Homeostatic Control of Membrane Fatty Acid Composition in the Rat After Dietary Lipid Treatment

R.A. GIBSON,*[a] E.J. MCMURCHIE,[b] J.S. CHARNOCK[b] and G.M. KNEEBONE,[a]
[a]Department of Pediatrics, Flinders Medical Center, Bedford Park, South Australia 5042, Australia, and [b]CSIRO, Division of Human Nutrition, Glenthorne Laboratories, Majors Road, O'Halloran Hill, South Australia 5158, Australia

ABSTRACT

Diets in which both the lipid content and composition (polyunsaturated to saturated fatty acid ratio) were varied were fed to rats for 20 weeks, and the effects on the tissue lipid profiles were determined. The fatty acid profile of the plasma lipids, and the phospholipid fatty acids of the mitochondrial and microsomal fractions of liver, heart, kidney and brain, as well as erythrocyte membranes were determined. Despite large differences in the level and type of lipid present in the experimental diets and in the proportion of saturated fatty acids in the plasma lipids in response to the various diets, there was little effect on the proportion of saturated to unsaturated fatty acids in the phospholipids of the various membranes examined. The major effect of altering the dietary level of polyunsaturated to saturated fatty acids was on the ratio of the $\omega6/\omega3$ series of unsaturated fatty acids in the membrane lipids. This change occurred in all tissues except the brain, in which only a small response to altered dietary lipid intake was observed. The $\omega6/\omega3$ ratio was elevated upon feeding a diet rich in $\omega6$ polyunsaturated fatty acids, but decreased when a diet rich in saturated fatty acids was fed. The failure to significantly alter membrane lipid saturation/unsaturation in the tissues examined would suggest that a homeostatic mechanism is operative in biological membranes and may act to buffer membranes from the effects of changes in the nature of the dietary lipid intake.
Lipids 19:942-951, 1984.

INTRODUCTION

Dietary lipids are known to influence the fatty acid composition of tissue lipids (1-3) including those of heart (4), brain (5), liver (6) and blood (7-9). Most studies have concentrated on the dietary-induced changes in the fatty acid distribution of either total lipids or the individual phospholipids from unfractionated tissues and organs (10,11). However, recent studies have focused on dietary-induced changes in specific subcellular membranes (12-15). These changes are now being recognized as functionally important as the specific role of subcellular membrane lipids in modulating membrane function becomes more clear (16-18). This is particularly so with regard to the effects of dietary lipids on the physical properties of membrane lipids and the effect they have on the functioning of the various membrane enzymes associated with many physiological processes (19-22).

As part of a larger survey on the effects of dietary lipids on the physical and biochemical properties of cellular membranes and associated processes, we have examined the changes in subcellular membrane lipid composition following changes in the nature of the dietary lipid intake. Of particular interest have been the effects of diets of widely differing lipid composition on both the level of membrane lipid saturated/unsaturated fatty acids and the nature of the unsaturated fatty acids present, particularly with regard to the relationship between the $\omega9$ (oleate), $\omega6$ (linoleate) and $\omega3$ (linolenate) series of unsaturated fatty acids. In addition to the biophysical effects of changing membrane lipid composition, the established role of these fatty acid families in prostanoid metabolism (23-25) supports the relevance of such a study.

We show that in the rat, subcellular membrane phospholipid fatty acid composition can be altered by dietary lipid treatment. However, for any one organ the change is predominantly in the type of membrane unsaturated fatty acids present rather than in differences in the ratio of the saturated to unsaturated fatty acids. The subcellular membranes from tissues that do respond significantly to changes in the dietary lipid intake appear to exhibit considerable homeostasis in that a constant level is maintained for both the proportion of lipid unsaturation and the value of the unsaturation index, despite wide differences in the values of these two parameters in the various experimental diets used.

MATERIALS AND METHODS

Male rats (Hooded Wistar), weighing be-

*To whom correspondence should be addressed.

tween 242 and 315 g (mean 277 g) were maintained on the various lipid-supplemented diets described below for 20 weeks. At the time of sacrifice the body weights were, REF. group 438 ± 7.5 g (mean ±SE; n = 18), SAT. group 497 ± 7.8 g (n = 18) and UNSAT. group 495 ± 7.2 g (n = 18). Rats were killed by decapitation and the tissues removed immediately for preparation of subcellular membrane fractions.

Rat Diets

The basic rat ration was composed of 52% wheat starch, 18% milling byproducts (including starch, fiber and crude protein), 7% meat and bone meal, 6% cottonseed meal, 6% fishmeal, 5% soybean meal, 1% brewers yeast, 3% salt mix and 2% vitamin mix. Vitamins and minerals were added to the mixture in the following amounts per kilogram: choline, 525 mg; vitamin E, 36 mg; vitamin K_3, 18 mg; thiamine, 58 μg; vitamin A, 22,000 I.U.; pantothenic acid, 16 mg; niacin, 18 mg; vitamin B_{12}, 0.12 mg; vitamin D_3, 4,000 I.U.; biotin, 0.12 mg; riboflavin, 5 mg; pyridoxine, 5 mg; folic acid, 2 mg; magnesium, 415 mg; iron, 66 mg; manganese, 114 mg; zinc, 64 mg; copper, 5 mg; molybedenum, 1 mg; iodine, 2 mg, and cobalt, 0.4 mg. The fat content of the diet was either 4% or 16%, depending on the nature of the experimental diet. The fat was either a basal mixture of vegetable oils as used in normal rat ration (Australasian Feed Services, Australia) for the reference (REF.) diet or 4% basal fat plus 12% sheep perirenal fat for the saturated fatty acid (SAT.) diet or 4% basal fat plus 12% sunflower seed oil (Nuttelex Pty. Ltd., Australia) for the unsaturated fatty acid (UNSAT.) diet. The fat was added to the commercial mixture at the time of pelleting. Fatty acid analysis of the diets is shown in Table 1.

TABLE 1

Fatty Acid Composition of Normal and Lipid Supplemented Rat Diets

Fatty acid[1] (%; w/w)	REF.[2]	SAT.[2]	UNSAT.[2]
14:0	1.5	2.5	0.3
16:0	18.6	21.1	8.2
16:1	3.1	2.2	0.7
17:0	0.6	1.9	0.2
18:0	6.7	29.1	4.9
18:1	22.6	31.5	22.6
18:2ω6	33.2	8.6	60.3
18:3ω3	3.1	1.3	0.9
20:1	3.4	0.8	0.7
22:1/20:5	4.7	0.6	0.7
22:6ω3	2.4	0.3	0.4
SAT.	27.4	54.6	13.6
UNSAT.	72.6	45.4	86.4
UNSAT./SAT.	2.6	0.8	6.4

[1] Fatty acids are designated by the number of carbon atoms followed by the number of double bonds. The position of the first double bond relative to the methyl or omega (ω) end of the molecule also is indicated.

