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Dietary-induced changes in the fatty acid profile of rat pancreatic membranes are associated with modifications in acinar cell function and signalling

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The effects of dietary lipids on the fatty acid composition of rat pancreatic membranes and acinar cell function were investigated. Weaning rats were fed for 8 weeks on one of two diets which contained 100 g virgin olive oil (OO) or sunflowerseed oil (SO)/kg. Pancreatic plasma membranes were isolated and fatty acids determined. Amylase secretion and cytosolic concentrations of Ca^{2+} and Mg^{2+} were measured in pancreatic acini. Membrane fatty acids were profoundly affected by the diets; the rats fed OO had higher levels of 18 : 1*n*-9 (42·86 (SEM 1·99) %) and total MUFA compared with the animals fed SO (25·37 (SEM 1·11) %). Reciprocally, the SO diet resulted in greater levels of total and *n*-6 PUFA than the OO diet. The most striking effect was observed for 18 : 2*n*-6 (SO 17·88 (SEM 1·32) %; OO 4·45 (SEM 0·60) %), although the levels of 20 : 4*n*-6 were also different. The proportion of total saturated fatty acids was similar in both groups, and there was only a slight, not significant ($P=0·098$), effect on the unsaturation index. Compared with the OO group, acinar cells from the rats fed SO secreted more amylase at rest but less in response to cholecystokinin octapeptide, and this was paralleled by reduced Ca^{2+} responses to the secretagogue. The results confirm that rat pancreatic cell membranes are strongly influenced by the type of dietary fat consumed and this is accompanied by a modulation of the secretory activity of pancreatic acinar cells that involves, at least in part, Ca^{2+} signalling.

Dietary fat: Pancreatic acini: Membrane fatty acids: Cholecystokinin: Calcium signalling

It has been accepted for many years that the exocrine function of the pancreas is influenced by the type of dietary fat. The approach most frequently used to tackle this task has been the analysis of the enzyme content of the pancreas after feeding animals different dietary lipids (Sabb *et al.* 1986; Ricketts & Brannon, 1994). The exact mechanisms by which this adaptive effect is brought about are not clear. Moreover, the enzyme content does not necessarily reflect the secretory activity of the organ. In previous studies, the *in vivo* pancreatic response after medium- or long-term intake of diets differing in the type of fat source has been examined (Ballesta *et al.* 1990; Yago *et al.* 1997*a*). These investigations indicated that pancreatic adaptation to dietary fat type is mediated, at least in part, by changes in the circulating levels of some gastrointestinal hormones (Serrano *et al.* 1997; Yago *et al.* 1997*a*,*b*). However, whether the secretory activity of pancreatic acinar cells is influenced directly by dietary fat alteration is unknown.

Dietary fats have been demonstrated to exert profound effects on membrane lipids in several tissues and cell types. In newly weaned rat pups, diets containing 20 g fat from different sources/100 g affected the fatty acid composition of intestinal brush-border membranes after only 40 h of feeding (Wang *et al.* 1996). Also, the effects of olive-oil or sunflowerseed-oil diets (10 g fat/100 g) were evident in plasma lipids (Giron *et al.* 1995) and liver microsomes (Giron *et al.* 1996) after 6 d. The differences in fatty acid profiles were shown to be permanent, provided the animals were kept on the same diets (Wang *et al.* 1996). A time period of 6 weeks has been found to be enough to induce changes in adipocyte plasma membrane of weaning rats (Field *et al.* 1989). Also, some reports (Suarez *et al.* 1996*a*,*b*) indicate that the rat heart, kidney, lung, erythrocytes and brain are also very responsive to modifications in dietary fatty acids during early postnatal life (4–6 weeks after weaning). Changes in membrane fatty acid composition may, in turn, influence cell function (Neelands & Clandinin, 1983; Field *et al.* 1989; Clandinin *et al.* 1991; Vajreswari & Narayanareddy, 1992; Wang *et al.* 1996; Quiles *et al.* 2001). This is not an unexpected finding, since there is growing evidence that fatty acids, in addition

Abbreviations: BSA, bovine serum albumin; CCK-8, cholecystokinin octapeptide; cPLA2, cytosolic Ca^{2+}-dependent phospholipase A_2; IP3, inositol trisphosphate; PLC, phospholipase C; PSS, physiological salt solution; SFA, saturated fatty acids; UI, unsaturation index.

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to their role in determining membrane structure and fluidity, can participate in intracellular processes as diverse as signal transduction or the regulation of gene expression.

