Effect of Dietary Fats on Desaturase Activities and the Biosynthesis of Fatty Acids in Rat-Liver Microsomes

M.M. MAHFOUZ*, T.L. SMITH, and F.A. KUMMEROW, *Burnsider Research Laboratory, University of Illinois, Urbana, IL 61801*

ABSTRACT

Four groups of rats were fed diets containing 15% (w/w) high-oleic safflower oil (SFO, rich in *cis*-18:1 acids), a mixture of 80% partially hydrogenated soybean oil plus 20% corn oil (H + CO, rich in *trans*-18:1 acids), lard (L, rich in saturated fatty acids) and corn oil (Co, rich in 18:2ω6). Fatty acid composition of liver microsomes and activities of the Δ^5, Δ^6 and Δ^9 desaturases were determined. Microsomal Δ^6 desaturase activity and arachidonic acid were lower in the H + CO group compared with SFO of L. No difference was found in the Δ^5 or Δ^6 desaturase activity of CO and SFO groups. Thus, the oleic-acid level of the SFO diet had no effect on the metabolism of 18:2ω6. Fluorescent polarization studies, using *trans*-parinaric acid as a probe, showed no differences between the physical states of phospholipid vesicles made from lipids isolated from each group. We concluded that the *trans*-18:1 acids in partially hydrogenated soybean oil have a more inhibitory effect than saturated acids on EFA metabolism, even in the presence of adequate amounts of essential fatty acid.
Lipids 19:214-222, 1984.

INTRODUCTION

In technological societies, the consumption of partially hydrogenated fats has been increasing. These fats contain significant quantities of various positional isomers of octadecenoic acids with *trans* and *cis* double-bond configurations (1-3). These unnatural acids are now found in human tissues at levels as high as 14% of the fatty acids of certain lipids (4). In vitro studies using liver microsomes from animals deficient in essential fatty acids (EFA) (5,6) have shown that positional isomers of *cis*- and *trans*-octadecenoic acids inhibit liver microsomal desaturation of 18:2ω6 and 20:3ω6. In in vivo experiments, the isomeric octadecenoic acids present in partially hydrogenated soybean oil (PHSO) were found to aggravate the symptons of EFA deficiency, probably through the inhibitory effect of these isomers on the conversion of 18:2ω6 to 20:4ω6 (7). Also, feeding rats diets containing PHSO in the presence of a low level of 18:2ω6 (18% of the minimum nutrient requirement) was shown to affect the metabolism of EFA with a consequent change in the polyunsaturated fatty acid (PUFA) pattern of liver and heart phospholipids (8).

Previous studies were conducted to assess the effect of *cis*- and *trans*-18:1 acids (present in partially hydrogenated vegetable oils (PHVO)) on the essential fatty acid metabolism in vitro or in vivo. These studies used liver microsomal fractions or tissues from animals that had been fed diets deficient in EFA (5,6) or that contained levels of 18:2ω6 lower than the minimum nutrient requirement (8). Alfin-Slater and Aftergood have suggested that *trans*-siomeric fatty acids have no adverse effects in animal models when an adequate amount of EFA is provided (9). This suggestion has been based largely on weight gain, longevity and reproductive performance data. However, little is known about the effect of isomeric 18:1 acids present in PHVO on the EFA metabolism when an adequate amount of 18:2ω6 is supplied in the diet.

The present nutritional experiments were designed to investigate the effects of dietary isomeric *trans*-18:1 acids (present in PHSO) and saturated fatty acids on the Δ^5 and Δ^6 desaturase activities in rats with sufficient EFA. Desaturase activities were measured in incubation reactions using the microsomal fraction of the liver. The fatty acid pattern was also analyzed, especially for the ω6 acids of the hepatic microsomes, in order to determine relationships between the synthesis of ω6 PUFA and the role of dietary *trans* and saturated acids in these metabolic processes.

In the present study, we used corn oil (CO), high-oleic safflower oil (SFO), a mixture of 80% partially hydrogenated soybean oil and 20% corn oil (H + CO) and lard (L). All the diets contained adequate amounts of 18:2ω6. The membrane fluidity of the microsomal phospholipid vesicles was also measured to correlate changes in desaturase activities with any alteration of the physical state of the vesicular membranes. A preliminary account of portions of this work has appeared elsewhere (10).

*To whom correspondence should be addressed.

MATERIALS AND METHODS

Amimals and Diets

Male weanling Sprague-Dawley rats, divided into 4 groups of 12 rats each, were fed a modified AIN-76 semipurified diet (11). Fifteen percent of the diet, by weight, was comprised of CO, SFO, L or a mixture of H + CO, for 6 weeks. The composition of the basal diet and the fatty acid composition of the fat supplements are shown in Tables 1 and 2. Food was prepared as needed and stored at 4C. The rats were fed fresh food daily with food and water provided ad libitum. The rats were housed individually in suspended cages with wire mesh bottoms. Room lighting consisted of 12-hr periods of light and dark. The rats were weighed weekly and were not fasted before being killed so that the maximum activities of the liver desaturases could be measured (12). The dietary treatments showed no significant effect on net body weight. All animals were sacrificed at ca. 9 a.m. on the day of the experiment.

