well of 96-well plates (Falcon, Becton Dickinson, Oxnard, CA). After overnight culture, cells were labeled with 1 μCi/well of [^{3}H]thymidine (67 Ci/mmol, New England Nuclear, Boston, MA, USA) for 6 h, and then they were washed and collected with an automatic cell harvester on glass filter strips (Mash-II, Flow Laboratories, Inc., VA, USA), dried, and counted in Aquassure (New England Nuclear).

Exposure to HS and HSP Synthesis

After 72 h of incubation with the various FA, U937 cells were exposed to HS (44°) for 20 min, as previously described [16], and allowed to recover in the 37° incubator for 2 to 4 h. The cells were then labeled with 200 μCi L-[^{35}S]methionine (Amersham, Zürich, Switzerland) for 1 h. The cells were collected, washed twice in PBS (Gibco), and lysed in lysis buffer as described [17]. Protein aliquots were counted and resolved by gel electrophoresis using sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) (10% acrylamide) as described [16–18]. Proteins were revealed by autoradiography on X-Omat AR films (Kodak, Lausanne, Switzerland).

Exposure to Hydrogen Peroxide (H_2O_2)

After 72 h of incubation with the various FA, U937 cells were exposed to H_2O_2 at the indicated concentrations in RPMI with 10% fetal calf serum for 60 min at 37°. Cells were then centrifuged and washed, and cell viability was determined as described above. In the experiments aiming at establishing the effects of pre-exposure to HS on subsequent H_2O_2 toxicity, cells were first exposed to HS then to H_2O_2 after overnight recovery from HS, which allows maximum HSP accumulation [19].

Determination of Cytosolic Ca^{2+} Levels by Fura-2 Fluorescence

After a 72 h incubation period with eicosapentaenoic acid (EPA) (10^{-5} M) or medium alone, U937 cells were recovered and washed twice with PBS (Gibco). Cells (20×10^6/mL) were then loaded with Fura-2 by incubation with 2 μM Fura-2 AM for 45–60 min at 37° as previously described [20]. Fluorescence was measured with a Perkin-Elmer fluorimeter, at excitation wavelengths 340 nm and 380 nm and emission wavelength 510 nm, as described [21].

Statistics

Cells incubated with FA were compared to control cells by paired Student's *t* test. The various conditions presented in Table 3 were analyzed by unpaired Student's *t* test.

TABLE 1. Effect of incubation with various fatty acids (FA) on U937 cell membrane FA composition at 72 h (A) and 24 h (B)

	C	SA	OA	LA	AA	EPA	DCHA
A							
16:0	21.1	29.3	19.2	25.4	23.4	26.7	25.1
16:1	2.5	7.1	3.0	2.4	3.6	3.7	1.9
18:0	24.6	22.7	18.2	22.2	23.0	17.0	17.7
18:1 (n − 9)	21.7	24.3	37.1	15.4	18.7	16.7	14.9
18:1 (n − 7)	8.7	5.4	5.5	5.0	6.1	4.3	4.0
18:2 (n − 6)	3.6	5.6	2.6	19.5	3.7	2.8	2.8
20:2 (n − 6)	3.2	2.5	3.5	2.0	2.5	2.6	2.8
20:4 (n − 6)	5.7	2.1	3.3	4.8	9.6	1.8	3.6
20:5 (n − 3)	1.4	0.5	0.9	0.5	0.7	12.0	1.2
22:5 (n − 3)	2.5	0.9	2.0	1.1	2.2	11.0	2.9
22:6 (n − 3)	5.3	0.8	3.4	2.1	2.7	2.2	21.0
B							
16:0	27.9	44.3	25.3	24.1	26.9	37.4	26.7
16:1	3.8	1.6	2.4	3.6	4.1	4.1	4.6
18:0	25.6	23.7	21.4	18.4	22.3	17.5	18.7
18:1 (n − 9)	18.0	14.6	27.9	16.7	20.7	15.6	16.7
18:1 (n − 7)	6.3	4.0	5.0	4.9	6.1	3.6	4.5
18:2 (n − 6)	4.8	2.0	2.6	22.0	4.7	3.7	3.7
20:2 (n − 6)	2.8	2.8	2.5	4.8	3.1	8.5	2.0
20:4 (n − 6)	4.0	3.0	4.8	2.8	8.1	1.3	2.7
20:5 (n − 3)	2.8	—	2.3	—	—	3.0	1.9
22:5 (n − 3)	—	2.1	1.0	1.2	1.2	4.2	1.1
22:6 (n − 3)	4.1	1.9	5.8	1.7	2.8	1.2	17.5

