Membrane Lipid Composition of Pancreatic AR42J Cells: Modification by Exposure to Different Fatty Acids

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Dietary fat type influences fatty acids in rat pancreatic membranes, in association with modulation of secretory activity and cell signalling in viable acini. We aimed to confirm whether AR42J cells are a valid model to study the interactions between lipids and pancreatic acinar cell function. For this purpose we have (i) compared the baseline fatty acid composition of AR42J cells with that of pancreatic membranes from rats fed a standard chow; (ii) investigated if fatty acids in AR42J membranes can be modified in culture; and (iii) studied if similar compositional variations that can be evoked in rats when dietary fat type is altered occur in AR42J cells. Weaning Wistar rats were fed for 8 weeks either a commercial chow (C) or semi-purified diets containing virgin olive oil (VOO) or sunflower oil (SO) as fat source. AR42J cells were incubated for 72 hrs in medium containing unmodified fetal calf serum (FCS, AR42J-C cells), FCS enriched with 18:1 n-9 (AR42J-O cells), or FCS enriched with 18:2 n-6 (AR42J-L cells). Fatty acids in crude membranes from rat pancreas and AR42J cells were determined by gas–liquid chromatography. Differences in membrane fatty acids between C rats and AR42J-C cells can be explained in part by variations in the amount of fatty acids in the extracellular environment. Supplementation of FCS with 18:1 n-9 or 18:2 n-6 changed the fatty acid spectrum of AR42J cells in a manner that resembles the pattern found, respectively, in VOO and SO rats, although AR42J-L cells were unable to accumulate 20:4 n-6. The AR42J cell line can be a useful tool to assess the effect of membrane compositional changes on acinar cell function. However, differences in baseline characteristics, and perhaps fatty acid metabolism, indicate that results obtained in AR42J cells should be confirmed with experiments in the whole animal.

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Introduction

Recent research in nutritional science is trying to elucidate the effect of dietary lipids on membrane composition and function. Membrane fatty acid composition can be modified in many different types of mammalian cells. The changes can be of sufficient magnitude to affect a number of cellular functions, including carrier-mediated transport, transduction pathways, activity of membrane-bound enzymes, receptor binding, and exocytosis processes (1–4). Many of the functional responses are probably caused directly by the membrane lipid structural changes, which affect either bulk lipid fluidity or specific lipid domains. The conformation of some transporters, receptors, and enzymes may be sensitive to changes in lipid microenvironment, thus leading to changes in activity.

Studies in animal models have provided evidence about the effects of dietary fats on physiologic and pathologic processes. Work in our laboratory confirmed that the type of dietary fat strongly influences the fatty acid composition of rat (5) and rabbit (6) pancreatic membranes. In the rat study, this was accompanied by a change in the secretory activity and mobilization of intracellular Ca^{2+} stimulated by cholecystokinin-octapeptide (CCK-8) in viable pancreatic acini (5, 7). Interestingly, by using the method of direct cannulation of the pancreatic duct in anaesthetized rats, we have been able to find a modification of CCK-induced secretory output as a function of the type of fat previously fed (8), which indicates that the modulating effect of this nutrient on exocrine pancreatic function in not limited to the cellular level. In a more recent study (9), we have observed that chronic intake of diets differing in the fat type can also

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influence the responsiveness of acinar cells to acetylcholine, likely in relation to dietary-induced changes in cell membrane composition.

The effects of lipid modification on cell function are complex. Not only can they vary from one tissue to another, but they also are not exerted uniformly on all processes in a single cell line. For this reason, it is not possible to make generalizations or to predict how a given system will respond to a particular lipid alteration. Within this context, cell culture appears to be a good approach for investigating the molecular aspects of this problem. A much wider array of modifications is possible in cultured cells than in intact animals, and the environmental conditions can be controlled better. Because of homogeneity in morphology and composition, cell culture systems provide us with an elevated number of identical replicas, thus avoiding the serious problem of samples heterogeneity inherent to animal experimentation. Another valuable advantage, in terms of budget and time economy, is that animals require long periods of adaptation to dietary fats, whereas adaptation of cultured cells is achieved in a few days. In addition, ethical issues raised by animal experimentation support the use of cell culture. While cultured cells and tissues cannot replace the *in vivo* systems, they are valid alternative models in many situations.

The AR42J cell line is the only currently available cell line that maintains many characteristics of normal pancreatic acinar cells, such as the synthesis and secretion of digestive enzymes (10). AR42J cells show receptor expression and signal transduction mechanisms parallel to those of normal pancreatic acinar cells (11), and have been widely used to study secretion, signal transduction, cytoskeleton function, apoptosis, and pancreatitis responses of the exocrine pancreas (12–18). Thus, we thought that this cell line could be a suitable *in vitro* model for our purpose of examining the molecular mechanisms of the effect of dietary lipids on membrane composition and cell function. Surprisingly, we found that, in spite of the wide usage in recent years, no information is available on the baseline lipid composition of AR42J cell membranes, and there are no attempts made in recorded scientific literature to modify the fatty acid composition of AR42J cells in culture. Our aim in the current study was to determine the membrane fatty acid composition of AR42J cells, to investigate whether these cells adapt their membranes after exposure to different fatty acids in the culture medium, and to confirm if this process is similar to the adaptation of the rat exocrine pancreas that occurs when dietary fat intake is modified (5, 8, 9). To achieve our objectives, two separate groups of weaning rats were fed over 8 weeks on diets containing either virgin olive oil or sunflower oil as the fat source. In turn, culture medium for AR42J cells was supplemented with 18:1 n-9 or 18:2 n-6, the two major components of olive oil and sunflower oil, respectively.

Materials and Methods

Materials. Unless otherwise stated, all chemicals and solvents of the highest grade available were obtained from Sigma (St. Louis, MO, USA) and Merck (Darmstadt, Germany).