Chemicals and Reagents

All cofactors, NADH, CoA (Lithium salt) and bovine serum albumin V fraction (essentially free of fatty acid), were obtained from Sigma Chemical Corporation (St. Louis, MO). All other chemicals were analytical grade. Palmitic, linoleic and eicosa-8,11,14-trienoic acids were obtained from NuChek Prep., Inc. (Elysian, MN). [$^{1-14}$C]-Palmitic acid (sp. act. 56 Ci/mol), [$^{1-14}$C] linoleic acid (sp. act. 51 Ci/mol) and [$^{1-14}$C] eicosa-8,11,14-trienoic acid (sp. act. 55 Ci/mol) were purchased from New Enagland Nuclear (Boston, MA).

Incubation Conditions and Assay of Desaturases

The liver microsomes were assayed for Δ^5, Δ^6, and Δ^9 desaturase activities as previously described (13). Each incubation in 1 ml of a 0.15 M KCL-0.25 M sucrose solution contained (in μmoles): ATP, 5.0; CoA, 0.25; NADH, 1.0; $MgCl_2$, 5.0; glutathione, 1.5; NaF, 45.0 nicotinamide, 0.5; phosphate buffer (pH 7.0), 100.0; and 2 mg protein of a microsomal suspension. The microsomal protein concentration was measured according to Lowry et al. (14). The microsomes were separated by centrifugation at 105,000 x *g* for 2 hr (5). One hundred nmol (containing an amount of ^{14}C labeled acid equivalent to 0.1 μCi) of palmitic or linoleic and 40 nmol eicosa-8,11,14-trienoic acid in the form of sodium salt bovine albumin complex (1 μg free fatty acid/11.5 μg bovine serum albumin) were used as substrates. Under these conditions, the enzymes were saturated by the substrates. The incubations were carried out for 20 min in a Dubnoff metabolic shaker at 37 C. The products of the reactions under the assay conditions as described were proportional to the 2 mg protein concentration and the 20 min reaction time.

TABLE 1

Composition of Semipurified Diets

Component	g/100 g
Casein[a]	20.0
Dextrose[b]	30.0
Cornstrach[c]	25.0
Cellulose[d]	5.0
Fat[e]	15.0
Mineral mix[f]	3.5
Vitamin mix[g]	1.0
DL-methionine[h]	0.3
Choline[i]	0.2

[a]Shamrock brand, Erie Casein Co., Erie, IL.
[b]Staleydex 333, A.E. Staley Co., Decatur, IL.
[c]Food grade, A.E. Staley Co., Decatur, IL.
[d]Solka-Floc, Brown Co., Berlin, NH.
[e]Corn oil, a mixture of 80% partially hydrogenated soybean oil plus 20% corn oil, lard and high-oleic acid safflower oil provided by Best Foods/CPC International, Union, NJ.
[f]AIN Mieral Mixture 76, ICN Nutritional Biochemicals, Cleveland, OH.
[g]AIN Mineral Mixture 76, ICN Nutritional Biochemicals, Cleveland. OH.
[h]ICN Nutritional Biochemicals, Cleveland, OH.
[i]Choline Bitartrate, ICN Nutritional Biochemicals, Cleveland, OH.

TABLE 2

Dietary Fatty Acid Composition

Diet[a]	CO	SFO	L	H + CO
Fatty acids[b]				
16:0	10.4	4.9	24.7	10.2
16:1	ND[c]	0.1	2.3	ND[c]
18:0	1.8	2.6	15.0	10.7
18:1 t[e]	ND[c]	ND[c]	0.8	35.1
18:1 c[e]	25.6	76.1	43.5	25.7[d]
18:2ω6	61.3	15.2	10.0	15.3
18:3ω3	0.9	ND[c]	0.3	0.3
20:0	ND[c]	0.5	0.2	0.3
20:1ω9	ND[c]	0.3	0.6	0.1
20:2ω6	ND[c]	ND[c]	0.4	ND[c]
22:0	ND[c]	0.4	ND[c]	0.3
24:0	ND[c]	0.2	ND[c]	0.2
Others	0.0	0.0	2.2	1.8

[a]Semipurified diet containing 15% fat as corn oil (CO), high-oleic safflower oil (SFO), Lard (L) or a mixture of 80% partially hydrogenated soybean oil + 20% corn oil (H + CO).
[b]Carbon number: number of bonds, ω = double bond position from the methyl end.
[c]ND = not detected (<0.1%).
[d]This value represents a mixture of 80% *cis* 18:1ω9 + 20% of other *cis* isomers.
[e]c = *cis*, t = *trans*.

The reaction was terminated by the addition of 5% HCl in methanol and the lipids were extracted with chloroform/methanol (2:1, v/v). The extract was dried under a stream of N_2 and transesterified with 3 N methanolic-HCl (Supelco, Inc., Bellefonte, PA) at 70 C for 2 hr (7). The HCl-methanol was evaporated under N_2. The methyl esters were dissolved in petroleum ether. A mixture of unlabeled carriers of methyl esters of 16:0 + 16:1, 18:2 + 18:3 or 20:3 + 20:4 were added to the incubation products. The esters were separated on 10% $AgNO_3$ Silica Gel H plates as previously described (6). The separated bands were scraped into scintillation vials, 10 ml of scintillation fluid (4 gm PPO + 84 mg POPOP/l toluene) was added, and the activity was counted in a Packard Scintillation Counter. The percentage of desaturation was calculated as the ratio of the counts in the desaturated products to the sum of the counts in the substrate plus product, corrected for background. The nmol of the product were then calculated. The recovery of the radioactivity was more than 85% of the amount used.