Cells were incubated for 72 h with FA (stearic acid (SA), oleic acid (OA), arachidonic acid (AA), linoleic acid (LA), and ω-3 fatty acid (EPA and DCHA) at 10 μM). Quantitation was performed as described in "Materials and Methods," and FA composition was expressed as a percent of the total FA identified. As expected, incubation with a given FA increases its own incorporation into cell membranes from two to five times, except for SA and AA. Numbers represent means of two experiments. Numbers obtained at 24 h (a single experiment) are indicated in Table 1B.

RESULTS

Effects of FA Supply on Membrane FA Composition

Incubation of U937 cells with the different FA (10^{-5} M) significantly altered the FA composition of cell phospholipids. In most cases, cell phospholipids were enriched in the FA with which they were incubated. However, stearic acid (18:0, SA) addition resulted in no elevation of this FA in cells. Instead, SA was associated with an increased content of its β-oxidation product, palmitic acid (16:0, PA), and a reduced PUFA content. Oleic acid (18:1, OA) addition increased OA content of cell membranes with little reduction of total PUFA. Linoleic acid (LA) addition resulted in marked elevations of 18:2, but there was no associated increase in the content of arachidonic acid (AA) and little or no increase in the elongation product 20:2 (n − 6). In contrast, EPA was extensively incorporated into cells and was associated with comparable levels of the elongation product docosapentaenoic acid (22:5). The U937 cells were capable of incorporating both docosahexaenoic acid (22:6, DCHA) and 20:4 into membrane phospholipids, although increases in AA were smaller after incubation with AA than increases in 22:6 after incubation with 22:6. In fact, incubation with AA did not increase the total PUFA content of U937 cells at either 24 h or 72 h, whereas both LA and ω-3 FA increased total PUFA. There was no evidence of retroconversion of PUFA to shorter chain PUFA. Most of the changes were similar at 24 h and 72 h (compare Table 1B with Table 1A); data at 72 h are means from data of two experiments; data at 24 h are from a single experiment. Similar results were also obtained in a different cell line, the porcine renal epithelial cell line LLC-PK1.

Effects of FA Supply on U937 Cell Viability, DNA Synthesis, and Cell Proliferation

PUFA have been shown to decrease cell proliferation in cultures of many different types of cells [22, 23]. Figure 1 shows one representative experiment out of six, each performed in triplicate, and indicates that the PUFA also inhibited cell proliferation in the U937 cells. This inhibition was maximal during the exponential growth phase. The inhibitory effects of FA on U937 cell growth increased with the degree of unsaturation and were significant ($p < 0.05$) for 18:2 (n − 6), 20:4 (n − 6), 20:5 (n − 3), and 22:6 (n − 3), while OA and SA had no significant effects on cell proliferation. When [^{3}H]thymidine incorporation into DNA was used as an index for cell proliferation, both EPA ($p < 0.001$; $n = 6$) and DCHA ($p < 0.01$; $n = 6$) induced significant inhibition of cell proliferation (Table 2). The effects of EPA on U937 cell proliferation were associated with morphological changes suggestive of cellular differentiation (dendritic processes). Indeed, control U937 cells grow in suspension, and EPA induced some degree of adherence and spreading on tissue culture plastic (Fig. 2). These morphological alterations were markedly