Thus, the aim of the present study was to investigate the effects of two dietary oils that differ markedly in their fatty acid profile (virgin olive oil and sunflowerseed oil) on the function of rat pancreatic acinar cells and if this could be related to the fatty acid composition of cell membranes. These oils were chosen because of their preferential use in the Granada geographical area. Furthermore, olive oil is a major component of the Mediterranean diet, and its role in human health is actively debated at present. In order to investigate the study's aim, diets containing either virgin olive oil or sunflowerseed oil were given to separate groups of rats for 8 weeks. Acinar cell function was assessed by determining basal and cholecystokinin octapeptide (CCK-8)-stimulated amylase release in suspensions of viable acini. The present study also examined the effect of these diets on the mobilisation of intracellular free Ca (Ca^{2+}), a key mediator of CCK-8-evoked enzyme secretion, and on the changes in the cytosolic concentration of free Mg (Mg^{2+}), an abundant cation that has been proposed to act as a Ca^{2+} antagonist.

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

All experimental procedures were approved by the ethical committee of the Spanish Ministry of Science and Technology. The animals were handled according to the guidelines of the Spanish Society for Laboratory Animal Sciences and killed humanely. Seventy male weaning Wistar rats initially weighing 40–55 g (supplied by the animal farm at the University of Granada) were allocated to two groups (thirty-five each) so that the average weight per group was the same. The rats were fed over an 8-week period with two semi-purified, isoenergetic and isonitrogenous diets that were essentially AIN-93G diets (Reeves *et al.* 1993) except that the total fat content was increased from 70 to 100 g/kg at the expense of carbohydrate. The composition of the diets (g/kg) was as follows: casein, 200; maize starch, 367·5; dyetrose, 132; sucrose, 100; cellulose, 50; fat, 100; L-cystine, 3; choline bitartrate, 2·5; AIN-93G mineral mixture, 35; AIN-93G vitamin mixture, 10. The two diets differed only in the nature of the fat source; virgin olive oil or sunflowerseed oil. Commercial edible oils were obtained locally (Fedeoliva, S.A., Jaén, Spain and Koipesol®; Koipe, S.A., Andújar, Juén, Spain). The fatty acid composition of the two oils (Table 1) was determined by GLC as described later for the membrane fractions. The experimental diets were prepared at the nutrition unit of the animal farm (University of Granada, Spain), packed in plastic bags, sealed, and sent to the laboratory, where they were stored at 4°C in the dark. During the 8-week adaptation period to the diets, the animals were housed individually in a temperature-controlled room (22 ± 1°C), kept on a 12 h light–dark cycle and given free access to water and food. Food intake was determined daily in a subset of rats from both groups (*n* 8 each). Body weight was recorded weekly. Feeding diets varying in the fat source did not affect daily food intake or body-weight gain during the 8-week adaptation period; accordingly, there was no difference between the groups in the weight of pancreases (data not shown). All rats in the present study were killed by severance of the vertebral column after an overnight fast (food was always withheld from 20.00–20.30 and the time of killing was 09.00–09.30 on the next morning).

Table 1. Fatty acid composition of experimental fats
(Mean values for five replicates)

	Olive oil (g/100 g fatty acids)	Sunflowerseed oil (g/100 g fatty acids)
16:0	11·44	7·31
16:1*n*-7	0·85	0·19
18:0	4·38	4·59
18:1*n*-9	74·88	32·62
18:2*n*-6	7·72	55·17
18:3*n*-3	0·62	0·10
Saturated fatty acids	15·82	11·90
Unsaturated fatty acids	84·18	88·10
MUFA	75·84	32·83
PUFA	8·34	55·27

Isolation and analysis of cell membranes

The rat pancreatic plasma membrane fractions were prepared from gland homogenates by differential and sucrose gradient centrifugation (Meldolesi *et al.* 1971). Next, the technique of Lepage & Roy (1986) was used, a method that combines lipid extraction and fatty acid methylation in a one-step reaction. A GLC system, model HP 5890 series II (Hewlett Packard, Palo Alto, CA, USA), equipped with an automatic injector and a flame ionisation detector, was used to analyse fatty acids as methyl esters. Chromatography was performed using a 60 m long capillary column (32 mm internal diameter and 20 mm thickness) impregnated with Sp 2330™ FS (Supelco Inc., Bellefonte, CA, USA).

Preparation of isolated rat pancreatic acini

Acini were isolated according essentially to the modification (Jensen *et al.* 1982) of the procedure published previously (Peikin *et al.* 1978). Following killing, the pancreas was rapidly removed and trimmed free of fat and nodes in a physiological salt solution (PSS, pH 7·45). The solution comprised (per litre): bovine serum albumin (BSA), 2 g; soyabean trypsin inhibitor, 1 g; glutamine, 0·3 g; vitamin mixture (BME vitamins solution 100x; Sigma, St Louis, MO, USA), 10 ml; amino acid mixture (BME Amino Acids Solution 50x; Sigma), 25 ml. The PSS comprised (mM): NaCl, 120; KCl, 7·2; sodium pyruvate, 6; sodium

fumarate, 7·1; sodium glutamate, 6; glucose, 14; KH_2PO_4, 2; $MgCl_2$, 1·2; HEPES, 24; $CaCl_2$, 2. Next, 2 ml PSS containing 44 U purified collagenase/ml (type CLSPA; Worthington Biochemical Corp., Lakewood, NJ, USA) were injected into the pancreas. The distended pancreas was incubated at 37°C for 30 min in a shaking water-bath, washed with fresh collagenase solution every 10 min. This was followed by vigorous manual agitation. The dispersed acini were placed in cold PSS containing 40 g BSA/l and centrifuged (50 ***g***, 4 min, 4°C). The pellet was then re-suspended in PSS containing 2 g BSA/l. Following gentle pippeting, the digested material was filtered and centrifuged (50 ***g***, 4 min, 4°C). Cell viability was, at different times, monitored by trypan blue exclusion.

