Uptake and activation of eicosapentaenoic acid are related to accumulation of triacylglycerol in Ramos cells dying from apoptosis

Hanne S. Finstad, Hanne Dyrendal, Mari C. Wik Myhrstad, Hilde Heimli, and Christian A. Drevon[1]

Institute for Nutrition Research, University of Oslo, P.O. Box 1046, Blindern, 0316 Oslo, Norway

Abstract **The present study investigates the mechanism behind induction of cell death by eicosapentaenoic acid (EPA) in leukemia cells. The PUFA-sensitive cell lines Raji and Ramos, which die by necrosis and apoptosis upon incubation with EPA respectively, had 2- to 3-fold higher uptake rate of EPA than the PUFA-resistant U-698 cell line. Furthermore, Ramos cells contained more lipid bodies and 3-fold more triacylglycerol than U-698 cells after 24 h incubation with 60 μM EPA. The mechanism behind the increased rate of EPA uptake in the PUFA-sensitive cell lines was examined by comparing the expression of 6 different fatty acid binding proteins (FABPs) and 3 acyl-CoA synthetases (ACSs) in U-698 and Ramos cells. Moreover, enzymatic activity of ACS and acyl-CoA:1,2-diacylglycerol acyltransferase (ADGAT) was investigated. The protein expression level of CD36 and p-FABPpm, the mRNA level of FABP, liver-FABP, heart-FABP, intestinal-FABP, ACS1, ACS2, and enzymatic ADGAT activity were similar in the two cell lines. However, an mRNA signal observed with a probe for ACS3 was 1.7 times higher in Ramos than in U-698 cells, and lysate from Ramos cells had a higher capacity to activate EPA to EPA-CoA than U-698 cell lysate. In conclusion, the present findings indicate that cellular uptake, activation and incorporation of EPA into lipids may be related to induction of cell death in leukemia cell lines.**—Finstad, H. S., H. Dyrendal, M. C. W. Myhrstad, H. Heimli, and C. A. Drevon. **Uptake and activation of eicosapentaenoic acid are related to accumulation of triacylglycerol in Ramos cells dying from apoptosis.** ***J. Lipid Res.*** **2000.** 41: **554–563.**

Supplementary key words eicosapentaenoic acid • apoptosis • lipid bodies • triacylglycerol • fatty acid uptake • fatty acid binding proteins • acyl-CoA synthetase • fatty acid activation

A reduced incidence and mortality of certain types of cancer have been reported in populations with a high dietary intake of n–3 fatty acids (1–4). Furthermore, reduced tumor growth and metastasis have been observed in animals fed n–3 fatty acids (5–7). These effects may be due to reduced cell multiplication, as observed in several cancer cell lines derived from breast, colon, prostate, and lung incubated with very long-chain polyunsaturated fatty acids (PUFA) (8–11). In some of these studies, various sensitivities to PUFA have been reported (12–14). This was also observed when we studied the sensitivity of 14 different leukemia cell lines to PUFA (15). In 10 of these cell lines, reduced cell multiplication was observed with arachidonic acid (AA, 20:4, n–6), eicosapentaenoic acid (EPA, 20:5, n–3) or docosahexaenoic acid (DHA, 22:6, n–3), whereas 4 cell lines were resistant to PUFA. The sensitivity to fatty acids was specific for PUFA as none of the cell lines were sensitive to oleic acid (OA, 18:1, n–9) or stearic acid (SA, 18:0). The reduction in cell number with PUFA could be ascribed to modulation of cell differentiation and cell proliferation as well as induction of apoptosis or necrosis. In the promyelocytic HL-60 cell line, differentiation was accompanied by both apoptosis and necrosis after incubation with EPA or AA (16). In Raji cells, EPA induced necrosis, whereas apoptosis predominantly was observed in Ramos and U937-1 cells (15, 17). Induction of apoptosis has also been observed in HepG2 cells incubated with AA, and this was related to generation of lipid peroxidation products (18). Induction of necrosis in Raji cells incubated with EPA was probably mediated by a similar mechanism, as cell death was counteracted by vitamin E (15). However, induction of apoptosis and necrosis in HL-60 cells, and apoptosis in U937-1 and Ramos cells incubated with EPA, were unaffected by antioxidants as well as inhibitors of eicosanoid synthesis (15–17).

In U937-1 cells, inhibition of proliferation and induction of apoptosis by EPA was accompanied by accumulation of intracellular triacylglycerol (TAG)-rich lipid bodies (17). The main fatty acids recovered in this lipid fraction were EPA and its elongation products docosapentaenoic acid (DPA, 22:5, n–3) and DHA. A sulfur-substituted EPA-analogue, on the contrary, was not incorporated into cellular TAG, did not affect cell viability, and had only minor effects on cell multiplication. These observations may sug-

Abbreviations: EPA, eicosapentaenoic acid; PUFA, polyunsaturated fatty acids; ADGAT, acyl-CoA:1,2-diacylglycerol acyltransferase; ACS, acyl-CoA synthetase; FABP, fatty acid binding protein; TAG, triacylglycerol.

[1] To whom correspondence should be addressed.

gest that formation of PUFA-rich lipid bodies was involved in the inhibition of cell multiplication and induction of cell death.

The aim of the present study was to search for mechanisms important for induction of cell death by EPA in leukemia cell lines. The PUFA-sensitive cell lines Raji and Ramos were compared with the PUFA-resistant cell line U-698, assuming that features important for induction of apoptosis or necrosis by EPA are different in these cell types. We first investigated whether the cellular uptake rate of EPA could be related to PUFA sensitivity. These experiments demonstrated that the sensitive cell lines Raji and Ramos had a higher rate of uptake and incorporation of EPA into cellular lipids than the resistant U-698 cell line, and this difference was reflected in the amount of TAG deposited in U-698 and Ramos cells. On the other hand, uptake and incorporation of OA were similar in all 3 cell lines. Expression of 6 different fatty acid binding proteins (FABPs), 3 acyl-coenzyme A synthetases (ACSs), and activity of ACS and acyl-CoA:1,2-diacylglycerol acyltransferase (ADGAT) were compared in U-698 and Ramos cells. We observed increased activation of EPA in the sensitive Ramos cells parallel with a higher level of a long fragment of mRNA for ACS3. The present findings indicate that cellular uptake, activation, and incorporation of EPA into lipids may be related to induction of cell death in leukemia cells.

MATERIALS AND METHODS

Materials

OA, EPA, fatty acid-free bovine serum albumin (BSA), butylated hydroxytoluene (BHT), adenosinetriphosphate (ATP), dithiothreitol (DTT), coenzyme A, 1,2-dioleoylglycerol, 2.7-dichlorofluorescein, and $K_4Fe(CN)_6$ were purchased from Sigma Chemical Co, St. Louis, MO. Phosphate-buffered saline (PBS), RPMI-1640, fetal bovine serum (FBS), l-glutamine, and gentamycin were bought from BioWhittaker, Walkersville, MD. Triacsin C was bought from Biomol Research Laboratories, Inc., Plymouth Meeting, PA. Dimethyldichlorosilane was purchased from Supelco Inc., Bellafonte, PA, whereas [9,10-^{3}H(N)]-OA [^{3}H]OA and [5,8,11,14,17(1-^{14}C)]EPA [^{14}C]EPA were obtained from DuPont, NEN Research Products, Boston, MA. 1,2–Di[1-^{14}C]oleoyl-*sn*-glycerophosphocholine was purchased from Amersham International, Amersham, Bucks, UK, and phospholipase C (grade I) was delivered by Boehringer Mannheim, Mannheim, FRG. Filters with 0.22 υm pore size were bought from MSI, Westboro, MA, and Insta-gel II Plus liquid scintillation fluid from Packard, Meriden, CT. Epon and 2.2-dimethoxypropane were obtained from Fluka, Buchs, Switzerland, and trypsin was purchased from Difco Laboratories, Detroit, MI. Enzymatic determination of cellular TAG and cholesterol was performed with reagents supplied by BioMérieux, Lyon, France. Triheptadecanoin was bought from Larodan, Malmö, Sweden. Cellular protein content was determined with BCA Protein Assay Reagent, Pierce, Rockford, IL. FITC-labeled CD36 antibody (clone FA6-152) was from Immunotech, Marseilles, France. Permeabilization solution (10×) and IgG-FITC antibody were purchased from Beckton Dickinson, San Jose, CA, and measurement of CD36 was performed by an FACScan flow cytometer from the same supplier. The probe for glyceraldehyde 3-phosphate dehydrogenase (G3DH) was purchased from Clontech, Palo Alto, CA. Other chemicals were analytical grade from commercial suppliers.

