Correlation Between Fatty Acyl Composition in Neutral and Polar Lipids and Enzyme Activities from Various Tissues of Calcium-Deficient Rats

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ABSTRACT: In this study we investigated the changes induced by feeding rats a calcium-deficient diet (0.5 g Ca/kg diet) during 65 d after weaning. Phospholipase A2, acyl-Co synthetase and FA Δ9-, Δ6-, and Δ5-desaturase activities were also determined. Calcium deficiency evoked a general alteration in the quality and proportion of the FA chains acylated to neutral and polar lipids from liver, lungs, spleen, brain, kidneys, fat, articular cartilage, erythrocyte ghosts, and plasmas, characterized by an increment of saturated FA and a significant depletion of polyunsaturated acids derived from linoleate and α-linolenate. Several interlipid and lipid/protein relationships were also modified in microsomes from calcium-deprived rats, with a concomitant reduction in the rotational mobility of the probe diphenylhexatriene. Phospholipase A_2 and acyl-CoA synthetase activities were also decreased and increased, respectively, in some tissues from calcium-deficient rats, whereas Δ9-, Δ6- and Δ5-desaturases were significantly depressed. We conclude that changes in tissue fatty acyl composition evoked by calcium deprivation are due to alterations in the acylation/deacylation cycles *via* inhibition of the phospholipase A_2. These changes were reflected in the physicochemical properties of the membranes, which in turn inhibits desaturase activities. A possible failure in the transcriptional rate for desaturase-mRNA was also discussed.

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Previous works from our laboratory have documented that the content of dietary calcium plays an important role in PUFA metabolism since a calcium-deficient (CaD) diet fed to rats for 65 d caused a significant inhibition of Δ5-, Δ6-, and Δ9-desaturase activities in liver microsomes (1). It was also reported by Huang *et al.* (2) that deprivation of calcium may cause a decrease in the rate of elongation of γ-linolenic acid (18:3n-6) (2). The authors supported this conclusion by the analysis of microsomal fatty acyl composition obtained from calcium-deprived rats whose diets were supplemented—or not—with 18:3n-6 acid. Previously, Bierenbaum *et al*. (3) suggested that calcium may limit the availability of 18:3n-6 for the elongase system by forming insoluble complexes with this cation. We also demonstrated that a CaD diet was able to produce a marked stimulation in acyl-CoA synthetase activity (4) and a significant reduction in phospholipase A_2 activity that evokes permanent alterations in the physicochemical properties of the liver microsomal membranes (1). The picture that emerges from those results is that the changes in the desaturase activities correlate well with both the rotational mobility of the diphenylhexatriene probe and the analytical changes produced on the microsomal lipids by the CaD diet. Whether these changes were completely developed in liver tissue or whether they were the consequence of a general alteration in the lipid metabolism that, in fact, involved various tissues of the animal is not clear. In addition, the importance of the inhibition of FA desaturase activities induced by calcium depletion on the FA composition of lipids from tissues other than liver still remains unexplored. The central role of liver FA desaturases in supplying PUFA for the rest of the body prompted us to investigate the possible correlation between calcium content and lipid composition among several tissues from both sufficient (S) and CaD rats. The aim of the present study was to obtain a general description of the changes induced by calcium deficiency and to evaluate the contribution of some key enzyme activities—involved in FA metabolism—to changes observed under the CaD condition.

MATERIALS AND METHODS

FA and other chemicals. The unlabeled FA used as standards for GLC or employed in the enzymatic determinations were obtained from Nu-Chek-Prep (Elysian, MN). ATP (disodium salt), NADH, *N*-acetylcysteine, CoA (lithium salt), snake venom *(Crotalus atrox)* Western Diamondback Rattlesnake, and sodium deoxycholate (grade II) were purchased from Sigma Chemicals Co. (St. Louis, MO). Unlabeled phospholipids were obtained from Serdary Research Laboratories (London, Ontario, Canada). The procedure to obtain labeled PC was developed in our laboratory after assaying different experimental conditions (1). The following radioactive FA were supplied by Amersham International (Buckinghamshire, UK) (specific activity as mCi/mmol and percent degree of radiochemical purity are indicated, respectively, in parentheses: [1-^{14}C]palmitic (58.0, 99), [1-^{14}C]linoleic (55.5, 99), and [1-^{14}C]eicosa-8,11,14-trienoic (58.5, 99). The concentration and degree of purity of the FA were routinely checked by liquid scintillation counting and GLC of their FAME prepared by using 14% boron trifluoride in methanol (Alltech Associ-

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Abbreviations: 18:3n-6, γ-linolenic acid; 20:4n-6, arachidonic acid; CaD, calcium-deficient; DBI, double bond index; DPH, 1,6-diphenyl-1,3,5-hexatriene; ED, elongation–desaturation index; PDIA, palmitoyl desaturase index activity; S, sufficient (control); SDIA, stearoyl desaturase activity index.

ates Inc., Deerfield, IL). Eicosa-11-monoenoic acid was used as internal standard. FA were appropriately diluted in absolute ethanol (Riedel-de-Haen, Seelze, Germany) and stored in the dark at −20°C under an atmosphere of N_2 until used. Solvents for HPLC were provided by Carlo Erba (Milano, Italy). 1,6-Diphenyl-1,3,5-hexatriene (DPH) probe was obtained from Fluka Chemie AG GmbH & Co. (Buenos Aires, Argentina). Other chemicals used were supplied by commercial sources.

Animal treatment. Female Wistar rats from Comisión Nacional de Energía Atómica (Buenos Aires, Argentina) weighing 170 ± 10 g were bred and maintained on a control diet (Cargill type "C," Rosario, Argentina) throughout gestation and lactation. The dams were housed in plastic cages (one animal per cage) in a vivarium kept at 22 ± 1°C with a 12-h light/dark cycle and a RH of 60 ± 10%. After weaning, 24 female pups (weighing 47 ± 4 g/animal) were randomly divided into two groups of 12 animals each and fed *ad libitum* either on a CaD diet (group CaD) or on a balanced one (group S). The composition of the CaD diet prepared in our laboratory was reported in detail in a previous paper (4). The Ca^{2+} content of the diet (0.5 g/kg) was determined in a Shimadzu Atomic Absorption Spectrophotometer AA-630-12 (Shimadzu Corp., Kyoto, Japan) following the mineralization procedure described elsewhere (5). Control animals were fed a standard balanced diet supplemented with 5.0 g/kg calcium in order to supply the mineral at a level equivalent to that recommended by The American Institute of Nutrition for the Formulation of the AIN-93 Purified Diets for Laboratory Rodents (6). The content of Ca^{2+} in drinking water (given *ad libitum*) was determined either by atomic absorption or by calcium-selective electrode (Orion model 93-20; Orion Research Inc., Cambridge, MA), and it was generally below 15 ppm. During the feeding period, body weight, water consumption, and food intake were determined every day. Samples of blood (100 to 150 µL) were collected from the tail vein once a week in order to determine plasma calcium levels. Animals were sacrificed on day 65 after feeding. In order to avoid individual differences among animals that might result from an *ad libitum* feeding, on day 64 all the rats were fasted for 24 h, refed with the corresponding diet for 2 h, and then killed by decapitation without prior anesthesia 12 h after the refeeding period. All the diet components used were purchased from Carlo Erba (Milano, Italy) or Mallinckrodt Chem. Works (Oneonta, NY). The casein was depleted of calcium by EGTA treatment and then defatted with boiling acetone. The calcium content in the extracted casein was negligible. Animal maintenance and handling were in accordance with the NIH guide for the care and use of laboratory animals (7).

Collection of samples and preparation of microsomal suspensions. Liver, heart, kidneys, skeletal muscle (psoas major and quadriceps), lungs, brain, and spleen from S and CaD rats were rapidly excised and immediately placed in an ice-cold homogenizing medium (8). The homogenates were processed individually at 1°C, and the microsomal fractions were separated by differential centrifugation at 110,000 × *g* as described previously (8). Microsomal pellets were resuspended in cold homogenizing solution up to a final protein concentration of 30–40 mg/mL. Other tissues such as perirenal fat, large bones (femur and tibiotarsal), and articular cartilage from S and CaD rats were also used to obtain homogenates from whole tissue samples because, in these cases, microsomal suspensions were not prepared. Cortical (parietal) and tibiotarsal bones—with intact articular and epiphyseal cartilages—were carefully excised; chilled on ice; and thoroughly cleaned of connective tissues, fatty inclusions, and vessels; weighed; and stored at −80°C under N_2 atmosphere until further use. Thin-sliced cartilage samples were prepared as shavings from the articular surfaces of femoral condyles and tibial plateaus from six S or CaD rats. They were processed and assayed separately. Epiphyseal and diaphyseal zones of one femur per rat were also excised and processed independently. They were carefully rinsed in ice-cold saline to remove adherent tissues and/or body fluids and homogenized using a powerful homogenizer with a rotating blade (Ultra-Turrax Type TP 18/10; Janke & Kunkel, Staufen, Germany). Blood was also collected after killing the rats by decapitation. Samples were individually dispensed into heparinized tubes and fractionated by centrifugation at 50 × *g* for 10 min. Plasmas were immediately processed for calcium determination and lipid analysis. Erythrocyte ghosts were prepared by hypotonic lysis according to the procedure of Dodge *et al.* (9), as modified by Berlin *et al.* (10). An aliquot from the erythrocyte membranes was processed for steady-state fluorescence anisotropy determinations (r_s) following the procedure described in Reference 11. The rest was employed for lipid analysis.