Amylase release

Amylase release was measured as described previously (Peikin *et al.* 1978; Jensen *et al.* 1982). In brief, acini were suspended in incubation medium (PSS containing 10 g BSA/l and 0·5 mM-$CaCl_2$). Samples (500 μl) were then incubated at 37°C for 30 min with either 0·1, 1 or 10 nM-CCK-8 ([Tyr(SO_3H)^{27}]CCK fragment 26-33 amide; Sigma) and this was followed by centrifugation at 350 ***g*** for 2 min (4°C). Acini exposed to the incubation medium alone served as unstimulated controls (basal release). Amylase activity in supernatant fractions was determined with the Phadebas reagent (Pharmacia & Upjohn, Barcelona, Spain) and expressed as a percentage of total amylase content at the beginning that was released into the extracellular medium during the incubation.

Measurement of intracellular free Ca^{2+} concentration

Acini were suspended in sodium HEPES solution (pH 7·40) containing (g/l): BSA, 2; soyabean trypsin inhibitor, 0·1. The HEPES solution contained (mM): $CaCl_2$, 1; NaCl, 130; KCl, 5; HEPES, 20; KH_2PO_4, 1·2; $MgSO_4$, 1; glucose, 10. Cells were loaded with 2 μM-fura-2 AM (Molecular Probes Europe BV, Leiden, The Netherlands) for 20 min at 37°C in a shaking water-bath, washed and re-suspended in the same solution without BSA or trypsin inhibitor. For quantification of fluorescence, samples of acinar suspension (2 ml) were placed in a quartz cuvette in a Perkin-Elmer LS 50B spectrofluorimeter (Perkin-Elmer, Beaconfield, Bucks, UK) and continuously stirred at 37°C. Fura-2-loaded cells were excited at 340 and 380 nm, and emission was monitored at 510 nm. After baseline measurement, CCK-8 was added into the cuvette to a final concentration of 0·1, 1 or 10 nM. At the end of each experiment, maximum and minimum fluorescence ratio values were obtained by the respective addition of digitonin (final concentration 80 μM) and ethylene-glycol-bis(a-aminoethyl)-N,N,N′,N′-tetra-acetic acid– tri(hydroxymethyl)-aminomethane pH 8·7 (ethylene-glycol-bis (a-aminoethyl)-N,N,N′,N′-tetra-acetic acid final concentration was 7 mM). Values for intracellular free Ca^{2+} concentration were calculated according to Grynkiewicz *et al.* (1985).

Measurement of intracellular free Mg^{2+} concentration

The same protocol and technique described for intracellular free Ca^{2+} concentration measurement was used, but in this case cells were loaded with 2 μM-magfura-2 AM (Molecular Probes Europe BV, Leiden, The Netherlands) and fluorescence recorded from cell populations at excitation wavelengths of 335 and 370 nm and emission at 510 nm. CCK-8 was assayed only at 1 and 10 nM, since the response to lower concentrations is hardly detectable by this technique.

Calculations and statistical analysis

In order to calculate the integrated response in the Ca^{2+} experiments, the basal intracellular free Ca^{2+} concentration values before the addition of CCK-8 were averaged to estimate the baseline and this was subtracted from each of the stimulation values. The numbers obtained in this way were then summed. The unsaturation index (UI) was calculated according to the formula: UI = (Σ (fatty acid content × number of double bonds))/total content of saturated fatty acids (SFA). The results presented in the text, tables and figures are means and standard errors of the mean. Before mean comparison, the normality of the distribution of values was checked using the Kolmogorov–Smirnov test. For variables with a normal distribution, the significance of mean differences among the dietary groups was assessed by the parametric Student's *t* test. For variables lacking a normal distribution, the non-parametric Mann–Whitney U test was applied. In all cases, SPSS software was used (SPSS for Windows, version 11.0.1, 2001; SPSS Inc., Chicago, IL, USA). Only values of $P<0·05$ were considered significant.