Cell culture

Raji and Ramos cells were purchased from BioWhittaker. U-698 cells were a gift from M.F. Greaves, Imperial Cancer Research, Fund Laboratories, London, UK. The cell lines were cultured in RPMI-medium supplemented with 10% heat-inactivated FBS, l-glutamine (2 mmol/L) and gentamycin (0.1 mg/ml). Cells were incubated with fatty acids complexed to BSA with a molar ratio of 2.5:1, and the cells were routinely kept in logarithmic growth phase at 0.2–1.2 × 10^6 cells/ml. For experiments, cells were seeded at a density of 0.2–0.6 × 10^6 cells/ml. Cell count experiments were performed in duplicate by use of Coulter Z1 (Coulter Electronics Limited, Luton, Beds, UK). Each experiment was performed at least 3 times. Cell cultures incubated without fatty acids were used as controls in all cell count experiments and defined as 100%. The effect of a certain treatment on cell number is presented as % of control. Apoptosis was studied by PI/HO342 staining as previously described (15).

Trypsination was performed with 25 μg trypsin and 1 × 10^6 cells/ml PBS for 10 min at 37°C. The reaction was stopped by addition of 20 ml ice-cold RPMI-medium containing 10% FCS and transfer of samples to ice. Thereafter, cells were sedimented, washed in 10 ml medium, re-sedimented, and suspended in RPMI-medium before cell density was adjusted to 0.6 × 10^6 cells/ml. Trypsination did not affect cell viability as assessed by trypan blue exclusion.

Cellular uptake of fatty acids

Uptake of [^{3}H]OA (1 μCi/ml) or [^{14}C]EPA (0.25 μCi/ml) was determined after incubation of the labeled fatty acids mixed with 60 μmol/L of unlabeled OA or EPA, respectively. Medium (0.2 ml) and cell homogenate (0.1 ml) were precipitated with equal volumes of ice-cold trichloroacetic acid (10% w/v, final concentration) in glass tubes silanized with dimethyldichlorosilane. The mixture was centrifuged at 1570 *g* for 10 min at 4°C. Samples from the supernatant were filtered through a 0.22-μm filter before counting acid-soluble products, mostly fatty acid degradation products. The precipitate was solubilized in 1 ml saline with SDS (70 mm) and Triton X-100 (10%). A cell-free control was analyzed together with the samples. The recovery of labeled fatty acids was 94 ± 3% (n = 9) after acid precipitation.

Incorporation of fatty acids into cellular lipids

The incorporation of [^{3}H]OA and [^{14}C]EPA into cellular lipids was determined after lipid extraction and thin-layer chromatography (TLC). From 350 μl of cell homogenate and 750 μl of incubation medium lipids were extracted (pH 2.0) in silanized glass tubes (19, 20). The lipid extracts were dissolved in 200 μl of chloroform–methanol 2:1 and separated by TLC using hexane–diethyl ether–acetic acid 65:35:1 (v/v/v). The different lipid fractions were identified by iodine vapor, transferred to 8 ml of Insta-gel II Plus liquid scintillation fluid (Packard, Meriden, CT), and counted in a WinSpectral 1414 Liquid Scintillation Counter (Wallac, Turku, Finland). The recovery of labeled fatty acids after extraction and TLC was 80 ± 7% (n = 9).

Expression of FABPs and ACSs

For CD36 measurement, an IgG-FITC antibody was used as isotype control to exclude background labeling. Cells were concentrated to 4 mill cells/ml and aliquotes of 50 μl were incubated with 20 μl CD36 or IgG-FITC antibody in the dark for 30 min. Thereafter, cells were washed and resuspended in 1.5% BSA. From each sample 10,000 cells were analyzed by flow cytometry, and the markers were set by including 1% of the isotype-labeled cells. The data were analyzed with the Cell Quest software (Beckton Dickinson, San Jose, CA). Measurement of p-FABP-pm was done by Western blot analysis with rabbit polyclonal antiserum

(21). Expression of FATP, L-FABP, I-FABP, H-FABP, ACS1, ACS2, and ACS3 was investigated by mRNA analysis (17). Each membrane was rehybridized up to 6 times. The signals were calibrated to that of the G3PDH transcript (22).

Assay of acyl-CoA synthetase activity

Source of enzyme was whole cell lysate (25 μg protein/sample) which was added to ice-cold reaction mixture (250 μl) containing 150 mm Tris, pH 7.4, 6.2 mm $MgCl_2$, 2.5 mm EDTA, 2.3 mm ATP, 0.5 mm CoA, 0.9 mm DTT, and 0.02% Triton X-100. The reaction was initiated by addition of 1.5 mm [^{3}H]OA (1 μCI/ml) or [^{14}C]EPA (0.25 μCi/ml) and transferred to 37°C. The reaction was terminated and samples were extracted as previously described (23). A 1-ml sample of the aqueous phase was transferred to 10 ml Insta-gel II Plus liquid scintillation fluid (Packard, Meriden, CT) and counted in a WinSpectral 1414 Liquid Scintillation Counter (Wallac, Turku, Finland).

Electron microscopy

Cells subjected to electron microscopy (24) were prefixed in 2% glutaraldehyde in 0.1 m cacodylate buffer overnight at 4°C, and postfixed in 2% OsO_4 in 1.5% $K_4Fe(CN)_6$, prior to 30 min incubation in 1.5% uranylacetate in the dark. The specimens were thereafter dehydrated in graded ethanol and embedded in Epon before they were sectioned and stained with lead citrate. The sections were examined in a JEOL JEM 1200 EX transmission microscope (JEOL, Tokyo, Japan).

Cellular triacylglycerol, fatty acids, and cholesterol

From each sample, 25 × 10^6 cells were lysed and sonicated in 600 μl of H_2O. Lipids were extracted from 500 μl of cell homogenate in silanized glass tubes (19, 20). The lipid extracts were dissolved in 50 μl of isopropanol, and 10-μl aliquots were used for enzymatic determination of cellular content of cholesterol and TAG. The cellular lipid content was related to the protein concentration in each sample, as determined by the BCA protein assay. For analysis of the fatty acids incorporated into cellular TAG, 4 μl of triheptadecanoin (1.1 mg/ml) was used as internal standard, and the lipids were extracted as described above in the presence of BHT (0.1 mg/ml). The samples were then solubilized in 200 μl methanol–chloroform 2:1, (v/v), and separated by TLC in the presence of argon, using hexane–diethyl ether–acetic acid 80:20:1 (v/v/v). The TAG bands were detected in UV light after spraying the plates with 0.2% 2.7-dichlorofluorescein and scraped into new glass tubes. Hydrolysis and transmethylation were performed by incubation in the dark at room temperature overnight in a mixture of 1 ml benzene, 2 ml methanol-HCl (3 m) and 200 μl 2.2-dimethoxypropane. The samples were thereafter neutralized with 4 ml $NaHCO_3$ (0.7 m), extracted twice with 2 ml hexane, and solubilized in 50 μl hexane. The fatty acids were separated on a GC-14A gas chromatograph (Shimadzu, Kyoto, Japan) equipped with a 100 m polar SP 2560 capillary column with 0.25 mm diameter (Supelco, Bellafonte, PA). Helium was used as carrier gas. The oven temperature was programmed to rise from 135°C to 180°C at 5°C/min, remain at 180°C for 30 min and rise to 193°C at 6°C/min and finally stay at this temperature for 70 min. Retention times and peak areas were automatically computed with a Shimadzu C-R4AX Chromatopac. Identification of the individual methyl esters was performed by frequent comparison with authentic standard mixtures analyzed under the same conditions.