[2] REF. (Reference, standard laboratory diet); SAT. (sheep kidney fat supplemented diet); UNSAT. (sunflower seed oil supplemented diet). Trace amounts (less than 0.3%) also were detected for the fatty acids 20:0 and 22:0.

Combustion calorimetry of the REF., SAT. and UNSAT. diets gave energy values of 17.1, 20.1 and 20.0 KJ/g respectively. Animals fed the SAT. and UNSAT. diets consumed about 20 g of food per day compared with approximately 25 g of food per day for the animals in the REF. group. All diets were supplied ad libitum.

Blood and Plasma Collection

Immediately after the animals were killed, blood was collected and centrifuged to separate plasma and red blood cells. The red blood cells were washed 3 times by centrifugation in phosphate buffered saline.

Liver, Kidney and Brain Mitochondrial Fractions

For preparation of mitochondria from the above tissues, about 4 g from one liver, both kidneys or all the brain tissue from one animal were chopped and rinsed in ice-cold medium containing 250 mM sucrose, 2 mM Hepes (4-(2-hydroxy-ethyl)-1 piperazine ethane-sulphonic acid), 0.5 mM EGTA and 0.05% (w/w) delipidated bovine serum albumin (BSA), pH 7.4 and then homogenized in 40 ml of the above medium using a Polytron tissue homogenizer (Kinematica, GmbH, Switzerland) at setting 3.5 for 2 bursts each of 6 seconds. The homogenate was filtered through cheesecloth and centrifuged at 500 g for 12 min and the supernatant saved. The resulting pellet was resuspended in medium to the original volume and recentrifuged at 500 g for 12 min. The supernatant from this and the previous centrifugation were combined and centrifuged at 6000 g for 15 min. The resulting supernatant was saved for preparation of the microsomal fraction described below. The mitochondrial pellet from the above centrifugation was washed twice by centrifugation in the above medium at 6000 g for 15 min. and finally resuspended in this medium.

Heart Mitochondrial Fraction

Ventricular tissue from one animal was chopped and rinsed in ice-cold medium contain-

ing 100 mM 50 mM Mops (4-Morpholinepropanesulphonic acid), 2 mM EGTA and 0.2% (w/v) delipidated BSA, pH 7.2 and then homogenized in 40 ml of the above medium in a manner identical to that described for the preparation of mitochondria from the other tissues. The procedure for the subsequent isolation of heart mitochondria by differential centrifugation was as described for the other tissues, except that the first mitochondrial pellet was resuspended in the above medium without BSA. After washing the mitochondrial pellet by centrifugation, the pellet was then finally resuspended in the buffer without BSA. The supernatant resulting from the first centrifugation at 6000 g for 15 min was saved for the preparation of the heart microsomal fraction.

Liver, Kidney, Brain and Heart Microsomal Fractions

The supernatants from the first 6000 g centrifugation described above were centrifuged at 110,000 g for 60 min and the pellet collected and referred to as the microsomal membrane fraction.

Lipid Analysis

Prior to lipid analysis, the mitochondrial and microsomal membrane preparations from the liver, kidney, brain and heart were diluted in 50 volumes of 20 mM Tris, 2 mM EDTA, pH 7.2 and centrifuged at 250,000 g for 60 min to remove sucrose or KCl. The resulting membrane pellets were resuspended in glass distilled water, prior to extraction of lipids using a modification of a method developed for erythrocyte membranes (26). Briefly, to one volume of plasma, washed red blood cells or washed mitochondrial or microsomal membrane preparations, 4 volumes of boiling 2-propanol were added and the mixture was boiled for 30 seconds. After cooling, 8 volumes of chloroform containing the antioxidant butylated hydroxyanisole (0.1% of the estimated lipid weight) were added. Following the addition of 1 volume of glass distilled water, the samples were shaken, centrifuged and the organic phase collected. After re-extracting the aqueous phase with a further 4 volumes of chloroform, the organic phases were combined and dried using anhydrous sodium sulphate. For all but the plasma lipids, phospholipids were separated from the total extracted lipids by thin-layer chromatography (TLC) on silica gel H plates developed in petroleum ether/diethyl ether/acetic acid, (90:15:1). The phospholipids remaining at the origin were removed and methylated in 1% (v/v) H_2SO_4 in methanol by heating at 70 C for 3 hr. For plasma, the fatty acids of the total lipids were methylated. For analysis of the fatty acids present in the various dietary lipid supplements, the diet pellets were extracted and fatty acid analysis was performed on the total lipid extract.

Gas Chromatography

Fatty acid methyl esters were analyzed using a Hewlett-Packard 5850A gas chromatograph fitted with dual FID detectors. Operating conditions were as follows: the FID was set at 300 C, the injection port at 200 C and the oven temperature programmed from 125 to 225 C at a heating rate of 4 C/min. Glass columns were packed with 5% SP2310 on chromasorb WAW (Supelco Inc. Bellefonte, Pennsylvania). This packing allows for the separation of up to 4 double bonds of one homologous series of fatty acids before encountering the parent saturate of the next series, thus resulting in minimal overlap of the major membrane phospholipid fatty acids. For example, 18:3ω3 was clearly separated from 20:0 and 20:4ω6 emerged before 22:0. Two overlaps which did occur in this system were 20:3ω9 with 20:2ω6, and also 20:5ω3 with 22:1, but as none of these compounds were present in large amounts in the samples examined, no attempt was made to estimate the amounts of the individual fatty acids by rechromatography on different packings.

RESULTS

The fatty acid composition of the various lipid-supplemented diets is shown in Table 1. Addition of sheep kidney perirenal fat (SAT. diet) to the normal or REF. diet increased the proportion of myristic (14:0), palmitic (16:0), stearic (18:0) and oleic (18:1ω9) acids. Sunflower seed oil (UNSAT. diet) increased lipid unsaturation to about 86%, mainly by elevating the proportion of linoleic acid (18:2ω6). The total caloric energy derived from linoleic acid represented 2.5, 12.4 and 23.9% of the dietary energy intake for the SAT., REF. and UNSAT. diets, respectively. These levels are all above the minimum requirement for linoleic acid of 1.3% of the total dietary energy intake in male rats (27). All diets also contained 0.9% or greater linolenic acid (18:3ω3), but no diet contained any measurable quantity of arachidonic (20:4ω6) or eicosapentaenoic (20:5ω3) acids. Only trace amounts of docosahexaenoic acid (22:6ω3) were present in the SAT. and UNSAT. diets.