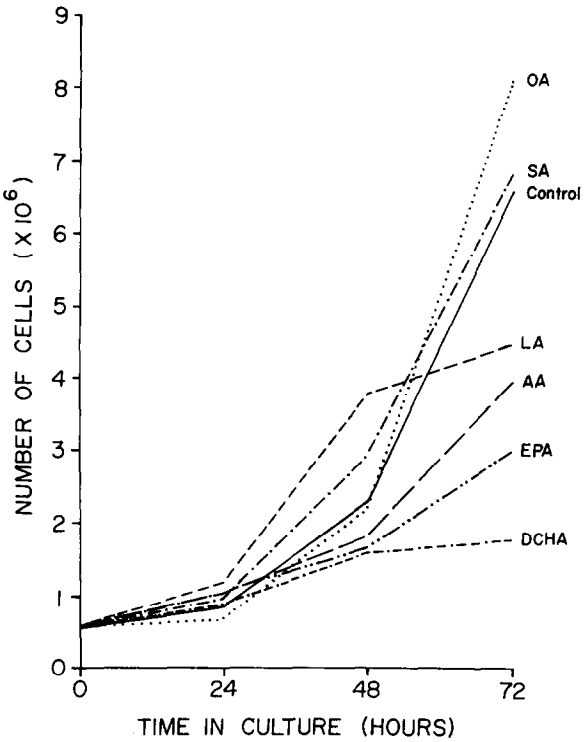

FIG. 1. Effects of FA supply on U937 cell growth. Cells were maintained for 72 h in culture medium containing the various FA (stearic acid, oleic acid, arachidonic acid, linoleic acid, and ω-3 fatty acid (EPA and DCHA) (10^{-5} M). At the times indicated, the number of cells was assessed using a Coulter Counter. One representative experiment out of six is shown. Inhibition on U937 cell proliferation increased with the degree of unsaturation and was significant at 72 h ($p < 0.05$) for all FA except OA and SA.

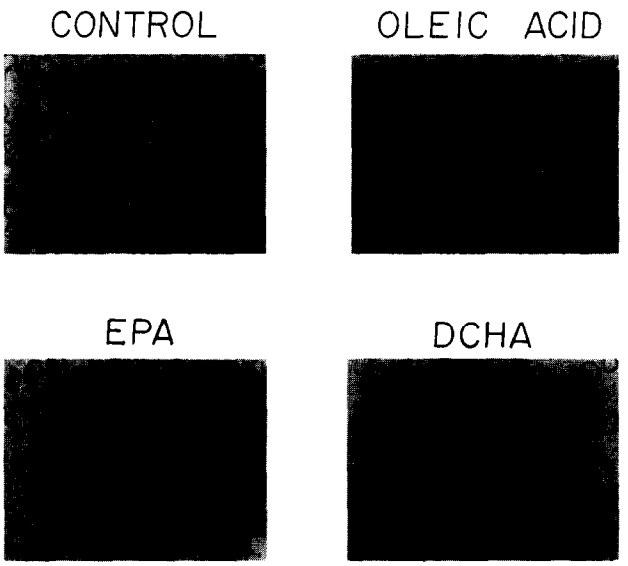

FIG. 2. Effects of OA and ω-3 fatty acid (EPA and DCHA) on U937 cell morphological alterations. U937 cells maintained for 72 h in culture medium containing the various FA (10^{-5} M) expressed some degree of adherence to the plastic culture dish.

increased when U937 cells were cultured with EPA for up to 6 days (not shown). The inhibition of cell proliferation by EPA or DCHA was not associated with an increase in cell death as assessed by two different techniques, i.e. trypan blue exclusion and protein synthesis. Under all preincubation conditions, trypan blue exclusion was >90%. Under all preincubation conditions except for DCHA, [^{35}S]methionine incorporation into proteins corresponded to 100% of control cells; for DCHA-preincubated cells, however, there was a 25% reduction in [^{35}S]methionine incorporation, which indicates that the latter method is more sensitive than trypan blue exclusion.