Lipid analysis. Product identification and quantification after FA desaturase activity assays were performed by RP-HPLC according to previously described methods (1,12). GLC of the FAME was performed as indicated in a previous paper (13) except that in this case we used a capillary column mounted in a Hewlett-Packard HP 6890 Series GC System Plus (Avondale, PA) equipped with a terminal computer integrator. The FAME were identified by comparison of their relative retention times with authentic standards, and the mass distribution was calculated electronically by quantification of the peak areas. Total lipids were extracted from S and CaD samples by the method of Folch *et al.* (14). Phospholipid and neutral lipid fractions were separated from the Folch extracts by a micro-column chromatography method described elsewhere (15). Cholesterol content was enzymatically measured according to Allain *et al.* (16). Total lipids and neutral lipids were estimated gravimetrically after evaporation of an aliquot of the corresponding lipid extract (Folch or silicic acid subfraction, respectively) up to constant weight (17). Phospholipids were measured as phosphorus content (18) after mineralization of an aliquot from the silicic acid partition.

Enzymatic determinations. Phospholipase A_2 activity was determined in microsomal fractions from various tissues with [^{14}C]PC (24.0 mCi/mmol, 99% pure) as substrate according to the method of Hirata *et al.* (19) with the modifications described in a previous paper (1). To determine the FA desaturase activities in microsomal suspensions from various tissues of S

and CaD rats, each FA used as substrate ([1-^{14}C]16:0, [1-^{14}C]18:2n-6, or [1-^{14}C]20:3n-3) was diluted to a specific activity of 0.20 to 0.25 μCi/mol with the respective pure unlabeled FA. In order to compare results, the enzymatic assays were conducted at saturated substrate concentrations. Each assay was performed by incubation of 1.25 mg of liver microsomal protein or 5 mg of brain, kidney, lung, heart, or spleen microsomal protein in an open test tube with $5 \cdot 10^3$ pmol of diluted labeled substrate for liver or 10^4 pmol for the other tissues, in a Dubnoff shaker at 37°C for 10 min. The total volume of the incubation medium was 0.8 mL. Details of the assay procedure were described in previous papers (1,20,21). Acyl-CoA activity assays were performed on cytosol fractions obtained as supernatants of 110,000 × *g*, according to the method of Tanaka *et al.* (22) as modified previously (4).

Measurement of Δ6-desaturase mRNA. Total liver RNA of six S or CaD animals were isolated by means of Wizard RNA Isolation System (Promega, Madison, WI) according to the manufacturer's instructions. Total RNA (20 μg) was size-fractionated on a 1% formaldehyde gel and then transferred to a Zeta-Probe nylon membrane (Bio-Rad, Hercules, CA). The Δ6-desaturase and β-actin probes were prepared by incorporating [^{32}P]dCTP by random primer labeling. Northern blot hybridization analysis was performed as described by Sambrook *et al.* (23). The auto radiographic signal for Δ6-desaturase mRNA was quantified and normalized to mRNA for β-actin, with all mRNA probed on the same gel.

Other analytical determinations. Calcium content in liver microsomal suspensions was determined after mineralization (5) by atomic absorption or calcium-sensitive electrode as described elsewhere (1,4). The protein content was determined by the micromethod of Lowry *et al.* (24) with crystalline serum albumin as standard. Fluorescence anisotropy measurements 352 nm excitation, 435 nm emission were done at 37°C following the procedure of Shinitzky and Barenholz (25,26). The apparatus used, a detailed description of the method employed, and the calculations/corrections made appeared in a previous paper (11).

Graphic software and statistical treatment of the data. All values represent the mean of 3 to 6 individual determinations (assayed in duplicate or triplicate) ± 1 SEM. To test the statistical significance of numerical differences in results, data were analyzed by either the Student *t*-test or by ANOVA, with the aid of Systat (version 8.0 for Windows) from SPSS Science (Chicago, IL). Data were also plotted and analyzed using Sigma Scientific Graphing Software (version 8.0) from Sigma Chemical Co. and/or GB-STAT Professional Statistics Program (version 4.0) from Dynamic Microsystems Inc. (Silver Spring, MD). The autoradiographic signal for Δ6-desaturase mRNA was quantified using 1-D Image Analysis Software (Kodak, Rochester, NY) from multiple exposures. It was normalized to mRNA for β-actin, with all mRNA probed on the same gel.

RESULTS

In our experimental model of calcium deficiency, we observed that both S and CaD rats grew at a similar rate for the initial 15 d after feeding. Then, the CaD group grew at a rate that was reduced—but not statistically significant—until day 65, when differences between groups became significant (Fig. 1). Water intake expressed as mL/rat was very similar in both groups of animals throughout the entire experimental period (Fig.1, inset). As shown in Figure 2, food intakes, expressed as grams of food per rat or relative to body weight (inset), were not significantly different between both groups of rats despite the fact that in the initial 10 d of feeding, CaD animals exhibited an increased food intake compared to that of S ones. After the first week of feeding, a progressive decline in plasma calcium concentration was observed in CaD rats (from 2.63 ± 0.10 mM at day 0 to 2.11 ± 0.15, 1.73 ± 0.12, or 1.58 ± 0.07 mM at days 8, 16, and 24, respectively) up to day 28 in which the values remained essentially constant and significantly lower (1.50 ± 0.08 mM) than those from the S group (2.63 ± 0.10 mM). Table 1 shows calcium content determinations in various tissues on the day of sacrifice.

With the exception of fat, brain, and lung, the rest of the tissue homogenates showed an important decrease in Ca content. In large bones the loss of calcium represented *ca.* 60% decrease with respect to control samples. Liver, kidney, erythrocytes, cortical bone, and articular cartilage showed 30 to 50% decreases in calcium content, but heart and skeletal muscle were affected to a minor extent (20%). When the microsomal suspension of some tissues was examined for their calcium content, only liver, kidney, and spleen showed significant decrease in the concentration of this cation (a loss of 42 to 47% with respect to control determinations). These findings were reflected in the total calcium content of plasma, which decreased 43% with respect to that of S rats. The proportion of ionic calcium in the plasma of CaD animals also decreased 66% with respect

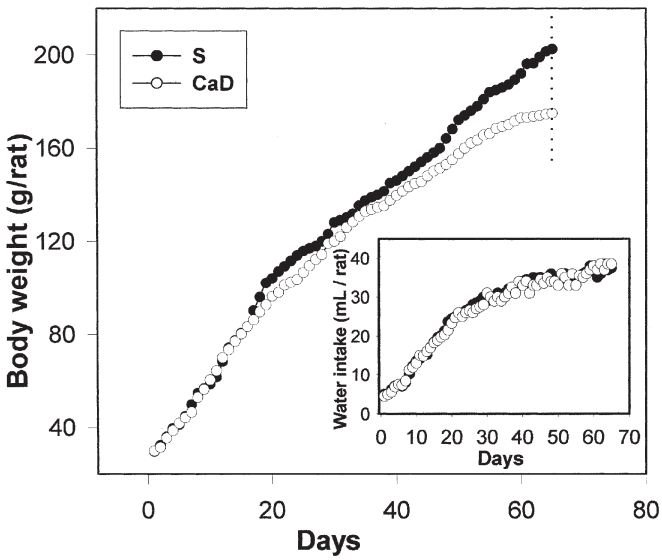

FIG. 1. Body weight (expressed as g per rat) and water intake (mL/rat, insert) were registered during the entire experimental period. Each datum is the mean of six rats per group. SEM, omitted for simplicity, never exceeded 6% of the corresponding mean value. The vertical dotted line represents the first significant difference between control (S) and calcium-deficient (CaD) rats.

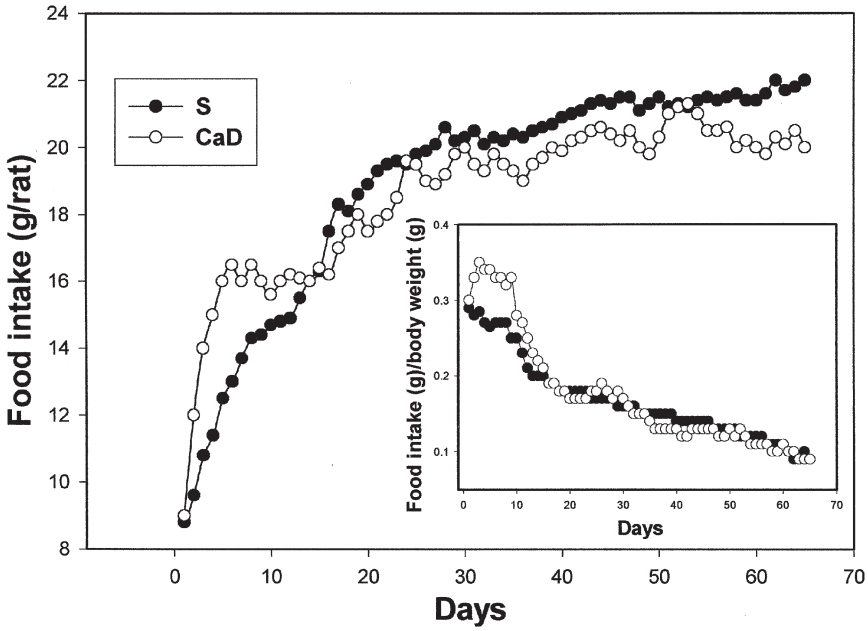

FIG. 2. Food intake was registered during the entire experimental period and expressed as g of aliment per rat or as the ratio g of food/g of body weight (insert). Each datum is the mean of six rats per group. SEM, omitted for simplicity, never exceeded 8% of the corresponding mean value. Points from days 2 to 9 (inclusive) were significantly different between S and CaD groups. For abbreviations see Figure 1.

to control ones, and the percentage of bound calcium in the blood of treated animals (78.0 ± 2.1) was significantly higher than that found in the control group (62.2 ± 1.8).