Extraction of Lipids and Analysis of Fatty Acids

In order to study the effect of the different dietary treatments on the fatty acid composition of the total lipids and the phospholipids of the liver microsomal fractions, the microsomes were extracted according to the Folch method (15) and the lipid extract was divided into 2 portions. One portion was used for preparing the methyl ester of the total lipids following saponification and methylation (16). The phospholipid fraction was seaprated from the other portions of the lipid extract by polysilicic acid gel-impregnated glass fiber sheets (ITLC), using a solvent system of petroleum ether/diethyl ether (90:10, v/v). The phospholipids were transesterified by BF_3-methanol complex (17). BHT was added as antioxidant in a concentration of 0.005% (w/v) to all the solvents used for lipid extraction and chromatography. All operations were carried out under N_2.

A Packard Model 428 gas chromatograph (Hewlett Packard Instrument Co., Inc., Chicago, IL), equipped with an all-glass injection splitter and a flame ionization detector (FID) was used to separate the methyl esters on a Quadrex, 60 m × 0.25 mm ID SP 2340 wall-coated, open-tubular (WCOT) glass column (Quadrex Inc., New haven, CT). The oven temperature was programmed from 160-220 C at 1 C/min and the injector and detector temperatures were 280 C and 300 C, respectively. The N_2 flow rate was 0.7 ml/min with a split ratio of 110:1. Retention time, peak areas and peak relative area percentages were determined electronically using a Hewlett Packard Model 3390A Reporting Integrator. Identification of methyl esters of fatty acids was accomplished by comparing relative retention times with authentic standards (NuChek Prep., Elysian, MN, and Supelco, Inc., Bellefonte, PA). A test of statistical significance was applied using a one-way analysis of variance with mean separation by Duncan's multiple range test (18).

Preparation of Microsomal Phospholipid Vesicles and Fluorescence Measurement

The microsomes isolated above were subjected to a modified Bligh and Dyer extraction (19). To 0.8 ml of a concentrated suspension of microsomes was added 3 ml chloroform/methanol (1:2, v/v), which was vortexed and then centrifuged at 200 × g to precipitate the residue. The supernatant was saved and the residue reextracted with 1 ml of chloroform. After centrifugation, the chloroform was collected with the previous supernatant. To the combined supernatants, 1 ml 0.1 M KCl was added. After centrifugation, the upper layer and any interfacial material was discarded. The extracts were dried, weighed and suspended in 1 ml chloroform.

Phospholipids were isolated by Unisil (activated silicic acid 200-325 mesh) chromatography. For each mg of lipid, 0.1 g of acid-washed, activated Unisil was put in a gooch funnel (minimum of 0.5 g) and washed with 5 ml of chloroform. The sample was applied in chloroform to the Unisil and then wased 3 times with 5 ml portions of chloroform to remove any neutral lipids. The phospholipids were eluted with 5 ml of methanol. Methanol was removed under vacuum with chloroform added to reduce the boiling point. The dry lipid was dissolved in 2 ml chloroform and the phospholipid molar concentration determined using ammonium ferrothiocyanate (20) with egg yolk phosphatidylcholine as a standard.

trans-parinaric acid was obtained from Molecular Probes (Junction City, OR) and stored in ethanol with .001% BHT at -20 C in the dark under N_2. To make vesicles, 1.2 μmol phospholipid were transferred to a screw-cap test tube and dried in a thin film on the side of the tube under a stream of N_2. Six ml potassium phosphate buffer (50 mM, pH 7.2) were added, the tube closed under N_2 and heated to 65 C. The tube was then vortexed at full speed for 15 sec and allowed to cool. Three ml were set aside for use as a scattering blank. To the remaining 3 ml was added 3 nmol of *trans*-parinaric acid (probe-to-lipid ratio of 1/200), then the tube was incubated at 37 C for 40 min.

Fluorescence polarization studies were done on a Perkin-Elmer spectrofluorimeter, model 650-10S (Norwalk, CT), fitted with a Hitachi model 650-0139 thermostatted turret cell holder, cell stirrer and polaroid filter rotator (C.N. Wood Manufacturing, Newtown, PA). Excitation occurred at 325 nm. Emission was read at 420 nm and fluorescence intensity measured for parallel ($I_{||}$) and perpendicular ($T_{\perp}$) orientations of the emission filter. The polarization ratio was calculated as $(I_{||}-I_{||}s)/((I_{\perp}-I_{\perp}s)\times G)$, where $I_{||}s$ and $I_{\perp}s$ are corrections for scattering and G is the grating factor equal to HH/HV (21). Readings were made while heating the cuvette from below the lowest temperature to the highest. Heating rates were less than 1 C/min.

RESULTS AND DISCUSSION

The percentage of the components of the dietary fatty acids used in this study are shown in Table 2. SFO, L and H + CO diets contained about 82% of their total fatty acids as 18:1 plus saturated fatty acids. The SFO diet contained 76% of the total fatty acids as *cis* Δ^9 18:1 and 7.5% as saturated fatty acids (16:0 + 18:0). The H + CO diet contained 60.8% as 18:1 acids and 20.9% saturated acids. The 60.8% present as 18:1 acids was distributed as 25.7% 18:1 *cis*-isomers (80% *cis*-Δ^9 18:1 and 20% other 18:1 isomers) and as 35.1% *trans*-18:1 isomers. SFO and H + CO diets are clearly high in 18:1 acids, but they are different in the double-bond configuration of these 18:1 acids. *Trans*-18:1 acids are more like saturated fatty acids than *cis*-18:1 acids in their structure and melting point. The lard fat, which contained 40% saturated fatty acids and 43% *cis*-Δ^9 18:1 acid, was used as a control for the effect of saturated fatty acids. These diets allow comparison between the effect of *trans*-18:1 acids and the effect of saturated fatty acids on the linoleic acid metabolism when this acid is present in sufficient amounts because these 3 diets contained adequate amounts of 18:2ω6 at comparable levels.