Assay of acyl-CoA:1,2-diacylglycerol acyltransferase (ADGAT) activity

1,2-Di[1-^{14}C]oleoylglycerol was prepared from 1,2-di[1-^{14}C]oleoyl-*sn*-glycerophosphocholine by treatment with purified phospholipase C (25, 26). More than 90% of the labeled phosphatidylcholine was converted to 1,2-dioleoylglycerol by this method. The dioleoylglycerol was isolated and purified by TLC (n-hexane–diethyl ether–acidic acid 80:20:1 (v/v/v)), redissolved in absolute ethanol, mixed with a stock solution of unlabeled dioleoylglycerol (1.25 mm) and stored at −70°C. More than 98% of the ^{14}C-labeled material comigrated with 1,2-dioleoylglycerol. Acyl-CoA esters were synthesized via the corresponding acylimidazoles (27). The ADGAT activity in cell lysates containing 90 μg of cell protein was measured at 23°C in 500 μl 175 mm Tris, pH 7.8, with 10 mm $MgCl_2$, defatted BSA (1 mg/ml), 20 μm acyl-CoA, and 1,2-dioleoylglycerol (125 μm) dissolved in ethanol (10% v/v final concentration). Adding 20 volumes of chloroform–methanol 2:1 (v/v) terminated the incubations. Serum (20 μl) was added to supply TAG as unlabeled carrier. The lipids were extracted and the amount of synthesized TAG was determined by TLC and liquid scintillation counting as described above.

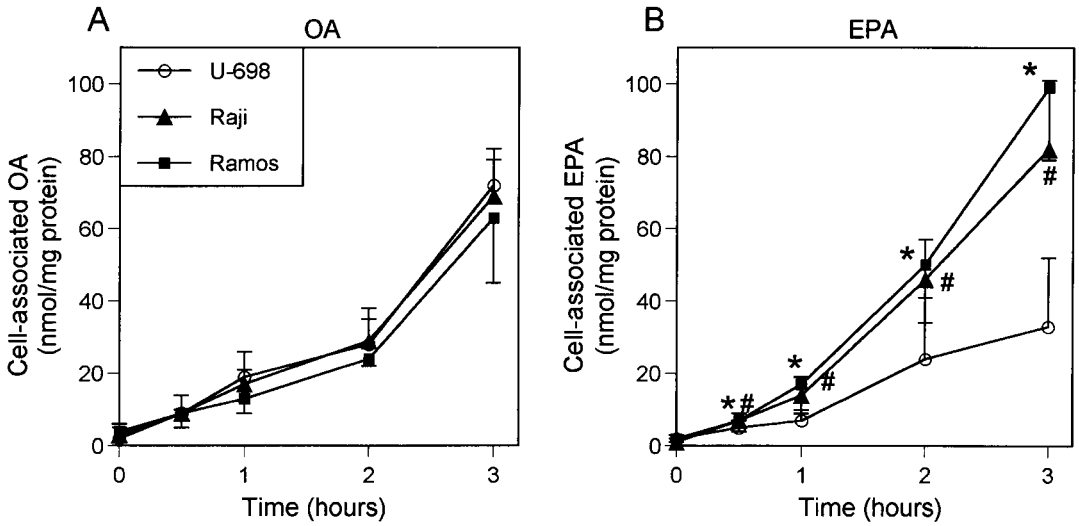

Fig. 1. Cellular uptake of radiolabeled EPA and OA. Cell-associated acid-precipitable radioactivity of [^{3}H]OA (A) and [^{14}C]EPA (B) was monitored in U-698, Raji, and Ramos cells after incubation with 60 μm of the fatty acids. The means ± SD from 3 separate experiments with triplicates are presented. # $P \leq 0.05$ Raji cells compared to U-698 cells. * $P \leq 0.05$ Ramos cells compared to U-698 cells.

Statistics

Mann-Whitney analysis was used to determine the significance level of differences among sample groups. The level of significance was set at $P \leq 0.05$. Results are presented as means ± standard deviation (SD).

RESULTS

Increased cell association of EPA in Raji and Ramos cells

Cellular uptake and incorporation into lipids of 60 μmol/L radiolabeled EPA and OA were measured in U-698, Raji, and Ramos cells. During 3 h the cell number and viability were unaffected in all 3 cultures, making it unlikely that changes in viability would affect the cellular incorporation of fatty acids. The amount of cell-associated OA was similar in all 3 cell lines (**Fig. 1A**), but there was significantly more EPA associated with Raji and Ramos cells than with U-698 cells at all time points (Fig. 1B). After 3 h, 82 and 99 nmol EPA/mg protein was associated with Raji and Ramos cells, respectively, whereas only 33 nmol EPA/mg protein was associated with U-698 cells. The amount of cell-associated OA and EPA was in agreement with the disappearance of the respective fatty acids from the medium (data not shown). Approximately 0.3% of the radioactivity in the cells and in the medium was acid soluble. There was no difference in the amount of acid-soluble radioactivity in the 3 cell lines (data not shown).

Increased incorporation of EPA into lipid fractions in Raji and Ramos cells

OA was incorporated to a similar extent into the different lipid fractions in all the 3 cell lines (**Fig. 2A**), whereas more EPA was recovered in diacylglycerol (DAG), free fatty acids (FFA), and TAG in Raji and Ramos cells than in U-698 cells (Fig. 2B). This difference was most prominent for the TAG fraction, where Raji and Ramos cells contained 3- to 4-fold more EPA than U-698 cells after 3 h of incubation. In the phosphoacylglycerol (PL) fraction, only Raji cells contained significantly more EPA than U-698 cells after 3 h of incubation. Only 1% of the labeled EPA recovered in cellular lipids was in the cholesteryl ester (CE) fraction. After 3 h, there was no significant difference in the incorporation of EPA in this fraction in the 3 cell lines. In further experiments, features related to the different incorporation of EPA in cellular lipids were investigated more closely by studying U-698 and Ramos cells.

Expression of FABPs and effect of trypsination on EPA uptake in U-698 and Ramos cells

The difference in cellular uptake and incorporation of EPA might be due to expression of specific FABPs in cell membranes or in the cytosol. The expression of several FABPs was therefore measured in U-698 and Ramos cells. The proteins CD36 (28, 29), p-FABP-pm (21) and the mRNA for L-FABP (30) were not detected, whereas the mRNA levels for FATP (31), H-FABP (32), and I-FABP (33, 34) were similar in Ramos and U-698 cells (**Table 1**). The different incorporation rates of EPA in the two cell lines could therefore not be related to differences in the expression of any of these FABPs. To investigate whether membrane-associated proteins could be involved in the higher incorporation of EPA in Ramos versus U-698 cells, the effect of pre-treating the cells with trypsin on cellular uptake of EPA was studied (**Fig. 3**). No significant differ-