The fatty acyl composition of neutral and polar lipids was profoundly modified under CaD conditions. The most important changes were found in liver, followed by kidney, spleen, and cartilage. Table 2 shows the result obtained when we analyzed microsomal neutral lipids from liver, brain, kidney, and heart. Neutral lipids from brain microsomes exhibited a decreased content of arachidonate and docosahexaenoate (n-3). By contrast, heart microsomes showed no significant changes. Palmitate and stearate content were increased in microsomal neutral lipids from both liver and kidney, with no concomitant modifications in the proportion of the corresponding monoenoates. In liver, the content of linoleate was diminished by 47% with respect to control values, and the proportion of 20:4n-6 (arachidonate) was dramatically reduced with a simultaneous increase in the level of its direct

TABLE 1
Calcium[a] Content in Tissues[b] from Control (S) or Calcium-Deficient (CaD) Rats

	Whole tissue		Microsomes	
	S	CaD	S	CaD
Liver[a]	1.51 ± 0.05	0.90 ± 0.02*	2.62 ± 0.04	1.51 ± 0.05*
Heart[a]	2.22 ± 0.05	1.80 ± 0.04*	5.53 ± 0.11	5.10 ± 0.20
Skeletal muscle[a]	3.10 ± 0.12	2.55 ± 0.08*	6.06 ± 0.20	5.74 ± 0.31
Brain[a]	2.30 ± 0.08	2.15 ± 0.10	4.12 ± 0.07	3.60 ± 0.15
Kidney[a]	1.23 ± 0.02	0.77 ± 0.03*	1.74 ± 0.05	0.92 ± 0.06*
Fat[a]	0.95 ± 0.04	0.80 ± 0.10	ND	ND
Erythrocytes[a]	1.88 ± 0.11	1.10 ± 0.07*	ND	ND
Lung[a]	2.12 ± 0.06	1.93 ± 0.11	3.05 ± 0.09	2.68 ± 0.13
Spleen[a]	1.76 ± 0.05	0.84 ± 0.03*	1.97 ± 0.06	1.15 ± 0.08*
Cortical bone[b]	18.6 ± 0.7	11.7 ± 0.5*	ND	ND
Epiphyseal bone[b]	15.9 ± 0.4	7.4 ± 0.6*	ND	ND
Diaphyseal bone[b]	21.5 ± 0.5	10.1 ± 0.3*	ND	ND
Articular cartilage[b]	6.3 ± 0.4	4.2 ± 0.1*	ND	ND
Plasma (total)[c]	2.63 ± 0.10	1.50 ± 0.08*	ND	ND
Plasma (ionic)[c]	0.98 ± 0.03	0.33 ± 0.02*	ND	ND

[a]Calcium contents were determined by atomic absorption from samples collected on day 65 after feeding. For technical details see the Materials and Methods section.
[b]Results were expressed as [a]nmol/mg of protein; [b]percentage of total wet weight; or [c]mM. Each value is the mean ± 1 SEM of four to six independent determinations assayed in duplicate. *Significantly different ($P < 0.01$) with respect to the corresponding S value.

TABLE 2
Fatty Acyl Composition[a] of Microsomal Neutral Lipids from Tissues of Control (S) or Calcium-Deficient (CaD) Rats

	Liver		Brain		Kidney		Heart	
FA	S	CaD	S	CaD	S	CaD	S	CaD
14:0	0.6 ± 0.1	0.6 ± 0.1	0.2 ± 0.0	0.3 ± 0.0	0.8 ± 0.1	0.5 ± 0.1	1.6 ± 0.2	2.2 ± 0.1
16:0	38.9 ± 1.2	45.9 ± 1.5*	30.3 ± 2.0	31.5 ± 3.1	25.9 ± 1.3	33.0 ± 2.0*	40.1 ± 3.0	39.0 ± 2.5
16:1n-7	3.3 ± 0.1	4.2 ± 0.2	6.5 ± 0.2	7.2 ± 0.3	4.0 ± 0.5	3.9 ± 0.1	2.4 ± 0.2	1.5 ± 0.1
18:0	7.8 ± 0.2	11.6 ± 0.9*	7.1 ± 0.1	6.8 ± 0.2	18.4 ± 2.5	24.3 ± 1.9*	36.1 ± 2.1	38.7 ± 1.9
18:1n-9	16.5 ± 0.6	18.5 ± 1.8	39.8 ± 2.7	41.0 ± 3.5	21.2 ± 1.4	20.7 ± 1.3	13.2 ± 0.7	14.3 ± 0.7
18:2n-6	25.4 ± 1.3	13.6 ± 0.7*	20.1 ± 1.6	19.5 ± 1.5	17.0 ± 1.8	15.0 ± 1.0	6.4 ± 0.3	5.8 ± 0.1
18:3n-6	0.1 ± 0.0	Trace	0.1 ± 0.0	0.2 ± 1.5	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.2 ± 0.0
18:3n-3	0.1 ± 0.0	Trace	0.2 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	Trace	0.1 ± 0.0	0.2 ± 0.0
18:4n-3	0.1 ± 0.0	Trace	0.3 ± 0.1	0.2 ± 0.0	Trace	Trace	0.1 ± 0.0	Trace
20:3n-6	0.3 ± 0.1	1.1 ± 0.1*	0.8 ± 0.1	0.5 ± 0.1	0.4 ± 0.1	0.5 ± 0.1	0.3 ± 0.1	0.1 ± 0.0
20:4n-6	4.5 ± 0.1	Trace*	5.5 ± 0.2	4.0 ± 0.1*	6.3 ± 0.1	3.5 ± 0.1*	2.9 ± 0.1	2.1 ± 0.1
20:5n-3	0.1 ± 0.0	Trace	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	Trace	0.1 ± 0.0	0.1 ± 0.0
22:0	Trace	0.2 ± 0.0*	0.1 ± 0.0	Trace	0.1 ± 0.0	Trace	0.3 ± 0.0	0.1 ± 0.0
22:1n-9	0.1 ± 0.0	Trace	0.1 ± 0.0	Trace	0.1 ± 0.0	Trace	0.1 ± 0.0	Trace
22:2n-6	0.2 ± 0.0	Trace	0.2 ± 0.0	Trace	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0
22:3n-3	0.1 ± 0.0	Trace	0.2 ± 0.0	Trace	Trace	0.1 ± 0.0	Trace	Trace
22:4n-6	0.1 ± 0.0	Trace	0.1 ± 0.0	Trace	Trace	Trace	Trace	Trace
22:5n-6	0.2 ± 0.0	Trace	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	Trace	Trace
22:4n-3	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.2 ± 0.0	Trace	Trace
22:5n-3	Trace	Trace	0.3 ± 0.1	0.1 ± 0.0	Trace	0.1 ± 0.0	Trace	Trace
22:6n-3	0.1 ± 0.0	0.1 ± 0.0	0.8 ± 0.1	0.3 ± 0.1*	0.1 ± 0.0	0.2 ± 0.0	0.2 ± 0.0	Trace
24:1	Trace	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	Trace	Trace	Trace	Trace

[a]GLC of the FAME was performed as indicated in the Materials and Methods section. Each value is expressed as μg/mg protein and corresponds to the mean ± 1 SEM of three independent determinations performed on randomly selected samples. Other FA not listed in the table are present in minor amounts. *Significantly different ($P < 0.01$) with respect to the corresponding control value. Amounts below 0.1 μg/mg protein are indicated as "Trace." SEM below 0.1 are indicated as "0.0."

precursor (20:3n-6). The fall in 20:4n-6 was also observed in neutral lipids from kidney microsomes, but it was of lesser extent (44%) than that of liver (98%). Within the C_{22} FA homologs, we observed in liver microsomes a minor increase in the proportion of 22:0. Phospholipid fatty acyl composition was also modified by calcium deficiency (Table 3). The composition of brain and heart microsomes showed no significant modifications, whereas liver and kidney exhibited an altered pattern. The extent of these changes, especially in liver microsomes, was more pronounced in both number and extension than that observed in neutral lipids. The saturated FA 14:0, 16:0, 18:0, and 22:0 were increased in liver phospholipids from CaD rats, whereas kidney phospholipids contained elevated levels of palmitate and stearate relative to those found in S rats. The most striking difference between the phospholipid fatty acyl composition from CaD and S animals was the higher proportion of linoleate in liver and kidney (72 and 40% over control data, respectively). Arachidonate was decreased *ca.* 60% in liver without any modification in the proportion of its metabolic precursor (20:3n-6). In contrast, the content of 20:4n-6 in phospholipids from kidney microsomes remained unchanged. In phospholipids of microsomes from CaD rats, the highest homologs derived from α-linolenate, such as 20:5n-3 and 22:6n-3, were decreased 75 and 48%, respectively, with a concomitant increase of 50% in their metabolic precursor (18:3n-3).

Tables 4 and 5 summarize analytical data of fatty acyl composition from neutral and polar lipids of spleen and lung microsomes, fat, and articular cartilage. In neutral lipids from spleen microsomes (Table 4), saturated FA were increased but arachidonate was decreased *ca.* 83% with respect to the values obtained in S rats. An important increase of 20:3n-6 (400%) was also observed. Neutral lipids of fat from CaD rats showed an increased proportion of arachidonate that was the only change associated with dietary manipulation. Lung microsomes evidenced no significant changes under calcium restriction, but in the case of articular cartilage, an increase in 20:3n-9 and 22:1n-9 FA with a simultaneous decrease in 20:4n-6 and 22:2n-6 were observed (Table 4). The phospholipid fraction from spleen microsomes (Table 5) showed alterations similar to those described for the neutral lipids, that is, an increased proportion of 16:0, 18:0, and 20:3n-6 with a reduced relative amount of 20:4n-6. Contrary to what was seen with neutral lipids, in phospholipids of fat from CaD rats an 86% reduction in arachidonate content was observed. Table 5 also shows that phospholipids from both lung and articular cartilage exhibited a marked decrease in arachidonate proportion. It is also important to remark that, in articular cartilage, we found a large amount of 20:3n-9 FA and low levels of n-6 PUFA (<10%), as previously reported by other authors (27,28). This particular composition is more evident in phospholipids than in neutral lipids of S rats. Interestingly, 20:3n-9 accumulated in neutral and polar lipids of articular cartilage under the CaD condition (Table 5).