Animals and Diets. Twenty-seven male weaning Wistar rats (weight 40–55 g; Animal Farm of the University of Granada, Granada, Spain) were divided into three groups (9 each) so that the average weight per group was the same. A first group, which served as control (C), was fed for 8 weeks with a standard cereal-based chow for this species (Panlab A04, Panlab Laboratories, Barcelona, Spain). The chow was composed of 15.4% protein, 2.9% fat, 60.5% carbohydrates, 3.9% fiber, 5.3% minerals, and 12% water (data from the manufacturer's analyses). The other two groups of rats were fed over the same period with two semi-purified diets that were a modified version of the AIN-93G diet (19). These semi-purified diets were prepared in powdered form by mixing the fresh, chemically defined ingredients, according to (%): casein, 20; maize starch, 36.75; dextrose, 13.2; sucrose, 10; cellulose, 5; fat, 10; L-cystine, 0.3; choline bitartrate, 0.25; AIN-93G mineral mixture, 3.5; AIN-93G vitamin mixture, 1. The sources of fat were commercial edible oils obtained locally: virgin olive oil (VOO rats, Fedeoliva, S.A., Jaén, Spain) or sunflower oil (SO rats, Koipe, S.A., Jaén, Spain). The VOO and SO diets were prepared at the Nutrition Unit of the Animal Farm (University of Granada, Granada, Spain), packed in plastic bags, sealed, and sent to our laboratory. Upon arrival, aliquots were taken for determination of water content by drying to constant weight in oven at 105°C. Average water content was 10% in both semi-purified diets. All diets were stored in darkness at 4°C until used to avoid peroxidation. The fatty acid composition of the diets (Table 1) was determined by gas–liquid chromatography (GLC) as described later for the membrane fractions.

During the 8-week adaptation period to the diets, animals were housed individually in a temperature-controlled room (22 ± 1°C), kept on a 12:12-hr light:dark cycle and given free access to water and food. All procedures were approved by the Ethics Committees of the University of Granada and the Spanish Ministry of Science and Technology. Animals were handled according to the guidelines of the Spanish Society for Laboratory Animal Sciences and killed humanely.

Cell Culture. Rat pancreatoma AR42J cell line, proceeding from the European Collection of Cell Cultures (ECACC N°: 93100618), was supplied by the Cell Bank of the Scientific Instrumentation Centre at the University of Granada (CIC-UGR, Granada, Spain). Cells were routinely cultured at 37°C in a 5% CO_2/95% air atmosphere in RPMI-1640 medium containing 10% fetal calf serum (FCS) and 2 m*M* glutamine. AR42J cells were used following treatment with 100 n*M* dexamethasone in culture medium for 72 hrs to induce differentiation (13, 14).

Maintenance, culture, and chronic treatments (dexamethasone and fatty acids) of AR42J cells were carried out in the Tissue and Cell Culture Unit of the CIC-UGR (Granada, Spain) in order to optimize cell production while avoiding the risk of bacterial contamination. Cells were transferred to our laboratory for analyses.

Membrane fatty acid modifications in AR42J cells were evoked during the 72-hr differentiation period according to a simple protocol based on a modification of the method of Chow et al. (20). Briefly, fetal calf serum (FCS) was enriched in either 18:1 n-9 or 18:2 n-6 by addition of necessary volume of stock solutions (100 m*M* in absolute ethanol). This mixture was equilibrated for 1 hr at 37°C and 5% CO_2. During this time, 3 sonication pulses of 30 secs each were applied to facilitate binding of fatty acids to FCS proteins. FCS enriched with 18:1 n-9 or 18:2 n-6 was then added to the culture medium (AR42J-O and AR42J-L cells, respectively). Final concentration of added fatty acids in medium was 50 μ*M*. Fatty acids were obtained from Sigma and were approximately 99% pure and cell culture tested. The pH of the medium did not differ with the addition of fatty-acid enriched serum. Control cells (AR42J-C cells) were fed the culture medium containing the intact, unmodified FCS. The viability of fatty acid–supplemented and not supplemented cells was found to be 95%–98% by trypan blue exclusion method.

Collection of Crude Membranes. At the end of the 8-week feeding period, overnight-fasted rats were killed by cervical dislocation. Each pancreas was quickly removed; trimmed free of fat, connective tissue, and lymph nodes in cold saline solution (0.9% NaCl); blotted, and weighed. AR42J cells were detached mechanically from culture flasks by gentle pippeting and washed twice in cold phosphate-buffered saline (PBS). Collection of crude membranes from rat pancreas and AR42J pancreatic acinar cells was accomplished by established methods (21).

Analysis of Cell Membranes. Lipid extraction and fatty acid methylation was done in a one-step reaction (22). A GLC system, model HP 5890 series II (Hewlett Packard, Palo Alto, CA, USA), equipped with an automatic injector and a flame ionization detector, was used to analyze fatty acids as methyl esters. GLC was performed using a 60-m–long capillary column (32 mm i.d. and 20 mm thickness) impregnated with Sp 2330 FS (Supelco, Bellefonte, PA, USA).

Electron Microscopy. AR42J cells were processed by conventional procedures for electron microscopy (23) at the Biological Sample Preparation Laboratory of the CIC-UGR (Granada, Spain). In brief, cells were prefixed for 2 hrs at 0°–4°C in 1.5% glutaraldehyde and 1% formaldehyde in 50 m*M* sodium cacodylate buffer (pH 7.4). The cultures were postfixed with 1% OsO_4 and 1% potassium ferricyanide for 1 hr at 0°–4°C in the dark, and passed through 0.15% tanic acid in the above buffer, followed by treatment with 2% uranyl acetate for 2 hrs in darkness. Increasing concentrations of ethanol (50, 75, 90, 95, and 3× 100%) were used (5 mins each, 0°–4°C) in the process of dehydration. The samples were then embedded in Epon epoxy resin through an ethanol:Epon series. Ultra-thin sections (500–700 μm) were cut, collected on copper grids, poststained with uranyl acetate and lead citrate, and examined with a Zeiss EM 902 transmission electron microscope (Zeiss, Oberkochen, Germany) at 80 kV by personnel of the Microscopy Service of the CIC-UGR (Granada, Spain).

Table 1. Fatty Acid Composition of the Experimental Diets and Fetal Calf Serum (FCS) Used, Respectively, in the Rat and Cell Culture (AR42J) Studies[a]

	Rat study			AR42J study
Fatty acid	VOO diet	SO diet	Commercial chow	FCS[b]
16:0	9.81	6.35	20.27	28.24
16:1 n-7	0.63	0.12	0.86	2.40
18:0	3.96	4.37	6.43	17.28
18:1 n-9	76.75	31.19	28.08	21.37
18:2 n-6	6.81	55.92	34.49	6.78
18:3 n-3	0.66	0.07	2.64	0.00
SFA	14.02	10.87	29.21	57.06
MUFA	77.67	31.48	31.21	25.68
PUFA	8.31	57.65	39.58	17.26
UFA	85.98	89.13	70.79	42.94
SFA/UFA	0.16	0.12	0.41	1.33

[a] Values are percentages of total fatty acid content (mean values of four replicates). VOO, virgin olive oil; SO, sunflower oil; FCS, fetal calf serum; SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; UFA, unsaturated fatty acids.
[b] Intact, unmodified FCS.