The fatty acid composition of the plasma lipids and the red blood cell (erythrocyte) phospholipids, as well as the phospholipids from the various subcellular membrane fractions of the tissues examined, are shown in Tables 2 to 6. A summary of all these tables in

TABLE 2

Major Fatty Acids of Plasma Lipids and Red Blood Cell Phospholipids from Rats Fed Lipid-Supplemented Diets for 20 Weeks

Fatty acid (%; w/w)	Plasma			Red blood cells		
	REF.	SAT.	UNSAT.	REF.	SAT.	UNSAT.
14:0	0.6 ± 0.04	0.8 ± 0.07	0.2 ± 0.02	0.3 ± 0.03	0.2 ± 0.01	0.2 ± 0.01
DMA 16:0	0.1 ± 0.01	0.1 ± 0.01	0.1 ± 0.01	3.3 ± 0.04	2.4 ± 0.17	2.3 ± 0.10
16:0	18.7 ± 0.70	15.5 ± 0.72	14.5 ± 1.00	28.4 ± 0.14	23.6 ± 0.52	25.3 ± 0.58
16:1	4.0 ± 0.18	2.9 ± 0.09	0.3 ± 0.28	–	–	–
17:0	0.8 ± 0.04	1.1 ± 0.07	0.5 ± 0.07	1.2 ± 0.05	1.5 ± 0.06	1.1 ± 0.04
DMA 18:0	0.4 ± 0.02	0.3 ± 0.04	0.1 ± 0.03	3.1 ± 0.13	3.1 ± 0.17	2.6 ± 0.14
DMA 18:1	–	–	–	1.2 ± 0.09	1.3 ± 0.05	1.2 ± 0.06
18:0	11.1 ± 0.55	13.8 ± 0.42	13.3 ± 1.13	13.1 ± 0.22	17.0 ± 0.29	17.1 ± 0.35
18:1	14.9 ± 0.43	25.8 ± 1.33	13.8 ± 0.68	9.3 ± 0.14	11.3 ± 0.20	8.1 ± 0.13
18:2ω6	19.6 ± 0.57	15.1 ± 0.17	30.1 ± 0.72	8.7 ± 0.08	9.7 ± 0.30	11.4 ± 0.32
18:3ω6	0.3 ± 0.20	0.2 ± 0.16	0.3 ± 0.27	–	–	–
18:3ω3	0.9 ± 0.07	0.9 ± 0.05	0.5 ± 0.15	–	–	–
20:0	0.4 ± 0.04	0.8 ± 0.04	0.5 ± 0.02	0.2 ± 0.01	0.2 ± 0.02	0.3 ± 0.02
20:1	1.1 ± 0.08	1.0 ± 0.04	0.9 ± 0.04	0.3 ± 0.02	0.3 ± 0.02	0.4 ± 0.03
20:2ω6+20:3ω9	0.4 ± 0.03	0.4 ± 0.03	0.5 ± 0.04	0.2 ± 0.02	0.3 ± 0.04	0.5 ± 0.02
20:3ω6	0.7 ± 0.05	0.6 ± 0.08	0.4 ± 0.05	0.1 ± 0.03	0.5 ± 0.03	0.3 ± 0.01
20:4ω6	20.1 ± 1.11	14.3 ± 1.13	20.7 ± 1.55	21.4 ± 0.30	20.1 ± 0.56	21.9 ± 0.52
22:0	–	–	–	–	–	–
22:1+20:5ω3	1.3 ± 0.16	1.1 ± 0.16	0.6 ± 0.19	0.7 ± 0.01	0.9 ± 0.03	0.1 ± 0.01
22:4ω6	0.3 ± 0.05	0.2 ± 0.05	0.5 ± 0.06	1.1 ± 0.4	0.7 ± 0.05	1.9 ± 0.07
24:0	0.4 ± 0.06	0.3 ± 0.03	0.7 ± 0.05	1.4 ± 0.09	1.1 ± 0.04	1.7 ± 0.11
24:1+22:5ω6	–	–	–	0.6 ± 0.05	0.7 ± 0.04	0.6 ± 0.07
22:5ω3	0.7 ± 0.08	0.3 ± 0.04	0.1 ± 0.02	1.4 ± 0.04	1.4 ± 0.07	0.5 ± 0.02
22:6ω3	3.0 ± 0.22	3.4 ± 1.05	1.3 ± 0.10	3.7 ± 0.05	3.8 ± 0.19	2.6 ± 0.20

Fatty acids are designated as described in Table 1. Data are presented as the mean relative percentage ±SEM for n = 6 animals in each dietary group. (–) = not detected or present at less than 0.1%.

TABLE 3

Major Fatty Acids of the Phospholipids of the Mitochondrial and Microsomal Membrane Fractions from the Liver of Rats Fed Lipid-Supplemented Diets for 20 Weeks

Fatty acid (%; w/w)	Mitochondria			Microsomes		
	REF.	SAT.	UNSAT.	REF.	SAT.	UNSAT.
14:0	0.2 ± 0	0.1 ± 0	0.1 ± 0	0.2 ± 0	0.1 ± 0	0.1 ± 0
DMA 16:0	0.1 ± 0	0.1 ± 0	0.1 ± 0	0.1 ± 0	0.1 ± 0	0.1 ± 0
16:0	15.1 ± 0.2	11.1 ± 0.3	11.7 ± 0.1	16.4 ± 0.2	11.6 ± 0.2	12.8 ± 0.3
16:1	2.6 ± 0.1	1.3 ± 0	0.1 ± 0	2.5 ± 0.1	1.3 ± 0	0.01 ± 0
17:0	0.7 ± 0.1	0.8 ± 0	0.7 ± 0	0.9 ± 0	0.9 ± 0	0.7 ± 0
DMA 18:0	0.5 ± 0	0.3 ± 0	0.3 ± 0	0.5 ± 0	0.4 ± 0	0.4 ± 0
DMA 18:1	–	–	–	–	–	–
18:0	18.4 ± 0.2	23.1 ± 0.8	21.3 ± 0.3	21.1 ± 0.5	23.9 ± 0.4	24.9 ± 0.3
18:1	9.5 ± 0.3	11.0 ± 0.5	8.3 ± 0.2	9.6 ± 0.2	11.1 ± 0.4	7.3 ± 0.1
18:2ω6	15.0 ± 0.3	12.4 ± 0.7	14.0 ± 0.7	12.1 ± 0.1	10.7 ± 0.4	10.7 ± 0.2
18:3ω6	0.3 ± 0.3	0.4 ± 0.2	–	0.3 ± 0.2	0.4 ± 0.3	0.1 ± 0.1
18:3ω3	–	–	–	–	–	–
20:0	0.2 ± 0	0.2 ± 0	0.3 ± 0	0.2 ± 0	0.4 ± 0	0.3 ± 0
20:1	0.5 ± 0.1	0.4 ± 0	0.7 ± 0	0.4 ± 0	0.6 ± 0.1	0.7 ± 0
20:2ω6+20:3ω9	0.4 ± 0	0.4 ± 0	1.2 ± 0.1	0.4 ± 0	0.6 ± 0	0.9 ± 0
20:3ω6	1.2 ± 0.1	1.1 ± 0.1	0.5 ± 0	1.3 ± 0.1	1.3 ± 0.1	0.5 ± 0
20:4ω6	24.5 ± 0.4	25.8 ± 1.1	31.3 ± 0.5	24.6 ± 0.4	25.2 ± 0.5	32.1 ± 0.3
22:0	–	–	–	–	–	–
22:1+20:5ω3	1.4 ± 0	0.8 ± 0.2	–	1.2 ± 0.1	1.4 ± 0.1	0.2 ± 0.2
22:4ω6	0.2 ± 0	0.2 ± 0	0.5 ± 0	0.3 ± 0	0.2 ± 0	0.5 ± 0
24:0	0.5 ± 0	1.5 ± 0.1	1.5 ± 0.1	0.6 ± 0.1	0.4 ± 0	1.3 ± 0.1
24:1+22:5ω6	0.1 ± 0	–	–	0.1 ± 0	0.1 ± 0	0.1 ± 0
22:5ω3	0.5 ± 0	0.3 ± 0	0.3 ± 0	0.4 ± 0	0.4 ± 0	0.2 ± 0
22:6ω3	8.2 ± 0.3	9.2 ± 0.2	7.4 ± 0.2	6.7 ± 0.1	8.3 ± 0.2	6.0 ± 0.2

