Influence of dietary fat on the concentration of long-chain unsaturated fatty acid families in rat tissues

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Abstract The relative concentration of long-chain unsaturated fatty acids (chain length C_{20} and greater) of the (n − 6), (n − 7), and (n − 9) families in the cholesteryl esters and phospholipids of rat adrenals, liver, heart, and plasma lipoproteins was measured after the feeding of hydrogenated fat, milk fat, beef tallow, corn oil, and fat-free diets. Barely optimal levels of dietary linoleate were found to result in the same order of concentration of the (n − 6) series of fatty acids as was obtained with excess dietary linoleate. The linoleate-poor or deficient diets—hydrogenated fat and fat-free diets—gave almost identical levels and trends with respect to the concentration of the (n − 9) and (n − 7) series of acids. With these two diets, the concentrations of the total (n − 9) long-chain acids were several times greater than the amounts obtained by feeding either the linoleate-rich diet or the barely linoleate-adequate diets. It is concluded from the results that the linoleate-deficient nature of the hydrogenated fat, rather than its high content of *trans* acids, would explain the high tendency of this fat to induce the accumulation of long-chain (n − 9) fatty acids in the cholesteryl esters and phospholipids of the tissues studied.

Supplementary key words phospholipids · cholesteryl esters · plasma lipoproteins · *trans* fatty acids

We have previously reported the accumulation of unusual eicosadienoic acids in the liver phospholipids, and docosadienoic acids in the adrenal cholesteryl esters and phospholipids, in animals fed diets containing 20% hydrogenated soybean fat for 20 wk (1, 2). The probable role of dietary elaidate in the accumulation of these acids and the possibility that these acids might have originated from some unusual elongation–desaturation reactions involving the (n − 9) and (n − 7) parent fatty acids[1] were commented on. It could be ascertained from our data (2) that concomitant with the accumulation of these acids there were significantly greater levels of long-chain polyenoic acids of the (n − 9) family in the tissue lipids of these animals, especially in the adrenal cholesteryl esters. It was not clear, however, to what extent, if any, the *trans* nature of this dietary fat induced the observed trends.

We have now used four different types of dietary fats, hydrogenated soybean fat (HF), milk fat (MF), beef tallow (BT), and corn oil (CO), as well as a fat-free diet, to study further the relationships between the nature of dietary fat and the relative concentrations of long-chain unsaturated fatty acids of the (n − 6), (n − 7), and (n − 9) families in the lipids of rat adrenals, liver, heart, and plasma lipoproteins.

MATERIALS AND METHODS

Male Holtzman strain rats, 21 days old, were divided into five groups of eight animals each. Groups 1–4 were fed different fat diets at 20% (by weight) level for 15–20 wk; group 5 received a fat-free diet (Nutritional Biochemicals Corp., Cleveland, Ohio) which had earlier been shown to contain insignificant traces of fat (1). We had reason to believe (from our unpublished data) that 15 wk would be enough time to more than allow for an equilibrium stage in the fatty acid composition of the

Abbreviations: HF, partially hydrogenated soybean fat; MF, milk fat; BT, beef tallow; CO, corn oil; FFD, fat-free diet; CE, cholesteryl ester; PL, phospholipid; VLDL, very low density lipoprotein; LDL, low density lipoprotein; HDL, high density lipoprotein; GLC, gas–liquid chromatography; TLC, thin-layer chromatography.

[1] Position of the first double bond from the methyl end of the chain is indicated.

TABLE 1. Fatty acid composition of dietary fats[a]

Fatty Acids[b]	Corn Oil	Milk Fat	Beef Tallow	Hydrogenated Fat
10:0		2.1		0.2
12:0		4.6	1.1	trace
14:0		8.3	7.4	0.2
14:1		4.4	1.0	trace
16:0	11.5	23.3	24.1	8.1
16:1	0.5	3.7	4.4	0.2
16:2			1.8	
17:0		0.2	1.7	trace
18:0	3.2	18.4	17.0	13.2
18:1	25.1	30.4	39.6	76.5[c]
18:2	58.5	3.1	2.0	1.0
18:3	1.3			0.4
20:0		1.4		
20:1				0.2
20:2				trace

[a] Wt % of total fatty acids. Each value is mean of two individual determinations.

[b] Number of carbon atoms: number of double bonds.

[c] Average *trans* fatty acid content (elaidate) from IR and capillary GLC analyses is 48.4% of total fatty acids. The *trans* unsaturation was found from previous degradative studies (2) to be essentially at the 9,10 position.

cholesteryl esters and phospholipids of the tissues studied here, while 20 wk would represent a period well past the equilibrium stage. The fats used in the diets were as follows: corn oil (Mazola, Best Foods, Englewood Cliffs, N.J.), milk fat (Land O'Lakes Creameries, Inc., Minneapolis, Minn.), beef tallow,[2] and partially hydrogenated soybean fat (kindly prepared and supplied by Humko Co., Champaign, Ill.). These fats were added at the expense of carbohydrate in the fat-free stock diet.[3] The major fatty acids of these dietary fats, as determined by GLC of the corresponding methyl esters, are presented in Table 1. Diets (routinely stored at 0–4°C) and water were provided ad lib. over the entire feeding period and the rats were inspected regularly. Growth and general well-being were satisfactory in all cases.

After the animals had been fasted for 12 hr, blood was taken from the animals in each group, under light ether anesthesia, via heart puncture using a heparinized syringe. The blood from each group was pooled and stored at 0°C until processed (usually within 1 hr). Livers, hearts, and adrenals were then quickly removed and placed temporarily in 0.9% saline at 0°C. Extraction of total lipids with chloroform–methanol 2:1 (v/v) was carried out on four individual livers, four pairs of hearts, and two sets of pooled adrenals (four animals per set).