Statistical Analysis. Unless otherwise expressly noted, results in the text, tables, and graphs are reported as means ± SEM. One-way analysis of variance (ANOVA) was performed to compare membrane fatty acid content between groups, and Tukey's HSD (honestly significant difference) was used for all *post hoc* comparisons (SPSS for Windows, version 13.0.1, 2005; SPSS Inc., Chicago, IL, USA). Values were considered to be significantly different when $P < 0.05$.

Results

Transmission Electron Microscopy (TEM). As a part of our objective of finding a cultured line of pancreatic acinar cells that could serve as an appropriate *in vitro* model to study the interaction between dietary lipids and membrane composition and function, it was pertinent to confirm dexamethasone-induced differentiation of AR42J cells. Figure 1 shows a typical AR42J cell cultured with unmodified, intact, fetal calf serum in the absence (A) or presence (B) of 100 n*M* dexamethasone. At the ultrastructural level, the most striking effect of dexamethasone treatment for 72 hrs concerned the secretory granules. The results from our analysis showed that whereas in non-treated

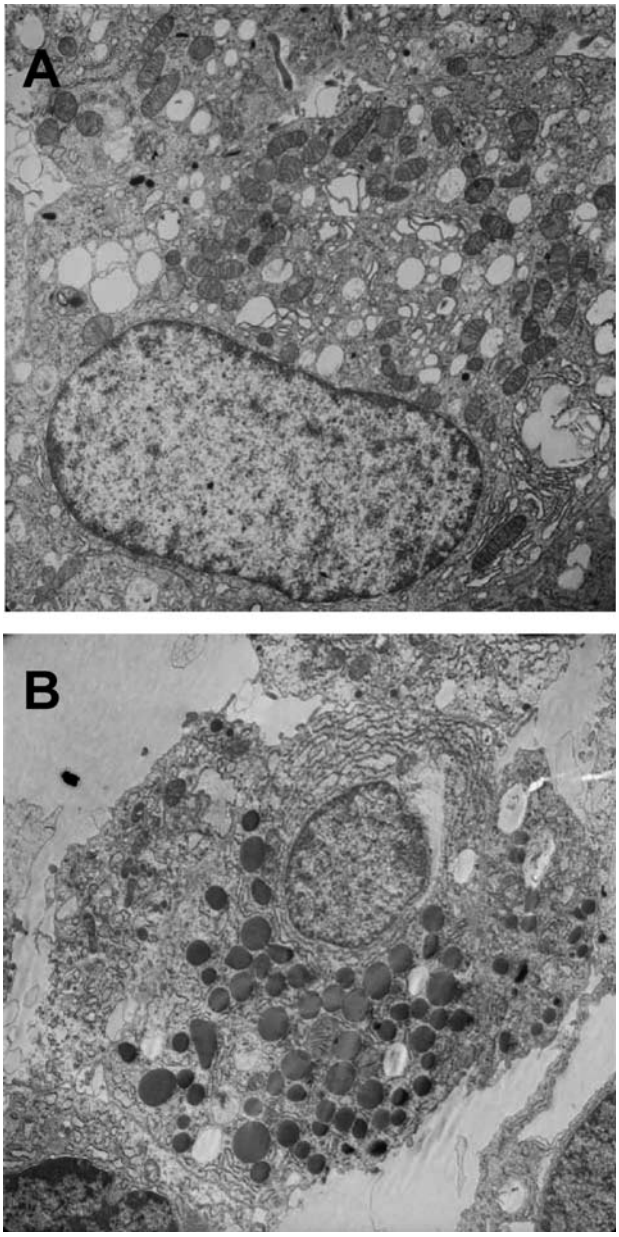

Figure 1. Electron micrograph of a typical AR42J cell cultured with unmodified fetal calf serum in the absence (A) or presence (B) of 100 n*M* dexamethasone for the 72 hrs prior to sample collection. Magnification: ×4400 for both A and B.

cells zymogen granules were rarely seen (Fig. 1A), in dexamethasone-treated cells the granules were very prominent, both in number and size (Fig. 1B). In addition, untreated cells looked relatively undifferentiated, with sparse rough endoplasmic reticulum consisting of vesicles and isolated small cisternae (Fig. 1A). Dexamethasone treatment, however, increased the amount of rough endoplasmic reticulum, which appeared as large stacked cisternae (Fig. 1B). So, the results from our analyses indicate that dexamethasone induced in this study the differentiation of AR42J cells towards an exocrine phenotype similar to that of a rat pancreatic acinar cell. There was no difference in the ability of dexamethasone to induce AR42J cell differentiation in the presence of fatty acid-supplemented serum compared with the unsupplemented one.

Baseline Fatty Acid Profiles of Crude Membranes from Rat Pancreas and AR42J Cells. Fatty acid profile of membranes from differentiated control AR42J cells (AR42J-C cells) and control rat (C rat) pancreas is detailed in Table 2. Major membrane fatty acids in AR42J-C cells, listed in decreasing order of abundance, were (mean values): 16:0 (28.05%), 18:1 n-9 (25.69%), and 18:0 (16.41%). Content of 18:3 n-3, 20:4 n-6, 16:1 n-7, 14:0, 18:2 n-6, and 20:0 was between 2%–4%. Among minor fatty acids (<2%) in membranes from AR42J-C cells, those that should be noted include: 22:6 n-3 (mean value of 1.87%), 20:3 n-6 (1.44%), 22:0 (1.39%), and 24:0 (1.19%).

Fatty acids in pancreatic membranes from C rats, listed in decreasing order of abundance, were (mean values): 16:0 (31.14%), 20:4 n-6 (18.10%), 18:2 n-6 (14.52%), 18:1 n-9 (13.84%), 18:0 (10.07%), and 16:1 n-7 (5.45%). For the rest of fatty acids detected, content was lower than 2% (see Table 2).