Data are as described in Table 2.

TABLE 4

Major Fatty Acids of the Phospholipids of the Mitochondrial and Microsomal Fraction from the Heart of Rats Fed Lipid-Supplemented Diets for 20 Weeks

Fatty acid (%; w/w)	Mitochondria			Microsomes		
	REF.	SAT.	UNSAT.	REF.	SAT.	UNSAT.
14:0	0.1 ± 0	0.1 ± 0	0.1 ± 0	0.1 ± 0.1	0.1 ± 0	0.1 ± 0.1
DMA 16:0	2.3 ± 0.1	1.9 ± 0.1	1.9 ± 0.1	2.6 ± 0.2	2.2 ± 0.2	2.0 ± 0.2
16:0	9.3 ± 0.2	7.9 ± 0.2	6.6 ± 0.2	9.9 ± 0.8	8.8 ± 0.5	8.3 ± 0.8
16:1	0.9 ± 0.2	1.6 ± 1.2	0.2 ± 0.2	–	–	–
17:0	0.6 ± 0	0.6 ± 0	0.3 ± 0.1	0.3 ± 0.1	0.4 ± 0	0.3 ± 0.1
DMA 18:0	1.1 ± 0	2.0 ± 0.1	1.4 ± 0.2	1.7 ± 0.1	2.3 ± 0.3	1.7 ± 0.1
DMA 18:1	0.1 ± 0.1	0.5 ± 0.1	0.7 ± 0.1	0.3 ± 0.1	0.6 ± 0.1	0.6 ± 0.1
18:0	19.9 ± 0.4	21.1 ± 0.2	23.3 ± 0.3	20.6 ± 1.0	21.1 ± 0.3	21.8 ± 0.7
18:1	9.0 ± 0.1	9.2 ± 0.1	7.2 ± 0.3	12.2 ± 0.7	11.4 ± 0.2	10.0 ± 0.5
18:2ω6	21.9 ± 0.9	15.1 ± 0.5	17.7 ± 0.5	16.9 ± 0.6	13.4 ± 0.6	16.0 ± 0.9
18:3ω6	–	–	–	–	–	–
18:3ω3	–	–	–	–	–	–
20:0	0.2 ± 0	0.2 ± 0	0.2 ± 0	0.2 ± 0	0.1 ± 0	0.2 ± 0
20:1	0.4 ± 0	0.3 ± 0.1	0.3 ± 0.1	0.8 ± 0.2	0.4 ± 0	0.5 ± 0.1
20:2ω6+20:3ω9	0.2 ± 0	0.2 ± 0	0.2 ± 0	0.3 ± 0	0.2 ± 0	0.4 ± 0
20:3ω6	0.3 ± 0	0.4 ± 0	0.3 ± 0	0.6 ± 0.1	0.5 ± 0	0.5 ± 0.1
20:4ω6	15.9 ± 0.3	18.6 ± 0.3	21.8 ± 0.3	16.4 ± 1.1	19.2 ± 0.7	21.1 ± 1.0
22:0	–	–	–	–	–	–
22:1+20:5ω3	0.4 ± 0.1	0.5 ± 0.1	0.2 ± 0.2	0.6 ± 0.3	0.6 ± 0.2	0.7 ± 0.3
22:4ω6	0.4 ± 0	0.3 ± 0	1.6 ± 0	1.4 ± 0.2	0.8 ± 0	1.9 ± 0.3
24:0	0.6 ± 0	0.4 ± 0	3.5 ± 0.1	1.3 ± 0.3	1.0 ± 0.2	3.0 ± 0.5
24:1+22:5ω6	–	–	–	–	–	–
22:5ω3	1.1 ± 0	1.4 ± 0	0.8 ± 0	1.2 ± 0.1	1.6 ± 0.1	0.9 ± 0.1
22:6ω3	15.2 ± 0.4	17.3 ± 0.3	11.5 ± 0.3	12.7 ± 0.9	15.4 ± 0.3	9.9 ± 0.6

Data are as described in Table 2.

TABLE 5

Major Fatty Acids of the Phospholipids of the Mitochondrial and Microsomal Membrane Fractions from the Kidney of Rats Fed Lipid-Supplemented Diets for 20 Weeks