The data listed in Table 3 shows the fatty acid profile of the liver microsomal lipids from rats fed CO, SFO, L or H + CO diets. Following a 6-week diet containing 15% fat, of which 35% were *trans*-18:1 acids, the amount of *trans*-18:1 acids deposited in the microsomal lipids of the H + CO group reached 12.8%. The presence of sufficient amounts of 18:2ω6 in the H + CO diet used in the present study did not prevent the uptake of the *trans*-acids into the microsomal lipids (8).

The total saturated fatty acids (16:0 + 18:0) supplied in the H + CO diet (20.9%) was much higher than the amount supplied in the SFO diet (7.5%) (Table 2), but the total amount of

TABLE 3

The Effect of Dietary Fats on Rat-Liver Microsomal Tatal Lipid Composition

Diet⁺	CO (n=12)	SFO (n=12)	L (n=12)	H + CO (n=12)
Fatty acids*				
16:0	17.3 ± 0.0^{c}	16.7 ± 0.53^{b,d}	18.3 ± 0.63^{a,b}	13.5 ± 0.61^{a,c,d}
16:1 ω7	0.7 ± 0.1⁺⁺	0.7 ± 0.07	1.1 ± 0.30	1.0 ± 0.33
18:0	17.0 ± 0.8^{b,d,e}	19.2 ± 0.34^{c,d}	20.5 ± 0.50^{a,b}	13.9 ± 0.22^{a,c,e}
18:1 t	—	—	—	12.8 ± 0.70
18:1 c	6.8 ± 0.4^{a,d,f}	15.3 ± 1.0^{a,b,c}	11.8 ± 0.53^{c,d,e}	9.6 ± 0.50^{b,e,f}
18:2c,t + t,c	—	—	—	0.3 ± 0.01
18:2 ω6	15.2 ± 0.7^{a,b,c}	6.1 ± 0.36^{a,d}	6.3 ± 0.62^{b,e}	10.3 ± 0.57^{c,d,e}
18:3 ω6	0.3 ± 0.01	0.2 ± 0.02	0.1 ± 0.00	0.3 ± 0.04
20:2 ω6	1.0 ± 0.1	0.3 ± 0.03	0.2 ± 0.03	0.2 ± 0.02
22:0 + 20:3ω9	0.4 ± 0.02	0.7 ± 0.05	1.0 ± 0.12	0.3 ± 0.03
20:3 ω6	0.6 ± 0.06^{a}	0.6 ± 0.03^{b}	0.8 ± 0.12	0.9 ± 0.10^{a,b}
20:4 ω6	29.8 ± 0.5^{b}	30.5 ± 0.38^{a}	29.0 ± 0.74^{c}	24.6 ± 0.40^{a,b,c}
22:4 ω6	1.3 ± 0.1^{a,b,c}	0.8 ± 0.06^{c,d,e}	0.6 ± 0.05^{b,e}	0.5 ± 0.02^{a,d}
22:5 ω6	2.5 ± 0.3^{c}	4.3 ± 0.22^{a,b,c}	2.4 ± 0.26^{b}	1.8 ± 0.30^{a}
22:5 ω3	0.5 ± 0.05	—	0.4 ± 0.04	0.3 ± 0.02
22:6 ω3	3.3 ± 0.2^{c,d}	1.5 ± 0.01^{a,d,e}	4.0 ± 0.38^{a,b,c}	3.2 ± 0.10^{b,e}
20:4	0.4 ± 0.1	0.5 ± 0.04	0.4 ± 0.08	0.3 ± 0.02
Other		2.6	3.1	6.1

t = *trans*, c = *cis*.

⁺Animals fed 15% corn oil (CO), high-oleic safflower oil (SFO), lard (L) or a mixture of 80% partially hydrogenated soybean oil + 20% corn oil (H + CO).

*All fatty acids are of the *cis* configuration unless otherwise noted.

⁺⁺Mean ± SEM. Means within the same line with a superscript letter in common are statistically different at the p<0.05 level, as tested by Duncan's multiple-range test procedure.

saturated fatty acids that accumulated in the microsomal lipids of the H + CO group (27.4%) was lower than in the SFO group (35.9%). This can be attributed to the accumulation of the *trans*-18:1 acids by the microsomes of the H + CO group, which compete with the saturated fatty acids at the α-position of the acyl lipids. Also, the proportion of saturated fatty acids in the microsomes (including *trans*-18:1 acids) of rats fed different diets did not vary widely (Table 3), although the quantity taken in was different, depending on the diet given (Table 2) (22).

Table 4 shows the distribution of ω6 PUFA in the liver microsomes of the SFO, L or H + CO group. The content of 18:2ω6 (the substrate for Δ^6 desaturase) was the same in the microsomes of the SFO and L groups ($p>0.05$) but increased significantly in the microsomes of the H + CO group compared with the SFO or L groups. The 20:4ω6 acid, which is considered the major product in the synthesis of ω6 PUFA in the microsomal lipids, decreased significantly in the H + CO group compared with the L or SFO groups, whereas no significant difference was observed between the L and SFO groups.