Results

Fatty acid profile of pancreatic cell membranes

The fatty acid profile of pancreatic cell membranes (Table 2) was profoundly influenced by the dietary treatment. Membranes of the olive-oil-fed group were characterised by higher proportions of 18 : 1*n*-9 ($P<0·001$) and total MUFA ($P<0·001$) as compared with the sunflowerseed-oil group. Reciprocally, feeding the polyunsaturated sunflowerseed-oil diet for 8 weeks resulted in enhanced levels of total PUFA ($P<0·001$) in pancreatic membranes, particularly the *n*-6 series ($P<0·001$), in comparison with olive-oil-fed animals. The elevated proportion of *n*-6 PUFA in the membranes of the sunflowerseed-oil group was due to the contribution of 18 : 2*n*-6, the major fatty acid in sunflowerseed oil ($P<0·001$ *v.* olive-oil group), but also of 20 : 4*n*-6 ($P<0·05$ *v.* olive-oil group). The total amount of SFA was the same regardless of the diet. It is worth noting that, despite marked differences in the content of a number of individual fatty acids as well as in total MUFA and PUFA (Table 2), neither the SFA:unsaturated fatty acid value nor the UI were significantly different among the dietary groups.

Table 2. Fatty acid profile of rat pancreatic cell membranes after the 8-week adaptation period to the experimental diets (g/100 g total fatty acids)†

(Mean values and standard errors of the mean)

	Olive-oil group		Sunflowerseed-oil group	
	Mean	SEM	Mean	SEM
14:0	7·76	2·67	3·02	1·15
16:0	25·25	0·49	27·05	1·31
16:1*n*-7	5·15	0·41	4·21	0·41
18:0	6·69**	0·62	10·25	0·86
18:1*n*-9	42·86***	1·99	25·37	1·11
18:2*n*-6	4·45***	0·60	17·88	1·32
18:3*n*-3	0·39	0·04	0·31	0·08
20:4*n*-6	5·54*	0·70	9·83	1·34
20:5*n*-3	0·629	0·061	0·455	0·134
22:6*n*-3	0·580	0·090	0·913	0·215
Saturated fatty acids	39·71	2·33	40·34	2·02
MUFA	48·11***	2·29	29·72	1·41
PUFA	12·17***	1·41	29·94	2·25
n-6 PUFA	10·50***	1·28	28·15	2·18
n-3 PUFA	1·67	0·14	1·80	0·24
MUFA : PUFA	4·47***	0·48	1·12	0·15
Unsaturation index	2·35	0·22	3·04	0·30

*Mean values were significantly different from those of the sunflowerseed-oil group: * $P<0·05$, ** $P<0·005$, *** $P<0·001$ (*n* 12 for both groups).
† For details of diets and procedure, see Table 1 and p. 228.

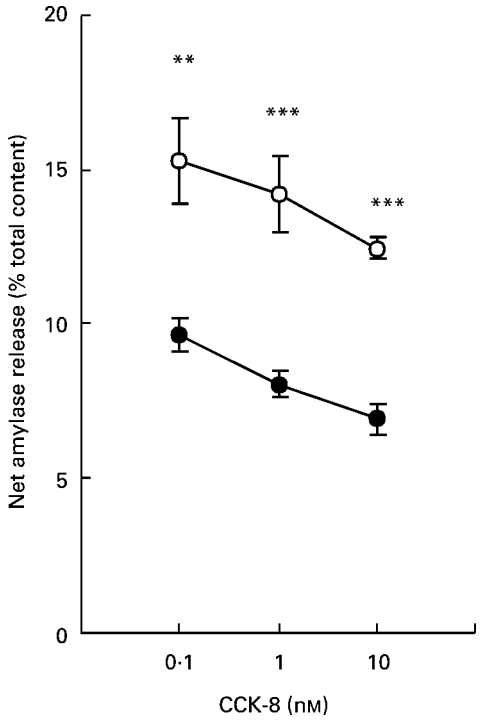

Fig. 1. Net amylase release (increase above basal) stimulated by cholecystokinin octapeptide (CCK-8) in pancreatic acini isolated from rats fed diets containing either virgin olive oil (-○-) or sunflowerseed oil (-●-) as the fat source. Amylase released during the incubation with the secretagogue (30 min, 37°C) is expressed as a percentage of total initial content. Results are mean values of fifteen to thirty-seven separate experiments (in each experiment values were determined in duplicate). Standard errors of the mean are represented by vertical bars. Mean values were significantly different between the dietary groups: ** $P<0·005$, *** $P<0·001$.

Amylase release

Basal values of amylase release were significantly higher ($P<0·001$) in acini from the rats fed sunflowerseed oil (12·2 (SEM 0·5) %; *n* 13) than in acini from the animals fed olive oil (5·9 (SEM 0·6) %; *n* 9). In both groups, net amylase release (above basal) in response to 0·1–10 nM-CCK-8 showed the highest values at 0·1 nM (Fig. 1) and decreased gradually with higher concentrations. However, at any particular concentration of the secretagogue, feeding the sunflowerseed-oil diet for 8 weeks was associated with a significant attenuation in CCK-8-stimulated net amylase release in rat pancreatic acini compared with the olive-oil diet (Fig. 1).