Fatty acid (%; w/w)	Mitochondria			Microsomes		
	REF.	SAT.	UNSAT.	REF.	SAT.	UNSAT.
14:0	0.1 ± 0	0.1 ± 0	0.1 ± 0	0.2 ± 0	0.1 ± 0	0.1 ± 0
DMA 16:0	1.8 ± 0.1	1.3 ± 0.1	1.3 ± 0	2.8 ± 0.1	2.0 ± 0	2.4 ± 0.2
16:0	13.6 ± 0.2	13.6 ± 0.2	13.3 ± 0.3	19.2 ± 0.3	19.7 ± 0.4	19.1 ± 1.1
16:1	1.8 ± 0.1	2.0 ± 0	0.1 ± 0	–	–	–
17:0	0.5 ± 0.1	0.5 ± 0	0.5 ± 0	0.6 ± 0	0.8 ± 0	0.6 ± 0.1
DMA 18:0	0.9 ± 0	1.1 ± 0	0.9 ± 0	1.5 ± 0.1	1.5 ± 0.1	1.5 ± 0.1
DMA 18:1	0.2 ± 0.1	0.3 ± 0.1	0.4 ± 0	0.1 ± 0.1	0.6 ± 0	0.5 ± 0.1
18:0	17.3 ± 0.2	17.5 ± 0.3	18.5 ± 0.3	19.4 ± 0.6	17.9 ± 0.3	20.5 ± 0.8
18:1	11.1 ± 0.2	12.8 ± 0.2	9.9 ± 0.1	10.3 ± 0.3	12.1 ± 0.3	10.1 ± 0.2
18:2ω6	14.1 ± 0.3	13.9 ± 0.3	15.8 ± 0.4	7.2 ± 0.2	7.5 ± 0.3	8.5 ± 0.2
18:3ω6	–	–	–	0.3 ± 0.2	0.1 ± 0	0.3 ± 0.2
18:3ω3	–	–	–	–	–	–
20:0	0.2 ± 0	0.2 ± 0	0.2 ± 0	0.2 ± 0	0.2 ± 0	0.2 ± 0
20:1	0.6 ± 0.2	0.4 ± 0.1	0.3 ± 0	0.5 ± 0.2	0.3 ± 0	0.5 ± 0.1
20:2ω6+20:3ω9	0.2 ± 0	0.2 ± 0	0.5 ± 0	0.3 ± 0	0.2 ± 0	0.3 ± 0
20:3ω6	0.8 ± 0.1	0.7 ± 0.1	0.5 ± 0	0.9 ± 0.1	0.6 ± 0	0.6 ± 0
20:4ω6	31.7 ± 0.4	30.5 ± 0.2	34.3 ± 0.4	30.5 ± 0.6	30.2 ± 0.4	29.7 ± 1.6
22:0	–	–	–	–	–	–
22:1+20:5ω3	1.5 ± 0.2	1.3 ± 0.1	0.1 ± 0.1	0.6 ± 0.2	0.9 ± 0.1	0.1 ± 0
22:4ω6	0.2 ± 0	0.2 ± 0	0.4 ± 0	0.5 ± 0.1	0.4 ± 0	0.8 ± 0.1
24:0	0.6 ± 0.1	0.6 ± 0.1	0.9 ± 0.1	2.0 ± 0.6	1.4 ± 1.0	1.7 ± 0.1
24:1+22:5ω6	0.1 ± 0	0.1 ± 0	0.1 ± 0	0.4 ± 0.1	0.4 ± 0	0.5 ± 0.1
22:5ω3	0.1 ± 0	0.2 ± 0	0.1 ± 0	0.2 ± 0	0.3 ± 0	0.1 ± 0
22:6ω3	2.5 ± 0.1	2.4 ± 0.1	2.1 ± 0.1	2.4 ± 0.2	2.9 ± 0.2	2.2 ± 0.2

Data are as described in Table 2.

TABLE 6

Major Fatty Acids of the Phospholipids of the Mitochondrial and Microsomal Membrane Fractions from the Brain of Rats Fed Lipid-Supplemented Diets for 20 Weeks

Fatty acid (%; w/w)	Mitochondria			Microsomes		
	REF.	SAT.	UNSAT.	REF.	SAT.	UNSAT.
14:0	0.1 ± 0	0.1 ± 0	0.1 ± 0	0.1 ± 0	0.2 ± 0	0.1 ± 0
DMA 16:0	2.3 ± 0.2	2.4 ± 0.1	2.5 ± 0.1	2.3 ± 0.1	2.5 ± 0.2	2.4 ± 0.1
16:0	17.0 ± 0.6	16.9 ± 0.8	15.8 ± 0.1	26.2 ± 0.3	26.0 ± 1.5	26.8 ± 1.9
16:1	1.9 ± 0.4	2.3 ± 0.1	2.0 ± 0.1	–	–	–
17:0	0.4 ± 0	0.5 ± 0	0.4 ± 0	0.5 ± 0	0.5 ± 0	0.4 ± 0.1
DMA 18:0	3.0 ± 0.2	2.9 ± 0.1	3.2 ± 0.2	3.6 ± 0.2	3.0 ± 0.2	3.2 ± 0.3
DMA 18:1	–	–	–	–	–	–
18:0	19.3 ± 0.2	18.9 ± 0.3	19.9 ± 0.4	17.8 ± 0.1	17.3 ± 0.5	17.7 ± 0.5
18:1	25.1 ± 0.2	24.7 ± 0.2	23.8 ± 0.2	18.1 ± 0.1	18.3 ± 0.2	17.7 ± 0.4
18:2ω6	2.4 ± 0.3	2.1 ± 0.1	2.4 ± 0.1	1.5 ± 0.2	1.5 ± 0.1	1.9 ± 0.1
18:3ω6	0.2 ± 0.2	0.7 ± 0.2	0.4 ± 0.2	0.3 ± 0.1	0.6 ± 0.2	0.1 ± 0.1
18:3ω3	–	–	–	–	–	–
20:0	0.3 ± 0.1	0.3 ± 0	0.3 ± 0	0.2 ± 0	0.2 ± 0	0.2 ± 0
20:1	2.3 ± 0.1	2.3 ± 0.1	2.3 ± 0.1	1.3 ± 0.1	1.4 ± 0.2	1.1 ± 0.1
20:2ω6+20:3ω9	0.2 ± 0	0.3 ± 0	0.3 ± 0	0.2 ± 0	0.2 ± 0	0.2 ± 0
20:3ω6	0.3 ± 0	0.3 ± 0	0.3 ± 0	0.2 ± 0	0.3 ± 0.1	0.2 ± 0.1
20:4ω6	8.4 ± 0.3	8.3 ± 0.2	9.1 ± 0.2	8.1 ± 0	8.0 ± 0.1	8.5 ± 0.2
22:0	–	–	–	–	–	–
22:1+20:5ω3	0.5 ± 0.1	0.5 ± 0.1	0.5 ± 0	0.1 ± 0.1	0.3 ± 0.2	0.2 ± 0.2
22:4ω6	2.6 ± 0.2	2.5 ± 0.1	2.5 ± 0.1	2.7 ± 0.1	2.6 ± 0.1	2.6 ± 0.1
24:0	0.5 ± 0.2	0.9 ± 0.1	1.1 ± 0.1	0.4 ± 0.1	0.5 ± 0.2	1.1 ± 0.1
24:1+22:5ω6	0.9 ± 0.1	1.0 ± 0.2	0.9 ± 0.1	0.1 ± 0	0.2 ± 0	0.2 ± 0
22:5ω3	–	–	–	0.1 ± 0	0.1 ± 0	0.1 ± 0
22:6ω3	12.1 ± 0.3	12.0 ± 0.3	11.9 ± 0.2	16.1 ± 0.3	16.3 ± 0.6	15.2 ± 0.9

Data are as described in Table 2.

relation to changes in the total saturated, unsaturated, ω6 and ω3 unsaturated fatty acids and the unsaturation index is shown in Table 7.