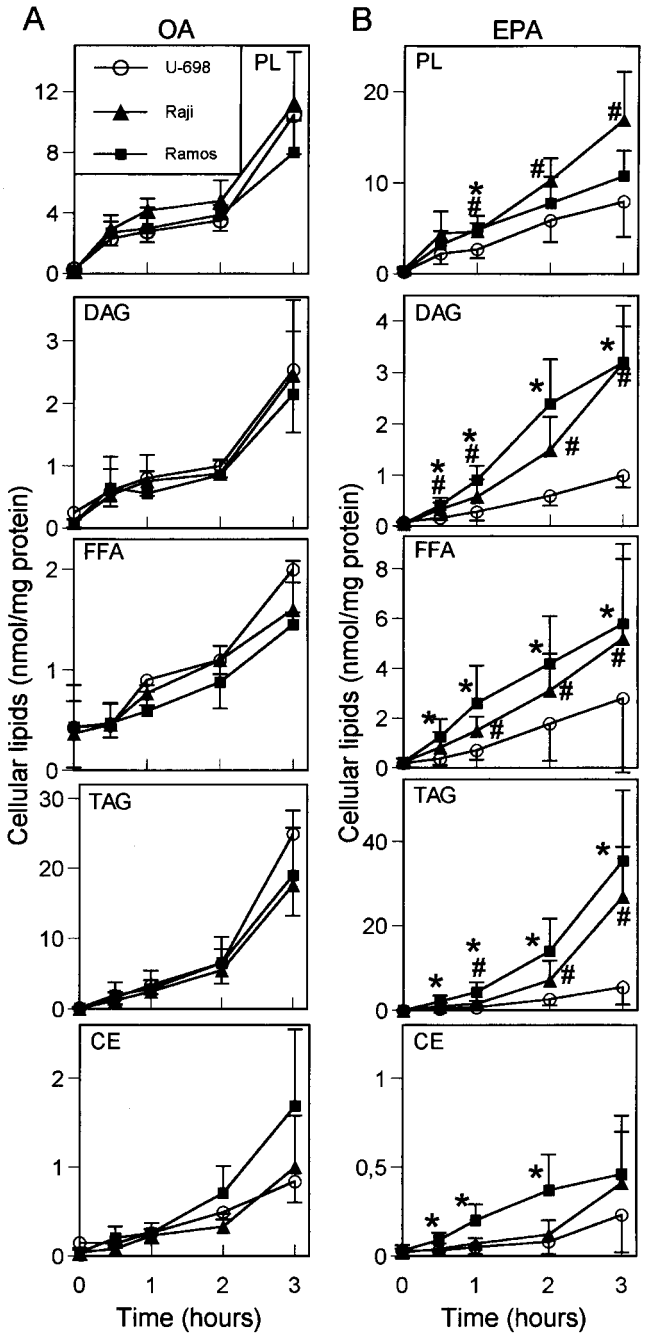

Fig. 2. Incorporation of radiolabeled EPA and OA into cellular lipids. Radioactivity of [^{3}H]OA (A) and [^{14}C]EPA (B) was recovered in cellular lipids in U-698, Raji, and Ramos cells after incubation with 60 μm of the fatty acids. We measured phosphoacylglycerol (PL), diacylglycerol (DAG), free fatty acids (FFA), triacylglycerol (TAG), and cholesteryl esters (CE). The means ± SD from 3 separate experiments with triplicates are presented. # $P \leq 0.05$ Raji cells compared to U-698 cells. * $P \leq 0.05$ Ramos cells compared to U-698 cells.

TABLE 1. Expression of mFABPs, cFABPs, and ACSs in U-698 and Ramos cells

	U-698	Ramos
mFABPs		
CD36 protein	nd	nd
p-FABP-pm protein	nd	nd
FATP mRNA	100	114 ± 11
cFABPs		
L-FABP mRNA	nd	nd
H-FABP mRNA	100	87 ± 9
I-FABP mRNA	100	109 ± 16
ACSs		
ACS1	100	90 ± 25
ACS2	nd	nd
ACS3 3.7 kb	100	64 ± 11
ACS3 7.0 kb	100	179 ± 38

Means ± SD are presented from two separate experiments performed in duplicate. The density of the mRNA signal observed in U-698 cells was defined as 100% and signals observed in Ramos cells are presented as % of the U-698 signal. FABP, fatty acid binding protein; ACS, acyl-CoA synthetase; nd, not detected.

ence was observed in the amount of radiolabeled EPA recovered after 1 h of incubation in trypsinated cells, as compared to untreated control cultures.

ACS expression and activity in U-698 versus Ramos cells

To compare the expression pattern of ACSs in U-698 and Ramos cells, the relative mRNA expression levels of ACS1, 2, and 3, were examined (35–37). ACS2 was not detected, whereas a similar mRNA expression level of ACS1 was observed in both cell lines (Table 1). Furthermore, two mRNA signals with sizes 3.7 and 7.0 kb were detected after hybridization with the ACS3 probe. The shorter transcript was expressed to a lower extent in Ramos than in U-698 cells, whereas the intensity of the 7.0 kb transcript was almost 2-fold stronger in Ramos than in U-698 cells.

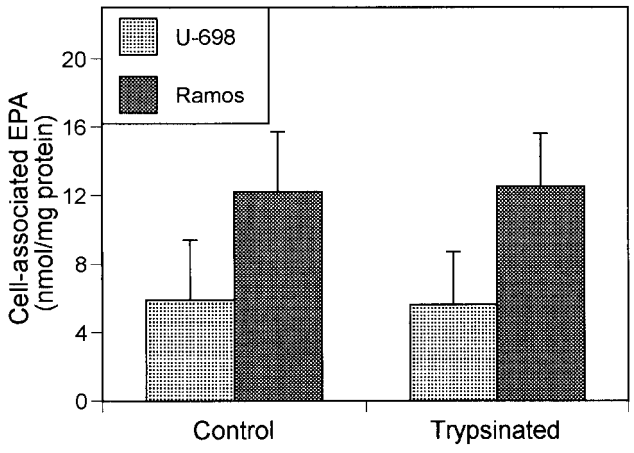

Fig. 3. Effect of trypsination. The amount of cell-associated EPA in trypsinated cells was compared to that recovered in control cultures after 1 h of incubation with 60 μm of radiolabeled (^{14}C)EPA. Means ± SD from 7 separate experiments each performed in triplicates are presented.

When ACS activity was measured in lysate from U-698 and Ramos cells, lysate from Ramos converted twice as much EPA to EPA-CoA as that of U-698 cells, whereas activation of OA was similar for Ramos and U-698 cells (**Fig. 4**). Furthermore, addition of 4.8 μm of the ACS inhibitor Triacsin C counteracted the cell death induced by 60 μm EPA in Ramos cells. After 24 h of incubation with 60 μm EPA, 45% of the cells were apoptotic, whereas only 5% had died in cultures given Triacsin C together with EPA.

High accumulation of lipid bodies in Ramos cells

We investigated whether the observed difference in cell association rate of EPA could be reflected in the formation of intracellular lipid bodies in U-698 and Ramos cells. Electron microscopy of cells incubated with 60 μm of OA or EPA for 12 h revealed accumulation of intracellular lipid bodies in both cell lines (Fig. 4). However, a semiquantitative comparison of the number of lipid bodies in the two cell types showed that Ramos cells incubated with EPA contained more lipid bodies than U-698 cells (**Fig. 5A** and **B**). After incubation with OA there was similar accumulation of lipid bodies in the two cell lines (Fig. 5C and D). In control cultures, none or one lipid body was observed per cell section (data not shown). No other major changes in cellular morphology were observed.

Lipid bodies contain TAG

Lipid bodies usually contain mostly cholesteryl ester or TAG (38), and our control cultures contained 24 nmol TAG/mg protein (**Fig. 6A**). After incubation with 60 μm of OA or EPA for 12 h, an increase in cellular TAG was observed in both cell lines (Fig. 5A). Ramos cells incubated with EPA contained 156 nmol TAG/mg protein and U-698 cells contained 64 nmol TAG/mg protein, whereas Ramos and U-698 cells incubated with OA contained 102 and 119 nmol TAG/mg protein, respectively. The cellular cholesterol content was unaffected by OA and EPA (Fig. 6B), indicating that the lipid droplets mainly consisted of TAG and not cholesteryl esters.

Lipid extraction and analysis by GC determined the type of fatty acids incorporated into the cellular TAG-fraction. After incubation with OA, a similar accumulation of monounsaturated fatty acids (MUFA), was observed in both cell lines. U-698 cells contained 334 nmol MUFA/mg protein and Ramos cells contained 310 nmol MUFA/mg protein (**Table 2**). Incubation with EPA caused accumulation of PUFA in TAG of both cell lines. However, Ramos cells contained 3-fold more PUFA in cellular TAG than U-698 cells, and most of this was EPA and DPA.