Intracellular free Ca^{2+} concentration

Basal values of intracellular free Ca^{2+} concentration (considering all the series) were 191·0 (SEM 9·4) nM (*n* 17) in acini from the olive-oil group and slightly lower in those from the sunflowerseed-oil group (175·9 (SEM 12·3) nM; *n* 18). The time-course changes in intracellular free Ca^{2+} concentration evoked by CCK-8 (Fig. 2) showed the typical responses to CCK-8 in acinar cell suspensions, i.e. a large transient increase followed by a slow decline towards a suprabasal level. Cells from both groups reacted with a very prompt rise in intracellular free Ca^{2+} concentration in response to 1 and 10 nM-CCK-8 (Fig. 2 (B) and (C)) whereas the time between the administration of the secretagogue until the intracellular free Ca^{2+} concentration maximum was reached increased when a concentration of 0·1 nM was used (Fig. 2 (A)). Data in Fig. 2 also indicate that 0·1, 1 and 10 nM-CCK-8 resulted in intracellular free Ca^{2+} concentration values that, not only at the peak response but also at many time points of the sustained phase, were significantly ($P<0·05$) higher in cells from the rats fed olive oil than in those isolated after sunflowerseed-oil feeding. The differences were, however, less pronounced at 10 nM-CCK-8 (Fig. 2 (C)). In fact, when the intracellular free Ca^{2+} concentration response is expressed as a peak increase relative to basal (Fig. 3 (A)) or as an integrated response (Fig. 3 (B)), the values attained with this concentration of the secretagogue were similar in both experimental groups.

Intracellular free Mg^{2+} concentration

Resting intracellular free Mg^{2+} concentration was comparable in both groups (Table 3). When CCK-8 was added, intracellular free Mg^{2+} concentration decreased gradually in cells from both dietary groups to reach a lower level 2–3 min later that persisted until the end of the experiment. This pattern of response was similar regardless of the antecedent diet and it is illustrated in Fig. 4, which reproduces an original trace of the changes evoked by 1 nM in cells prepared from a sunflowerseed-oil-fed animal. The responses and corresponding decreases below basal after the stimulation of cells from both groups with 1 and 10 nM-CCK-8 are summarised in Table 3. No significant differences were revealed between the experimental groups except for the decrease below basal evoked by 10 nM-CCK-8.

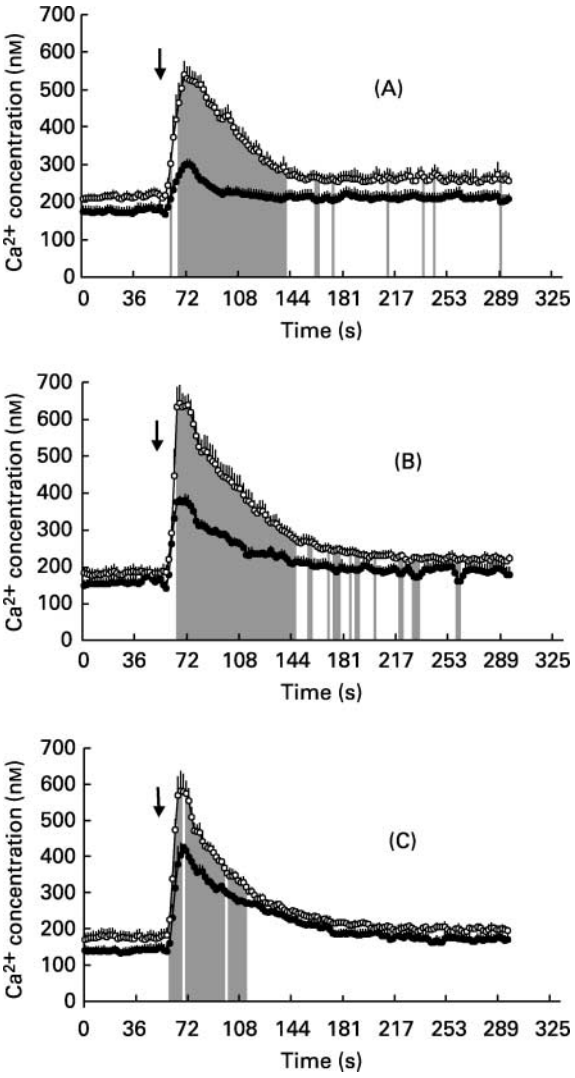

Fig. 2. Time-course changes in intracellular free Ca^{2+} concentration evoked by (A), 0·1 nM-; (B), 1 nM-; or (C), 10 nM-cholecystokinin octapeptide (CCK-8) in suspensions of fura-2-loaded pancreatic acinar cells isolated from rats fed diets containing either virgin olive oil (-○-) or sunflowerseed oil (-●-) as the fat source. Results are mean values of five to seven independent experiments. Standard errors of the mean are represented by vertical bars. (↓), The point of addition of the secretagogue; (■), significant differences between the groups at individual time points ($P<0·05$).