In general, the dietary lipid treatments had little effect on the ratio of saturated to unsaturated fatty acids in the lipids of the various samples examined, despite large differences in this ratio in the diet. For the plasma lipids, the increase in total unsaturated fatty acids upon feeding the more unsaturated diet was due primarily to an increase in the proportion of 18:2ω6. Although small changes in the value of the unsaturation index in both the SAT. and UNSAT. fed animals were seen in some membranes of the heart and liver, generally little change in this parameter was evident. The various dietary lipid treatments had little effect on the overall level of unsaturated fatty acids, but they did have a significant effect on the type of unsaturated fatty acids present in the membrane lipids, particularly with regard to the proportions of the ω6 and ω3 series of unsaturated fatty acids. In general, the changes observed in the ω6 and ω3 unsaturated fatty acids were in opposite directions, the UNSAT. diet elevating the proportion of the ω6 unsaturated fatty acids and the SAT. diet elevating the proportion of the ω3 unsaturated fatty acids.

With regard to the changes in the proportion of the ω6 unsaturated fatty acids as a result of the 2 dietary lipid treatments, it was apparent that the greatest change occurred in the plasma lipids (25% net change). Relatively smaller changes were evident in the various subcellular membrane lipid samples, with the extent of change for the heart, liver and erythrocyte membrane ω6 unsaturated fatty acids (2 to 12% net change) being greater than that observed for the kidney and the brain (Table 7).

Certain tissue-specific responses also were observed in the type of 6 unsaturated fatty acids undergoing the greatest change. Thus, in response to increased dietary 18:2, the levels of linoleic acid increased in plasma and red blood cells without any change in 20:4 levels. In contrast, the SAT. diet induced reductions in plasma 18:2 and 20:4 levels. The mitochondrial and microsomal membranes of heart and liver shifted in a similar way in response to diet, in that both SAT. and UNSAT. diets resulted in decreased levels of 18:2 and increased levels of 20:4. The UNSAT. diet evoked the greatest response. Although kidney membranes showed little response to the SAT. diet, small increases in both 18:2 and 20:4 were observed upon feeding the UNSAT. diet.

The predominant fatty acid of the ω3 series to undergo change as a result of the 2 dietary

TABLE 7

Fatty Acid Composition of the Plasma Lipids and the Phospholipids Isolated from Various Membrane Fractions from Rats Fed Lipid-Supplemented Diets for 20 Weeks

Parameter	Diet					
	REF.		SAT.		UNSAT.	
	Blood lipids					
	Plasma	RBC	Plasma	RBC	Plasma	RBC
Total sat. FA	32.5	50.9	32.8	49.0	29.9	50.5
Total unsat. FA	67.5	49.1	67.2	51.0	70.1	49.5
Total ω6 unsat. FA	41.1	31.7	30.3	31.0	51.8	35.5
Total ω3 unsat. FA	4.6	5.1	4.6	5.2	1.8	3.1
Total DMA	0.5	7.6	0.5	6.8	0.2	6.1
ω6/ω3	8.9	6.2	6.6	6.0	29.0	11.4
Unsat. index	171	151	149	149	174	149
	Liver membranes					
	Mito.	Micro.	Mito.	Micro.	Mito.	Micro.
Total sat. FA	35.6	40.0	36.3	37.9	36.0	40.7
Total unsat. FA	64.4	60.0	63.7	62.1	64.0	59.3
Total ω6 unsat. FA	41.4	38.6	39.8	37.8	46.2	43.9
Total ω3 unsat. FA	8.7	7.2	9.5	8.7	7.6	6.2
Total DMA	0.6	0.7	0.4	0.6	0.4	0.4
ω6/ω3	4.7	5.4	4.2	4.3	6.0	7.0
Unsat. index	200	186	206	197	214	202
	Heart membranes					
	Mito.	Micro.	Mito.	Micro.	Mito.	Micro.
Total sat. FA	34.0	36.7	34.0	35.8	37.2	37.4
Total unsat. FA	66.0	63.3	66.0	64.2	62.8	62.6
Total ω6 unsat. FA	38.5	35.3	34.4	34.0	41.3	39.6
Total ω3 unsat. FA	16.3	13.8	18.7	17.0	12.2	10.8
Total DMA	3.4	4.6	4.4	5.2	4.0	4.3
ω6/ω3	2.4	2.6	1.8	2.0	3.4	3.7
Unsat. index	218	203	232	222	213	203
	Kidney membranes					
	Mito.	Micro.	Mito.	Micro.	Mito.	Micro.
Total sat. FA	35.0	45.8	35.1	43.7	35.6	46.0
Total unsat. FA	65.0	54.2	64.9	56.3	64.4	54.0
Total ω6 unsat. FA	46.9	39.4	45.3	38.8	51.0	39.9
Total ω3 unsat. FA	2.6	2.6	2.6	3.1	2.1	2.3
Total DMA	2.9	4.4	2.7	4.1	2.6	4.4
ω6/ω3	18.1	15.1	17.5	12.4	23.8	17.2
Unsat. index	190	170	185	173	197	168
	Brain membranes					
	Mito.	Micro.	Mito.	Micro.	Mito.	Micro.
Total sat. FA	42.9	51.2	42.9	50.3	43.3	52.0
Total unsat. FA	57.1	48.8	57.1	49.7	56.7	48.0
Total ω6 unsat. FA	14.0	12.9	13.9	12.9	14.8	13.3
Total ω3 unsat. FA	12.1	16.2	12.0	16.3	11.9	15.2
Total DMA	5.3	5.9	5.3	5.6	5.7	5.6
ω6/ω3	1.2	0.8	1.2	0.8	1.2	0.9
Unsat. index	155	165	155	167	157	160

Data are as described in Table 2. The unsaturation index is $\Sigma[(a)(b)]$ where a is the relative percentage of each unsaturated fatty acid and b is the number of double bonds for that particular fatty acid. DMA, dimethyl acetal derivative.

lipid treatments was docosahexaenoic acid (22:6ω3) (Tables 2 to 6). In comparison to the UNSAT. dietary animals, the SAT. diet increased the proportion of the ω3 series unsaturated fatty acids, with the greatest difference being observed in the phospholipids of the 2 membrane fractions from liver, heart and kidney. Differences in the proportion of the ω3 unsaturated fatty acids in brain membrane phospholipids were relatively small.