The blood samples were centrifuged in 15-ml centrifuge tubes at 4,000 *g* for 30 min to sediment the red blood cells. The supernatant plasma was pipetted off using glass disposable pipettes and was pooled according to diet groups. Plasma lipoproteins—chylomicrons plus VLDL, LDL, and HDL—were separated from the pooled plasma of each group essentially by the serial polyanion precipitation procedure of Burnstein, Scholnick, and Morfin (3). Sodium dextran sulfate 500 (Pharmacia Fine Chemicals Inc., Piscataway, N.J.) was used as the precipitant. The only modification of the procedure was the use of Mg^{2+} ions (0.1 M) and 0.01% of the precipitant to selectively precipitate the chylomicron + VLDL fraction as a first step. After washing each lipoprotein preparation thoroughly (3), the lipids were extracted in a hand glass homogenizer with 5 vol of chloroform–methanol 2:1 (v/v), using the general extraction techniques described previously (1).

Total lipid extracts were made up to known volumes in 5- or 10-ml volumetric flasks, and aliquots from each sample were taken for determination of the total weight (2). Fractionation of known aliquots from the total lipids into the major lipid classes by TLC, recovery from plates, and transesterification of each fraction for GLC were performed by standard procedures described previously (1, 2).

Gas–liquid chromatographic analysis (Barber-Colman chromatograph, model 5000) was performed on a 1.8-m U-shaped glass column, 3.2 mm I.D., containing ethylene glycol succinate polyester, 15% on Chromosorb W AW, 80–100 mesh. The details of the analysis and the technique for peak identification and quantification have been described before (1, 2). The structures of the unusual fatty acids encountered in these studies had earlier been studied by degradative procedures (2).

The methyl esters prepared from the CE and PL of each of the four liver and heart total lipid samples, each of the two adrenal total lipid samples, or the total lipids of each plasma lipoprotein type (VLDL, LDL, and HDL) were analyzed in duplicate. For each duplicate set of analyses, the total concentration of the unsaturated fatty acids (as methyl esters) of the same family and of chain length greater than C_{18} was obtained by summation, and the mean was used as the value for the sample. Arithmetic means were then calculated from the number of samples analyzed for each tissue, and where applicable, standard deviations were calculated (liver and heart samples). In view of the small number of samples involved in the case of the other tissues, no calculations for the standard error of the mean were made.

[2] Prepared in bulk (4.0 kg) by rendering 8.2 kg of locally purchased fresh beef back fat and filtering off the tissue debris through cheesecloth.

[3] Prepared according to order by Nutritional Biochemicals. Composition (wt %): sucrose, 64.5; casein, 29.5; cellulose, 2.0; salt mix, 4.0; plus vitamin B_{12}, 0.8 mg, and all the vitamin supplements listed in fat-free diet of Ref. 1.

TABLE 2. Content of total lipids, cholesteryl esters, and phospholipids in the different tissues

	15-wk Feeding				
	CO	MF	BT	HF	FFD
Liver[a]					
Total lipids[b]	45.0 ± 1.8	46.1 ± 1.4	48.0 ± 1.6	46.0 ± 1.7	35.2 ± 0.7
Cholesteryl esters	4.7 ± 0.5	4.7 ± 0.6	7.5 ± 0.6	5.3 ± 0.2	5.1 ± 0.5
Phospholipids	33.0 ± 0.9	31.0 ± 0.8	31.8 ± 1.0	35.0 ± 1.2	25.8 ± 1.1
Heart[a]					
Total lipids[b]	48.7 ± 2.1	40.6 ± 0.5	42.0 ± 1.6	62.6 ± 1.8	58.8 ± 0.8
Cholesteryl esters	4.0 ± 0.6	4.0 ± 0.7	2.5 ± 0.2	5.3 ± 0.5	2.0 ± 0.1
Phospholipids	33.0 ± 0.9	26.0 ± 0.7	22.0 ± 0.6	18.0 ± 0.6	22.0 ± 1.1
Adrenals[c]					
Total lipids[b]	13.0	13.3	14.0	15.0	14.5
Cholesteryl esters	9.4	15.6	9.4	13.1	8.3
Phospholipids	4.0	4.2	4.8	4.5	1.5
VLDL[d]					
Total lipids[b]	70.5	65.8	87.1	48.0	85.1
Cholesteryl esters	10.7	11.3	12.5	11.0	36.7
Phospholipids	13.3	12.5	17.9	16.0	42.0
LDL[d]					
Total lipids[b]	50.5	61.2	51.5	88.2	67.2
Cholesteryl esters	36.7	35.0	23.2	28.7	20.5
Phospholipids	36.0	38.8	34.0	37.5	35.5
HDL[d]					
Total lipids[b]	42.8	40.2	65.0	41.3	39.5
Cholesteryl esters	20.3	19.0	25.4	13.0	15.0
Phospholipids	22.3	16.3	22.9	9.7	16.1
			20-wk Feeding		
Liver[a]					
Total lipids[b]	58.1 ± 2.2	48.7 ± 2.0	52.3 ± 2.1	45.8 ± 1.1	37.2 ± 1.0
Cholesteryl esters	3.9 ± 0.4	2.9 ± 0.1	3.5 ± 0.3	2.8 ± 0.1	2.5 ± 0.2
Phospholipids	30.8 ± 1.4	27.5 ± 1.0	30.7 ± 0.9	31.5 ± 0.8	25.2 ± 0.7
Heart[a]					
Total lipids[b]	52.0 ± 2.1	42.1 ± 1.6	24.0 ± 1.3	30.9 ± 1.1	32.6 ± 1.2
Cholesteryl esters	1.5 ± 0.1	3.3 ± 0.2	2.6 ± 0.2	2.9 ± 0.2	2.7 ± 0.1
Phospholipids	30.8 ± 1.4	18.9 ± 1.1	18.4 ± 0.6	23.1 ± 1.3	20.7 ± 1.4
Adrenals[c]					
Total lipids[b]	13.3	13.6	13.8	36.0	15.7
Cholesteryl esters	20.0	19.5	20.2	25.5	18.7
Phospholipids	6.5	6.2	5.2	5.0	3.6
VLDL[d]					
Total lipids[b]	220.5	150.0	295.0	220.5	145.1
Cholesteryl esters	23.0	27.3	17.7	19.3	18.3
Phospholipids	39.7	31.8	17.9	24.4	40.8
LDL[d]					
Total lipids[b]	151.2	131.2	239.1	151.2	121.2
Cholesteryl esters	35.8	37.8	18.7	17.5	18.8
Phospholipids	40.0	38.2	33.9	33.7	38.2
HDL[d]					
Total lipids[b]	113.2	61.8	174.1	113.2	66.3
Cholesteryl esters	25.1	20.5	30.3	15.4	17.8
Phospholipids	29.3	17.5	28.3	20.3	20.0