Discussion

Previous observations from the authors' research group in Spain (Quiles *et al.* 1999), along with those by other researchers (Neelands & Clandinin, 1983; Clandinin *et al.* 1991; Vajreswari & Narayanareddy, 1992), indicate that dietary fat composition influences the fatty acid profile of phospholipids in both cellular and subcellular membranes of different organs such as the heart, liver, muscle, etc. Reported changes reflect dietary variation but not always to the same extent, suggesting that this process does not depend only on fatty acid availability. Indeed, changes in the dietary fat consumed have been shown to 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 Clandinin *et al.* 1991). In addition, a clear influence of age, tissue and physiological state is to be considered. The present study confirms that the pancreas is very sensitive to dietary fat changes. Thus, after 8 weeks on the diets, pancreatic membranes in the olive-oil group had significantly higher levels of 18 : 1*n*-9 and total MUFA, whereas a higher level of PUFA, particularly *n*-6 PUFA such as 18 : 2*n*-6 and 20 : 4*n*-6, was found in the sunflowerseed-oil group. To the authors' knowledge, only two groups have investigated the influence of the type of dietary fat on the lipid composition of the pancreas (Begin *et al.* 1990; Soriguer *et al.* 2000) and they also show an enrichment in the pancreas of those fatty acids most abundant in the fat ingested. The work by Soriguer *et al.* (2000) is especially interesting, given that their olive-oil and sunflowerseed-oil diets (8 g/100 g) are very similar to those of the present study (10 g/100 g). A discrepancy between their results and the present results concerns arachidonic acid (20 : 4*n*-6); while Soriguer *et al.* (2000) found comparable levels, the present results indicate a higher proportion in the animals fed sunflowerseed oil. Differences in the study design probably account for this. Thus, Soriguer *et al.* (2000) used adult rats that were fed with the experimental diets for 1 month only whereas the present study employed weaning rats and a longer feeding period (8 weeks). Early-life feeding experiences with diets of varying fatty acid composition may affect the ability of the intestine to adapt to an altered lipid intake in later life (Thomson *et al.* 1993). In addition, the minimal time to induce diet-induced changes in tissue lipids has been shown to be influenced by the characteristics of the antecedent diet (Berlin *et al.* 1998). Another aspect to take into account is that Soriguer *et al.* (2000) analysed the fatty acid composition of phospholipids extracted from whole-pancreas homogenates, but plasma membranes were used in the present study. Whether the difference in 20 : 4*n*-6 proportion relates to this particularity remains to be elucidated.

In the present study, the proportion of total SFA was similar in the membranes of the two groups. In fact, except for 18 : 0 values, no further differences could be detected. This resistance of the SFA fraction to dietary-induced alterations is a common feature in different tissues (Begin *et al.* 1990; Vajreswari & Narayanareddy, 1992; Suarez *et al.* 1996*b*; Quiles *et al.* 1999; Soriguer *et al.* 2000). In addition, feeding diets rich in virgin olive oil or sunflowerseed oil did not alter significantly the total SFA:unsaturated fatty acid value or the UI. This suggests that membranes display a considerable degree of homeostasis with respect to these parameters (Vajreswari & Narayanareddy, 1992), and that changes in the proportion of several major fatty acids are associated to some metabolic compensation in order to keep plasma membrane fluidity within a certain range of values.

The dose–response curve for CCK-8-induced amylase release in pancreatic acinar cells is typically bell-shaped, reaching a maximum at around 0·1 nM (Akiyama *et al.* 1998; Lajas *et al.* 1998). The lowest concentration of CCK-8 employed in the present study was 0·1 nM, so only data regarding the second phase of the curve are available. Still, the present results are consistent with those in the literature since, in the conditions used in the present