ADGAT activity in Ramos versus U-698 cells

ADGAT might be involved in the higher TAG formation in Ramos cells, as it is responsible for the last step in TAG synthesis. Hence, the enzymatic activity of ADGAT was compared in U-698 and Ramos cells from lysate of cells in logarithmic growth. The homogenates from U-698 and Ramos cells exhibited a similar rate of TAG synthesis with EPA-CoA and OA-CoA as substrates (**Fig. 7**). It is therefore unlikely that the observed difference in accumulation of

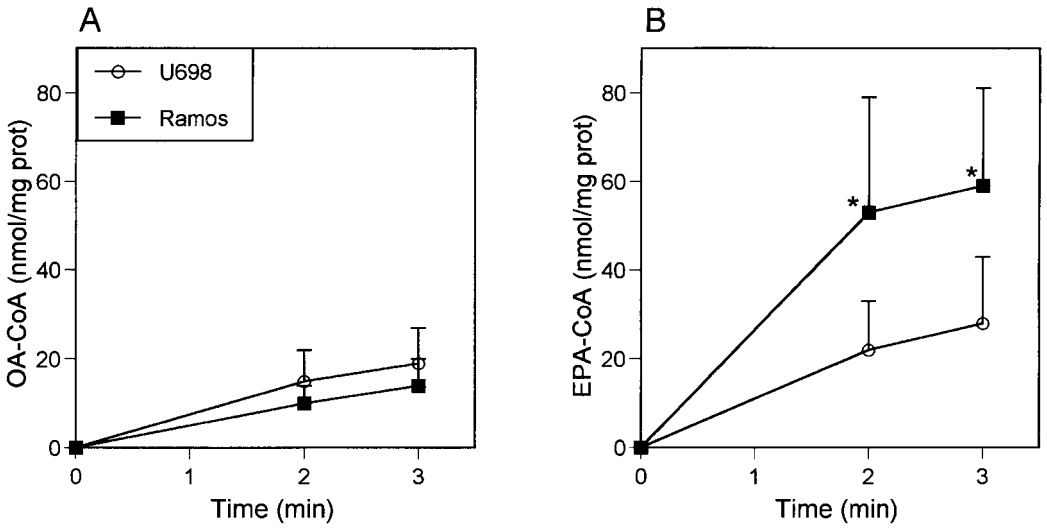

Fig. 4. ACS activity in U-698 and Ramos cell lysate. Cells in logarithmic growth were harvested and lysed and the amount of OA-CoA (A) and EPA-CoA (B) formed from radiolabeled [^{3}H]OA and [^{14}C]EPA as substrate was determined. The means ± SD from 3 separate experiments, each performed in triplicate, are presented (n = 9). * $P \leq 0.05$ as compared to U-698 cells.

TAG-rich lipid bodies was caused by different ADGAT activity in the two cell lines.

DISCUSSION

Our findings indicate that the rate of uptake and incorporation of EPA into cellular lipids is related to the cellular capacity for activation of EPA to EPA-CoA. The PUFA-sensitive cell lines Raji and Ramos had a 2- to 3-fold higher rate of uptake and incorporation of EPA into cellular lipids than the PUFA-resistant U-698 cell line, whereas uptake and incorporation of OA were similar in all 3 cell lines (Figs. 1 and 2). In accordance with these findings, lysate from Ramos cells had a 2-fold higher capacity to activate EPA than lysate from U-698 cells, whereas the capacity for activation of OA was similar in both cell lines (Fig. 4). Rat ACS3 activates EPA and AA most efficiently among C16–C20 PUFA, and has a 4-fold higher capacity for utilization of C20 PUFA than rat ACS1 (37). Human ACS3 has also been cloned (39), and mRNA expression of this gene is observed as two transcripts with size similar to those observed in the present study (Dr. Tokuo Yamamoto, Tohoku University Gene Research Centre, Japan, personal communication). Hence, the higher activation of EPA in lysate from Ramos cells may be attributable to the higher steady-state level of ACS3 7.0 kb mRNA observed in this cell line. However, a fourth rat ACS, named ACS4, has been cloned, with a preference for activation of AA and EPA as compared to other C_8–C_{22} fatty acids (40). A human homologue to rat ACS4, named FACL4, has also been identified (41, 42). Hence, the cellular capacity for activation of EPA in human cells may also be influenced by ACS4.

Some studies show that the cellular capacity for uptake of PUFA may be different from that of OA (43, 44), and involvement of ACS in regulating cellular uptake of endogenous fatty acids has been described in ACS-deleted yeast mutants (45). However, to our knowledge, only one previous study has indicated a role for ACS in regulation of PUFA uptake (46). In this work, a mutant fibrosarcoma cell line, selected for defective AA uptake, was demonstrated to lack arachidonate-specific ACS, whereas uptake and activation of OA in the mutant cell type were similar to that of the parent cell. In addition, Schaffer and Lodish (31) showed that ACS may be important for fatty acid transport during expression cloning in adipocytes.

Ramos cells incubated with EPA accumulated more TAG-rich lipid bodies than U-698 cells subject to the same treatment (Figs. 5 and 6). Lipid bodies may store fatty acids as energy source or play a role in inflammatory processes (47, 48). During cell activation, an increase of intracellular AA associated with cellular TAG in mast cells, eosinophils, monocytes and platelets has been observed. TAG might function as an early acceptor and reacylation pool for AA when released from PL. Results obtained in the present study and in our previous study on U937-1 cells (17) indicate a role for lipid bodies in regulation of the cellular suicide program as well. It is possible that the high number of TAG-rich bodies in Ramos cells, containing 2–3 molecules of EPA or DPA per glycerol backbone, may cause marked changes in intracellular signal transduction and thereby initiate apoptosis. Such a mechanism may operate in mast cells where appearance of AA in TAG was associated with cytoplasmic lipid bodies and reduction in cell density (49).

FABPs, located in the cell membrane (mFABP) or cytosol (cFABP), may be important for regulating the uptake of fatty acids across the lipid bilayer and targeting fatty acids to specific intracellular sites (50). However, the proteins p-FABP-pm and CD36 and the mRNA coding for