[a] Results for liver and heart are expressed as mg/g wet tissue and are means ± SD for four animals.

[b] Includes primarily triglycerides, but also free cholesterol and free fatty acids. In the interest of simplicity and clarity, the values for the individual classes of lipids are not included in the table.

[c] Results are expressed as percentage of wet weight of tissue, and each is the mean of two samples (two pairs of adrenals per sample), with duplicate determinations on each sample.

[d] Values are expressed as mg/100 ml of plasma, and each represents the mean for triplicate determinations on a plasma sample pooled from four rats.

RESULTS

Tissue lipid concentrations

The tissue concentrations of the lipids relevant to this study are presented in Table 2. The dietary fats did not give any consistent trends with respect to the concentrations of the total lipids and the lipid classes, thus precluding any general statements or conclusions about their effects. However, the high concentrations of phospholipids in all the tissues (except adrenals) and of cholesteryl esters (all tissues except liver and heart) are noteworthy. This is particularly true of the adrenals,

especially after 20 wk, when, on the average, adrenal cholesteryl esters accounted for about 20% of the wet weight of the tissue.

(n − 9) and (n − 6) acids of cholesteryl esters

The fatty acids of chain length greater than C_{18} as well as the total concentrations of (n − 9) and (n − 6) unsaturated acids of cholesteryl esters of liver, heart, adrenals, and the three major plasma lipoprotein classes as affected by feeding the different fats for 15 and 20 wk are presented in Table 3. This partial fatty acid composition is given in the interest of completeness and clarity. Attention should be directed more specifically to the total concentrations of the two families of acids presented in the last two columns of the table. In the liver CE, with the exception of a moderately high level of total (n − 9) acids induced by feeding beef tallow for 15 wk (18.5%), the concentrations obtained for this fatty acid family were low (< 10%), irrespective of the diet and duration of feeding. With respect to the (n − 6) series of this lipid class, only the corn oil diet (after 20 wk of feeding) induced a high total concentration (33%).

Regarding the heart CE, the data show that only HF and fat-free diets led to high levels (35%) of the (n − 9) acids (after 15 wk of feeding). After 20 wk, the corresponding values were about 20% and 18%, respectively, with these diets; the values in all other cases still remained low. Only the MF diet after 20 wk induced a high total concentration of the (n − 6) fatty acids (38%) in heart CE fraction; an intermediate value of 16% was observed with the fat-free diet after 20 wk of feeding. In the latter case, the summation included the (n − 7) acids, which are not readily separated from the (n − 6) acids under our GLC operating conditions.

A striking accumulation of the (n − 9) series of acids was noted in the adrenal CE, especially when HF or fat-free diet was fed. For instance, HF and fat-free diets induced total concentrations of (n − 9) acids amounting to 44% and 42%, respectively, after 15 wk of feeding. At this time period, the intermediate values of 17% and 19%, respectively, were observed with CO and BT diets. After 20 wk, the value was still 42% with the fat-free diet, while with the HF diet the concentration was now approximately 49%. With respect to the (n − 6) acids, only those diets which were adequate in linoleate induced elevated concentrations of these acids in the adrenal cholesteryl esters. For instance, with CO and MF diets (after 20 wk), the values were, respectively, 35% and 32%; with the BT diet, the value was 20% after 15 wk of feeding, dropping to an intermediate value of 16% after 20 wk.

Regarding the lipoprotein CE, after 15 wk of feeding the total concentrations of the (n − 9) family of acids in each lipoprotein class were generally low, with the exception of the intermediate values of 18%, 15%, and 12%, respectively, seen with BT, HF, and fat-free diets for the HDL cholesteryl esters. However, for LDL CE, when HF and fat-free diets were fed for 20 wk the values were strikingly high, amounting, respectively, to 43% and 46%. In the VLDL CE, only MF and fat-free diets (20 wk) resulted in intermediate concentrations, amounting to 23% and 17%, respectively.

The total concentrations of the (n − 6) acids in the CE of the various lipoprotein classes after 15 wk of feeding were generally low (10% and under), except for the adrenals when CO was fed (20%). Some strikingly high values were, however, obtained after 20 wk of feeding. For instance, MF diet resulted in the value of 39% for total (n − 6) acids of LDL CE. With CO diet, the values were 42% and 38%, respectively, for VLDL CE and HDL CE, while the LDL CE contained a moderately high value of total (n − 6) acids (25%). Also moderately high were the values obtained in the case of total (n − 6) acids of VLDL CE (fat-free diet) and LDL CE (HF diet), amounting to 27% and 22%, respectively. It must be pointed out, however, that in these last two cases the (n − 7) acids were, for the reason given earlier, included in the summation.

(n − 9) and (n − 6) acids of phospholipids

The fatty acids of chain length greater than C_{18} as well as the total concentrations of (n − 9) and (n − 6) unsaturated acids of phospholipids of liver, heart, adrenals, and the three major plasma lipoproteins as affected by feeding the different fats for 15 and 20 wk are presented in Table 4. Again looking at the last two columns of the table, it is seen that only HF and fat-free diets induced moderately elevated concentrations of total (n − 9) acids in liver phospholipids, amounting, after 15 wk, to 27% and 25%, respectively, for the two diets. After 20 wk, the corresponding values were about 20% and 15%, respectively. Regarding the concentration of total (n − 6) acids, only CO diet resulted in the moderately high value of 25% in the liver phospholipids after 20 wk of feeding.