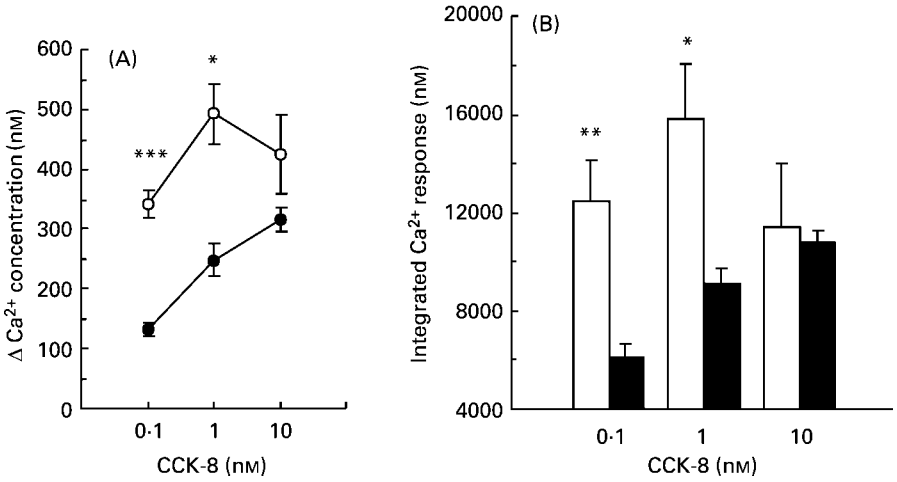

Fig. 3. Effect of graded concentrations of cholecystokinin octapeptide (CCK-8) on intracellular free Ca^{2+} concentration in suspensions of fura-2-loaded pancreatic acinar cells isolated from rats fed diets containing either virgin olive oil (-○-, □) or sunflowerseed oil (-●-, ■) as the fat source. (A), Ca^{2+} response expressed as increase above basal (difference between peak and basal value); (B), the integrated response calculated as described on p. 229. Results are mean values of five to seven independent experiments. Standard errors of the mean are represented by vertical bars. Mean values were significantly different between the dietary groups: * $P<0{\cdot}05$, ** $P<0{\cdot}005$, *** $P<0{\cdot}001$.

study, the strongest secretory effect was observed at 0·1 nM-CCK-8 and a characteristic decrease occurred after the addition of higher concentrations of the secretagogue. This pattern was followed in cells from both the olive-oil and sunflowerseed-oil groups. Quantitatively, however, marked differences were revealed between the two dietary groups. Thus, values for basal (unstimulated) amylase release in acini from rats fed the olive-oil diet were similar to those reported by most authors (Akiyama *et al.* 1998; Lajas *et al.* 1998) whereas release in cells from the sunflowerseed-oil-fed rats was markedly higher. The reason for this effect is unknown. High basal values of amylase release have been found in acinar cells from pancreatitic rats and the authors related this finding to increased acinar intracellular free Ca^{2+} concentration (Bragado *et al.* 1996). Basal intracellular free Ca^{2+} concentration values were comparable in acini from the present study's two dietary groups, so they cannot account for the differences in amylase release in unstimulated conditions. The possibility exists that different membrane composition is modifying the permeability for amylase. Were this true, it would be happening without a negative effect on membrane integrity, as indicated by the trypan blue exclusion test.

In contrast to the observations in basal conditions, net amylase secretion in response to all concentrations of CCK-8 was drastically reduced after sunflowerseed-oil feeding. This diminished secretory activity may be explained, without excluding other components of the secretory pathway, by the attenuation of CCK-8-evoked Ca^{2+} responses in the sunflowerseed-oil group. Moreover, the fact that not only the absolute value of the intracellular free Ca^{2+} concentration peak but also the peak increase over basal is lower in cells from the sunflowerseed-oil-fed rats suggests a reduction in the filling state of CCK-8-releasable Ca^{2+} pools and/or a limitation in the production or effectiveness of the mediators that participate in the Ca^{2+} mobilisation pathways.

The differences in acinar secretory activity and intracellular free Ca^{2+} concentration mobilisation in the present study are most probably related to the dietary-induced changes in cell membrane composition. Dietary fats

Table 3. Intracellular free Mg^{2+} concentration in unstimulated conditions and effect of cholecystokinin octapeptide (CCK-8) in pancreatic acinar cells from rats fed diets containing either virgin olive oil or sunflowerseed oil†

(Mean values and standard errors of the mean)

	Intracellular free Mg^{2+} concentration (mM)											
	Olive-oil group (*n* 4-7)						Sunflowerseed-oil group (*n* 5-6)					
	Basal		Response‡		Decrease below basal§		Basal		Response‡		Decrease below basal§	
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
1 nM-CCK-8	0·907	0·022	0·741	0·027	0·166	0·044	0·894	0·036	0·720	0·068	0·174	0·041
10 nM-CCK-8	1·017	0·120	0·826	0·106	0·156	0·022	0·939	0·113	0·674	0·081	0·265*	0·033

* Decrease below basal mean value was significantly different, for the same concentration of CCK-8, from that for the olive-oil group ($P<0{\cdot}05$).
† For details of diets and procedures, see Table 1 and pp. 228–229.
‡ Responses were calculated by averaging values once the new steady state had been reached.
§ The decrease below basal represents the difference between the basal and the corresponding response.

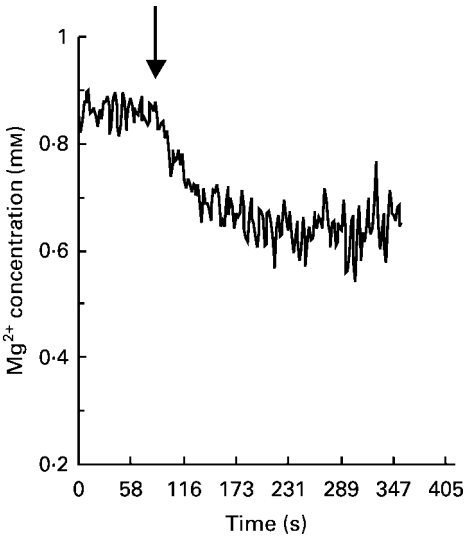

Fig. 4. Original trace-recording showing the effect of 1 nM-cholecystokinin octapeptide on intracellular free Mg^{2+} concentration in suspensions of magfura-2-loaded pancreatic acinar cells isolated from rats fed a diet containing sunflowerseed oil as the fat source. (↓), The point of addition of the secretagogue.