Effects of Membrane FA Composition on the HS Response

To investigate the effects of specific modifications of membrane FA composition on the induction of a HS response in U937 cells, the cells were incubated with various FA or carrier alone for 72 h at 37° and then exposed to HS, allowed to recover at 37°, and labeled as described under "Materials and Methods." All cells incorporated identical amounts of radioactivity and synthesized the same proteins, both quantitatively and qualitatively (see in particular the band just below 46 kDa, corresponding to actin), than under control conditions (Fig. 3; compare all the − lanes to the control − lane). After HS (44°), the major 70-kDa and 83–90-kDa HSP as well as the 110-kDa HSP were induced in the control U937 cells, whereas there was no detectable change in normal protein synthesis at this temperature (see again, in particular, actin). There was no difference in HSP synthesis between control and cells incubated with FA (Fig. 3); protein synthesis after exposure to 44°, and specifically the induction of the neosynthesized HSP (70 kDa, 83–90 kDa, and 110 kDa, were identical,

TABLE 2. Effects of oleic acid (OA) and ω-3 fatty acid (EPA and DCHA) on U937 cell proliferation

	[^{3}H]Thymidine incorporation into DNA (cpm × 10^3)	
Control	558 ± 20*	
Oleic acid	568 ± 57	NS
EPA	372 ± 16	$p < 0.001$
DCHA	471 ± 3	$p < 0.01$

Cells were incubated with FA (10^{-5} M) for 3 days and then labeled with 1 μCi [^{3}H]thymidine for 6 h. All numbers represent means ± SEM for $n = 6$. Both EPA and DCHA decreased U937 cell proliferation as estimated by [^{3}H]thymidine uptake.
*, SEM.

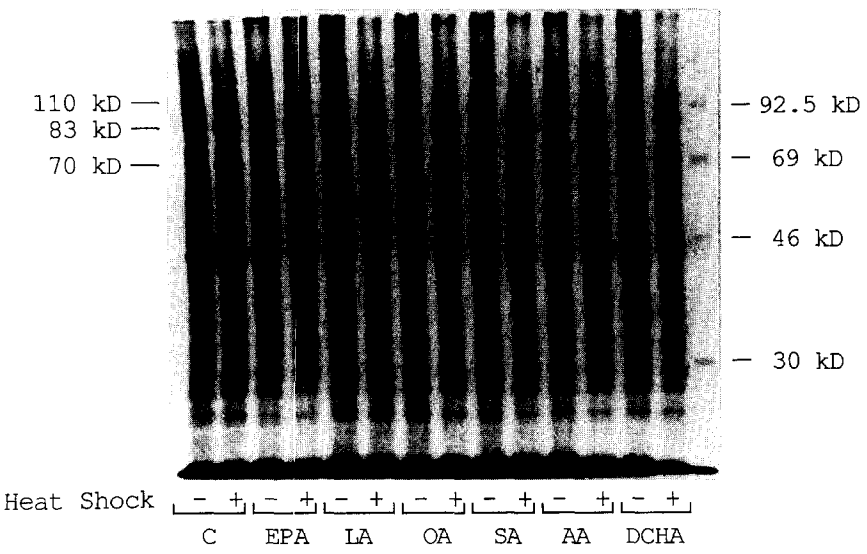

FIG. 3. SDS-PAGE analysis of protein synthesis by U937 cells after HS (20 min at 44°) (autoradiography). Cells were incubated for 72 h in culture medium containing the various FA (stearic acid, oleic acid, arachidonic acid, linoleic acid, and ω-3 fatty acid (EPA and DCHA) (10^{-5} M)). Cells were then exposed (+) or not (−) to HS (44°) and labeled as described. The neosynthesis of both normal proteins and of HSP (HS+) was identical under all conditions tested.

whatever the preincubation conditions (compare all the FA {HS+} lanes to the control {HS+} lane). Again, similar results were found in the porcine renal epithelial cell line LLC-PK1.*

When U937 cells were exposed to 47° for 20 min, there was a mean 10% decrease in cell viability as assessed by trypan blue exclusion. After exposure to higher temperatures (ranging from 50° to 53°), cell viability rapidly decreased to 0% but with some interexperiment variability. Preincubation with the various FA had no effect on thermosensitivity in U937 cells (8% to 10% decrease in cell viability after exposure to 47° under all conditions; not shown).