In heart phospholipids, only the fat-free diet induced a moderately high concentration (21%) of total (n − 9) acids after 15 wk; after 20 wk the corresponding value was 22%. In the case of the total concentration of the (n − 6) acids after 15 wk, only CO diet led to a high value (30%), while after 20 wk the values were about 23%, 32%, and 31%, respectively, with the CO, MF, and BT diets. The values with the HF and fat-free diets were low (18% and 13%, respectively).

As was the case with the adrenal cholesteryl esters, HF and fat-free diets after 15 wk resulted in the highest level of the (n − 9) series of acids in the adrenal phospholipids, amounting to 42% and 47%, respectively.

TABLE 3. Dietary fat and fatty acid composition of cholesteryl esters from various tissues:

	20:1 (n − 9)	20:1 (n − 6)	20:2 (n − 9)	20:2 (n − 6)	20:2 (n − 6 + n − 7)	20:3 (n − 9)	20:3 (n − 6)
Liver							
Cholesteryl esters, 15 wk							
Corn oil				0.6 ± 0.1		1.4 ± 0.2	
Milk fat				6.4 ± 0.1			
Beef tallow	12.0 ± 0.3					1.0 ± 0.1	
Hydrogenated fat		2.4 ± 0.2					
Fat-free					2.9 ± 0.2	3.9 ± 0.2	
Cholesteryl esters, 20 wk							
Corn oil						5.3 ± 0.1	
Milk fat						1.3 ± 0.2	
Beef tallow					0.5 ± 0.2	1.9 ± 0.1	
Hydrogenated fat						3.4 ± 0.1	
Fat-free					1.0 ± 0.1	1.0 ± 0.2	
Heart							
Cholesteryl esters, 15 wk							
Corn oil					2.9 ± 0.2	3.0 ± 0.3	
Milk fat						10.7 ± 0.1	
Beef tallow				4.0 ± 0.5		13.0 ± 0.8	
Hydrogenated fat					2.1 ± 0.6	21.2 ± 1.0	
Fat-free						20.3 ± 1.1	
Cholesteryl esters, 20 wk							
Corn oil			4.3 ± 0.2	0.8 ± 0.1			
Milk fat	1.2 ± 0.6					6.1 ± 0.3	
Beef tallow							
Hydrogenated fat	2.6 ± 0.3					8.4 ± 0.6	
Fat-free						15.1 ± 0.3	
Adrenals							
Cholesteryl esters, 15 wk							
Corn oil	5.0		2.4			3.3	1.5
Milk fat	7.6						
Beef tallow	4.0		4.3			10.7	2.2
Hydrogenated fat	5.0		4.1			15.3	
Fat-free	2.4		2.1			18.3	
Cholesteryl esters, 20 wk							
Corn oil	1.7			1.2		3.9	1.1
Milk fat	1.8		0.3			2.8	2.1
Beef tallow		1.5				3.7	
Hydrogenated fat	9.9		0.2		2.1	16.7	
Fat-free	14.1		3.5			8.4	
VLDL							
Cholesteryl esters, 15 wk							
Corn oil	0.6			trace		4.0	2.0
Milk fat	1.0			0.8		3.8	
Beef tallow	0.8	1.0		1.0		4.2	
Hydrogenated fat					0.8		
Fat-free		0.8			1.8		1.0
Cholesteryl esters, 20 wk							
Corn oil		trace		1.7			
Milk fat	3.5					15.7	
Beef tallow				3.5			
Hydrogenated fat			1.7		1.4	2.3	
Fat-free	7.1					8.3	
LDL							
Cholesteryl esters, 15 wk							
Corn oil	4.0	2.0	1.5	1.8		2.4	
Milk fat	3.8	0.5	1.2	1.4		1.5	
Beef tallow	3.0	1.8	2.0	2.0		2.0	
Hydrogenated fat		0.5					
Fat-free		0.3					
Cholesteryl esters, 20 wk							
Corn oil							3.3
Milk fat	0.7	0.4		trace		2.3	
Beef tallow				0.7		6.3	
Hydrogenated fat	16.0					21.9	
Fat-free	15.8			trace		27.0	
HDL							
Cholesteryl esters, 15 wk							
Corn oil	1.2						
Milk fat	0.5					4.0	1.0
Beef tallow	3.0			1.3		9.0	
Hydrogenated fat	0.3		14.0	0.8			
Fat-free			3.1	1.4		8.1	
Cholesteryl esters, 20 wk							
Corn oil	0.7					1.1	3.0
Milk fat		8.1					1.7
Beef tallow	0.3			6.5		0.9	0.4
Hydrogenated fat			1.2			7.3	
Fat-free	0.8				1.5	9.8	

[a] Values, expressed as wt % of total fatty acids of the lipid class, are arithmetical means as described in text. Standard deviations are included for data on liver and heart lipids.

percentage of (n − 9) and (n − 6) acids with chain length greater than C_{18}[a]