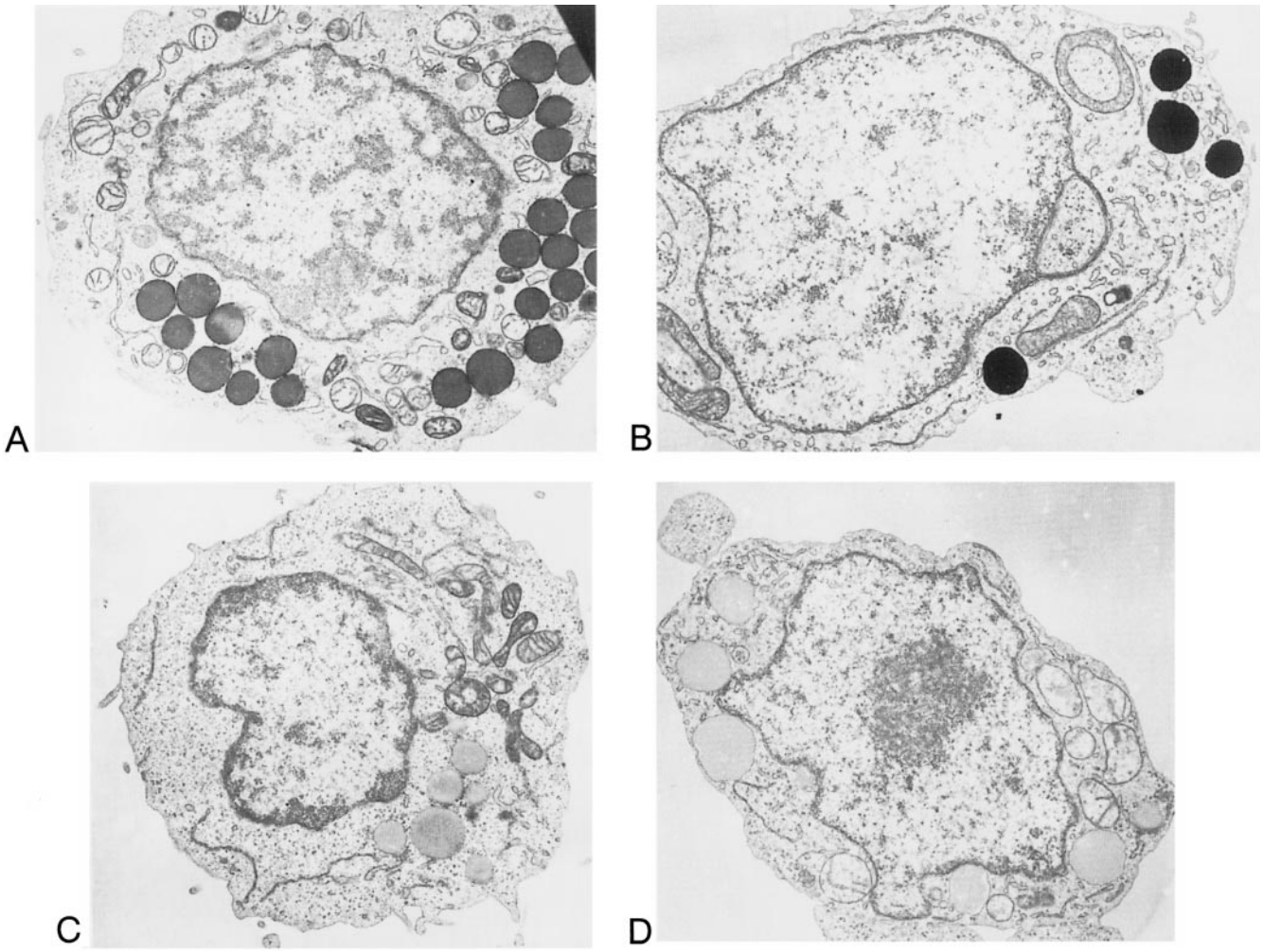

Fig. 5. Electron micrographs. Ramos and U-698 cells were cultured for 12 h in the presence of 60 μm EPA; Ramos (A) and U-698 (B), or 60 μm OA; Ramos (C) and U-698 (D). The magnification is 5000×.

L-FABP were not detected in either U-698 nor Ramos cells (Table 1). Furthermore, the mRNA levels for FATP, H-FABP and I-FABP were similar in both cell lines. It is therefore unlikely that any of these 6 FABPs were responsible for the observed difference in uptake of EPA. The results obtained with trypsinated cells (Fig. 3) also suggest

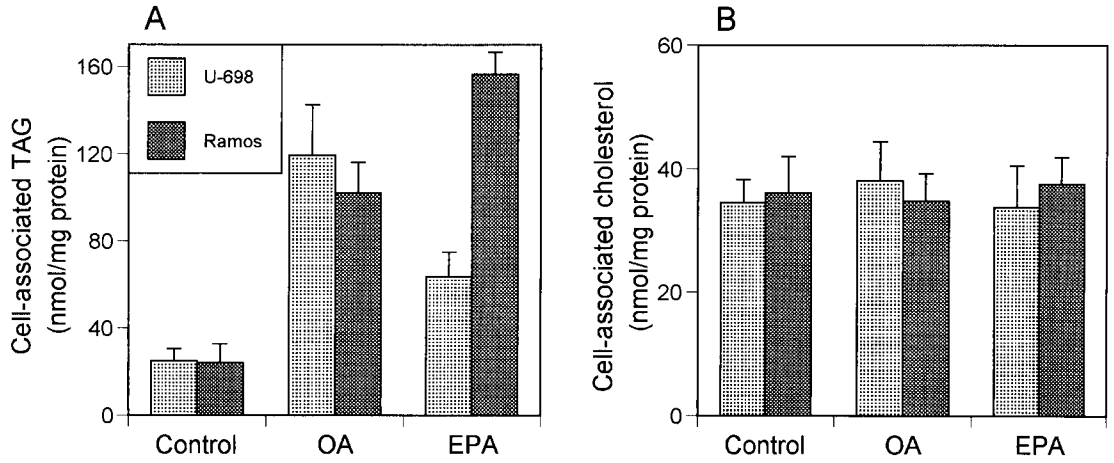

Fig. 6. Triacylglycerol content in cells incubated with EPA and OA. The cells were cultured for 12 h in the presence of 60 μm of EPA or OA, and the cellular content of TAG (A) and cholesterol (B) was measured and compared to control cells. Means ± SD from 3 separate experiments performed in duplicate are presented.

TABLE 2. Fatty acids recovered in TAG in U-698 and Ramos cells incubated with 60 μm of EPA or OA for 12 h

	U-698			Ramos		
	Control	OA	EPA	Control	OA	EPA
	nmol/mg protein					
SAFA	42 ± 3	70 ± 6	50 ± 12	54 ± 17	52 ± 10	57 ± 11
MUFA	55 ± 5	334 ± 32	48 ± 16	45 ± 4	310 ± 74	50 ± 9
PUFA	nd	nd	169 ± 76	nd	nd	531 ± 31
OA (18:1, n–9)	41 ± 3	317 ± 32	34 ± 10	33 ± 3	295 ± 69	34 ± 5
EPA (20:5, n–3)	nd	nd	107 ± 50	nd	nd	442 ± 37
DPA (22:5, n–3)	nd	nd	59 ± 24	nd	nd	79 ± 17

Means ± SD from 3 separate experiments are presented. TAG, triacylglycerol; nd, not detected; SAFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; OA, oleic acid; EPA, eicosapentaenoic acid; DPA, docosapentaenoic acid.

that cellular regulation of EPA uptake is not mediated by membrane-associated proteins. However, a different result may have been obtained using isolated plasma membrane fractions as was used when studying p-FABP-pm (51).

Because ADGAT catalyzes the final step in TAG biosynthesis and has been associated with lipid bodies (52), it was of interest to investigate whether the higher uptake of EPA and accumulation of lipid bodies in our cells could be related to different ADGAT activity in the two cell lines. ADGAT has also been reported to have variable affinity for different acyl-CoAs (53–55). However, results obtained studying ADGAT activity suggest that the rate-limiting step important for the observed difference in cellular uptake of EPA is located prior to TAG formation as equal amounts of TAG were formed using OA-CoA or EPA-CoA as substrate (Fig. 7).

In summary, the present findings suggest that a PUFA-specific ACS was responsible for the higher uptake rate of EPA in Ramos cells as compared to U-698 cells. Furthermore, as Ramos cells die by apoptosis when incubated with 60 μm EPA, whereas U-698 cells are unaffected by this PUFA, our results suggest that ACS expression may influence cellular sensitivity to PUFA. This conclusion is supported by the observation that inhibition of ACS activity in Ramos cells with the ACS inhibitor Triacsin C counteracts EPA-induced apoptosis. In future studies, it should be investigated whether ACS3 or ACS4 is responsible for the higher uptake of EPA in Ramos cells as compared to U-698 cells. Furthermore, a possible relationship between ACS expression and sensitivity to PUFA should be studied in other cell types.

We have greatly appreciated discussing our data with Dr. Asim Dutta-Roy and Dr. Fiona Campbell, Rowett Research Institute, Aberdeen, Scotland, and are very grateful for their analysis of p-FABP-pm. Dr. Jean E. Schaffer, Washington University, MO and Dr. Jeffrey Rottman, Vanderbilt University, TN kindly provided probes for FATP and H-FABP, respectively. Dr. Tokuo Yamamoto, Tohoku University Gene Research Centre, Japan, generously gave us probes for ACS1, ACS2, and ACS3. We also wish to thank Ane Gedde-Dahl, University of Oslo, Norway, for providing probes for L-FABP and I-FABP, Dr. Arild C Rustan, University of Oslo, for his measurement of ADGAT activity, and Endre Dyrøy, Haukeland Hospital, Bergen for synthesizing

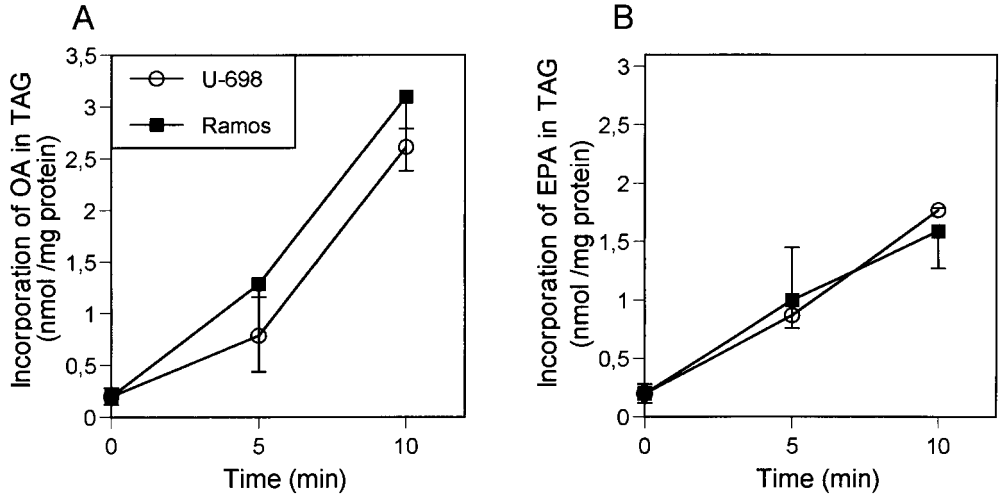

Fig. 7. Acyl-CoA:diacylglycerol acyltransferase activity in U-698 and Ramos cell lysate. Cells in logarithmic growth were harvested and lysed, and the amount of TAG formed with OA-CoA (A) and EPA-CoA (B) as substrate was determined. The means ± SD of triplicate experiments are presented (n = 3).