The amount of 22:5ω6 acid was significantly less in the microsomes of the L and H + CO groups than the SFO group ($p<0.05$). The decrease of 22:5ω6 acid in the microsomes of the L and H + CO groups can be related to the inhibitory effect of 22:6ω3, which was present in significantly higher amounts in the microsomes of the L (4.0%) and H + CO (3.2%) groups than in the SFO (1.5%) group (Table 3). The 22:6ω3 acid, which was present at a higher level in the microsomes of the L and H + CO groups, was not present in the diets fed the those groups (Table 2), so it must be derived from the desaturation and elongation of 18:3ω3 acid, which was 0.3% of the fatty acids of the L and H + CO diets (Table 2). This observation is consistent with the higher affinity of the Δ^6 desaturase for 18:3ω3 compared with 18:2ω6 reported by Brenner (23). However, the conversion of linoleic acid to arachidonic acid in the microsomes of the L group was not significantly different from the SFO group ($p>0.05$) (Table 4). This result was expected given that 18:3ω3, as substrate for the same enzymes, was present in a very small amount (0.3%) compared with 18:2ω6 acid (10%) in the L diet.

The small but significant decrease in the total amount of ω6 acids in the microsomes of the L group compared with the SFO group must be attributed to the lower content of dietary 18:2ω6 supplied to the L group (10%) compared with the SFO group (15%). Although the amount of dietary 18:2ω6 supplied to the SFO and H + CO groups was the same (15%), the amount of ω6 acids incorporated into the microsomal lipids of the H + CO group was significantly less than in the SFO group. The low level of total ω6 acids in the H + CO group can be related to the presence of *trans*-18:1 acids in the microsomes of this group as the amount of ω6 acids in liver phospholipid decreased as the amount of *trans*-acids increased (8). The amount of ω6 metabolites, which represent the desaturation and chain elongation products of 18:2ω6, was significantly less in the microsomes of the L group than in the SFO group; however, as mentioned before, this decrease is caused by the lower dietary level of

TABLE 4

ω6 Polyunsaturated Fatty Acids Distribution and the Ratios Between the ω6 Metabolits in Liver Microsomes of Rats Fed SFO, L or H + CO Diets

Diet+	SFO	L	H + CO
Fatty acids			
18:2 ω6	6.1 ± 0.36[a]	6.3 ± 0.62[b]	10.3 ± 0.57[a,b]
18:3 ω6	0.2 ± 0.02*	0.1 ± 0.00	0.3 ± 0.04
20:2 ω6	0.3 ± 0.03	0.2 ± 0.03	0.2 ± 0.02
20:3 ω6	0.6 ± 0.03[a]	0.8 ± 0.12	0.9 ± 0.10[a]
20:4 ω6	30.5 ± 0.38[a]	29.0 ± 0.74[b]	24.6 ± 0.40[a,b]
22:4 ω6	0.8 ± 0.06[a,b]	0.6 ± 0.05[b]	0.5 ± 0.02[a]
22:5 ω6	4.3 ± 0.22[a,b]	2.4 ± 0.26[b]	1.8 ± 0.30[a]
Total ω6 acids	42.8 ± 0.29[a,b]	39.4 ± 0.70[b]	38.4 ± 0.65[a]
Total ω6 metabolites	36.7 ± 0.46[a,b]	33.1 ± 0.57[b,c]	28.1 ± 0.41[a,c]
(ω6 metabolites)/(ω6 acids)	0.86 ± 0.01[a]	0.84 ± 0.01[b]	0.73 ± 0.01[a,b]
20:4ω6/18:2ω6	5.0 ± 0.31[a]	5.2 ± 0.17[b]	2.4 ± 0.11[a,b]

—Results at the same line with common superscript are statistically different from each other for $p<0.05$ as tested by Duncan's multiple-range procedure.

+Animals fed 15% high oleic safflower oil (SFO), lard (L) or a mixture of 30% partially hydrogenated soybean oil + 20% corn oil (H + CO).

*Mean ± SEM.

18:2ω6 supplied to the L group. Yet, even though the H + CO group was supplied with the same level of dieatry 18:2ω6 as the SFO group, which was higher than that of the L group, the amount of ω6 metabolites in the microsomes of the H + CO group was still significantly lower than in the L and the SFO groups (Table 4).

The ratio of (ω6 metabolites)/(ω6 acids) was significantly lower in the H + CO group than in the SFO or L groups, but no significant difference was observed between the L and SFO group ($p>0.05$). The conversion of 18:2ω6 to 20:4ω6, which involves Δ^6 desaturation, chain elongation and Δ^5 desaturation, was measured by the ratio of 20:4ω6/18:2ω6 (24). This ratio was significantly decreased in the H + CO group compared with the L or SFO groups. Again, no significant difference was found between the L and SFO groups ($p>0.05$).

The results from the in vitro measurements of Δ^6, Δ^5, and Δ^9 desaturase activities in liver microsomes from rats fed the different experimental diets are presented in Table 5. The rats in the present study were subjected to controlled lighting conditions and were killed at a fixed time to reduce the influence of possible diurnal changes on the measured enzyme activities (25). The Δ^6 desaturase activity in the microsomes of the L group was lower than in the SFO group, but this decrease was not significant ($p>0.05$). In the microsomes of the H + CO group, the Δ^6 desaturase activity was significantly lower than in the SFO or L groups. The in vivo effects of the *trans*-18:1 acids present in the H + CO diet on the conversion of 18:2ω6 to 20:4ω6, as well as on the distribution of ω6 PUFA in the microsomal lipids (Table 4), do correlate with the microsomal desaturase activities obtained in vitro. The accumulation of 18:2ω6, the substrate of Δ^6 desaturase and the decreased level of 20:4ω6 observed in the microsomal lipids of the H + CO group compared with the SFO group, are consistent with the tendency toward decreased microsomal Δ^6 desaturase activity obtained in vitro (Table 5).