Comparison among C rats and AR42J-C cells showed that the percentage of most fatty acid differed significantly ($P < 0.05$), with only minor fatty acids such as 15:0, 17:0, 20:2 n-6, and 21:0 being similar. The most abundant fatty acid in both AR42J-C cells and pancreatic membranes from C rats was 16:0, although the content in rats (31.14 $\pm$ 0.32%) was significantly greater ($P < 0.05$) than that in cultured cells (28.05 $\pm$ 0.21%). After 16:0, leading fatty acids in the rat were (mean values): 20:4 n-6 (18.10% vs. 3.80% in AR42J-C cells, $P < 0.05$) and 18:2 n-6 (14.52% vs. 3.33% in AR42J-C cells, $P < 0.05$). In turn, leading fatty acids (after 16:0) in cultured AR42J-C cells were: 18:1 n-9 (25.69 % vs. 13.84 % in the rat, $P < 0.05$) and 18:0 (16.41% vs. 10.07 % in the rat, $P < 0.05$).

Percent values for fatty acids were also distributed differently in rat and AR42J membranes. In the rat, five fatty acids exhibited percent values higher than 10% (16:0, 20:4 n-6, 18:2 n-6, 18:1 n-9, and 18:0), then there was 16:1 n-7 with approximately 5%, and the remaining fatty acids showed values lower than 1.5%. In contrast, there are three major fatty acids in AR42J-C cells with values higher than 10% (16:0, 18:1 n-9, and 18:0), and six fatty acids in the range of 2%–4%, the rest being minor fatty acids.

Differences in individual fatty acids between rat and cultured cells are reflected in indices, as summarized in Figure 2. AR42J-C cells had a higher ($P < 0.05$) proportion of saturated fatty acids (SFA), monounsaturated fatty acids (MUFA), and n-3 polyunsaturated fatty acids (n-3 PUFA), whereas total PUFA and, particularly, n-6 PUFA percentages were higher ($P < 0.05$) in rat pancreas (Fig. 2A). All percent ratios were also significantly ($P < 0.05$) different (Fig. 2B).

Effect of Feeding the Experimental Diets for 8

Table 2. Fatty Acid Profile of Pancreatic Crude Membranes from Control Rats (C Rats) and Control AR42J Cells (AR42J-C Cells)[a]

Fatty acid	C rats[b]	AR42J-C cells[c]
6:0	0.00 ± 0.00 *	0.37 ± 0.04
10:0	0.00 ± 0.00 *	0.45 ± 0.06
12:0	0.08 ± 0.01 *	0.46 ± 0.03
14:0	1.36 ± 0.04 *	3.40 ± 0.20
14:1 n-5	0.09 ± 0.01 *	0.00 ± 0.00
15:0	0.21 ± 0.01 [d]	0.34 ± 0.02
16:0	31.14 ± 0.32 *	28.05 ± 0.21
16:1 n-7	5.45 ± 0.12 *	3.47 ± 0.20
17:0	0.23 ± 0.01 [d]	0.34 ± 0.02
18:0	10.07 ± 0.10 *	16.41 ± 0.26
18:1 n-9	13.84 ± 0.30 *	25.69 ± 0.41
18:2 n-6	14.52 ± 0.85 *	3.33 ± 0.12
18:3 n-6	0.39 ± 0.03 *	0.00 ± 0.00
18:3 n-3	0.13 ± 0.01 *	3.83 ± 0.20
20:0	0.22 ± 0.02 *	2.29 ± 0.17
20:1 n-9	0.35 ± 0.04 *	0.00 ± 0.00
20:2 n-6	0.19 ± 0.01 [d]	0.19 ± 0.02
20:3 n-6	0.43 ± 0.02 *	1.44 ± 0.15
20:4 n-6	18.10 ± 0.23 *	3.80 ± 0.11
20:5 n-3	0.35 ± 0.01 *	0.58 ± 0.02
21:0	0.00 ± 0.00 [d]	0.00 ± 0.00
22:0	0.24 ± 0.03 *	1.39 ± 0.10
22:1 n-9	0.00 ± 0.00 *	0.50 ± 0.03
22:6 n-3	1.04 ± 0.04 *	1.87 ± 0.10
23:0	0.05 ± 0.01 *	0.33 ± 0.02
24:0	0.48 ± 0.02 *	1.19 ± 0.06
24:1 n-9	1.06 ± 0.01 *	0.85 ± 0.03

[a] Values are percentages of total fatty acid content (means ± SEM) C rats: $n = 9$; AR42J-C cells: $n = 18$ (from 6 batches of cells).
[b] C rats were fed a commercial chow for 8 weeks after weaning.
[c] AR42J-C cells were fed the culture medium containing unmodified fetal calf serum for the 72-hr period (differentiation period) before the experiments.
[d] Not significant.
* $P < 0.05$ compared with membranes from AR42J-C cells.

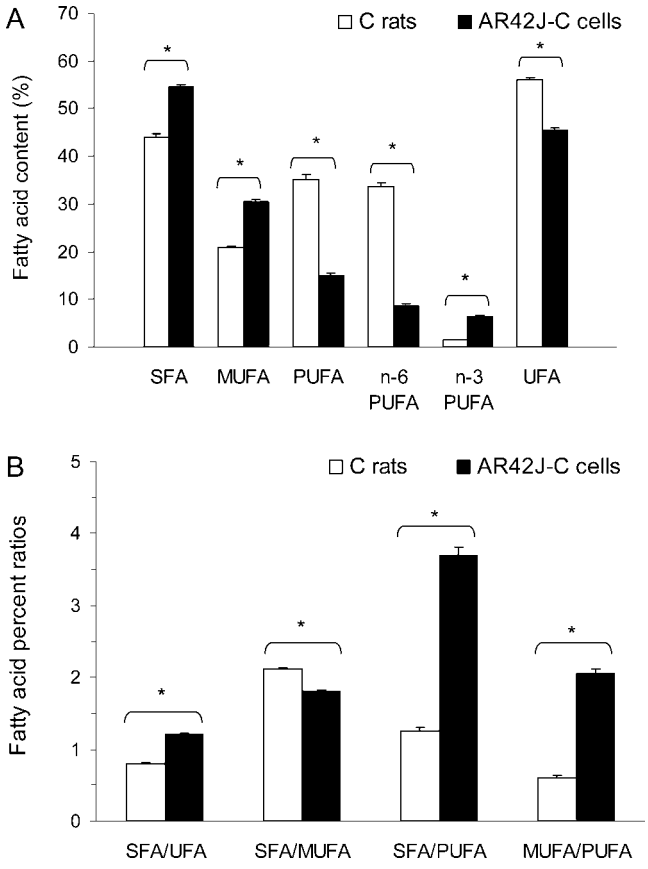

Figure 2. Fatty acid profile of pancreatic crude membranes from control rats (C rats) and control AR42J cells (AR42J-C cells). C rats were fed with a commercial chow for 8 weeks after weaning. AR42J-C cells were fed the culture medium containing the unmodified fetal calf serum for the 72 hrs prior to sample collection. SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; UFA, unsaturated fatty acids. (A) Fatty acid indices are expressed as percentages of total fatty acid content. (B) Fatty acid indices are expressed as percent ratios. Values are means ± SEM. C rats: $n = 9$; AR42J-C cells: $n = 18$ (from 6 batches of cells). Asterisks above the bars denote significant differences ($P < 0.05$) between C rats and AR42J-C cells.