Effects of Membrane FA Composition on H_2O_2 Toxicity and Calcium Flux

Although incubation with FA did not modulate thermal injury, H_2O_2-induced cell death increased with increasing FA desaturation. Figure 4 shows one representative experiment out of six, each performed in triplicate, and indicates a significant potentiation of oxidative injury in U937 cells preincubated with EPA or DCHA ($p < 0.05$). Preincubation with the less unsaturated FA had no significant effect on H_2O_2-induced cell death.

We have previously demonstrated that exposure of U937 cells to H_2O_2 induces a rapid rise in $[Ca^{2+}]_f$, which participates in cell death [20]. We therefore examined whether the increased H_2O_2 toxicity observed in EPA-treated U937 cells was related to an increase in $[Ca^{2+}]_f$. As shown in Fig. 5 (one representative experiment out of 4), incubation with 10^{-5} or 10^{-6} M EPA for 72 h altered neither basal levels of $[Ca^{2+}]_f$ nor the rise in $[Ca^{2+}]_f$ induced by H_2O_2.

*Polla BS, and Robinson DR, unpublished data.

Effects of Membrane FA Composition on H_2O_2 Cytotoxicity in HS Pre-exposed Cells

Pre-exposure to HS protects the cells from oxidative injury [19, 20]. We therefore examined whether HS could also protect EPA-treated U937 cells from an increase in H_2O_2 toxicity and found that, indeed, pre-exposure of U937 cells to HS partially but significantly ($n = 3$, $p < 0.01$) protected EPA-treated U937 cells from H_2O_2-induced cell death (Table 3).

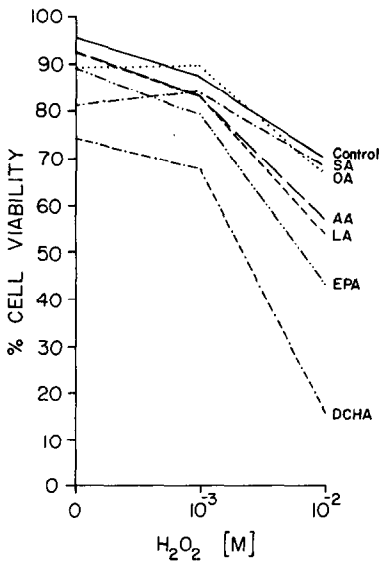

FIG. 4. Effects of membrane FA composition on H_2O_2 toxicity. Cells cultured with the various FA (stearic acid, oleic acid, arachidonic acid, linoleic acid, and ω-3 fatty acid (EPA and DCHA) (10^{-5} M)) were exposed to H_2O_2 at the indicated concentrations. One representative experiment out of six is shown. Cell viability was determined as described in "Materials and Methods." H_2O_2-induced cell death increased with increasing FA desaturation, but the potentiation of oxidative injury was significant for EPA and DCHA only ($p < 0.05$).