20:4 (n − 6)	20:4 (n − 6 + n − 7)	22:1 (n − 9)	22:1 (n − 6)	22:2 (n − 9)	22:2 (n − 6)	22:3 (n − 9)	22:3 (n − 6)	22:4 (n − 6)	Total (n − 9)[b]	Total (n − 6)[c]
2.5 ± 0.5	0.3 ± 0.1				3.5 ± 0.6				1.4	6.9
2.8 ± 0.2				0.5 ± 0.2	1.0 ± 0.2				0.5	10.2
2.9 ± 0.4				5.5 ± 0.6					18.5	2.9
	2.4 ± 0.5			3.8 ± 0.1					3.8	4.8
	1.3 ± 0.7								3.9	4.2
5.8 ± 0.4					27.3 ± 0.7				5.3	33.1
5.1 ± 0.5				2.5 ± 0.1	1.0 ± 0.3	1.0 ± 0.3			4.8	6.1
	3.4 ± 0.2		2.2 ± 0.4						1.9	6.1
	1.3 ± 0.6		1.5 ± 0.2	2.4 ± 0.4		4.0 ± 0.5			9.8	2.8
	1.6 ± 0.8								1.0	2.6
4.4 ± 0.1									2.9	7.4
2.3 ± 0.2				3.0 ± 0.2		1.0 ± 0.3			14.7	2.3
8.4 ± 0.5									13.0	12.4
				13.5 ± 0.5					34.7	2.1
6.2 ± 0.1				14.3 ± 0.7					34.6	6.2
7.9 ± 0.3						2.6 ± 0.2			6.9	8.7
9.4 ± 0.1			29.1 ± 0.4	2.1 ± 0.1					9.4	38.5
4.9 ± 0.4				3.1 ± 0.3		1.2 ± 0.6			4.3	4.9
	5.5 ± 0.7			8.2 ± 0.1		0.6 ± 0.3	1.0 ± 0.4	1.0 ± 0.3	19.8	7.5
				3.0 ± 0.1		16.5 ± 0.2			18.1	16.5
7.0		3.6		2.7					17.0	8.5
7.8		1.4							9.0	7.8
10.9					2.3			5.2	19.0	20.6
	7.7	3.0		6.6		10.0			44.0	7.7
	5.0	1.2		7.2		10.8			42.0	5.0
16.4			1.8		2.2		2.0	10.8	5.6	35.5
14.2				1.6		6.6	3.3	12.2	13.1	31.8
6.5				7.0		2.6	3.7	4.5	13.3	16.2
	2.9	6.6		4.0		11.5	1.1	2.9	48.9	11.1
	1.3			3.6		12.4			42.0	1.3
8.2						0.2			4.8	10.2
5.2			0.2			0.4			5.2	6.2
5.2			0.3			2.5			7.5	7.5
	4.0									4.8
	8.0		trace		1.2					12.8
35.0								5.9		42.6
7.0			1.9	3.7				3.2	22.9	12.1
10.4			0.5				2.1	1.9		18.4
	5.3			1.8				1.1	5.8	7.8
	25.2	1.2					2.3		16.6	27.5
4.1									7.9	7.9
4.6		0.4	0.5						6.9	7.0
3.6		0.5							7.5	7.4
	4.5		0.5		0.6					6.1
	3.5		0.4							4.2
22.3										25.6
37.6							0.8		3.0	38.8
40.0				0.5	4.1				6.8	44.8
	21.0	1.0	trace	1.2		3.0		1.2	43.1	22.2
	5.0					3.3			46.1	5.0
27.6			1.4						1.2	29.0
			0.2						4.5	1.2
				1.0		4.8			17.8	1.3
	2.0			0.5				1.1	14.8	3.9
	1.6		1.0			1.0			12.2	4.0
18.6			3.1		2.0		2.8	8.8	1.8	38.3
3.0					1.0					13.8
37.7			2.0	0.3				2.3	1.5	48.9
	10.3			3.5		6.5		2.5	18.5	12.8
		1.1	1.9	2.0		0.9		2.0	14.6	5.4

[b] Includes, in the case of HF diet, the unusual C_{20} or C_{22} dienes described previously (1, 2).
[c] Includes fatty acids of the (n − 7) series, especially in the case of HF and fat-free diets.

TABLE 4. Dietary fat and fatty acid composition of phospholipids from various tissues:

	20:1 (n − 9)	20:1 (n − 6)	20:2 (n − 9)	20:2 (n − 6)	20:2 (n − 6 + n − 7)	20:3 (n − 9)	20:3 (n − 6)
Liver							
Phospholipids, 15 wk							
Corn oil		0.9 ± 0.4		0.9 ± 0.3			0.7 ± 0.1
Milk fat	4.2 ± 0.2						2.4 ± 0.2
Beef tallow				0.2 ± 0.1			6.8 ± 0.4
Hydrogenated fat		0.2 ± 0.1			2.4	27.6	0.1 ± 0.0
Fat-free	0.3 ± 0.2				0.4	17.0	
Phospholipids, 20 wk							
Corn oil	trace						trace
Milk fat						1.1 ± 0.2	1.3 ± 0.2
Beef tallow						1.0 ± 0.1	0.5 ± 0.2
Hydrogenated fat						20.6 ± 0.1	2.7 ± 0.6
Fat-free						15.5 ± 0.6	4.0 ± 0.7
Heart							
Phospholipids, 15 wk							
Corn oil	trace					trace	
Milk fat						trace	0.3 ± 0.2
Beef tallow				1.0 ± 0.3		1.5 ± 0.2	
Hydrogenated fat						5.2 ± 0.6	
Fat-free			1.0 ± 0.1			18.2 ± 0.8	
Phospholipids, 20 wk							
Corn oil							4.6 ± 0.1
Milk fat							1.5 ± 0.7
Beef tallow	1.2 ± 0.2			0.9 ± 0.3		2.7 ± 0.1	1.4 ± 0.3
Hydrogenated fat			0.7 ± 0.2		1.4 ± 0.3	1.5 ± 0.3	1.4 ± 0.4
Fat-free					1.3 ± 0.3	22.4 ± 0.4	
Adrenals							
Phospholipids, 15 wk							
Corn oil				2.0		0.8	2.0
Milk fat	0.6					5.0	
Beef tallow	7.8			1.2			
Hydrogenated fat	10.4		6.5		5.8	18.6	
Fat-free	13.2		7.2			22.5	
Phospholipids, 20 wk							
Corn oil	2.5			3.0		1.5	
Milk fat	1.6			2.5		11.0	
Beef tallow	0.7			1.0		8.0	
Hydrogenated fat					5.0	13.0	
Fat-free	1.2				4.4	21.5	
VLDL							
Phospholipids, 15 wk							
Corn oil	1.3		2.5	1.0		1.7	
Milk fat		trace		0.5		5.0	
Beef tallow		trace		0.5		1.0	
Hydrogenated fat	1.0	0.4	2.9	0.3		2.6	
Fat-free	1.5	0.6				4.0	
Phospholipids, 20 wk							
Corn oil	2.3		3.0	1.5		4.0	4.4
Milk fat	0.3		1.5			2.6	
Beef tallow			1.3	1.0		2.8	1.2
Hydrogenated fat	trace		1.0		1.7	6.5	
Fat-free	0.5	0.5	1.7	0.5	2.5	4.9	
LDL							
Phospholipids, 15 wk							
Corn oil			1.0	0.2		6.0	
Milk fat				trace		2.0	0.6
Beef tallow		trace		0.6		3.6	
Hydrogenated fat			2.0		3.6	5.9	
Fat-free			0.6	0.4		7.6	
Phospholipids, 20 wk							
Corn oil	3.2					5.5	
Milk fat		3.0				4.3	
Beef tallow	0.8	1.3	5.3			11.7	
Hydrogenated fat		0.9	3.5		3.0	15.7	
Fat-free	0.9		2.1		1.5	15.2	
HDL							
Phospholipids, 15 wk							
Corn oil	2.8		2.5			4.7	
Milk fat	2.5		1.2			5.3	0.8
Beef tallow			0.4			5.0	0.4
Hydrogenated fat		0.2	0.6		1.6	7.5	
Fat-free		0.2	0.3		1.0	7.5	0.3
Phospholipids, 20 wk							
Corn oil	2.5		2.0			1.0	1.8
Milk fat						13.7	
Beef tallow						5.7	2.0
Hydrogenated fat					3.5	5.8	
Fat-free	2.5		1.5		3.3	3.4	