The composition of fatty acyl chains in neutral lipids from erythrocyte ghosts or plasmas obtained from CaD animals remained unchanged with respect to that of S rats (Table 6). However, in phospholipids from erythrocyte ghosts a

TABLE 3
Fatty Acyl Composition[a] of Microsomal Phospholipids from Tissues of Control (S) or Calcium-Deficient (CaD) Rats

	Liver		Brain		Kidney		Heart	
FA	S	CaD	S	CaD	S	CaD	S	CaD
14:0	0.2 ± 0.0	0.9 ± 0.1*	0.2 ± 0.0	0.3 ± 0.1	0.1 ± 0.0	0.4 ± 0.1	1.1 ± 0.2	1.9 ± 0.2
16:0	17.5 ± 1.3	24.6 ± 1.3*	33.7 ± 1.9	35.4 ± 2.0	18.7 ± 0.9	25.5 ± 0.7*	14.0 ± 0.9	17.0 ± 1.3
16:1n-7	1.0 ± 0.1	0.7 ± 0.1	7.8 ± 0.1	8.1 ± 0.4	1.8 ± 0.1	1.3 ± 0.1	3.2 ± 0.1	3.0 ± 0.2
18:0	20.2 ± 0.9	28.8 ± 1.3*	28.5 ± 2.0	32.1 ± 3.1	16.2 ± 0.7	22.2 ± 1.1*	21.7 ± 1.6	25.4 ± 2.1
18:1n-9	5.5 ± 0.3	5.7 ± 0.2	22.3 ± 1.2	20.3 ± 2.0	9.3 ± 0.5	10.3 ± 0.8	5.8 ± 0.2	4.9 ± 0.2
18:2n-6	4.6 ± 0.2	7.9 ± 0.2*	14.5 ± 0.2	15.9 ± 1.0	9.6 ± 0.4	13.4 ± 0.3*	12.6 ± 1.1	11.7 ± 0.9
18:3n-6	0.3 ± 0.1	0.4 ± 0.1	0.3 ± 0.1	0.3 ± 0.1	0.2 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.2 ± 0.0
18:3n-3	0.2 ± 0.0	0.4 ± 0.0*	0.2 ± 0.0	0.1 ± 0.0	0.4 ± 0.1	0.3 ± 0.0	0.2 ± 0.0	0.2 ± 0.0
18:4n-3	0.1 ± 0.0	0.1 ± 0.0	0.5 ± 0.1	0.3 ± 0.0	0.3 ± 0.1	0.4 ± 0.1	0.3 ± 0.0	0.2 ± 0.0
20:3n-6	2.7 ± 0.1	2.0 ± 0.2*	1.7 ± 0.1	2.2 ± 0.1	1.5 ± 0.1	0.6 ± 0.1	0.8 ± 0.1	0.7 ± 0.1
20:4n-6	27.4 ± 1.5	11.8 ± 2.0*	6.9 ± 0.2	6.0 ± 0.2	24.7 ± 0.8	21.5 ± 1.3	18.7 ± 0.3	15.5 ± 0.4
20:5n-3	0.4 ± 0.1	0.1 ± 0.0*	0.2 ± 0.0	0.3 ± 0.1	0.3 ± 0.1	0.2 ± 0.0	0.3 ± 0.1	0.2 ± 0.0
22:0	0.2 ± 0.0	0.5 ± 0.0*	0.1 ± 0.0	0.1 ± 0.0	0.4 ± 0.1	0.2 ± 0.0	0.2 ± 0.0	Trace
22:1n-9	0.2 ± 0.0	0.1 ± 0.0	0.2 ± 0.0	0.1 ± 0.0	0.2 ± 0.0	Trace	0.2 ± 0.0	0.3 ± 0.1
22:2n-6	0.3 ± 0.1	Trace	0.5 ± 0.1	0.3 ± 0.1	0.1 ± 0.0	Trace	0.1 ± 0.0	0.1 ± 0.0
22:3n-3	0.1 ± 0.0	Trace	0.3 ± 0.0	0.2 ± 0.0	0.2 ± 0.0	Trace	Trace	Trace
22:4n-6	0.3 ± 0.0	0.1 ± 0.0	0.4 ± 0.1	0.2 ± 0.0	0.3 ± 0.1	0.1 ± 0.0	Trace	Trace
22:5n-6	0.2 ± 0.0	Trace	1.1 ± 0.2	0.9 ± 0.1	0.2 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0
22:4n-3	0.2 ± 0.0	Trace	0.3 ± 0.1	0.2 ± 0.0	0.3 ± 0.1	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0
22:5n-3	0.1 ± 0.0	Trace	0.8 ± 0.1	1.1 ± 0.1	0.3 ± 0.0	0.1 ± 0.0	0.2 ± 0.0	0.1 ± 0.0
22:6n-3	10.1 ± 0.3	5.3 ± 0.2*	9.2 ± 0.3	10.2 ± 0.2	2.6 ± 0.2	2.0 ± 0.1	7.0 ± 0.2	6.3 ± 0.2
24:1	Trace	0.1 ± 0.0	0.2 ± 0.0	0.3 ± 01	Trace	Trace	0.2 ± 0.0	0.1 ± 0.0

[a]GLC of the FAME was performed as indicated in the Materials and Methods section. Each value is expressed as μg/mg protein and corresponds to the mean ± 1 SEM of three independent determinations performed on randomly selected samples. Other FA not listed in the table are present in minor amounts. *Significantly different ($P < 0.01$) with respect to the corresponding control value. Amounts below 0.1 μg/mg protein are indicated as "Trace." SEM below 0.1 are indicated as "0.0."

TABLE 4
Fatty Acyl Composition[a] of Microsomal Neutral Lipids from Tissues of Control (S) or Calcium-Deficient (CaD) Rats

	Spleen		Lung		Fat		Cartilage	
FA	S	CaD	S	CaD	S	CaD	S	CaD
14:0	0.8 ± 0.1	0.6 ± 0.1	1.6 ± 0.3	1.0 ± 0.1	1.5 ± 0.2	1.1 ± 0.2	2.0 ± 0.2	2.3 ± 0.3
16:0	33.5 ± 1.2	39.7 ± 1.3*	47.0 ± 2.5	40.5 ± 1.8	22.4 ± 1.0	21.2 ± 1.1	30.4 ± 1.9	29.6 ± 2.0
16:1n-7	2.5 ± 0.1	3.1 ± 0.2	4.0 ± 0.1	4.7 ± 0.2	7.2 ± 0.2	6.8 ± 0.2	6.1 ± 0.2	5.7 ± 0.4
18:0	13.3 ± 0.3	16.9 ± 0.7*	11.8 ± 0.4	13.4 ± 0.3	6.9 ± 0.3	9.0 ± 0.2	7.3 ± 0.2	8.6 ± 0.4
18:1n-9	28.0 ± 1.7	22.2 ± 2.3	12.5 ± 0.4	10.0 ± 0.5	35.7 ± 2.0	33.1 ± 2.0	47.4 ± 2.5	45.0 ± 1.9
18:2n-6	15.0 ± 1.0	15.3 ± 0.9	10.0 ± 0.5	14.3 ± 0.5	22.1 ± 1.7	23.5 ± 2.5	2.6 ± 0.1	3.0 ± 0.2
18:3n-6	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.2 ± 0.0	0.1 ± 0.0	0.2 ± 0.0	0.1 ± 0.0	0.1 ± 0.0
18:3n-3	0.2 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.2 ± 0.0	0.1 ± 0.0	0.2 ± 0.0
18:4n-3	0.1 ± 0.0	0.1 ± 0.0	Trace	0.1 ± 0.0	0.2 ± 0.0	0.3 ± 0.0	0.2 ± 0.0	0.3 ± 0.0
20:3n-9	Trace	Trace	Trace	0.1 ± 0.0	Trace	Trace	2.2 ± 0.3	3.8 ± 0.2*
20:3n-6	0.2 ± 0.1	1.0 ± 0.1*	1.5 ± 0.1	1.8 ± 0.2	0.5 ± 0.1	0.9 ± 0.1	0.1 ± 0.0	0.1 ± 0.0
20:4n-6	5.3 ± 0.2	0.9 ± 0.2*	7.0 ± 0.2	9.7 ± 0.6	1.5 ± 0.1	2.7 ± 0.1*	0.3 ± 0.0	Trace*
20:5n-3	Trace	Trace	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.2 ± 0.0	0.1 ± 0.0	0.2 ± 0.0
22:0	Trace	Trace	0.3 ± 0.0	0.2 ± 0.0	0.3 ± 0.0	0.4 ± 0.1	0.4 ± 0.1	0.5 ± 0.1
22:1n-9	Trace	Trace	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.2 ± 0.1	0.2 ± 0.0	0.5 ± 0.1*
22:2n-6	0.2 ± 0.0	Trace	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.5 ± 0.1	0.1 ± 0.0*
22:3n-3	Trace	Trace	0.1 ± 0.0	0.2 ± 0.0	Trace	Trace	Trace	Trace
22:4n-6	0.1 ± 0.0	Trace	1.5 ± 0.2	1.3 ± 0.1	0.1 ± 0.0	0.1 ± 0.0	Trace	Trace
22:5n-6	0.4 ± 0.1	Trace	1.0 ± 0.1	0.9 ± 0.1	0.1 ± 0.0	Trace	Trace	Trace
22:4n-3	Trace	Trace	0.2 ± 0.0	0.2 ± 0.0	Trace	Trace	Trace	Trace
22:5n-3	Trace	Trace	0.3 ± 0.0	0.1 ± 0.0	0.2 ± 0.0	Trace	Trace	Trace
22:6n-3	0.3 ± 0.0	Trace	0.7 ± 0.1	0.8 ± 0.1	0.7 ± 0.1	Trace	Trace	Trace
24:1	Trace	Trace	Trace	0.1 ± 0.0	0.1 ± 0.0	Trace	Trace	Trace

[a]GLC of the FAME was performed as indicated in the Materials and Methods section. Each value is expressed as μg/mg protein and corresponds to the mean ± 1 SEM of three independent determinations performed on randomly selected samples. Other FA not listed in the table are present in minor amounts. *Significantly different ($P < 0.01$) with respect to the corresponding control value. Amounts below 0.1 μg/mg protein are indicated as "Trace." SEM below 0.1 are indicated as "0.0."