Weeks on Rat Pancreatic Crude Membranes. The fatty acid profile of pancreatic membranes (Table 3 and Fig. 3) was profoundly influenced by dietary treatments. Compared to control (C) rats, pancreatic membranes of rats fed the virgin olive oil (VOO) diet displayed higher ($P < 0.05$) levels of 18:1 n-9 and 20:5 n-3, and lower ($P < 0.05$) levels of 16:0, 16:1 n-7, 18:2 n-6, and 20:4 n-6 (Table 3). Content of 14:0, 18:0, 18:3 n-3, and 22:6 n-3 did not vary among C rats and VOO rats. As shown in Fig. 3A, the VOO diet caused a significant increase in MUFA (due to 18:1 n-9) compared to C rats, which was balanced by a decrease ($P < 0.05$) in SFA (due to 16:0) and a decrease in n-6 PUFA (due to both 18:2 n-6 and 20:4 n-6).

When compared with rats fed the standard chow (C rats), pancreatic membranes of rats given the sunflower oil diet (SO) for 8 weeks showed (Table 3) a significant ($P < 0.05$) increase in 18:0, 18:2 n-6, and 20:4 n-6, together with a significant decrease in 16:0, 16:1 n-7, 20:5 n-3, and 22:6 n-3. Values for 14:0, 18:1 n-9, and 18:3 n-3 were similar in C and SO rats. As summarized in Figure 3A, feeding rats the SO diet instead of a commercial chow (C) increased n-6 PUFA in membranes, not only due to 18:2 n-6, the most abundant fatty acid in sunflower oil, but also through the contribution of its main metabolite, 20:4 n-6. The increase in n-6 PUFA (Fig. 3A) was achieved at the expense of SFA (16:0) and MUFA (16:1 n-7).

Compared with the other two groups, VOO rats showed the highest values for 18:1 n-9, MUFA, and MUFA/PUFA and the lowest values for SFA/MUFA. In turn, membranes prepared from SO rats displayed the highest values for 18:2 n-6, PUFA, n-6 PUFA, and SFA/MUFA. The SFA/UFA ratio, which showed a mean value of 0.78 in rats fed a standard chow (C rats), was significantly reduced after intake of the VOO and SO diets (Fig. 3B), reaching comparable values of 0.65 and 0.67, respectively. It is interesting that, despite marked differences in the content of a number of individual fatty acids as well as in total MUFA

Table 3. Selected Fatty Acids in Pancreatic Membranes from Rats Fed a Commercial Chow (C), a Virgin Olive Oil Diet (VOO) or a Sunflower Oil Diet (SO) for 8 Weeks After Weaning[a]

Fatty acid	C rats	VOO rats	SO rats
14:0	1.36 ± 0.04 [a]	0.95 ± 0.06 [a]	0.84 ± 0.04 [a]
16:0	31.14 ± 0.32 [a]	27.01 ± 0.24 [b]	25.36 ± 0.20 [c]
16:1 n-7	5.45 ± 0.12 [a]	2.84 ± 0.44 [b]	1.93 ± 0.17 [b]
18:0	10.07 ± 0.10 [a]	9.92 ± 0.30 [a]	12.26 ± 0.32 [b]
18:1 n-9	13.84 ± 0.30 [a]	32.41 ± 1.06 [b]	11.49 ± 0.72 [a]
18:2 n-6	14.52 ± 0.85 [a]	7.45 ± 0.14 [b]	22.21 ± 0.69 [c]
18:3 n-3	0.13 ± 0.01 [a]	0.34 ± 0.03 [a]	0.13 ± 0.01 [a]
20:4 n-6	18.10 ± 0.23 [a]	14.36 ± 0.93 [b]	21.46 ± 0.94 [c]
20:5 n-3	0.35 ± 0.01 [a]	0.69 ± 0.02 [b]	0.03 ± 0.00 [c]
22:6 n-3	1.04 ± 0.04 [a]	1.06 ± 0.07 [a]	0.12 ± 0.01 [b]

[a] Values are percentages of total fatty acid content (means ± SEM, $n = 9$ for all groups). For a particular row, values with different superscript letters are significantly different at $P < 0.05$.

and PUFA, the value for SFA/UFA was the same in VOO and SO animals.

Effect of Supplementation of Culture Medium with 18:1 n-9 or 18:2 n-6 on the Fatty Acid Profile of AR42J Cells. Table 4 and Figure 4 show the changes in membrane fatty acid composition of AR42J cells grown for 72 hrs with fetal calf serum (FCS) supplemented with either 18:1 n-9 (AR42J-O) or 18:2 n-6 (AR42J-L). Enrichment with those fatty acids profoundly influenced the fatty acid composition of AR42J cell membranes.

Compared to control cells (AR42J-C), addition of 18:1 n-9 to culture medium (AR42J-O) evoked a significant ($P < 0.05$) increase in the membrane content of 14:0, 18:1 n-9, and 18:3 n-3, and a significant ($P < 0.05$) decrease in 18:0, 20:4 n-6, 20:5 n-3, and 22:6 n-3. The proportion of 16:0, 16:1 n-7, and 18:2 n-6 did not vary among AR42J-O and AR42J-C cells (Table 4). Fatty acid indices (Fig. 4) summarize the effects of enrichment with 18:1 n-9. With this fatty acid in culture medium, the MUFA content of membranes increased significantly (due to 18:1 n-9) in comparison with control cells (AR42J-C), an increase that was compensated by a significant ($P < 0.05$) decrease in SFA (due to 18:0) and a significant decrease in n-6 PUFA (mainly due to 20:3 n-6 [data not shown] and 20:4 n-6).