[a] Values, expressed as wt % of total fatty acids of the lipid class, are arithmetical means as described in text. Standard deviations are included for data on liver and heart lipids.

percentage of (n − 9) and (n − 6) acids with chain length greater than C_{18}[a]

20:4 (n − 6)	20:4 (n − 6 + n − 7)	22:1 (n − 9)	22:1 (n − 6)	22:2 (n − 9)	22:2 (n − 6)	22:3 (n − 9)	22:3 (n − 6)	22:4 (n − 6)	Total (n − 9)[b]	Total (n − 6)[c]
6.1 ± 0.4				0.4 ± 0.2	0.2 ± 0.1				0.4	8.8
6.6 ± 0.4				12.1 ± 0.1			0.2 ± 0.1		16.3	9.2
1.7 ± 0.1			2.5 ± 0.1				0.8 ± 0.2			12.0
	0.5 ± 0.3								27.6	3.2
		5.2 ± 0.2				2.2 ± 0.2			24.7	0.4
24.4 ± 0.5								0.6 ± 0.3		25.0
17.7 ± 1.1				0.9 ± 0.3					2.0	19.0
6.0 ± 1.0									1.0	6.5
	2.7 ± 0.3		0.5 ± 0.1						20.6	5.9
	3.4 ± 0.5								15.5	7.4
27.6 ± 0.4			2.7 ± 0.3							30.3
2.8 ± 0.2										3.1
4.2 ± 0.1									1.5	5.2
	4.6 ± 0.4								5.2	4.6
		1.5 ± 0.5							20.7	
11.6			1.0 ± 0.2				2.6 ± 0.3	3.8 ± 0.6		23.6
18.5								12.0 ± 0.8		32.0
23.1		1.0 ± 0.2					1.3 ± 0.2	4.9 ± 0.2	4.9	31.6
	15.0 ± 0.1			0.8 ± 0.1		0.3 ± 0.2			3.3	17.8
	11.5 ± 0.2								22.4	12.8
18.7					4.7			8.6	0.8	36.0
4.0									5.6	4.0
14.1					4.3		0.7		7.8	20.3
	4.5					6.3			41.8	10.3
	2.0					4.3			47.2	2.0
8.8								7.3	4.0	19.1
10.2					1.2		2.5	7.8	12.6	25.1
9.8							0.9	2.8	8.7	14.5
	25.5			1.0		10.0	1.9		24.0	32.4
	13.8		1.0				2.0	3.0	22.7	24.2
10.2								1.0	5.5	12.2
4.5								1.0	5.0	6.0
8.5								1.1	1.0	10.1
	3.0		1.0					0.5	6.5	5.2
	3.5		0.5			0.7	0.6	0.6	6.2	5.8
30.8		1.4	0.7		2.0		2.8	4.8	10.7	47.0
12.6			trace						4.4	12.6
25.9					3.1			3.7	4.1	34.9
	9.9								7.5	11.6
	10.0								7.1	13.5
5.8			1.0						7.0	7.0
3.0									2.0	3.6
6.0									3.6	6.6
	11.8			2.5	0.6	1.5	1.5		11.9	16.5
	11.0			1.8	0.5	1.0	1.1		10.5	13.5
8.0		1.4		2.0	3.0			3.1	12.1	14.1
20.6					1.8				4.3	25.4
8.7		1.0		2.0	1.3				20.8	11.3
	8.1	1.0		1.8		3.7	1.6		25.7	13.6
	2.5	1.3		2.0		3.4			24.9	4.0
6.1						1.0		1.0	11.0	7.1
9.8				2.5	1.0			2.0	11.5	13.6
2.0									5.4	2.4
	5.2								8.1	7.0
	4.5		0.4						7.8	6.4
15.8		1.0		1.1		1.7		8.1	9.3	25.7
8.9			2.7					1.6	13.7	13.2
16.0		trace	2.2		1.8			3.2	5.7	25.2
	10.7			3.5		2.7			12.0	14.2
	6.4		3.6	1.6		2.4			11.4	13.3

[b] Includes, in the case of HF diet, the unusual C_{20} or C_{22} dienes described previously (1, 2).
[c] Includes fatty acids of the (n − 7) series, especially in the case of HF and fat-free diets.