TABLE 5
Fatty Acyl Composition[a] of Microsomal Phospholipids from Tissues of Control (S) or Calcium-Deficient (CaD) Rats

	Spleen		Lung		Fat		Cartilage	
FA	S	CaD	S	CaD	S	CaD	S	CaD
14:0	Trace	0.1 ± 0.0	1.0 ± 0.1	0.9 ± 0.1	1.2 ± 0.2	1.1 ± 0.1	0.3 ± 0.0	0.3 ± 0.1
16:0	21.5 ± 1.1	27.2 ± 0.8*	23.2 ± 2.2	24.5 ± 2.1	25.4 ± 0.9	27.3 ± 1.5	12.5 ± 0.3	15.4 ± 0.3*
16:1n-7	3.0 ± 0.1	2.7 ± 0.1	6.1 ± 0.5	7.2 ± 0.3	4.5 ± 0.2	4.9 ± 0.2	9.5 ± 0.2	8.0 ± 0.4
18:0	12.0 ± 0.5	14.8 ± 0.6*	10.0 ± 0.4	8.8 ± 0.2	12.0 ± 0.3	13.4 ± 0.4	17.0 ± 0.5	19.8 ± 0.6*
18:1n-9	18.7 ± 0.6	16.3 ± 1.0	15.5 ± 0.6	17.3 ± 0.6	24.1 ± 1.0	26.5 ± 2.1	40.4 ± 2.0	31.8 ± 1.3*
18:2n-6	14.6 ± 0.5	15.7 ± 0.8	13.1 ± 0.7	14.0 ± 0.4	17.5 ± 0.4	17.0 ± 0.9	3.5 ± 0.1	4.0 ± 0.1
18:3n-6	0.5 ± 0.1	0.6 ± 0.1	0.3 ± 0.0	0.5 ± 0.1	0.2 ± 0.0	0.3 ± 0.0	0.1 ± 0.0	0.1 ± 0.0
18:3n-3	0.3 ± 0.0	0.2 ± 0.0	0.2 ± 0.0	0.2 ± 0.0	0.3 ± 0.0	0.4 ± 0.1	0.2 ± 0.0	0.1 ± 0.0
18:4n-3	0.2 ± 0.0	0.2 ± 0.0	0.2 ± 0.0	0.2 ± 0.0	0.1 ± 0.0	0.3 ± 0.0	0.1 ± 0.0	0.2 ± 0.0
20:3n-9	Trace	0.1 ± 0.0	0.2 ± 0.0	0.2 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	8.5 ± 0.2	13.0 ± 0.5*
20:3n-6	2.4 ± 0.1	3.6 ± 0.1*	2.6 ± 0.2	3.0 ± 0.2	2.2 ± 0.1	2.6 ± 0.1	0.5 ± 0.1	1.0 ± 0.1*
20:4n-6	18.5 ± 0.3	12.1 ± 0.3*	14.1 ± 0.8	10.0 ± 0.2*	7.4 ± 0.1	1.0 ± 0.1*	4.8 ± 0.1	2.3 ± 0.1*
20:5n-3	0.2 ± 0.0	0.1 ± 0.0	0.4 ± 0.0	0.5 ± 0.1	0.3 ± 0.0	0.3 ± 0.0	0.3 ± 0.0	0.4 ± 0.1
22:0	0.1 ± 0.0	0.1 ± 0.0	0.2 ± 0.0	0.2 ± 0.0	0.4 ± 0.1	0.5 ± 0.1	0.3 ± 0.0	0.3 ± 0.0
22:1n-9	0.2 ± 0.0	0.2 ± 0.0	0.2 ± 0.0	0.4 ± 0.1	0.2 ± 0.0	0.2 ± 0.0	1.6 ± 0.1	2.9 ± 0.1*
22:2n-6	0.3 ± 0.0	0.2 ± 0.0	0.5 ± 0.1	0.4 ± 0.1	0.4 ± 0.1	0.3 ± 0.0	Trace	Trace
22:3n-3	0.1 ± 0.0	0.1 ± 0.0	0.2 ± 0.0	0.3 ± 0.0	0.2 ± 0.0	0.3 ± 0.1	0.3 ± 0.0	0.4 ± 0.1
22:4n-6	3.3 ± 0.1	2.5 ± 0.2	4.3 ± 0.2	3.8 ± 0.1	0.3 ± 0.0	0.2 ± 0.0	0.1 ± 0.0	Trace
22:5n-6	1.5 ± 0.1	1.1 ± 0.3	3.5 ± 0.1	3.0 ± 0.1	0.2 ± 0.0	0.3 ± 0.0	Trace	Trace
22:4n-3	0.2 ± 0.0	0.1 ± 0.0	0.4 ± 0.1	0.5 ± 0.1	0.2 ± 0.0	0.2 ± 0.0	Trace	Trace
22:5n-3	1.0 ± 0.1	0.8 ± 0.1	0.8 ± 0.1	0.9 ± 0.1	0.8 ± 0.1	0.7 ± 0.1	Trace	Trace
22:6n-3	1.3 ± 0.1	1.1 ± 0.2	2.9 ± 0.1	3.1 ± 0.1	1.8 ± 0.2	1.9 ± 0.1	Trace	Trace
24:1	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.2 ± 0.0	0.2 ± 0.0	Trace	Trace

[a]GLC of the FAME was performed as indicated in the Materials and Methods section. Each value is expressed as μg/mg protein and corresponds to the mean ± 1 SEM of three independent determinations performed on randomly selected samples. Other FA not listed in the table are present in minor amounts. *Significantly different ($P < 0.01$) with respect to the corresponding control value. Amounts below 0.1 μg/mg protein are indicated as "Trace." SEM below 0.1 are indicated as "0.0."

TABLE 6
Fatty Acyl Composition[a] of Neutral and Polar Lipids from Erythrocyte Ghosts and Plasmas from Control (S) or Calcium-Deficient (CaD) Rats

	Neutral lipids				Phospholipids			
	Erythrocyte ghosts		Plasmas		Erythrocyte ghosts		Plasmas	
FA	S	CaD	S	CaD	S	CaD	S	CaD
14:0	0.7 ± 0.1	0.5 ± 0.1	0.1 ± 0.0	0.1 ± 0.0	Trace	0.1 ± 0.0	0.3 ± 0.1	0.3 ± 0.1
16:0	48.8 ± 3.1	47.2 ± 4.4	37.5 ± 2.2	41.0 ± 3.9	30.1 ± 2.0	29.8 ± 3.0	23.1 ± 0.8	26.9 ± 0.5*
16:1n-7	0.5 ± 0.1	0.7 ± 0.1	1.6 ± 0.1	1.3 ± 0.2	2.1 ± 0.1	1.7 ± 0.2	2.5 ± 0.1	2.3 ± 0.1
18:0	45.1 ± 4.2	48.1 ± 4.0	12.6 ± 1.7	13.2 ± 1.0	16.4 ± 0.8	17.5 ± 1.1	23.0 ± 2.2	24.4 ± 1.1
18:1n-9	10.9 ± 1.1	9.7 ± 0.4	8.9 ± 1.5	9.0 ± 0.5	10.6 ± 0.5	11.3 ± 0.7	6.6 ± 0.2	5.9 ± 0.3
18:2n-6	3.0 ± 0.3	4.5 ± 0.4	32.8 ± 2.6	33.3 ± 4.0	16.8 ± 1.1	15.0 ± 0.9	11.3 ± 0.2	19.8 ± 0.3*
18:3n-6	0.1 ± 0.0	0.2 ± 0.0	0.1 ± 0.0	0.2 ± 0.0	0.2 ± 0.0	0.1 ± 0.0	0.4 ± 0.1	0.3 ± 0.0
18:3n-3	0.2 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.3 ± 0.1	0.2 ± 0.0	0.1 ± 0.0
18:4n-3	0.1 ± 0.0	0.1 ± 0.0	0.2 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.2 ± 0.0	0.3 ± 0.0	0.2 ± 0.0
20:3n-6	0.1 ± 0.0	0.1 ± 0.0	0.2 ± 0.0	0.2 ± 0.0	1.0 ± 0.1	0.8 ± 0.1	1.0 ± 0.1	0.3 ± 0.0
20:4n-6	0.8 ± 0.1	0.5 ± 0.1	5.1 ± 0.1	4.7 ± 0.1	7.6 ± 0.1	4.2 ± 0.1*	14.9 ± 0.2	8.8 ± 0.3*
20:5n-3	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.3 ± 0.1	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0
22:0	Trace	0.1 ± 0.0	Trace	Trace	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	Trace
22:1n-9	Trace	0.1 ± 0.0	Trace	Trace	0.1 ± 0.0	0.1 ± 0.0	0.3 ± 0.0	0.2 ± 0.0
22:2n-6	0.1 ± 0.0	Trace	Trace	Trace	0.2 ± 0.0	Trace	0.2 ± 0.0	0.1 ± 0.0
22:3n-3	Trace	Trace	Trace	Trace	0.1 ± 0.0	Trace	0.3 ± 0.1	0.2 ± 0.0
22:4n-6	0.1 ± 0.0	Trace	0.1 ± 0.0	Trace	0.3 ± 0.0	Trace	0.2 ± 0.0	Trace
22:5n-6	0.1 ± 0.0	Trace	0.1 ± 0.0	Trace	0.2 ± 0.0	Trace	0.1 ± 0.0	0.1 ± 0.0
22:4n-3	Trace	Trace	Trace	Trace	0.3 ± 0.0	Trace	0.1 ± 0.0	0.1 ± 0.0
22:5n-3	Trace	Trace	Trace	Trace	0.1 ± 0.0	Trace	0.1 ± 0.0	Trace
22:6n-3	0.1 ± 0.0	Trace	0.3 ± 0.1	0.1 ± 0.0	7.9 ± 0.1	5.0 ± 0.1*	3.2 ± 0.1	1.9 ± 0.2
24:1	Trace	Trace	0.1 ± 0.0	Trace	0.1 ± 0.0	Trace	Trace	0.1 ± 0.0

[a]GLC of the FAME was performed as indicated in the Materials and Methods section. Each value is expressed as μg/mg protein and corresponds to the mean ± 1 SEM of three independent determinations performed on randomly selected samples. Other FA not listed in the table are present in minor amounts. *Significantly different ($P < 0.01$) with respect to the corresponding control value. SEM below 0.1 are indicated as "0.0." Amounts below 0.1 μg/mg protein are indicated as "Trace."

decrease of 45% in the relative content of arachidonate and a 37% reduction in the 22:6n-3 level were observed. In the plasma phospholipid fraction we found much increased percentages of palmitate and α-linoleate and decreased arachidonate levels relative to those of control animals (Table 6).