Compared with cells grown in unmodified serum (AR42J-C), membranes of cells cultured in the presence of 50 μM 18:2 n-6 showed a significant ($P < 0.05$) increase in the proportions of 14:0 and 18:2 n-6 and a significant ($P < 0.05$) decrease in 18:0, 18:1 n-9, 20:4 n-6, 20:5 n-3, and 22:6 n-3. Percent values of 16:0, 16:1 n-7, and 18:3 n-3 did not change (Table 4). Indices (Fig. 4) outline the changes in membrane fatty acids evoked by supplementation with 18:2 n-6. We can see that compared with control cells, AR42J-L suffered a large increase in n-6 PUFA (Fig. 4A). This increase was due to the incorporation of 18:2 n-6 itself, given that 18:2 n-6 metabolites either remained unchanged (20:3 n-6, data not shown) or even decreased significantly

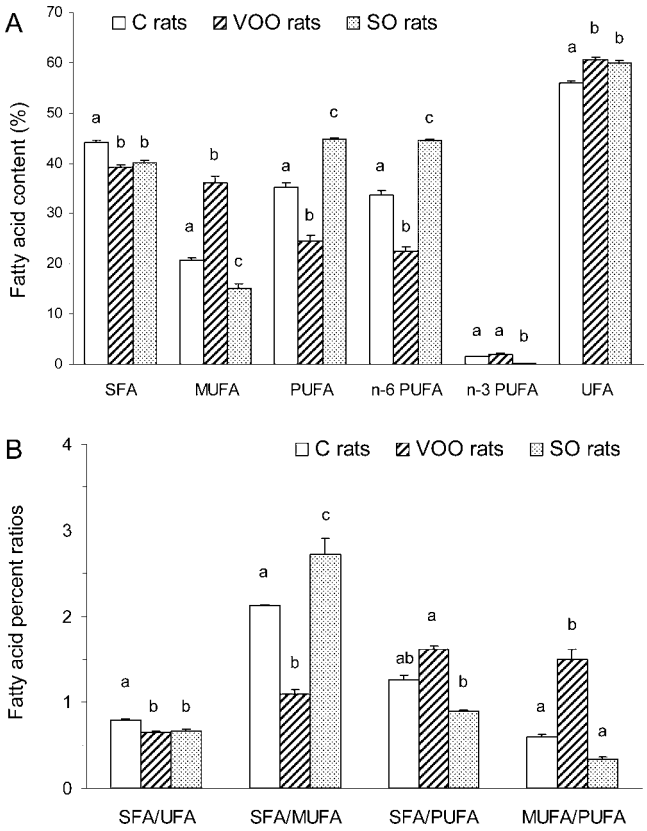

Figure 3. Fatty acid profile of pancreatic crude membranes from rats fed a commercial chow (C rats), a virgin olive oil diet (VOO rats) or a sunflower oil diet (SO rats) for 8 weeks after weaning. SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; UFA, unsaturated fatty acids. (A) Fatty acid indices are expressed as percentages of total fatty acid content. (B) Fatty acid indices are expressed as percent ratios. Values are means ± SEM. $n = 9$ for all groups. For a given index, different letters above the bars denote significant differences ($P < 0.05$) between dietary groups.

(20:4 n-6, see Table 4). Increase in n-6 PUFA was done at the expense of SFA (18:0) and MUFA (18:1 n-9).

In comparison with the remaining two treatments, the presence of 18:1 n-9 in the culture medium (AR42J-O) evoked in cells the highest values for 18:1 n-9, MUFA, MUFA/PUFA, and SFA/PUFA and the lowest values for SFA/MUFA. In turn, membranes prepared from AR42J-L cells showed the highest values for 18:2 n-6, PUFA, n-6 PUFA, and SFA/MUFA and the lowest values for SFA/PUFA and MUFA/PUFA. The effect of adding 18:1 n-9 or 18:2 n-6 was similar in that both fatty acids decreased the SFA/UFA ratio of AR42J-C cells to a comparable degree (Fig. 4B), from a mean value of 1.20 (AR42J-C) to 1.04 in AR42J-O and 1.10 in AR42J-L.

Discussion

Our previous experience with viable rat pancreatic acini (5, 7, 9, 24) indicated that a cultured line of pancreatic acinar cells would be beneficial to completely understand the interactions between dietary lipids and the exocrine

Table 4. Selected Fatty Acids in Membranes from Differentiated AR42J Cells Cultured for 72 Hours in Medium Containing Unmodified Serum (AR42J-C), Serum Enriched in 18:1 n-9 (AR42J-O), or Serum Enriched in 18:2 n-6 (AR42J-L)[a,b]

Fatty acid	AR42J-C	AR42J-O	AR42J-L
14:0	3.40 ± 0.20 [a]	4.58 ± 0.08 [b]	4.43 ± 0.09 [b]
16:0	28.05 ± 0.21 [a]	27.39 ± 0.10 [a]	28.14 ± 0.38 [a]
16:1 n-7	3.47 ± 0.20 [a]	3.85 ± 0.19 [a]	3.70 ± 0.16 [a]
18:0	16.41 ± 0.26 [a]	12.35 ± 0.33 [b]	13.04 ± 0.30 [b]
18:1 n-9	25.69 ± 0.41 [a]	32.40 ± 1.11 [b]	15.95 ± 0.22 [c]
18:2 n-6	3.33 ± 0.12 [a]	2.39 ± 0.03 [a]	17.03 ± 0.21 [b]
18:3 n-3	3.83 ± 0.20 [a]	5.11 ± 0.36 [b]	3.78 ± 0.24 [a]
20:4 n-6	3.80 ± 0.11 [a]	2.01 ± 0.11 [b]	1.86 ± 0.06 [b]
20:5 n-3	0.58 ± 0.02 [a]	0.36 ± 0.02 [b]	0.39 ± 0.06 [b]
22:6 n-3	1.87 ± 0.10 [a]	1.08 ± 0.06 [b]	0.88 ± 0.07 [b]

[a] Values are percentages of total fatty acid content (means ± SEM). AR42J-C: $n = 18$ (from 6 batches of cells); AR42J-O: $n = 15$ (from 5 batches); AR42J-L: $n = 15$ (from 5 batches).
[b] For a particular row, values with different superscript letters are significantly different at $P < 0.05$.

pancreas. For example, when working with the rat pancreas we observed that the yield of viable cells can be quite variable, which is likely due to the inability to rigidly control day-to-day differences in collagenase and mechanical digestion. Therefore, apart from other important advantages previously mentioned (see the Introduction section), switching to a continuous cell line could result in easier preparation and greater reproducibility. A main goal of this research was to find a cell model for studies on the influence of changes in membrane lipid composition upon transduction pathways and cell function.