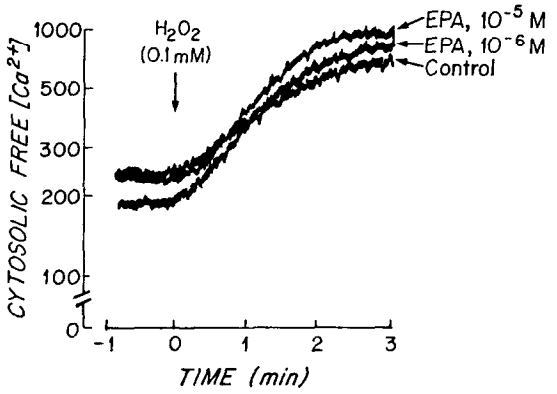

FIG. 5. Calcium flux in EPA treated-cells exposed to H_2O_2 (0.1 mM). After 72 h of incubation in the presence of the indicated concentrations of eicosapentaenoic acid (EPA), cytosolic Ca^{2+} levels were measured as described in "Materials and Methods." There were no differences whether the cells had been preincubated with EPA or not at two different concentrations. Shown is one representative experiment of four.

DISCUSSION

Here, we have used the human premonocytic line U937 to investigate the effects of specific modifications of membrane FA composition on thermal and oxidative injury. Our study demonstrates that under the culture conditions used, the various FA readily incorporated into U937 cell membranes. We found that the more polyunsaturated FA EPA and DCHA decreased cell proliferation without altering cell viability and induced a number of functional alterations compatible with differentiation such as adherence. Furthermore, the PUFA did not alter the HS response but increased hydrogen peroxide toxicity.

It has previously been shown that the FA composition of various cell types is readily modified *in vitro* by addition of FA to culture media. Our study indicates that this was also the case for the human premonocytic line U937. The numbers we show (i.e. a 2- to 5-fold increase in the relevant FA after 72 h of incubation (except for SA and AA) and of the elongation products for EPA) are generally considered as adequate evidence for a significant effect under our incubation conditions. Interestingly, similar results were also found in the porcine renal epithelial cell line LLC-PK1 (Polla BS, and Robinson DR, unpublished data).

TABLE 3. Effects of eicosapentanenoic acid (EPA) and heat shock on U937 viability (%) of cells exposed, or not, to H_2O_2

		Preincubation			
		Control		EPA	
Heat Shock		−	+	−	+
H_2O_2	0	94	92	95	94
	10^{-3} M	93	92	51*	83**

Cells preincubated or not (control) with EPA were exposed (+) or not (−) to HS (44°, 20 min) and then, after overnight recovery, to H_2O_2 (10^{-3} M, 37°, 60 min). Cell viability was assessed using trypan blue exclusion as described in "Materials and Methods." HS significantly protected EPA-treated cells from the PUFA-induced potentiation in H_2O_2-induced cell death ($n = 3$; $p < 0.01$).

*$p < 0.01$ as compared with control cells exposed to the same concentration of H_2O_2.

**$p < 0.01$ as compared with cells preincubated with EPA and exposed to the concentration of H_2O_2.

Modification of FA composition of cells has been associated with changes in their physical properties, their functions, and their intracellular enzyme activities [22, 27]. Increased membrane fluidity accompanying increased contents of PUFA has been observed in some cells, including monocytes, but not in others [28]. Furthermore, incubation of rat peritoneal mast cells with either 20:4 or 20:5 modulates their production of ROS, while *in vivo* enrichment of dietary PUFA modulates both monocyte and neutrophil functions [29]. Enhanced binding of lipopolysaccharides to macrophage membranes has been reported after incubation with PUFA but not with saturated FA [30, 31]. We also observed increased production of superoxide by human neutrophils and differentiated U937 cells treated with EPA when the respiratory burst was activated in these cells (Polla BS, unpublished data).

In the U937 cells, we observed that PUFA decreased cell proliferation. Similar effects on cell proliferation have been described earlier for other cell types and have been ascribed to the initiation of lipid peroxidation [14]. In our studies, the decrease in cell proliferation did not appear to be related to a direct toxic effect, since there was no decrease in cell viability or normal protein synthesis. Although increased production of prostaglandin E_2 could explain that AA decreased U937 cell proliferation, this is unlikely for EPA [14]. Furthermore, along with the decrease in cell proliferation, EPA induced morphological alterations in U937 cells, suggesting differentiation such as adherence and spreading. Whether or not the antiproliferative effects of PUFA are related to U937 cell differentiation remains, however, to be established.