The analytical changes described above correlate well with various metabolic indicators that account for the rate of supply and/or utilization of the different FA chains acylated to lipids (29,30). In Table 7 are summarized some of these parameters for liver and kidney, calculated from the results in Tables 2 and 3. The double-bond index (DBI), obtained as PUFA/saturated FA ratio, was significantly decreased (50 to 60%) in neutral and polar lipids from both liver and kidney microsomes. One possible explanation is that this result may be the consequence of a general failure in FA desaturation capacity together with an accumulation of saturated FA. In all fractions studied, the decrease in the PUFA relative content was evoked through a similar contribution of the n-3 and n-6 FA, resulting in a very similar n-3/n-6 ratio for control and treated animals. However, it was not the same with the essential/nonessential FA ratio. This parameter was significantly diminished in both neutral and polar lipids from the liver and kidney of CaD rats. This alteration may be produced through both a selective loss of unsaturated FA derived from linoleate and/or α-linolenate and the increment in the FA members of the n-7 and n-9 series. In fact, results from Table 7 indicate that these two possibilities are in agreement with the results obtained. The inhibition in the metabolic transformation of linoleate was clearly indicated through the decreased values of the ED (elongation–desaturation) 18:2n-6 index. Moreover, comparison between this index and ED 20:4n-6 also indicates that the impairment for linoleate utilization was greater in the Δ6 desaturation step than in the Δ5 one. Similar conclusions can be derived from comparison between the sum of the desaturase index activity—Δ6 + Δ5 DIA—and the individual index for Δ6 and Δ5 desaturase enzymes. With regard to this metabolic pathway in kidney phospholipids, Δ5-desaturase activity not only remained at control levels under CaD conditions but also was stimulated (Table 7). The significant decrease in the palmitoyl- and stearoyl-desaturase activity indexes indicates that calcium deficiency also evoked an impairment in the metabolic utilization of both palmitate and stearate, the precursors of the n-7 and n-9 FA series, respectively.

Lipid relationships and data from fluorescence anisotropy measurement of various tissues from CaD rats are shown in Tables 8 and 9. Cholesterol/phospholipid ratio was diminished in heart and kidney microsomes and also in erythrocyte ghosts, articular cartilage, and plasmas. With the sole exception of brain microsomes, in all samples studied a significant reduction in the cholesterol/protein ratio was observed. In most of the tissues, the total lipid/cholesterol ratio was also diminished, indicating that the reduction in the proportion of total lipids was greater than that of cholesterol. The total lipid/protein ratio was significantly reduced in spleen, erythrocyte ghosts, cartilage, and plasmas from CaD rats. Articular cartilage was the only tissue that exhibited a significant decrease in the proportion of total lipids to phospholipids, whereas all the other examined tissues showed an important increase in neutral lipid content relative to polar lipids. Measurements of neutral and polar lipid contents relative to the amount of total protein demonstrated that the general increase observed in the neutral lipid/polar lipid ratio was evoked primarily by the reduction in the phospholipid/protein ratio rather than the increase in the neutral lipid/protein ratio (Table 7).

Steady-state DPH fluorescence anisotropy determinations

TABLE 7
Analytical Parameters from Fatty Acyl Composition of Microsomal Lipids

Analytical parameters	Phospholipids[a]				Neutral lipids[a]			
	Liver		Kidney		Liver		Kidney	
	S	CaD	S	CaD	S	CaD	S	CaD
Saturated acids	38.1 ± 1.1	54.6 ± 0.8*	35.4 ± 1.4	48.1 ± 3.1*	47.3 ± 2.1	57.3 ± 1.8*	45.2 ± 1.8	57.8 ± 2.5*
PUFA	47.0 ± 2.0	27.9 ± 0.7*	41.2 ± 2.6	38.9 ± 2.9	31.4 ± 3.3	15.2 ± 2.0*	24.5 ± 2.4	19.9 ± 1.1*
DBI[a]	1.23 ± 0.03	0.51 ± 0.02*	1.16 ± 0.10	0.80 ± 0.02*	0.66 ± 0.05	0.32 ± 0.02*	0.54 ± 0.04	0.34 ± 0.03*
Total n-3	11.1 ± 0.2	5.90 ± 0.06*	4.20 ± 0.10	3.10 ± 0.10*	0.50 ± 0.02	0.20 ± 0.01*	0.70 ± 0.01	0.50 ± 0.02*
Total n-6	35.9 ± 1.9	22.2 ± 0.5*	36.8 ± 3.3	35.8 ± 4.0	30.9 ± 1.9	14.7 ± 2.4*	24.0 ± 2.0	19.4 ± 1.4*
(n-3)/(n-6)	0.31 ± 0.03	0.27 ± 0.03	0.11 ± 0.02	0.09 ± 0.01	0.02 ± 0.01	0.02 ± 0.01	0.03 ± 0.0	0.03 ± 0.0
EFA/non-EFA[b]	7.01 ± 0.2	4.28 ± 0.1*	0.88 ± 0.02	0.65 ± 0.01*	0.47 ± 0.02	0.18 ± 0.01*	0.35 ± 0.01	0.24 ± 0.01*
ED 18:2n-6[c]	6.59 ± 0.2	1.75 ± 0.1*	2.75 ± 0.03	1.66 ± 0.02*	4.81 ± 0.5	0.08 ± 0.01*	0.40 ± 0.03	0.27 ± 0.02*
ED 20:4n-6[d]	0.02 ± 0.0	0.01 ± 0.0	0.02 ± 0.0	0.01 ± 0.0	0.07 ± 0.02	—*	0.02 ± 0.0	0.02 ± 0.0
(Δ6 + Δ5) DIA[e]	5.96 ± 0.10	1.49 ± 0.11*	2.57 ± 0.10	1.60 ± 0.10*	0.18 ± 0.04	—*	0.37 ± 0.02	0.23 ± 0.01*
(Δ6) DIA[f]	0.59 ± 0.02	0.25 ± 0.01*	0.16 ± 0.02	0.04 ± 0.0*	0.01 ± 0.0	0.08 ± 0.01*	0.02 ± 0.0	0.03 ± 0.0
(Δ5) DIA[g]	10.2 ± 0.3	5.90 ± 0.2*	16.4 ± 1.5	35.8 ± 2.9*	15.0 ± 0.9	—*	15.8 ± 1.7	7.00 ± 0.9*
PDIA[h]	0.06 ± 0.003	0.03 ± 0.0*	0.10 ± 0.01	0.05 ± 0.01*	0.08 ± 0.01	0.09 ± 0.02	0.15 ± 0.02	0.12 ± 0.02
SDIA[i]	0.27 ± 0.02	0.20 ± 0.0*	0.57 ± 0.01	0.46 ± 0.0*	2.12 ± 0.1	1.68 ± 0.1*	1.15 ± 0.03	0.85 ± 0.01*

[a]Results are expressed as the mean ± 1 SEM of three independent determinations obtained from data in Tables 2 and 3. Parameters a, b, f, h, and i were calculated according to Lepage *et al.* (30). Calculations for parameters c and d were done according to Martínez and Ballabriga (29). [a]PUFA/saturated, [b][(n-6) + (n-3)]/[(n-7) + (n-9) + saturated], [c][20:3n-6 + 20:4n-6 + 22:5n-6]/18:2n-6, [d][22:4n-6 + 22:5n-6]/20:4n-6, [e]20:4n-6/18:2n-6, [f]20:3n-6/18:2n-6, [g]20:4n-6/20:3n-6, [h]16:1n-7/16:0, [i]18:1n-9/18:0. DBI, double bond index; ED, elongation–desaturation index; DIA, desaturase index activity; PDIA, palmitoyl desaturase index activity; SDIA, stearoyl desaturase index activity; for other abbreviations see Table 1. *Significantly different with respect to the corresponding control values. Amounts below 0.01 are indicated as "—." SEM <0.01 are indicated as "0.0."