The dietary-induced changes in the type of unsaturated fatty acids present in the membrane phospholipids are best viewed in terms of changes in the ω6/ω3 ratio of unsaturated fatty acids (Table 7). In all instances this ratio was elevated on feeding the UNSAT. diet and decreased on feeding the SAT. diet relative to the value obtained on feeding the REF. diet. The extent of change in the ω6/ω3 unsaturated fatty acid ratio differed between the various tissues examined. The greatest response was observed in the fatty acids of the plasma lipids, where a 4-fold change in the value of this ratio between SAT. and UNSAT. dietary treated rats was evident. In contrast, the phospholipid fatty acids of the mitochondrial and microsomal membranes from all organs exhibited a one- to 2-fold change in the ω6/ω3 unsaturated fatty acid ratio, with the exception of the lipids from the membrane fractions from brain tissue which were virtually unchanged (Table 7). In comparison to the REF. diet, the lipid-supplemented diets were equally effective in altering the ω6/ω3 unsaturated fatty acid ratio away from the value exhibited by the REF. group for each of the respective samples examined, except those of the erythrocyte membrane. For erythrocyte membrane phospholipid fatty acids, the ω6/ω3 ratio was altered only in response to the UNSAT. diet.

DISCUSSION

This study was designed to examine the effects of dietary lipids on the fatty acid composition of microsomal and mitochondrial membrane phospholipids. In most of the tissues examined the same general findings were observed, and these can be summarized as follows. First, the proportion of saturated fatty acids in the membrane phospholipids was affected only marginally by dietary lipid manipulation. Where small changes did occur, it was apparent that increased dietary lipid saturation often caused small decreases in the proportion of saturated fatty acids, while increases in dietary polyunsaturates induced an opposite effect. Second, increasing the ratio of one class of dietary unsaturated fatty acid increased the ratio of that class of unsaturated fatty acids in the membrane lipids. For example, the diet with the highest ω6/ω3 unsaturated ratio, i.e. the UNSAT. diet, induced a change in the membrane unsaturated fatty acids toward an increased ω6/ω3 ratio. The diet characterized by the lower ω6/ω3 ratio, the SAT. diet, had the opposite effect. Third, the value for the unsaturation index of the membrane phospholipids was independent of the nature of the dietary lipid intake.

An increase in the dietary 18:2 intake increased the proportion of 6 unsaturated fatty acids in the membrane phospholipids, but did not lead to an increase in the total proportion of unsaturated fatty acids in the various membranes. In general terms, although the level of ω6 unsaturated fatty acids increased in certain membrane lipids, this was balanced by decreases in the proportion of both monounsaturated fatty acids and the ω3 series of unsaturated fatty acids, particularly docosahexaenoic acid (22:6). The effect of increasing dietary 18:2ω6 on total ω6 fatty acids was predictable with regard to the results obtained by other workers (2,4,15). The increase in ω6 fatty acids in most tissues was due mainly to increases in 20:4ω6, with the exception of samples from plasma and brain. For the brain, which is noted for its lack of response to dietary manipulation (5,15), only minimal changes in the proportion of 18:2ω6, 20:4ω6 and 22:4ω6 were observed. Of the other tissues examined, the liver elicited the greatest response, followed by the lipids from the various membranes from the heart and kidney. These results confirm the results of other workers (2,7,8,10,13-15) who have demonstrated that the composition of the membrane lipids is modulated by dietary long chain fatty acids even when the diet is adequate in all nutrients.

It is well established that several distinct pathways exist for the metabolic conversion of unsaturated fatty acids, these being denoted as the ω9 (oleate), ω6 (linoleate) and ω3 (linolenate) pathways. Important features of these pathways are, first, they are noninterconvertible and the fatty acids of one series cannot give rise to fatty acids of another series. Second, the competition for further unsaturation of 18 carbon unsaturated fatty acids is known to be in the order of ω3 > ω6 > ω9 (28). Third, to a large extent the factors controlling the level of polyunsaturated fatty acids in tissue lipids are a combination of substrate affinity and availability. The effects of substrate concentrations on the relative rates of synthesis by these 3 pathways of polyunsaturated fatty acid synthesis have been investigated both in vivo and in

vitro (29-33).

In neither of the 2 dietary manipulated groups was the level of ω3 fatty acids intentionally varied, and both lipid-supplemented diets contained a level of 18:3ω3 at about 1% (w/w) of the total fatty acid content. However, by increasing the level of the saturated fatty acid in the SAT. diet at the expense of ω6 polyunsaturated fatty acids, the ω6/ω3 ratio of the SAT. diet was lowered to 5 from about 50 in the UNSAT. diet. This reinforced the fact that in addition to the change in the proportion of saturated fatty acids, the change in the dietary lipids was a change primarily in the ω6/ω3 ratio rather than in the individual proportions of unsaturated fatty acids from each class. The effect of the SAT. diet on all tissues was to increase the proportion of ω3 unsaturated fatty acids and lower the proportion of ω6 unsaturated fatty acids. In all cases, any increase in the level of ω3 polyunsaturates due to this dietary treatment was the result of increases in the level of 22:6ω3 which presumably was synthesized from dietary 18:3ω3.

Where the supply of dietary ω3 fatty acids is nonlimiting, the role of 22:6ω3 in modulating the unsaturation index requires special comment. The compensatory variation in the levels of docosahexaenoic acid, in response to oscillating levels of ω6 fatty acids in particular, has been reported by other workers (10,34) and is confirmed by our own results. Although monounsaturates are involved in the maintenance of the unsaturation index, their contribution is relatively small. By far the greatest contributors to the unsaturation index are the ω6 and ω3 fatty acids. Since 22:6 constitutes over 90% of the total ω3 acids present in most membranes, the key regulatory role of docosahexaenoate is apparent. Obviously where dietary ω6 and ω3 fatty acids are unavailable, such as in fat-free diets, this compensatory role is taken over by monounsaturated fatty acids and the ω9 unsaturated fatty acid, 20:3.

The mechanism by which the levels of the ω6 fatty acids, principally 20:4, and the ω3 fatty acids, principally 22:6, act to maintain the unsaturation index is unclear; the process does not appear to involve a dilution of one or more fatty acids by the other. Although in vitro studies have established the effectiveness of 22:6ω3 as an inhibitor of the various desaturase enzymes (35), there is little direct evidence to support this concept from in vivo studies. The early work of Mohrhauer and Holman (29) clearly established the competitive nature of the inhibition of each of the 3 major pathways by the substrate of each fatty acid family. They concluded that the composition of the tissue polyunsaturated fatty acids is dependent upon the dietary supplies of their precursors and is regulated by competitive inhibition(s) of their metabolism. Whether any other feedback mechanism exists to allow for fine tuning of the membrane fatty acid profile and thus maintenance of the overall fluidity of the membrane is unknown. However, recent results obtained with cultured hamster kidney cells (31) indicate that while the presence of 18:2ω6 in the culture medium resulted in replacement of monoenes by 18:2ω6 at position 1 of membrane phosphatidylcholine, the presence of 20:3ω6 or 20:4ω6 resulted in these same monoenes being replaced by saturates. These results may help to explain the paradox whereby diets which are rich in 18:2ω6 and cause increased levels of 20:3ω6 and 20:4ω6 result in slightly higher levels of saturates in some membranes.