AR42J cells are widely used in exocrine pancreatic research. However, there have been no previous reports on their membrane fatty acid composition. As a first step in confirming the validity of this cell line for our purposes, we determined the fatty acid profile of crude membranes from AR42J cells and compared this profile to that from rat pancreas. Then we investigated whether these cells were able to adapt their membranes to medium lipids (i.e. whether the fatty acid composition of AR42J membranes could be modified by altering the fatty acids to which they were exposed).

The results of the current study showed marked differences in baseline fatty acid profiles of membranes from rat pancreas and AR42J cells. Differences concerned not only major fatty acids (which, in addition, were not coincident) but also minor fatty acids and fatty acid indices. Initially, this could be explained by variations in the quantity of fatty acids in the extracellular environment. In this sense, we should note here that the lipid composition of the rat chow and fetal calf serum (FCS) differed widely. Membrane fatty acids in AR42J-C cells correlated closely with the amount available in our culture medium, with proportions of SFA, MUFA, PUFA, and most individual

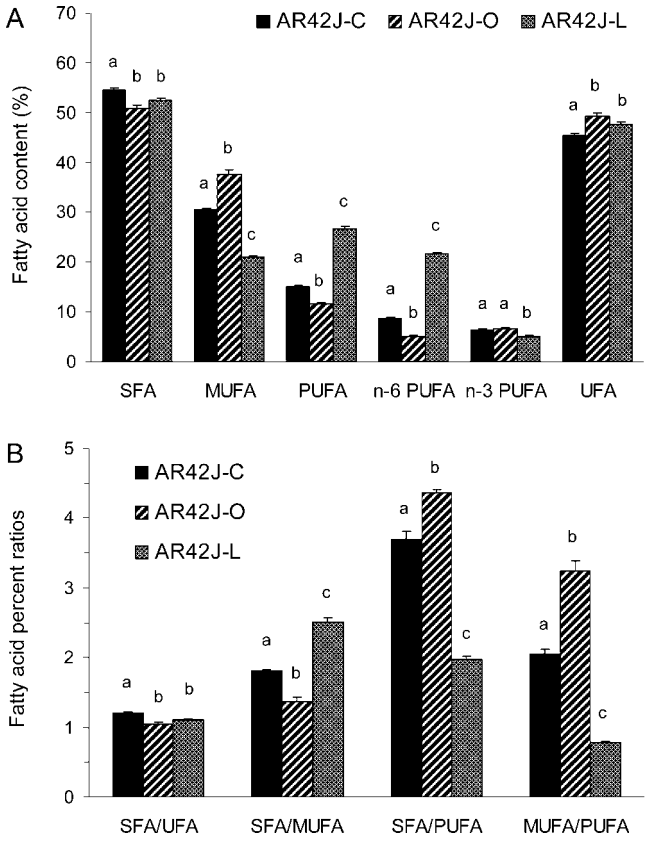

Figure 4. Fatty acid profile of crude membranes from differentiated AR42J cells cultured for 72 hrs in medium containing unmodified serum (AR42J-C), serum enriched in 18:1 n-9 (AR42J-O) or serum enriched in 18:2 n-6 (AR42J-L). SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; UFA, unsaturated fatty acids. (A) Fatty acid indices are expressed as percentages of total fatty acid content. (B) Fatty acid indices are expressed as percent ratios. Values are means ± SEM. AR42J-C: $n = 18$ (from 6 batches of cells); AR42J-O: $n = 15$ (from 5 batches); AR42J-L: $n = 15$ (from 5 batches). For a given index, different letters above the bars denote significant differences ($P < 0.05$) between dietary groups.

fatty acids reflecting to a high degree those in serum. This confirms the findings in other mammalian cells (25) that fatty acid *de novo* synthesis is inhibited when the culture medium contains an adequate supply of fatty acids. Under these conditions, most cellular fatty acids seem to be derived directly from the material that is taken up from the extracellular medium.

In contrast to AR42J-C cells, fatty acids in pancreatic membranes from rats fed the standard chow (C rats) reflected dietary lipid composition, but not with such fidelity. Indeed, apart from a clear influence of age, physiological state, and tissue (26, 27), many *in vivo* studies have indicated that adaptation of biological membranes to dietary fat type does not depend only on fatty acid availability, since changes in the dietary fat consumed do affect the rate of phospholipid synthesis *de novo*, the redistribution of fatty acyl chains, and the activity of desaturases and elongases (for a review, see Ref. 1).

Unsaturation of fatty acyl chains is a major determinant of the physical properties of biological membranes. A main feature of membrane lipid composition that determines membrane fluidity is the ratio of saturated to unsaturated fatty acids (SFA/UFA). Optimal SFA/UFA ratio in mammalian plasma membranes is 1:1 (28) and it is desaturases, at least in part, that work to maintain this ratio within certain limits. The commercial chow given in our study to C rats was very rich in unsaturated fatty acids (SFA/UFA of approx. 0.4). Membrane data in this group of animals show how the physiological mechanisms work to cause a decrease in UFA and an increase in SFA (compared to chow composition) in such way that a SFA/UFA ratio of approximately 0.8 is achieved in pancreatic membranes. Together with a modest MUFA percentage, the high content of 16:0 and 18:0 in C rat membranes suggest down-regulation of Δ9 desaturase by abundant linoleic acid in chow (29), with the subsequent accumulation of substrate. Contrary to MUFA and SFA, the proportion of total PUFA in membranes from C rats was similar to that in chow, with the particularity that 18:2 n-6, the major PUFA in diet (34.49%), was not preserved in pancreatic membranes (approx. 14%). Rather, the current results are indicative of efficient conversion of dietary 18:2 n-6 into 20:4 n-6, which accounted for approximately 18% of membrane fatty acids.

As previously observed by us (5, 8), in this study, adaptation of weaning rats to diets enriched in virgin olive oil (VOO) or sunflower oil (SO) markedly affected the fatty acid composition of pancreatic membranes. Thus, membranes in the VOO group were characterized by significantly higher levels of 18:1 n-9 and total MUFA compared with the other groups. In turn, consumption of the SO diet increased the proportion of n-6 PUFA such as 18:2 n-6 and 20:4 n-6. Membranes from both VOO and SO rats had a significantly lower SFA/UFA ratio compared to C rats. Still, SFA/UFA values kept within physiological limits (>0.6), which suggest that membranes display a good degree of homeostasis in relation to this parameter, with increases of several major fatty acids being always balanced by a change in the level of other fatty acids.