TABLE 8
Interlipid Relationships and Fluorescence Anisotropy of Microsomal Membranes from Liver, Heart, Kidney, and Brain of Control (S) or Calcium-Deficient (CaD) Rats[a]

	Liver		Heart		Kidney		Brain	
	S	CaD	S	CaD	S	CaD	S	CaD
Cholesterol/phospholipids (μmol/μmol)	0.32 ± 0.02	0.23 ± 0.01	0.29 ± 0.02	0.15 ± 0.02*	0.41 ± 0.02	0.26 ± 0.01*	0.52 ± 0.02	0.63 ± 0.04
Total lipids/phospholipids (mg/μmol)	0.79 ± 0.03	0.98 ± 0.04	0.96 ± 0.03	1.11 ± 0.10	0.56 ± 0.02	0.64 ± 0.03	1.25 ± 0.11	1.32 ± 0.20
Total lipids/cholesterol (mg/μmol)	2.48 ± 0.02	3.00 ± 0.03*	3.31 ± 0.04	7.40 ± 0.26*	1.37 ± 0.01	2.46 ± 0.02*	2.40 ± 0.15	2.09 ± 0.10
Phospholipids/protein (μmol/mg)	0.45 ± 0.03	0.30 ± 0.01*	0.40 ± 0.02	0.37 ± 0.03	0.60 ± 0.02	0.41 ± 0.01*	0.51 ± 0.05	0.32 ± 0.03*
Neutral lipids/protein (μg/mg)	0.06 ± 0.003	0.11 ± 0.01*	0.04 ± 0.005	0.06 ± 0.01	0.05 ± 0.004	0.13 ± 0.005*	0.03 ± 0.002	0.03 ± 0.005
Cholesterol/protein (μmol/mg)	0.14 ± 0.01	0.07 ± 0.01*	0.12 ± 0.01	0.06 ± 0.01*	0.25 ± 0.01	0.10 ± 0.01*	0.27 ± 0.02	0.20 ± 0.05
Total lipids/protein (mg/mg)	0.36 ± 0.03	0.29 ± 0.02*	0.38 ± 0.02	0.41 ± 0.03	0.34 ± 0.01	0.26 ± 0.03*	0.65 ± 0.05	0.42 ± 0.20
Neutral lipids/polar lipids (μg/μg)	0.13 ± 0.01	0.37 ± 0.02*	0.10 ± 0.01	0.16 ± 0.02*	0.08 ± 0.005	0.32 ± 0.01*	0.06 ± 0.01	0.09 ± 0.01
DPH fluorescence anisotropy	0.1405 ± 0.0010	0.1012 ± 0.0008*	0.2314 ± 0.0020	0.2001 ± 0.0015*	0.1971 ± 0.0012	0.1355 ± 0.0009*	0.1113 ± 0.0007	0.1204 ± 0.0011

[a]Results are expressed as the mean ± 1 SEM of three independent determinations. For details see the Materials and Methods section. Diphenylhexatriene (DPH) steady-state fluorescence anisotropy in membrane suspensions was measured at 37°C using the probe DPH as described in the Materials and Methods section. Results for DPH determinations are expressed as the mean ± 1 SEM of six independent determinations assayed in duplicate. *Significantly different ($P < 0.01$) from the respective control value.

TABLE 9
Interlipid Relationships and Fluorescence Anisotropy of Erythrocyte Ghosts, Whole Articular Cartilage, Plasma, and Microsomal Membranes from Spleen of Control (S) or Calcium-Deficient (CaD) Rats[a]

	Spleen		Erythrocyte ghosts		Articular cartilage		Plasma	
	S	CaD	S	CaD	S	CaD	S	CaD
Cholesterol/phospholipids (μmol/μmol)	0.55 ± 0.03	0.43 ± 0.04	0.86 ± 0.05	0.54 ± 0.02*	2.0 ± 0.1	0.32 ± 0.03	2.33 ± 0.08	2.05 ± 0.11*
Total lipids/phospholipids (mg/μmol)	0.61 ± 0.02	0.70 ± 0.03	0.52 ± 0.03	0.60 ± 0.04	7.6 ± 0.2	1.4 ± 0.1*	2.11 ± 0.09	2.34 ± 0.11
Total lipids/cholesterol (mg/μmol)	1.07 ± 0.06	1.64 ± 0.03*	0.60 ± 0.04	0.35 ± 0.03*	4.6 ± 0.1	4.4 ± 0.1*	0.80 ± 0.04	1.00 ± 0.03
Phospholipids/protein (μmol/mg)	0.50 ± 0.03	0.33 ± 0.03*	0.35 ± 0.03	0.12 ± 0.01*	0.03 ± 0.01	5.0 ± 0.2	0.04 ± 0.01	0.01 ± 0.00*
Neutral lipids/protein (μg/mg)	0.06 ± 0.004	0.10 ± 0.01	0.04 ± 0.003	0.06 ± 0.01	0.06 ± 0.01	0.01 ± 0.00*	0.02 ± 0.001	0.03 ± 0.001
Cholesterol/protein (μmol/mg)	0.28 ± 0.02	0.14 ± 0.01*	0.30 ± 0.03	0.19 ± 0.02*	0.05 ± 0.01	0.01 ± 0.00*	0.10 ± 0.02	0.02 ± 0.00*
Total lipids/protein (mg/mg)	0.30 ± 0.02	0.23 ± 0.03*	0.18 ± 0.01	0.07 ± 0.01*	0.23 ± 0.03	0.04 ± 0.01*	0.08 ± 0.01	0.02 ± 0.00*
Neutral lipids/polar lipids (μg/μg)	0.12 ± 0.01	0.30 ± 0.03*	0.11 ± 0.01	0.49 ± 0.04*	2.1 ± 0.2	3.7 ± 0.2*	0.50 ± 0.03	1.76 ± 0.05*
DPH fluorescence anisotropy	0.1202 ± 0.0005	0.1051 ± 0.0003*	0.1077 ± 0.0005	0.0831 ± 0.0006*	ND	ND	ND	ND

[a]Results are expressed as the mean ± 1 SEM of three independent determinations randomly selected from both experimental groups, whether S or CaD. For details see the Materials and Methods section. Diphenylhexatriene (DPH) steady-state fluorescence anisotropy in membrane suspensions was measured at 37°C using the probe DPH as described in the Materials and Methods section. Results for DPH determinations are expressed as the mean ± 1 SEM of six independent determinations assayed in duplicate. *Significantly different ($P < 0.01$) from the respective control value. ND, not determined.

were carried out in microsomal suspensions from both S and CaD rats (Tables 8 and 9). Experimental data show that calcium deficiency produces microsomal membranes in which acyl chain packing is disordered relative to the membranes of the S group. A similar conclusion was obtained from plasma membranes of erythrocyte ghosts from CaD rats. Fluorescence anisotropy determinations in suspensions from CaD brain microsomes showed no significant changes with respect to the control, as expected from the minor alterations observed in the proportion of membrane lipids under calcium deficiency (Tables 2, 3, and 8).

Some enzyme activities closely related to lipid metabolism were determined in various tissues from S and CaD rats (Table 10). As regards FA desaturases, the most striking difference observed was the very low levels of activities that all tissues studied exhibited with respect to that of liver microsomes. These differences were observed irrespective of the substrate assayed. Kidney and heart microsomes were more active than spleen, lung, or brain. However, specific activity in liver was much greater than that determined in kidney or heart microsomes. The CaD diet evoked an important reduction (54 to 59% respect to control values) in Δ5, Δ6, and Δ9 liver desaturase activities. This reduction was also observed for the rest of the tissues studied except for heart, in which only Δ9-desaturase capacity was affected (Table 10). Basal activity for phospholipase A_2 varied from a maximun for kidney microsomes to a minimum for spleen ones. Lung, heart, and liver displayed similar activities. Calcium deficiency did not modify phospholipase A_2 activity of brain, lung, or heart, but the activities in microsomes from kidney, liver, and spleen were significantly reduced: *ca.* 80% for the former tissues and 35% for the latter (Table 10). The most active tissue acylating free FA to CoA-SH was the liver, which exhibited a basal acyl-CoA synthetase activity three times greater than that of kidney, lung, or heart. Brain and spleen were the least active tissues studied, with a basal enzyme activity that was almost 80% smaller than that of control liver. In microsomes from CaD rats, an increased acyl-CoA synthetase activity was determined in both kidney and liver (32 and 76% higher, respectively, than that of controls).

Finally, we found a significant reduction in the transcription rate for mRNA that encodes for liver Δ6-desaturase (Fig. 3). The ratio mRNA (Δ6-desaturase)/mRNA (β-actin) was reduced from 1.62 ± 0.17 for S rats to 0.93 ± 0.06 for CaD ones (–43% with respect to control values).

DISCUSSION

From these results it is evident that calcium levels are, indeed, involved in FA metabolism, and that its deficiency evoked many alterations in several tissues from CaD rats. Taking into account that desaturases are key enzymes in FA metabolism, the main question arising from our findings is whether the analytical changes are the cause or the consequence of the altered desaturase activities. Several studies from this and other laboratories have extensively documented that alterations in desaturase activities, evoked by hormonal and/or dietary manipulations, are usually reflected in the acyl composition of cellular lipids (1,31–36). On the other hand, previous studies from our laboratory demonstrated that fasting and/or energy restriction profoundly modifies desaturase activities, and the gain in body weight is directly involved in this regulatory effect (31,32,37,38). It is generally accepted that desaturase activities determine the final FA profile of a tissue and, in turn, its particular lipid metabolism (31,32,34–36). As stated before, a modified desaturase activity can be ascribed to a change in the body weight gain under certain dietary conditions; therefore, subsequent alterations in the FA profile may be considered as a natural consequence of such a modification. However, in our experimental conditions changes ob-

TABLE 10
Enzyme Activities[a] in Various Tissues from Control (S) or Calcium-Deficient (CaD) Rats

Microsome source	Treatment	Desaturase activities[a]			Phospholipase A_2[b]	Acyl-CoA-synthetase[c]
		Δ9 (16:0)	Δ5 (20:3)	Δ6 (18:2)		
Brain	S	16 ± 1	23 ± 2	15 ± 2	3,112 ± 124	27.6 ± 0.9
	CaD	ND*	Trace*	ND*	3,410 ± 137	33.3 ± 1.5
Kidney	S	25 ± 3	34 ± 3	26 ± 4	7,241 ± 153	40.1 ± 2.6
	CaD	ND*	8 ± 2*	ND*	2,099 ± 111*	53.2 ± 1.9*
Lung	S	31 ± 3	16 ± 1	18 ± 3	2,114 ± 85	43.4 ± 2.8
	CaD	ND*	Trace*	Trace*	1,309 ± 94*	69.7 ± 3.2*
Heart	S	31 ± 4	48 ± 2	55 ± 3	2,485 ± 76	54.1 ± 3.0
	CaD	ND*	40 ± 3	49 ± 4	2,102 ± 127	57.7 ± 2.5
Liver	S	168 ± 7	402 ± 11	205 ± 12	3,501 ± 143	148.7 ± 3.9
	CaD	96 ± 5*	237 ± 8*	111 ± 9*	686 ± 75*	256.1 ± 4.4*
Spleen	S	15 ± 1	19 ± 3	17 ± 2	1,609 ± 90	29.8 ± 0.7
	CaD	ND*	Trace*	ND*	1,051 ± 107*	54.6 ± 1.4*

[a]Results are expressed as [a]pmol of substrate transformed/min·mg protein, [b]dpm/mg protein, or [c]nmol/min·mg protein. Data are given as the mean ± 1 SEM of four independent determinations performed on samples randomly selected from both experimental groups, whether S or CaD. *Significantly different with respect to the corresponding control value ($P < 0.01$). For technical details see the Materials and Methods section. Trace, below 5 pmol/min/mg protein; ND, nondetectable amount.