After 20 wk of feeding, the corresponding values were 24% and 23%, respectively. Corn oil diet induced the highest concentration of the total (n − 6) acids in adrenal phospholipids (36% after 15 wk), while BT diet led to a value of 20%. After 20 wk the values were as follows: 19% and 25%, respectively, with the CO and MF diets; 32% and 24%, respectively, with the HF and fat-free diets ([n − 7] acids included in the last two cases).

In the VLDL phospholipids, the concentrations of total (n − 9) acids were generally low, irrespective of dietary fat and duration of feeding. The same observation holds for the total (n − 6) concentrations, except that the CO and BT diets (after 20 wk) resulted in values of 47% and 35%, respectively.

After 15 wk of feeding, both the total (n − 9) and the total (n − 6) acid concentrations in the LDL phospholipids were generally low, irrespective of the dietary fat (upper limits: about 12% for the [n − 9] series; 16% for the [n − 6] acids). However, after 20 wk the total (n − 9) acids attained the concentrations of 21%, 26%, and 25% with the BT, HF, and fat-free diets, respectively. With respect to the (n − 6) acids after 20 wk, only the MF diet led to a moderately high concentration in the LDL phospholipids (25%); with CO, BT, and HF diets the values were intermediate at between 11% and 14%.

The data in Table 4 also show that after 15 wk of feeding, none of the diets induced even moderately high concentrations of the (n − 9) acids in the HDL phospholipids. After 20 wk the trend in the levels of the (n − 9) acids remained the same. Regarding the levels of the (n − 6) acids after 15 wk of feeding, the concentrations were low regardless of the dietary fat; however, after 20 wk CO and BT diets induced moderately high concentrations of these acids in the HDL phospholipids (26% and 25%, respectively), while intermediate values (13–14%) were obtained with the other diets.

Some rather unexpected general aspects of these data deserve mention. First, instances of very large increases in the values obtained at 15 and 20 wk are evident in Tables 3 and 4. For example, when rats were fed the CO diet, adrenal CE increased from 8.5% to 35.5% with respect to the total (n − 6) acids (Table 3). Under the same conditions, the increase was from 6.9% to 33.1% in the liver cholesteryl esters. Again, in animals fed HF and fat-free diets, LDL CE contained negligible amounts of total (n − 9) long-chain (> C_{18}) acids after 15 wk but 43.1% and 46.1%, respectively, after 20 wk (Table 3). Secondly, some of these changes were in the opposite direction to those expected. For instance, with the CO diet adrenal phospholipids had 36% total (n − 6) acids after 15 wk but 19.1% of these acids after 20 wk (Table 4); with respect to the total (n − 9) acids of CE, the decrease was from 17.0% to 5.6% under the same conditions (Table 3). Thirdly, in all cases no direct or predictable relationship between the concentrations of these acids and the duration of feeding could be established from the data. Lastly, the general tendency for the total (n − 6) acids of cholesteryl esters from tissues of animals fed the linoleate-adequate diets (especially CO) to be largely accounted for by relatively higher concentrations of 20:4 (n − 6), 22:2 (n − 6), and 22:4 (n − 6) is noteworthy. The CE of animals fed the linoleate-deficient diets (HF and fat-free) tended to contain higher levels of 20:3 (n − 9), 20:4 (n − 6 + n − 7), and 22:3 (n − 9). This tendency was, however, subject to modification, depending on the tissue. The phospholipids from the tissues of animals fed the linoleate-deficient diets contained 20:3 (n − 9) as the predominant polyunsaturated fatty acid.

DISCUSSION

The data on lipid composition presented in Table 2 seem to indicate variations in the distribution pattern and concentrations of total lipids, cholesteryl esters, and phospholipids dependent on tissue type, dietary fat, and duration of feeding. The data of Gidez, Roheim, and Eder (4), in which the concentrations of total cholesterol of rat VLDL, LDL, and HDL seemed to vary with lipoprotein type and dietary fat, would be comparable to this observation. Because of the hepatic origin of the plasma lipoproteins (5), it may be tempting to compare the lipid data for the liver with those for the lipoprotein classes. Although in several cases high hepatic phospholipid levels were matched by parallel elevated phospholipid levels in the lipoproteins, this correlation may be only fortuitous, since heart phospholipids were also elevated in a number of cases. The indication is that these dietary fats probably led to some complex effects on the physiological state of the animals, as noted by Narayan, Mary, and Kummerow (6), resulting in the observed variations in the lipid composition of the rat serum lipoprotein classes. The nature of these effects is not yet understood.

Before discussing the data on the total concentrations of the (n − 6) and (n − 9) long-chain acids, it should be recognized that the significance of a high level of a fatty acid or fatty acid series clearly depends on its identity, the lipid class involved, and the tissue. Consequently, cross-comparisons of the results on the levels of the (n − 6) and (n − 9) long-chain acids between tissues would seem neither warranted nor meaningful. The same seems to be true of the data from the two time periods, since the intervals of 15 and 20 wk were originally chosen to represent two periods describing stationary levels of the polyunsaturated fatty acids in the

tissues, the former closer to and the latter farther removed from the equilibrium state for fatty acid composition of the tissue lipid classes. The rather large changes in concentration seen in some instances between 15 and 20 wk and the fact that these changes were not always in the expected direction are not easily explained from our data. They may represent a clue to some complex effects on the physiological state of the animals brought about by prolonged fat feeding (6).