acyl-CoA esters. We are grateful for the skillful technical assistance of Anne Randi Alvestad, Eva Grabner, Mari-Ann Baltzersen, Anne Synstad, and Manigeh M. Beigi. This work was supported by grants from the Norwegian Cancer Society, Nansen Foundation, Familien Blix forskningsfond, Elisabeth og Knut Knutsens O.A.S Fond til Kreftforskning, Astri & Birger Torstedts Legat, Johan Throne Holst Fond and Freia Medicinske Fond. H.S.F. has been a fellow of the Norwegian Cancer Society.

Manuscript received 25 March 1999, in revised form 23 September 1999, and in re-revised form 7 January 2000.

REFERENCES

1. Kromhout, D. 1990. The importance of N−6 and N−3 fatty acids in carcinogenesis. *Med. Oncol. Tumor Pharmacother.* **7:** 173−176.
2. Kaizer, L., N. F. Boyd, V. Kriukov, and D. Tritchler. 1989. Fish consumption and breast cancer risk: an ecological study. *Nutr. Cancer.* **12:** 61−68.
3. Caygill, C. P., and M. J. Hill. 1995. Fish, n−3 fatty acids and human colorectal and breast cancer mortality. *Eur. J. Cancer Prev.* **4:** 329−332.
4. Caygill, C. P., A. Charlett, and M. J. Hill. 1996. Fat, fish, fish oil and cancer. *Br. J. Cancer.* **74:** 159−164.
5. Braden, L. M., and K. K. Carroll. 1986. Dietary polyunsaturated fat in relation to mammary carcinogenesis in rats. *Lipids.* **21:** 285−288.
6. Fritsche, K. L., and P. V. Johnston. 1990. Effect of dietary alpha-linolenic acid on growth, metastasis, fatty acid profile and prostaglandin production of two murine mammary adenocarcinomas. *J. Nutr.* **120:** 1601−1609.
7. Hendrickse, C. W., M. R. Keighley, and J. P. Neoptolemos. 1995. Dietary omega-3 fats reduce proliferation and tumor yields at colorectal anastomosis in rats. *Gastroenterology.* **109:** 431−439.
8. Mengeaud, V., J. L. Nano, S. Fournel, and P. Rampal. 1992. Effects of eicosapentaenoic acid, gamma-linolenic acid and prostaglandin E1 on three human colon carcinoma cell lines. *Prostaglandins Leukot. Essent. Fatty Acids.* **47:** 313−319.
9. Noguchi, M., M. Earashi, M. Minami, K. Kinoshita, and I. Miyazaki. 1995. Effects of eicosapentaenoic and docosahexaenoic acid on cell growth and prostaglandin E and leukotriene B production by a human breast cancer cell line (MDA-MB-231). *Oncology.* **52:** 458−464.
10. Pandalai, P. K., M. J. Pilat, K. Yamazaki, H. Naik, and K. J. Pienta. 1996. The effects of omega-3 and omega-6 fatty acids on in vitro prostate cancer growth. *Anticancer Res.* **16:** 815−820.
11. Schønberg, S. A., and F. Skorpen. 1997. Paracetamol counteracts docosahexaenoic acid-induced growth inhibition of A-427 lung carcinoma cells and enhances tumor cell proliferation in vitro. *Anticancer Res.* **17:** 2443−2448.
12. Rose, D. P., and J. M. Connolly. 1991. Effects of fatty acids and eicosanoid synthesis inhibitors on the growth of two human prostate cancer cell lines. *Prostate.* **18:** 243−254.
13. Chajes, V., W. Sattler, A. Stranzl, and G. M. Kostner. 1995. Influence of n−3 fatty acids on the growth of human breast cancer cells in vitro: relationship to peroxides and vitamin-E. *Breast Cancer Res. Treat.* **34:** 199−212.
14. Mæhle, L., E. Eilertsen, S. Mollerup, S. Schønberg, H. E. Krokan, and A. Haugen. 1995. Effects of n−3 fatty acids during neoplastic progression and comparison of in vitro and in vivo sensitivity of two human tumour cell lines. *Br. J. Cancer.* **71:** 691−696.
15. Finstad, H. S., M. C. W. Myhrstad, H. Heimli, J. Lømo, H. Kiil Blomhoff, S. O. Kolset, and C. A. Drevon. 1998. Multiplication and death-type of leukemia cell lines exposed to very long-chain polyunsaturated fatty acids. *Leukemia.* **12:** 921−929.
16. Finstad, H. S., S. O. Kolset, J. A. Holme, R. Wiger, A. K. Farrants, R. Blomhoff, and C. A. Drevon. 1994. Effect of n−3 and n−6 fatty acids on proliferation and differentiation of promyelocytic leukemic HL-60 cells. *Blood.* **84:** 3799−3809.
17. Finstad, H. S., C. A. Drevon, M. A. Kulseth, A. V. Synstad, E. Knudsen, and S. O. Kolset. 1998. Cell proliferation, apoptosis and accumulation of lipid droplets in U937-1 cells incubated with eicosapentaenoic acid. *Biochem. J.* **336:** 451−459.
18. Chen, Q., M. Galleano, and A. I. Cederbaum. 1997. Cytotoxicity and apoptosis produced by arachidonic acid in Hep G2 cells overexpressing human cytochrome P4502E1. *J. Biol. Chem.* **272:** 14532−14541.
19. Folch, J., M. Lees, and G. Sloane Stanley. 1957. A simple method for the isolation and purification of total lipids from animal tissues. *J. Biol. Chem.* **226:** 497−509.
20. Ranheim, T., A. Gedde-Dahl, A. C. Rustan, and C. A. Drevon. 1992. Influence of eicosapentaenoic acid (20:5, n−3) on secretion of lipoproteins in CaCo-2 cells. *J. Lipid Res.* **33:** 1281−1293.
21. Campbell, F. M., A. M. Clohessy, M. J. Gordon, K. R. Page, and A. K. Dutta-Roy. 1997. Uptake of long chain fatty acids by human placental choriocarcinoma (BeWo) cells: role of plasma membrane fatty acid-binding protein. *J. Lipid Res.* **38:** 2558−2568.
22. Arcari, P., R. Martinelli, and F. Salvatore. 1984. The complete sequence of a full length cDNA for human liver glyceraldehyde-3-phosphate dehydrogenase: evidence for multiple mRNA species. *Nucleic Acids Res.* **12:** 9179−9189.
23. Wilson, D. B., S. M. Prescott, and P. W. Majerus. 1982. Discovery of an arachidonoyl coenzyme A synthetase in human platelets. *J. Biol. Chem.* **257:** 3510−3515.
24. Parton, R. G., K. Prydz, M. Bomsel, K. Simons, and G. Griffiths. 1989. Meeting of the apical and basolateral endocytic pathways of the Madin-Darby canine kidney cell in late endosomes. *J. Cell Biol.* **109:** 3259−3272.
25. Mavis, R. D., R. M. Bell, and P. R. Vagelos. 1972. Effect of phospholipase C hydrolysis of membrane phospholipids on membranous enzymes. *J. Biol. Chem.* **247:** 2835−2841.
26. Coleman, R., and R. M. Bell. 1976. Triacylglycerol synthesis in isolated fat cells. Studies on the microsomal diacylglycerol acyltransferase activity using ethanol-dispersed diacylglycerols. *J. Biol. Chem.* **251:** 4537−4543.
27. Kawaguchi, A., T. Yoshimura, and S. Okuda. 1981. A new method for the preparation of acyl-CoA thioesters. *J. Biochem. (Tokyo)* **89:** 337−339.
28. Abumrad, N. A., M. R. el-Maghrabi, E. Z. Amri, E. Lopez, and P. A. Grimaldi. 1993. Cloning of a rat adipocyte membrane protein implicated in binding or transport of long-chain fatty acids that is induced during preadipocyte differentiation. Homology with human CD36. *J. Biol. Chem.* **268:** 17665−17668.
29. Alessio, M., L. De Monte, A. Scirea, P. Gruarin, N. N. Tandon, and R. Sitia. 1996. Synthesis, processing, and intracellular transport of CD36 during monocytic differentiation. *J. Biol. Chem.* **271:** 1770−1775.
30. Gordon, J. I., D. H. Alpers, R. K. Ockner, and A. W. Strauss. 1983. The nucleotide sequence of rat liver fatty acid binding protein mRNA. *J. Biol. Chem.* **258:** 3356−3363.
31. Schaffer, J. E., and H. F. Lodish. 1994. Expression cloning and characterization of a novel adipocyte long chain fatty acid transport protein [see comments]. *Cell.* **79:** 427−436.
32. Arlt, M. F., P. J. Goodfellow, and J. N. Rottman. 1996. Dinucleotide repeat in the third intron of the FABP3/MDGI putative tumor suppressor gene. *Dis. Markers.* **13:** 57−59.
33. Alpers, D. H., A. W. Strauss, R. K. Ockner, N. M. Bass, and J. I. Gordon. 1984. Cloning of a cDNA encoding rat intestinal fatty acid binding protein. *Proc. Natl. Acad. Sci. USA.* **81:** 313−317.
34. Lowe, J. B., J. C. Sacchettini, M. Laposata, J. J. McQuillan, and J. I. Gordon. 1987. Expression of rat intestinal fatty acid-binding protein in *Escherichia coli*. Purification and comparison of ligand binding characteristics with that of *Escherichia coli*-derived rat liver fatty acid-binding protein. *J. Biol. Chem.* **262:** 5931−5937.
35. Fujino, T., and T. Yamamoto. 1992. Cloning and functional expression of a novel long-chain acyl-CoA synthetase expressed in brain. *J. Biochem. (Tokyo)* **111:** 197−203.
36. Suzuki, H., Y. Kawarabayasi, J. Kondo, T. Abe, K. Nishikawa, S. Kimura, T. Hashimoto, and T. Yamamoto. 1990. Structure and regulation of rat long-chain acyl-CoA synthetase. *J. Biol. Chem.* **265:** 8681−8685.
37. Fujino, T., M. J. Kang, H. Suzuki, H. Iijima, and T. Yamamoto. 1996. Molecular characterization and expression of rat acyl-CoA synthetase 3. *J. Biol. Chem.* **271:** 16748−16752.
38. Drevon, C. A., and T. Hovig. 1977. The effects of cholesterol/fat feeding on lipid levels and morphological structures in liver, kidney and spleen in guinea pigs. *Acta Pathol. Microbiol. Scand. [A]* **85A:** 1−18.
39. Minekura, H., T. Fujino, M. J. Kang, T. Fujita, Y. Endo, and T. T. Yamamoto. 1997. Human acyl-coenzyme A synthetase 3 cDNA and