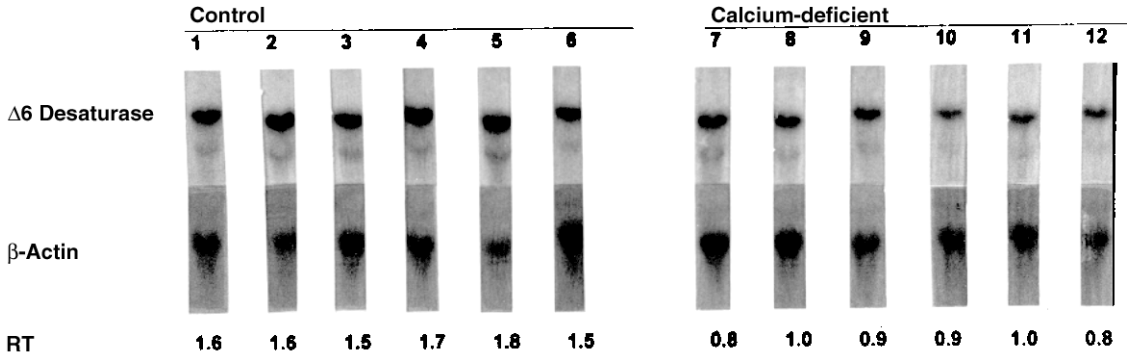

FIG. 3. Amount of mRNA of Δ6 desaturase from livers of S and CaD rats as measured by blot hybridization. Total mRNA isolated from livers was processed independently and subjected to electrophoresis in 1% formaldehyde gel. Blot hybridization was performed as described in the text with the indicated ^{32}P-labeled probe. The amount of radioactivity in each band was quantified and normalized by means of an image analysis software system. "RT" indicates the relative content of mRNA for Δ6-desaturase gene with respect to the mRNA for β-actin; the latter was chosen to normalize the results for loading differences. Individual ratios are shown below each blot. Means ± 1 SEM were 1.62 ± 0.12 and 0.93 ± 0.06 for S and CaD rats, respectively. For abbreviations see Figure 1.

served in both the FA profile of several tissues (Tables 2 to 5) and the activities of the desaturase enzymes (Table 10) can be ascribed to calcium deprivation (Table 1) rather than to a difference in food intake (Fig. 1) and/or body weight gain (Fig. 2) between CaD and S rats. Dietary calcium is known to make calcium soaps with FA in the gut, resulting in increased excretion of dietary fats. In the CaD diet group, fat absorption can be expected to be greater than in the S group, thus providing another possible mechanism for the observed differences in tissue FA composition. However, we think the diet employed in our experiments contained standard (normal) amounts of lipids, as previously demonstrated (7). If the phenomenon of low fat absorption plays a particular role in the changes we observed, the analytical and enzymatic modifications would be expected to result from a general lack of availability of calories from fat (or a simultaneous depletion in saturated FA and PUFA as diet precursors). On the contrary, our observations suggest a PUFA deficiency with a selective depletion of FA keeping constant the ratio (n-3)/(n-6). Moreover, previous works from this and other laboratories have demonstrated that low-fat diets evoked a stimulation of Δ9- and Δ6-desaturase activities rather than an inhibition (32,39,40).

Previous experiments clearly demonstrated that the regulatory step in the desaturation mechanism is located at the level of the desaturase protein itself (41,42). Additional evidence from our laboratory led us to discard, rather confidently, a direct effect of calcium ions on the desaturase enzyme itself or on another component involved in the transport of electrons to the terminal component of the desaturase system (1). At present, no experimental evidence that relates to the possible role of a calcium as a desaturase cofactor and/or any other function of this cation as an essential component during the catalytic process has been presented. The results of our previous experiments, performed on the Δ9 and Δ5 liver microsomal desaturase activities in CaD rats, also led us to discard the idea of the involvement of a soluble (cytosolic) factor or of a problem related to the activation of the substrate by acylation to CoA-SH (1). Moreover, in the present study we found a significant increase in the acyl-CoA synthetase activity in various tissues from CaD rats (Table 10) in which desaturase activities were simultaneously depressed. A similar conclusion was obtained in the case of the acyl-CoA synthetase activity from livers of CaD rats (1,4). From these considerations, it seems that the effect of calcium deficiency on the desaturase activities may result from an indirect modification that would be produced through a common metabolic disturbance that involves the three desaturase systems we have studied.

The CaD diet clearly induced important modifications in the quality and/or quantity of the FA chains acylated to neutral and polar lipid fractions from microsomal membranes of several tissues (Tables 2 to 5). These changes were closely related to the measured Ca level in tissues (Table 1), and they were observed even in tissues with FA profiles generally considered very stable, such as brain (Table 2) and cartilage (Tables 4 and 5), in which there is not much metabolic diversity owing to their very specialized functions. They were also reflected, though in minor extension, in lipids from erythrocyte ghosts and plasmas from CaD rats (Table 6). Calculations made from the analytical data demonstrated in general that these changes are characterized by an increased saturated FA proportion and a significant decrease in PUFA and DBI (Table 7). All the analytical parameters calculated for the estimation of the metabolic conversion of the saturated and unsaturated FA precursors (29,30) suggest a general loss of desaturation capacity in microsomes from CaD rats (Table 7). In all the tissues studied from CaD rats, we found a significant decrease in the membrane packing order with respect to S rats that was closely associated with several changes in the interlipid and lipid/protein ratios (Tables 8 and 9). It is generally accepted that the main factors that determine the fluidity of membrane lipids are the cholesterol/phospholipid molar ratio and the degree of unsaturation of the phospholipid acyl chains (43–45). In relation to this, the proportion of cholesterol—usually associated with a diminished membrane

fluidity—was significantly reduced in CaD rats with respect to S ones (1,2; Tables 8 and 9). We also found a decrease in those indices determined by the PUFA and DBI. The data presented in Tables 2 to 7 compared to those from Tables 8 and 9 may indicate an apparent contradiction since the CaD condition caused a large decrease in PUFA and an increase in saturated FA, while acyl packing order decreased as a result.

As is well known, the cholesterol/phospholipid ratio, together with DBI values, is the main determinant of acyl chain packing order in membranes (41–43). Tables 8 and 9 show a significant decrease in r_s values that directly correlates with a decrease of more than 40% in the cholesterol/phospholipid ratio. Apparently, two different changes in membrane composition produce opposite effects on acyl chain packing order: One of them decreases this physicochemical parameter and the other one increases it. The resulting DPH data show that the reduction in cholesterol drives the change measured in the packing state of the membrane. Previous work has documented that, in microsomes from animals fed a fat-deficient diet, the common feature is a depletion in the FA derived from the precursor linoleic and α-linolenic acids (46,47). The physicochemical state of the membrane is well-known as a key question in determining the activities of the desaturase systems (31,32,41,48–51). As was previously suggested (52), an increased fluidity leads to a depletion in PUFA biosynthesis, and vice versa. This is one aspect of a general regulatory mechanism that controls several membrane functions and that has been designated "viscotropic regulation" (47,52).

Additional experimental support for a central role of the decreased desaturase activities in the effects evoked by calcium deprivation was obtained by determining the transcriptional rate of the mRNA that encodes for rat liver Δ6-desaturase enzyme. In recent reports by Kan *et al*. (53) and Xing and Insel (54), it was demonstrated that a calcium increase in cytosol gives rise to the release of arachidonic acid into the nucleus by means of phospholipase A_2 translocation to the nuclear envelope. This fact is related to the stimulated transcription of desaturase genes described by Landschulz *et al.* (55). Thus, a decrease in calcium availability would imply a reduced transcription rate of FA desaturase from CaD rats.

We think that at least two different mechanisms leading to the same biochemical effect exist. In one, calcium deprivation in rats evoked an important alteration in acylation/deacylation cycles through the inhibition of the calcium-dependent phospholipase A_2. This primary effect results in a significant modification of the physicochemical properties of the microsomal membranes that, in turn, leads to a subsequent inhibition of the desaturase activities by viscotropic effect. In the other postulated mechanism, deprivation of calcium leads to a general failure in the transcriptional rate for the corresponding FA desaturase mRNA. As a result of these two mechanisms, the modifications in the analytical FA pattern and membrane composition would be the consequence of the adaptation to the CaD condition, which seems to be of general influence rather than limited to a central organ such as liver.

The relationship between FA metabolism in humans and calcium deprivation was recently reviewed (56). Especially in elderly people, calcium loss is related to altered membrane functions and several changes in lipid composition (57). Although this field of study has received some attention during recent years, we think that the interactions between FA metabolism and calcium availability deserve more investigation since they offer novel approaches for understanding human illnesses of increasing incidence such as osteoporosis (56).

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