In the U937 cells, the various FA had no effect on protein synthesis before or after exposure to HS and did not alter the induction and synthesis of the classical HSP, 70, 83–90, and 110 kDa. The HS response is an immediate, complex, and transient reprogramming of cellular metabolism after exposure to HS or other forms of cell injury. It is well established that HS is associated with the activation of the transcription factor HSF and its nuclear translocation and binding to the HSE, leading to the transcriptional activation of the HS genes [1]. The "cellular thermometer(s)", i.e. the signal(s) leading to HSF activation, however, are not yet fully elucidated, and the possibility that membrane lipids act as such in human cells has been suggested. Indeed, based on the observation that poikilotherms adjust their membrane lipids as the temperature increases, it has been suggested that membrane lipids play a role in thermosensitivity [24, 25], although other studies have not demonstrated such effects [26]. Our results in the U937 (as well as in other cell lines such as LLC-PK1, see above) indicate that *in vitro* modifications of cell membrane FA composition does not alter the cells' capacity to activate the pathways leading to HSP synthesis.

The various FA also had no effect on thermosensitivity. The lack of effect of the various FA on the HS response possibly relates to the capacity of cells, in particular mammalian cells, to adjust their microviscosity by various means after addition of FA; for example, during food intake, when cells are constantly exposed to different FA from the diet, they maintain a roughly constant fluidity [32].

The association between dietary marine lipids and low risk for cardiovascular diseases has been largely investigated, while their effects on oxidative injury remain more questionable [33, 34]. Earlier studies demonstrated that dietary enrichment with EPA and DCHA modulates monocyte function and may potentiate lipid peroxidation [28, 29, 35]. Our results indicate that although PUFA exerted no toxic effects by themselves, they did sensitize the U937 cells to H_2O_2-induced toxicity; in particular, EPA significantly increased H_2O_2 toxicity.

Because calcium has been shown to potentiate oxidative injury and exposure of U937 cells to H_2O_2 is associated with a rise in $[Ca^{2+}]_f$ [20], we examined the effects of EPA on $[Ca^{2+}]_f$, in resting and H_2O_2-exposed U937 cells. The increased H_2O_2 toxicity induced by PUFA appeared unrelated to a Ca^{2+}-dependent mechanism, which is in contrast with the increased toxicity of H_2O_2 we previously reported in 1,25-dihydroxy-vitamin D3-differentiated U937 cells [20].

As an alternative to membrane lipid changes, ROS have also been proposed as cellular thermometer [13]. However, in the U937 cells, it is unlikely that thermal injury was mediated by ROS since PUFA increased oxidative injury without altering the HS response. Although contrasting data have been obtained in yeast [9, 36], in human cells, the lack of superoxide production during exposure of neutrophils to similar temperatures [37] and the lack of effect of antioxidants on HSP induction during HS [18] are in agreement with our current results.

HSP, in particular the 70-kDa family, act as molecular chaperones, preventing aggregation of unfolded, misfolded, or altered proteins. ROS such as H_2O_2 induce protein alterations and the synthesis of HSP in human monocytes and U937 cells [13, 16]. Here, we show that pre-exposure to HS partially protected U937 from the increased H_2O_2 toxicity induced by EPA, further supporting a role for HSP in the protection of human cells against oxidative injury, as recently reported [19].

In conclusion, our data support the hypothesis that PUFA modify a number of functions of monocytic and other cells, in a way suggestive of a cell-differentiating effect, and are preferential targets for ROS-mediated cell lesions, likely by amplifying lipid peroxidation, as we previously suggested [35]. It should thus be taken into consideration that dietary marine lipids may potentiate ROS-mediated damage in the many human diseases or conditions (including aging) associated with an increased generation of ROS. The overexpression of HSP, although not a part of the PUFA's induced U937 cell modifications, can prevent the observed increase in ROS toxicity.

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