differing in the degree of unsaturation have been shown to modify insulin binding in rat adipocytes (Field *et al.* 1989; Clandinin *et al.* 1991) and the activity of membrane-associated enzymes in liver (Neelands & Clandinin, 1983), heart (Vajreswari & Narayanareddy, 1992) and submandibular glands (Alam *et al.* 1993). Many steps of the stimulus-secretion coupling process in acinar cells are membrane-dependent. Although the present results do not support gross modifications of whole-membrane fluidity, differential enrichment in certain fatty acids may influence the accessibility of the CCK receptor, the interaction with G proteins or the functionality of such enzymes as phospholipases and protein kinase C which are known to interact with cell membranes during their activation.

Apart from their structural role, membrane fatty acids participate themselves as mediators in signal transduction. The CCK receptor in rat pancreatic acinar cells (Bourassa *et al.* 1999) can display two binding (high and low) affinity states and, moreover, CCK occupancy of high and low affinity sites is thought to be related to the initiation of different intracellular events and consequent biological responses (Tsunoda *et al.* 1996; Gonzalez *et al.* 1999). The moderately high concentrations of CCK-8 used in the present study probably stimulate low-affinity sites and initiate a route linked to both phospholipase C (PLC) and phospholipase D (Gonzalez *et al.* 1999). PLC activation involves the hydrolysis of phosphatidylinositol bisphosphate and subsequent production of inositol trisphosphate (IP3), which initiates the Ca^{2+} signal, and diacylglycerol. The membrane modifications confirmed in the present study after olive-oil and sunflowerseed-oil intake could reasonably involve an alteration in the phosphoinositide turnover and a change in the supply of inositol lipid precursors of IP3. Reduced production of IP3 in acini from rats fed sunflowerseed oil might explain the diminished intracellular free Ca^{2+} concentration peaks in response to CCK-8, since it is well known that the initial rise in Ca^{2+} concentration transients is mainly due to Ca^{2+} released from IP3-sensitive internal stores. Alternatively, it is tempting to speculate that diacylglycerol, abundantly generated via PLC and phospholipase D (Gonzalez *et al.* 1999) and possibly with different acyl moieties as a consequence of the observed changes in the membrane, may have resulted in the present study in differential activation of protein kinase C, a crucial modulator of the secretory machinery of acinar cells. This is strongly supported by the finding in guinea-pig epidermis that diacylglycerol with a 18 : 2*n*-6 metabolite at the 2-position inhibited protein kinase C isozymes compared with 1,2-dioleylglycerol (Cho & Ziboh, 1994). On the other hand, the activation of high-affinity receptor sites by low CCK concentrations is coupled to cytosolic Ca^{2+}-dependent phospholipase A_2 (cPLA2) and, to a lower extent, to PLC (Gonzalez *et al.* 1999). Arachidonic acid (20 : 4*n*-6) liberated from membrane phospholipids via cPLA2 then slows the Ca^{2+} wave spreading elicited by CCK (Gonzalez *et al.* 1999). With the methodology of the present study it was not possible to assess the participation of the cPLA2 cascade but, if there is any, it would occur only after stimulation with 0·1 nM-CCK-8. In this case, the significantly higher availability of arachidonic acid in membranes of the sunflowerseed-oil group should be considered.

Concerning intracellular Mg^{2+} concentrations, the characteristic response to CCK-8 was found (Wisdom *et al.* 1996). It has been proposed that for optimal generation and maintenance of the Ca^{2+} signal and enzyme secretion in pancreatic acinar cells, a concomitant decrease in the cellular Mg^{2+} level is necessary (Wisdom *et al.* 1996) and, indeed, a fall in intracellular free Mg^{2+} concentration in both dietary groups was observed after the addition of CCK-8. From a quantitative point of view, however, the changes in intracellular free Mg^{2+} concentration do not provide an explanation for the different patterns of amylase release and Ca^{2+} mobilisation in cells isolated from the rats fed different dietary fats.

In conclusion, the results of the present study demonstrate that the type of dietary fat strongly influences the fatty acid composition of rat pancreatic cell membranes and this is associated with a change in the secretory activity and intracellular Ca^{2+} mobilisation stimulated by CCK-8 in viable pancreatic acini. Lipids are emerging as potent regulators of cell function. At the level of CCK-8-evoked transduction pathways in acinar cells, this regulatory action of lipids could potentially involve many steps of the cascade because of the properties they confer on biological membranes or due to their performance as intracellular mediators themselves. Experiments are in progress in order to better characterise the precise mechanisms for the observed effects.

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