The Δ^5 desaturase activity was slightly lower in the microsomes of the L and H + CO groups than in the SFO group, but no significant differences were observed between groups (Table 5). The 20:3ω6 acid, the substrate for Δ^5 desaturase, was also slightly higher in the microsomes of the L and H + CO groups than in the SFO group (Table 4). Undetectable differences in the rate of reaction over extended time may lead to larger, more easily detectable differences in the products incorporated and accumulated in structural lipids (8).

The Δ^5 desaturase activities obtained in vitro were higher than those of Δ^6 desaturase (23), as shown in Table 5. Consequently, the Δ^6 desaturase is likely to be the main regulatory enzyme in the synthesis of PUFA.

Although the Δ^9 desaturase is not directly involved in the metabolism of ω6 PUFA, it was included for comparison. No significant differences were observed for Δ^9 desaturase activity among the SFO, L and H + CO groups (Table 5). The amount of monounsaturated fatty acids (16:1 + 18:1) incorporated into liver microsomal lipids (Table 3) are more or less proportional to the amounts provided in the diets (Table 2). In a previous report, we found that the positional isomers of *trans*-18:1 acid inhibit liver microsomal Δ^9 desaturase (5). This previous study, however, was carried out in vitro and used liver microsomes of EFA-deficient rats. In a recent study, Svensson (26) showed that Δ^9 desaturase activity was higher in the liver microsomes of rats fed partially hydrogenated oils than in the control group. In his study, the control diet contained a high proportion of linoleic acid (37.8%) whereas the experimental, *trans*-containing diets contained a lower proportion of linoleic acid (11%). The high content of linoleic acid in the control diet tends to decrease Δ^9 desaturase activity in rat-liver microsomes, as reported by Jeffcoat and James (27). In the present study, the SFO, L and H + CO diets contained comparable levels of 18:2ω6, whereas the microsomal lipids of each group contained equivalent levels of 18:2ω6 and 20:4ω6 (acids that have been shown to inhibit the Δ^9 desaturase) (27,28).

The possibility cannot be excluded that the large proportion of oleic acid (76%) in the SFO diet could inhibit the synthesis of ω6 PUFA. Mohrhauer et al. (29) have shown that dietary oleic acid, fed in proportions as high as 22 cal % of the diet, did not significantly alter the conversion of 18:2ω6 to 20:4ω6 if 18:2ω6 was fed at or above the minimal dietary requirement of ca. 1 cal %. Shimp et al. (30) also indicated that oleic acid had little impact on the conversion of 18:2ω6 to 18:3ω6 when added as an inhibitor in a concentration of 400 nmol to 50 nmol of 18:2ω6 as substrate in vitro. However, in the present study, we included another group of rats, that had been fed 15% corn oil (CO), to clarify this point. Table 3 shows a comparison among the fatty acid composition of the microsomal lipids of SFO, L and CO groups. As shown in Table 3, the level of arachidonic acid did not increase in the liver microsomal lipids of the CO group, which was fed a diet containing 61% 18:2ω6, compared with the SFO or L groups, which

were fed 15% and 10% 18:2ω6 in their diet. This finding indicates that the CO diet supplied more than an adequate amount of 18:2ω6 to meet the requirement for EFA. Evidence of this adequacy is supplied by the fact that the levels of arachidonic acid in the microsomal lipids of the SFO, L and CO groups were essentially the same (ca. 30%), as seen in Table 3. Kurata and Privett (31) and Tahin et al. (32) made the same observation when they fed 2 groups of rats 2 different levels of adequate amounts of 18:2ω6.

The CO group was also supplied with an amount of dietary 18:3ω3 3 times the amount supplied to the L group (Table 2), but the amount of 22:6ω3 acid derived from the desaturation and elongation of 18:3ω3 and accumulated in the liver microsomal lipids of the CO group (3.3%) was significantly lower than in the L group (4%). Thus, diets rich in linoleic acid not only induce an increase in ω6 PUFA but also inhibit the metabolism and incorporation of ω3 acids into the liver microsomes (32). This supports the observation by de Schrijver and Privett (33) that the accumulation of 22:6ω3 is under metabolic control and that a high quantity of ω6 long-chain fatty acids in the microsomes seems to limit 22:6ω3 incorporation.

Table 5 shows that Δ^6 and Δ^5 desaturase activities were not significantly changed in the CO group compared with the SFO or L groups. The Δ^9 desaturase activity was significantly decreased in the CO group compared with the SFO group, possibly because of the high level of dietary 18:2ω6 in the CO diets, which can affect Δ^9 desaturase (27). The high oleic acid in the SFO diet used in the this study did not significantly affect the linoleic acid metabolism, probably because of the higher affinity of Δ^6 desaturase for 18:2ω6 compared with *cis*-18:1ω9 (23).

In order to determine whether changes in desaturase activity were related to alterations in the physical state of the microsomal membrane caused by changes in the microsomal phospholipid fatty acids between dietary treatments, we isolated the microsomal phospholipids which were then used for preparing vesicles. The vesicular membrane fluidity was measured using *trans*-parinaric acid as a fluorescent probe. The fatty acid composition of the liver microsomal phospholipids of the different groups are shown in Table 6, and the fluorescent polarization ratio results are shown in Table 7. With *trans*-parinaric acid, a polarization ratio of less than 1.6 indicates that no solid lipid domain is present (34). The lack of solid domain, even at 5 C, correlates well with the ratio of the double-bond index to saturated fatty acids of ca. 5 for all diets, as shown in Table 6 (35). To determine whether any difference exists between the diets, the polarization ratio were evaluated by the F test at each temperature. The results are shown in Table 8. No differences were found between diets at any temperature. This demonstrates that the physical state of lipids derived from liver microsomal fractions of rats fed different diets was essentially the same. Thus, any differences in desaturase activity cannot be attributed to differences in membrane fluidity.