localization of its gene (ACS3) to chromosome band 2q34-q35. *Genomics*. **42:** 181–182.
40. Kang, M. J., T. Fujino, H. Sasano, H. Minekura, N. Yabuki, H. Nagura, H. Iijima, and T. T. Yamamoto. 1997. A novel arachidonate-preferring acyl-CoA synthetase is present in steroidogenic cells of the rat adrenal, ovary, and testis. *Proc. Natl. Acad. Sci. USA*. **94:** 2880–2884.
41. Piccini, M., F. Vitelli, M. Bruttini, B. R. Pober, J. J. Jonsson, M. Villanova, M. Zollo, G. Borsani, A. Ballabio, and A. Renieri. 1998. FACL4, a new gene encoding long-chain acyl-CoA synthetase 4, is deleted in a family with Alport syndrome, elliptocytosis, and mental retardation. *Genomics*. **47:** 350–358.
42. Cao, Y., E. Traer, G. A. Zimmerman, T. M. McIntyre, and S. M. Prescott. 1998. Cloning, expression and chromosomal localization of human long-chain fatty acid-CoA ligase 4 (FACL4). *Genomics*. **49:** 327–330.
43. Uriel, J., J. M. Torres, and A. Anel. 1994. Carrier-protein-mediated enhancement of fatty-acid binding and internalization in human T-lymphocytes. *Biochim. Biophys. Acta*. **1220:** 231–240.
44. Schurer, N. Y., W. Stremmel, J. U. Grundmann, V. Schliep, H. Kleinert, N. M. Bass, and M. L. Williams. 1994. Evidence for a novel keratinocyte fatty acid uptake mechanism with preference for linoleic acid: comparison of oleic and linoleic acid uptake by cultured human keratinocytes, fibroblasts and a human hepatoma cell line. *Biochim. Biophys. Acta*. **1211:** 51–60.
45. Johnson, D. R., L. J. Knoll, D. E. Levin, and J. I. Gordon. 1994. Saccharomyces cerevisiae contains four fatty acid activation (FAA) genes: An assessment of their role in regulating protein N-Myristoylation and cellular lipid metabolism. *J. Cell Biol*. **127:** 751–762.
46. Neufeld, E. J., T. E. Bross, and P. W. Majerus. 1984. A mutant HSDM1C1 fibrosarcoma line selected for defective eicosanoid precursor uptake lacks arachidonate specific acyl-CoA synthetase. *J. Biol. Chem*. **259:** 1986–1992.
47. Triggiani, M., A. Oriente, G. de Crescenzo, G. Rossi, and G. Marone. 1995. Biochemical functions of a pool of arachidonic acid associated with triglycerides in human inflammatory cells. *Int. Arch. Allergy. Immunol*. **107:** 261–263.
48. Triggiani, M., V. De Marino, G. de Crescenzo, and G. Marone. 1997. Arachidonic acid remodeling in human inflammatory cells migrating to the lung in vivo. *Int. Arch. Allergy. Immunol*. **113:** 190–192.
49. Triggiani, M., A. Oriente, M. C. Seeds, D. A. Bass, G. Marone, and F. H. Chilton. 1995. Migration of human inflammatory cells into the lung results in the remodeling of arachidonic acid into a triglyceride pool. *J. Exp. Med*. **182:** 1181–1190.
50. Mangroo, D., B. L. Trigatti, and G. E. Gerber. 1995. Membrane permeation and intracellular trafficking of long chain fatty acids: insights from *Escherichia coli* and 3T3-L1 adipocytes. *Biochem. Cell Biol*. **73:** 223–234.
51. Campbell, F. M., S. Taffesse, M. J. Gordon, and A. K. Dutta-Roy. 1995. Plasma membrane fatty-acid-binding protein in human placenta: indentification and characterization. *Biochem. Biophys. Res. Commun*. **209:** 1011–1017.
52. Kamisaka, Y., S. Mishra, and T. Nakahara. 1997. Purification and characterization of diacylglycerol acyltransferase from the lipid body fraction of an oleaginous fungus. *J. Biol. Chem*. **121:** 1107–1114.
53. Little, D., R. Weselake, K. Pomeroy, T. Furukawa-Stoffer, and J. Bagu. 1994. Solubilization and characterization of diacylglycerol acyltransferase from microspore-derived cultures of oilseed rape. *Biochem. J*. **304:** 951–958.
54. Stals, H. K., W. Top, and P. E. Declercq. 1994. Regulation of triacylglycerol synthesis in permeabilized rat hepatocytes. Role of fatty acid concentration and diacylglycerol acyltransferase. *FEBS Lett*. **343:** 99–102.
55. Rustan, A. C., J. O. Nossen, E. N. Christiansen, and C. A. Drevon. 1988. Eicosapentaenoic acid reduces hepatic synthesis and secretion of triacylglycerol by decreasing the activity of acyl-coenzyme A:1,2-diacylglycerol acyltransferase. *J. Lipid Res*. **29:** 1417–1426.