A main objective of this work was to examine the validity of differentiated AR42J cells as a model to study the interactions between lipids and pancreatic acinar cell function. To fulfil this aim, we had to confirm (i) that the fatty acid composition of AR42J cells could be modified, and (ii) that similar compositional variations that can be evoked in rats when dietary fat type is altered occurred in AR42J cells. We now communicate for the first time that the fatty acid composition of crude membranes from AR42J cells can be altered in culture by adding specific fatty acids to the usual growth medium for 72 hrs. We included major fatty acids abundant in olive oil (18:1 n-9) or sunflower oil (18:2 n-6), that have been shown to alter pancreatic function in our previous *in vivo* and *in vitro* studies (5, 8). Compared with cells grown in standard conditions (AR42J-C cells), addition of 18:1 n-9 to the culture medium (AR42J-O cells) increased significantly the proportion of this fatty acid in the membrane fraction, and 18:2 n-6 supplementation (AR42J-L cells) of the culture produced a significant increase in the 18:2 n-6 content.

A number of techniques have been developed for modifying the fatty acid composition of cells in culture. Fatty acids are toxic in the unbound form and are also very poorly soluble in water, so they are usually provided in the bound form (either to albumin or other protein) or in the form of liposomes. The most widely used method consists of the preparation of a fatty acid-BSA complex that is then added to serum-free (30) or serum-containing (31) media. Given that our protocol did not consider removing serum from the culture medium, we bound fatty acids directly to serum proteins. The method is simple and rapid, and did not appear to alter the main characteristics of the culture. Fatty acid–supplemented and unsupplemented AR42J cells had similar growth patterns and degrees of differentiation. Our data in AR42J cells also indicate that the fatty acid delivery system, which was chosen to mimic physiological conditions and to avoid damaging effects of free fatty acids or solvents, is very effective in modifying membrane fatty acids in this cell model, since extensive changes were produced in 72-hr exposure. Moreover, the concentration of added fatty acids in the culture medium (50 μ*M*) was selected to approximate physiological concentrations. The total plasma free fatty acid concentration in the fed state is approximately 0.3 m*M*, so concentrations of individual fatty acids are expected to be in the range of 30–130 μ*M* (32).

Until now, no study has existed about the influence of changes in the extracellular lipid composition on membrane fatty acid profile of AR42J cells. Our results show that this cell line is very responsive to such changes and this has a first obvious implication. Given that the fatty acid composition of different kinds of commercially available serum differs widely (33), the fact that the membrane profile is likely to change should be taken into account when changes are contemplated in the serum used to grow AR42J pancreatic cells.

In the current study, the addition of 18:1 n-9 or 18:2 n-6 to the culture medium for 72 hrs profoundly influenced the fatty acid composition of AR42J cell membranes. Importantly, despite some differences in magnitude, the pattern and direction of changes was parallel to that found in rats fed virgin olive oil or sunflower oil (best illustrated in Figs. 3 and 4). For example, compared with baseline situation (C rats and AR42J-C cells) both virgin olive oil in rats and 18:1 n-9 in cells evoked a significant increase in membrane MUFA (due to 18:1 n-9) at the expense of SFA and PUFA. We also observed that both sunflower oil in rats and 18:2 n-6 in AR42J cells produced significant increases in total and n-6 PUFA at the expense of SFA and MUFA. Variations in the other fatty acid indices, including the SFA/UFA ratio, followed the same trend after feeding oils *in vivo* or growing the cells with the respective major fatty acid.

There is only one remarkable difference between rat

pancreas and AR42J cells in their ability to adapt to environmental fatty acids and it concerns 20:4 n-6. The enrichment of standard serum with 18:2 n-6 did not produce the expected increase in the 20:4 n-6 content of AR42J phospholipids. It is unknown whether the failure of these cells to accumulate 20:4 n-6 under our experimental conditions is due to an intrinsic inability of the cell to store an increased amount of 20:4 or, more likely, if these cells express a low desaturase activity, as shown by some continuously cultured cell lines (34, 35). Not only 20:4 n-6 did not increase, but it actually decreased in AR42J-L cells compared with those cultured with standard serum. Two factors may be responsible for this decrease and both could operate concurrently. One is that the cell has a limited capacity to convert 18:2 n-6 to 20:4 n-6. The other is that the incorporation of 20:4 n-6 into membrane phospholipids is reduced as the availability of 18:2 n-6 increases, presumably through competitive inhibition. Studies are being conducted in our laboratory to make clear this fundamental point. Preliminary results of time-course experiments[1] show that the membrane levels of 20:4 n-6 in AR42J cells are similarly low regardless of whether 18:2 n-6 is added to the medium for 72 hrs or 144 hrs. Nevertheless, the results of the present work indicate that this characteristic of AR42J cells should be taken into account, especially when using this line as a cellular model for pancreatitis studies, given the role of 20:4 n-6 in eicosanoid synthesis.

To summarize, the present work reveals the existence of significant differences between the membrane fatty acid profile of AR42J cells cultured with intact fetal calf serum and that of pancreas from rats fed a standard chow for this species. Supplementation of culture media with 18:1 n-9 or 18:2 n-6 changed in 72 hrs the fatty acid spectrum of AR42J cells and, despite some differences in magnitude, the pattern and direction of changes was parallel to that found in rats fed diets enriched in virgin olive oil or sunflower oil. A largely different content in 20:4 n-6 observed in membranes from rat pancreas and AR42J cells upon enrichment of diet and culture medium, respectively, with 18:2 n-6 might account for the role played by the liver in the rat.

Overall, our results suggest that the AR42J cell line can be a useful tool to assess the effects of specific membrane compositional changes on acinar cell function and signalling. However, because of differences in baseline characteristics, and perhaps fatty acid metabolism, we believe that *in vitro* work with this cell line is no substitute for *in vivo* studies. Thus, although research with AR42J cells can possibly save time, money and, more importantly, animals, we encourage other scientists to confirm results obtained in AR42J cells with experiments *in vivo*, because there can be no doubt that the whole animal is the "gold standard" to corroborate the events described at the cellular level.

[1]N. Audi, unpublished data, 2006.

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