From this study, we can conclude that the *trans*-18:1 acids in partially hydrogenated soybean oil can affect the metabolism of essential fatty acids even in the presence of adequate amounts of dietary 18:2ω6. Acyl-CoA desaturases are membrane-bound enzymes; lipid cofactors are important for the maximum activity of these desaturases (36.37). Brenner (23) also suggested that lipoporteins play a specific role in the Δ^6 desaturation. Therefore, the *trans*-18:1 acids may possibly affect the integrity of a lipoportein structure or the alteration of the composition of lipid cofactors required for maximum desaturation. Further studies are needed to determine the specific action of *trans*-18:1 acids in the inhibition of

TABLE 5

In vitro Desaturase Activities of Liver Microsomes Derived from Rats Fed CO, SFO, L or H + CO Diets

Diet	CO (n=12)	SFO (n=12)	L (n=12)	H + CO (n=12)
Δ^6 desaturase (18:2ω6 → 18:3ω6)	0.29 ± 0.02^b	0.34 ± 0.03^a	0.28 ± 0.03^c	$0.20 \pm 0.02^{a,b,c}$
Δ^5 desaturase (20:3ω6 → 20:4ω6)	0.55 ± 0.03	0.52 ± 0.03	0.45 ± 0.02	0.46 ± 0.03
Δ^9 desaturase (16:0 → 16:1ω7)	0.40 ± 0.03^a	0.55 ± 0.02^a	0.52 ± 0.09	0.45 ± 0.05

Results (mean ± SEM) are expressed as nmol of substrate converted per min per mg protein.

Results at the same line with a common supercript are statistically different from each other for $p<0.05$ as tested by Duncan's multiple-range test procedure.

TABLE 6

Fatty Acid Composition of Liver Microsomal Phospholipids from Rats Fed SFO, L or H + CO Diets

Diet[+]	SFO (n=6)	L (n=6)	H + CO (n=6)
Fatty acids*			
16:0	15.3 ± 0.28[b,c]	17.8 ± 0.68[a,b]	11.7 ± 0.42[a,c]
16:1 ω7	0.3 ± 0.02[++]	0.7 ± 0.16	0.6 ± 0.14
18:0	22.3 ± 0.73[b]	23.1 ± 0.40[a]	15.9 ± 0.55[a,b]
18:1 t	—	—	12.2 ± 0.4
18:1 c	10.2 ± 0.43[a,b]	8.1 ± 0.29[b,c]	6.9 ± 0.4[a,c]
18:2 c,t + t,c	—	—	0.2 ± 0.00
18:2 ω6	5.6 ± 0.41[a]	5.7 ± 0.60[b]	10.9 ± 0.46[a,b]
18:3 ω6	0.1 ± 0.01	0.1 ± 0.00	0.3 ± 0.03
20:2 ω6	0.4 ± 0.04	0.3 ± 0.05	0.4 ± 0.03
22:0 + 20:3ω9	0.9 ± 0.10	1.1 ± 0.17	0.8 ± 0.05
20:3 ω6	0.6 ± 0.03[a]	0.8 ± 0.13	1.0 ± 0.14[a]
20:4 ω6	32.7 ± 0.62[a]	30.9 ± 0.7[b]	27.0 ± 0.5[a,b]
22:4 ω6	1.0 ± 0.04[a,b]	0.7 ± 0.08[b]	0.6 ± 0.02[a]
22:5 ω6	5.1 ± 0.20[a,b]	2.5 ± 0.3[b]	1.9 ± 0.33[a]
22:5 ω3	0.1 ± 0.00[a,b]	0.3 ± 0.06[b]	0.3 ± 0.04[a]
22:6 ω3	1.5 ± 0.12[a,b]	4.9 ± 0.4[a]	4.2 ± 0.14[b]
24:0	0.6 ± 0.02	0.5 ± 0.05	0.4 ± 0.04
Other	3.3	2.5	4.7
Double bond index / Saturated fatty acids**	5.0 ± 0.11[a]	4.7 ± 0.08	4.6 ± 0.10[a]

Results at the same line with a common superscripts are *statistically different from each* other at $p<0.05$ level as tested by Duncan's multiple-range test procedure. t=*trans*, c=*cis*.

[+]Animals fed 15% high-oleic safflower oil (SFO), lard (L), or a mixture of 80% partially hydrogenated soybean oil + 20% corn oil (H + CO).

*All fatty acid are of *cis* configuration unless otherwise noted.

[++]Mean ± SEM.

***Trans*-18:1 acids were considered as saturated acid.

TABLE 7

Fluorescent Polarization Ratio of the Vesicular Membranes Prepared from the Liver Microsomal Phospholipids

Diet[a]	Polarization ratio (P)[b] SFO	L	H + CO
Temperature			
5	1.3	1.4	1.4
15	1.3	1.3	1.3
25	1.1	1.2	1.2
35	1.0	1.1	1.1

[a]Animals fed 15% high-oleic safflower oil (SFO), lard (L) or a mixture of 80% partially hydrogenated soybean oil + 20% corn oil (H + CO).