We believe our data illustrate an important homeostatic principle in which the level of membrane lipid saturation/unsaturation is buffered against transient changes which may be induced by alteration in the nature of the dietary lipid intake. Many reports (1-10,27-29, 34) confirm such a concept of lipid homeostasis. One recent study confirms our findings in the microsomes and mitochondrial membranes of the rat, although in that study the phospholipid fraction was not examined (15). Furthermore, even the work on rats raised on fat-free diets (34,36) confirms that there is a maintenance of both the level of lipid saturation and the unsaturation index to within a few per cent, despite the unavailability of both ω6 and ω3 unsaturated fatty acids in the diet. Although we did not attempt to determine the dynamic nature of these changes in membrane lipid composition, we have established in separate experiments that the changes that occur after 20 weeks do not change significantly after 12 mo of dietary lipid treatment (data not shown). It has been shown by others (37) that changes can occur in the fatty acid component of certain phospholipids of rat liver membranes within hours of dietary lipid changes.

In summary, our data clearly demonstrate that dietary fats can change the composition of membrane phospholipids in only a very restricted sense. The proportion of saturated fatty acids in membrane phospholipids appears to remain relatively constant, regardless of the dietary treatment, and may be dictated by the specificity of the *sn*-1 position of the phospholipid molecule for saturated fatty acids. Our data also suggest that there is competition for the *sn*-2 position of the phospholipid molecule

by long chain ω6 and ω3 polyunsaturated fatty acids, particularly 20:4ω6 and 22:6ω3. Whether such a homeostatic control of the unsaturation index of the membrane lipids allows for the maintenance of more than just the physical properties of the bulk lipid phase of the membrane remains to be determined. The question as to the consequences of this lipid homeostasis in terms of possible modulation of various membrane-associated enzyme activities, is also an important aspect for consideration with regard to the potential effects of dietary lipids on various physiological processes.

ACKNOWLEDGMENTS

The technical assistance of Helena McGee, Mark Neumann, Anita Holmes and Sharon Crouch is gratefully acknowledged. Mr. J. Greenfield is thanked for preparation of the diets and care of the experimental animals. This work was supported in part by a grant from the Channel 10 Children's Medical Research Foundation of South Australia.

REFERENCES

1. Holman, R.T. (1964) Fed. Proc. 23, 1062-1067.
2. De Schrijver, R., and Privett, O. (1982) J. Nutr. 112, 619-626.
3. FAO Food and Nutrition Paper No. 3 (1978).
4. Kramer, J.K.G. (1980) Lipids 15, 651-660.
5. Purvis, J.M., Clandinin, M.T., and Hacker, R.R. (1982) Comp. Biochem. Physiol. 72B, 195-199.
6. Caster, W.O., Andrews, J.W., Mohrhauer, H., and Holman, R.T. (1976) J. Nutr. 103, 1809-1816.
7. Farquhar, J.W., and Ahrens, E.H. (1963) J. Clin. Invest. 42, 675-685.
8. Mohrhauer, H., and Holman, R.T. (1963) J. Nutr. 81, 67-74.
9. Innis, S.M., and Clandinin, M.T. (1980) J. Nutr. 110, 1006-1013.
10. Paturneau-Jonas, M., Durand, G., Nouvelot, A., Masson, M., and Bouvre, J.M. (1982) Reprod. Nutr. Develop. 22, 193-200.
11. Morand, O., Chanez, C., Masson, M., Dumont, O., Flexor, M.A., Baumann, N., and Bouvre, J.M. (1982) Ann. Nutr. Metab. 26, 111-120.
12. Kurata, N., and Privett, O.S. (1980) Lipids 15, 1029-1036.
13. Kurata, N., and Privett, O.S. (1980) Lipids 15, 512-518.
14. Innis, S.M., and Clandinin, M.T. (1981) Biochem. J. 193, 155-167.
15. Tahin, Q.S., Blum, M., and Carafoli, E. (1981) Eur. J. Biochem. 121, 5-13.
16. Clandinin, M.T. (1978) J. Nutr. 108, 273-281.
17. Chapman, D. (1975) in Cell Membranes, Biochemistry, Cell Biology and Pathology (Weissman, G. and Claiborne, R., eds.) pp. 13-22, Hospital Practice Publishing Co., New York.
18. Abuirmeileh, N.M., and Elson, C.E. (1980) Lipids 15, 918-924.
19. Innis, S.M., and Clandinin, M.T. (1981) Biochem. J. 198, 167-175.
20. McMurchie, E.J., and Raison, J.K. (1979) Biochim. Biophys. Acta 554, 364-374.
21. McMurchie, E.J., Gibson, R.A., Abeywardena, M.Y., and Charnock, J.S., Biochim. Biophys. Acta 727, 163-169.
22. McMurchie, E.J., Abeywardena, M.Y., Charnock, J.S., and Gibson, R.A. (1983) Biochim. Biophys. Acta 760, 13-24.
23. Galli, C., Agradi, E., Petroni, A., and Tremoli, E. (1982) Lipids 16, 165-172.
24. Galli, C., Spagnuolo, C., Bosisio, E., Tosi, L., Folco, G.C., and Galli, G. (1978) in Advances in Prostaglandin and Thromboxane Research (Coceani, F. and Olley, P.M., eds.) Vol. 4, pp. 181-189, Raven Press, New York.
25. Olley, P.M., and Coceani, F. (1980) Am. J. Dis. Child. 134, 688-696.
26. Broekhuyse, R.M. (1974) Clin. Chim. Acta 51, 341-343.
27. Pudelkewicz, C., Seufert, J., and Holman, R.T. (1968) J. Nutr. 94, 138-146.
28. Brenner, R.R., and Peluffo, R.O. (1966) J. Biol. Chem. 241, 5213-5219.
29. Mohrhauer, H., and Holman, R.T. (1963) J. Nutr. 81, 67-74.
30. Mohrhauer, H., and Holman, R.T. (1963) J. Lipid Res. 4, 151-159.
31. Stern, W., and Pullman, M.E. (1983) J. Biol. Chem. 258, 11128-11135.
32. Brenner, R.R. (1974) Molec. Cell. Biochem. 3, 41-52.
33. Brenner, R.R., and Peluffo, R.O. (1969) Biochim. Biophys. Acta 176, 471-479.
34. Iritani, N., and Fujikawa, S. (1982) J. Nutr. Sci. Vitaminol. 28, 621-629.
35. Sprecher, H. (1977) in Polyunsaturated Fatty Acids (Kunau, W.H. and Holman, R.T., eds.) pp. 1-18, Am. Oil Chem. Soc., Champaign, IL.
36. Odutuga, A.A. (1981) Int. J. Biochem. 13, 1035-1038.
37. Flick, P.K. (1981) Nutr. Rep. Int. 24, 667-674.

[Received June 26, 1984]