That fatty acids occur in families, in which the terminal structures are similar, is now widely recognized. Original studies in this area, as well as further investigations of the metabolic conversions of several families of polyunsaturated fatty acids, have been reported by Klenk (7–9). Thus, the fatty acids considered here include the mono-, di-, and polyenes of the (n − 6), (n − 7), and (n − 9) families and of chain lengths C_{20}–C_{22}. With respect to the (n − 6) acids, our data indicate that diets very rich in linoleate (CO) or nutritionally adequate with respect to this acid (MF and BT) lead to an elevated level of (n − 6) long-chain unsaturated fatty acids in the cholesteryl esters and phospholipids in some of the tissues, especially after 20 wk. Dietary linoleate as corn oil is of course known to lead to increased levels of the members of the (n − 6) family, especially arachidonic acid (20:4 [n − 6]), in these lipid classes (10–12). The absolute (n − 6) concentrations were generally higher in the phospholipids than in the cholesteryl esters, especially in the adrenals, heart, and liver, and were largely accounted for by high levels of 20:4 (n − 6). Tissue fatty acid specificity was also evident; for instance, relatively high amounts of 22:4 (n − 6) were seen only in the adrenal cholesteryl esters (Table 3). This observation has been reported by other workers (13, 14). Furthermore, Walker has shown that corn oil supplementation of a fat-free diet resulted in increased levels of the (n − 6) acids with time of supplementation in the total lipids of rat liver, heart, and plasma (15, 16). There was a simultaneous decrease in the level of the (n − 9) series of fatty acids. However, because of differences in diet and experimental design, and above all, the fact that his data were for fatty acids from total lipids, no meaningful comparison of the reported data with ours can be made.

In these two lipid classes (Tables 3 and 4), diets nutritionally poor in linoleate but rich in palmitate and oleate (HF and fat-free diets) induced relatively high levels of the 20:4 acids—a mixture of (n − 6) and (n − 7) acids—especially in the phospholipids (1). This adaptation underscores the need for the 20:4 acids in tissue phospholipids, where they, like the more predominant eicosatrienoate 20:3 (n − 9), favor the 2 position of the phospholipids (17–22). Fatty acid tissue specificity was seen in the adrenal cholesteryl esters, with the appearance of relatively elevated amounts of 22:3 (n − 9). This is in accord with the reports by Gidez (14) and Walker (15). Our data (Table 3) indicated relatively low levels of 20:3 (n − 9) and other polyunsaturated acids in the liver cholesteryl esters of animals fed the fat-free diet. Furthermore, there were negligible amounts of 20:3 (n − 9) in the cholesteryl esters of VLDL and LDL after 15 wk, while the HDL cholesteryl esters were relatively rich in this acid at both time intervals. These findings are in accord with those of Gidez et al. (4), who suggested that this could be due to a process capable of a high degree of selectivity. However, at the end of 20 wk the levels of this acid were substantial in the cholesteryl esters of VLDL and LDL. The major difference in experimental design is that in Gidez's study the animals were fed a cholesterol-supplemented fat-free diet for a shorter period of 11 wk. It is not clear whether this difference was enough to modify the incorporation of 20:3 (n − 9) by the end of 20 wk to include the cholesteryl esters of VLDL and LDL.

Regarding the (n − 9) family of long-chain unsaturated acids, our results (Tables 3 and 4) show that the greatest accumulation of these acids was seen in the cholesteryl esters and phospholipids of adrenals, heart, and LDL when HF or fat-free diet was fed; furthermore, the fatty acid patterns of these lipid classes were in accord with the reports of other workers (20–22). Our data are also in agreement with the finding by Holman and Mohrhauer (23) that the enzyme substrate affinities of chain elongation–desaturation reactions are of the order 18:3 (n − 3) > 18:2 (n − 6) > 18:1 (n − 9), thus explaining why formation of the (n − 9) series of fatty acids is best when 18:3 (n − 3) and 18:2 (n − 6) are absent or limited in the diet. This trend can also be deduced from our data on adrenal lipids published earlier (2).

It would seem from our results that HF and fat-free diets had comparable potential to induce chain desaturation and elongation reactions involving palmitoleate and oleate as the parent fatty acids of the (n − 7) and (n − 9) families, respectively. Their ability to do this vis-à-vis the other diets appears to correlate well with their inadequacy in linoleate, an essential fatty acid and the parent acid of the (n − 6) series.

It is clear from our results that the sum total of the (n − 6) family of fatty acids in a given tissue and lipid class does not necessarily bear a direct relationship to the quantitative level of linoleic acid in the diet, since the CO diet and the two diets (MF and BT) that were just nutritionally adequate with respect to linoleate resulted in comparable degrees of accumulation of this family of fatty acids.

The formation of palmitoleate and oleate by the desaturation of palmitate and stearate, respectively,

arising from lipogenesis from acetate in fat-free rats is well known (24). Palmitoleate and oleate are, of course, the parent acids of the (n − 7) and (n − 9) families of fatty acids, respectively. Furthermore, these results indicate that our earlier observation (2) that HF diet led to an accumulation of (n − 9) acids in the adrenal cholesteryl esters (38.7%) and phospholipids (23.4%) was not due to the high *trans* content of this fat (48%), but rather was a reflection of the suboptimal level of linoleate in the 20% HF diet in the face of high levels (76%) of octadecanoates. This conclusion stems from the almost identical potential of HF and fat-free diets to induce an overall accumulation of (n − 9) long-chain unsaturated acids in most of the tissues examined in the study. However, the identities of the individual (n − 9) long-chain acids differ with the two types of diet (1, 2). The apparent influence of the *trans* nature of the HF was, as we pointed out earlier (1), to induce a series of unusual elongation–desaturation reactions leading to a number of eicosadienoates which still maintained the (n − 9) or (n − 7) terminal structure of the parent acids, oleate or palmitoleate, but with the second double bond not conforming to the natural isolated double bond system. It is concluded from this study that the linoleate-deficient nature of the HF, rather than its high *trans* content, would explain its ability to stimulate to a greater extent than could beef tallow or butter fat the accumulation of the oleic (and palmitoleic) families of long-chain unsaturated acids in tissue cholesteryl esters and phospholipids.

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