[b]Polarization ratio calculated as described in Methods. Values are the mean of at least 2 and generally 3 determinations.

Δ^6 desaturase under EFA-sufficient conditions and to determine if the changes are of such magnitude that they are able to affect prostaglandin synthesis.

ACKNOWLEDGMENT

This research was supported by the University of Illinois Foundation Burnsides Research Fund.

TABLE 8

Statistical Evaluation of Polarization Ratios at Each Temperature

Temperature	F value[a]	Significance P>[b]
5	0.29	0.75
15	0.06	0.95
25	0.03	0.97
35	0.08	0.92

[a]F value = treatment mean squares/error mean squares.

[b]No difference in polarization ratio value at any temperature.

REFERENCES

1. Schofield, C.R., Davison, V.L., and Dutton, H.J. (1967) J. Am. Oil Chem. Soc. 44, 648-651.
2. Carpenter, D.L., and Slover, H.T. (1973) J. Am. Oil Chem. Soc. 50, 372-376.
3. Reichwald, H.I., Ilsemann, K., and Mukherjee, K. (1979) J. Nutr. 109, 1051-1056.
4. Kummerow, F.A. (1974) J. Am. Oil Chem. Soc. 51, 255-259.
5. Mahfouz, M., Johnson, S., and Holman, R.T (1980) Lipids 15, 100-107.
6. Mahfouz, M., Johnson, S., and Holman, R.T. (1981) Biochim. Biophys. Acta 663, 58-68.
7. Hill, E.G., Johnson, S., and Holman, R.T. (1979)

J. Nutr. 109, 1759-1765.
8. Hill, E.G., Johnson, S., Lawson, L.D., Mahfouz, M., and Holman, R.T. (1982) Proc. Natl. Acad. Aci. USA 79, 953-957.
9. Alfin-Slater R.B., and Aftergood, L. (1979) in Geometrical and Positional Fatty Acids Isomers (Emken, E.A., and Dutton, H.J., eds.) pp. 53-74, Am. Oil Chem. Soc., Champaign, IL.
10. Mahfouz, M., Smith, T.L., Holmes, R.P., and Kummerow, F.A. (1983) Fed. Proc. 42, 1186, Abstr. No. 5236.
11. Bieri, J.G., Stoewasnd, G.S., Briggs, G.M., Phillips, R.W., Woodard, J.C., and Knapka, J.J. (1977) J. Nutr 107, 1340-1348.
12. Inkpen, C.A., Arris, R.A., and Quakenbush, F.W. (1969) J. Lipid Res. 10, 277-282.
13. Mahfouz, M., and Holman, R.T. (1980) Lipids 15, 63-65.
14. Lowry, O.H., Rosebrough, N.J., Farr, A.L., and Randall, A.J. (1951) J. Biol. Chem. 193, 265-275.
15. Folch, J., Lees, M., and Sloane-Stanley, G.H. (1957) J. Biol. Chem. 226, 497-509.
16. De Schrijver, R., and Privett, O.S. (1982) Lipids 17, 27-34.
17. Morrison, W.R., and Smith L.M. (1964) J. Lipid Res. 5, 600-608.
18. Steel, R.G., and Torrie, J.H. (1960) in Principles and Procedures of Statistics, pp. 107-114, McGraw-Hill Book Comp., Inc., N.Y.
19. Veerkamp, J.H., and Broekhuyse, R.M. (1976) in Techniques for the Analysis of Membrane Lipids in Biochemical analysis of Membranes, (Maddy, A.H., ed.) pp. 269-270, John Wiley & Sons, Inc., New York.
20. Stewart, J.C.M. (1980) anal. Biochem. 104, 10-14.
21. Chen, R.F., and Bowman, R.L. (1965) Science 147, 729-732.
22. Catherine, T.H., and Wills, E.D. (1978) Biochem. J. 174, 585-593.
23. Brenner, R.R. (1974) Mol. Cell. Biochem. 3, 41-52.
24. Cunnane, S.C., and Wahle, K.W. (1981) Lipids 16, 771-774.
25. Actis Dato, S.M., Catala, A., and Brenner, R.R. (1973) Lipids 8, 1-6.
26. Svensson, L. (1983) Lipids 18, 171-178.
27. Jeffcoat, R., and James, A.T. (1977) Lipids 12, 469-474.
28. Ullman, D., and Sprecher, H. (1971) Biochim. Biophys. Acta 248, 61-70.
29. Mohrhauer, H., Rahm, J.J., Seufert, J., and Holman, R.T. (1971) J. Nutr. 91, 521-527.
30. Shimp, J.L., Bruckner, G., and Kinsella, J.E. (1982) J. Nutr. 112, 722-735.
31. Kurata, N., and Privett, O.S. (1980) Lipids 15, 1029-1036.
32. Thain, Q.S., Blum, M., and Carafoli, E. (1981) Eur. J. Biochem. 121, 5-13.
33. DeSchrijver, R. and Privett, O.S. (1982) J. Nutr. 112, 619-626.
34. Sklar, L.A., Milijanich, G.P., and Dratz, E.A. (1979) Biochemistry 18, 1707-1716.
35. Lipiello, P.M., Holloway, C.T., Garfield, S.A., and Holloaway, P.W. (1979) J. Biol. Chem. 254, 2004-2009.
36. Jones, P.D., Holloway, P.W., Pellutto, R.O., and Wakil, S.J. (1969) J. Biol. Chem. 244, 744-754.
37. Holloway, P.W. (1971) Biochemistry 10, 1556-1560.

[Received May 26, 1983]