Influence of dietary fatty acids on phospholipid fatty acid composition in subcellular particles of rat liver

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SUMMARY Male rats were divided into three groups and fed a fat-free diet (Group A), a diet supplemented with 7% of ethyl linolenate (Group B), and as a control, a diet supplemented with 7% of ethyl linoleate (Group C) for 3 weeks. Fatty acid compositions of cardiolipin, "cephalin," and lecithin in column chromatographic fractions of the liver mitochondrial and microsomal lipids were determined.

In the cardiolipin fraction, the percentages of palmitoleic and oleic acids increased markedly in group A, as did that of linolenic acid in group B; linoleic acid decreased. In all groups the "cephalin" fraction was richer in highly unsaturated fatty acids than the lecithin fraction. In group A a marked decrease of arachidonic acid in the lecithin fraction matched the increases of oleic and eicosatrienoic acids. The increase of eicosatrienoic acid during linoleic acid deficiency was most markedly shown in the inositol-containing fraction. Mitochondrial and microsomal lipids were similar in all cases.

These changes may be related to the functional specificity of phospholipids and to the influence of linoleic acid deficiency on their different biosynthetic pathways.

KEY WORDS dietary fatty acid · rat · liver · microsomes · mitochondria · phospholipid fractions · fatty acid composition · linoleate · linolenate

OLEIC, LINOLEIC, and linolenic acids (18:1, 18:2, and 18:3) are the major unsaturated fatty acids widely distributed in plants. Among these, 18:2 is the only acid that has been shown to be indispensable for the growth of some animals (1, 2). Linoleic acid and more unsaturated fatty acids of its series are important constituents of biological membranes and are indispensable for the activity of some enzyme systems (3, 4). Recently it has been demonstrated that 18:2 plays an important role in the transport of lipids (5–7).

Oleic acid is synthesized from saturated fatty acids in the animal body (8), and ω9 fatty acids of its series are proportionately increased during linoleic acid deficiency (9, 10). Linolenic acid, though it can restore growth to a considerable degree, cannot prevent the development of dermal symptoms caused by the linoleic acid deficiency (9, 11).

Fatty acids are unevenly distributed among various lipid classes. Ethanolamine phospholipids have been shown to be richer in highly unsaturated fatty acids than are lecithins (12, 13), and cardiolipin contains a high percentage of linoleic acid (13–15). These differences in the fatty acid composition may possibly be related to the functions of the different phospholipids.

Dietary fatty acids affect the fatty acid composition of each lipid class to some degree. In this report data are presented concerning the influence of dietary fatty acids on the phospholipid fatty acid composition of subcellular particles of rat liver.

MATERIALS AND METHODS

Male albino rats of the Sprague-Dawley strain, average weight 63 g, which had been raised on a stock diet (Oriental Yeast, Osaka), were divided into three groups and fed on experimental diets for 3 weeks. The composition of the diets is shown in Table 1. Ethyl linoleate and ethyl linolenate were kindly provided by Ono Pharmaceutical Co., Osaka, Japan. The ethyl linoleate was almost 100% pure; the ethyl linolenate was contaminated with 3% of ethyl oleate. At the end of the feeding period, rats were sacrificed by decapitation after 20 hr of fasting. Livers were removed and homogenized in an isotonic sucrose solution at below 5°. Mitochondria and microsomes were isolated by the method of Schneider (16).

TABLE 1 COMPOSITION OF THE EXPERIMENTAL DIETS

	Group		
	A Fat-free	B Linolenate	C Linoleate
	mg	*mg*	*mg*
Casein	1500	1500	1500
Gelatin	1500	1500	1500
Sucrose	7800	6300	6300
Ethyl linolenate	—	700	—
Ethyl linoleate	—	—	700

Values are the amount of dietary components consumed daily by each rat.

Levels of other constituents were: 500 mg of salt mixture, 150 mg of vitamin mixture in lactose, 150 mg of cellulose, and 20 mg of choline chloride.

Lipids were extracted with chloroform–methanol (C–M) 2:1 (v/v) following the method of Folch et al. (17). The extract was washed with 0.2 volume of water and dried at 50° under a stream of nitrogen, and the residue was reextracted with chloroform. Lipid phosphorus was estimated by the method of Fiske and Subbarow (18). Phospholipids were chromatographed on silicic acid columns according to the method of Hanahan et al. (19). Fractions were eluted successively with C–M 8:1 (cardiolipin), C–M 4:1 ("cephalin"), and C–M 3:2 and 1:4 (inositol phospholipids plus lecithin). The recovery of lipid phosphorus averaged 92%.

Individual fractions were identified by silicic acid impregnated paper chromatography by the method of Marinetti (20). Hydrolysis products of phospholipids were identified by paper chromatography using the method of Dawson (21). The cardiolipin fraction contained no detectable amounts of choline, ethanolamine, or aldehyde components. The "cephalin" and inositol-containing fractions were almost completely free from choline phospholipids. The lecithin fraction was contaminated by less than 3% of serine, ethanolamine, and inositol glycerophosphatides.

Lipids were hydrolyzed in 2 N KOH in 50% ethanol at room temperature for 24 hr in the presence of hydroquinone. After the removal of unsaponifiable matter with petroleum ether, fatty acids were recovered with petroleum ether and methylated in diethyl ether with freshly distilled diazomethane (22).

TABLE 2 RATE OF INCREASE OF RAT BODY WEIGHTS DURING THE EXPERIMENTAL PERIOD

Feeding Period	Group		
	A Fat-free	B Linolenate	C Linoleate
days	%	%	%
12	+56.0	+68.3	+77.4
21	+96.6	+124.0	+137.4

TABLE 3 FATTY ACID COMPOSITION OF THE MITOCHONDRIAL CARDIOLIPIN FRACTION

Fatty Acid*	Group of Rats		
	A Fat-free	B Linolenate	C Linoleate
	%	%	%
14:0	0.41 ± 0.07	0.21 ± 0.10	0.30 ± 0.11
14:1	0.02	0.04	0.01
15:0	0.05	0.19 ± 0.09	0.09
16:0	2.26 ± 0.24	3.82 ± 0.50	2.15 ± 0.34
16:1	27.11 ± 1.03	11.42 ± 1.58	7.36 ± 1.88
17:0	1.10 ± 0.30	1.31 ± 0.66	0.25 ± 0.19
18:0	0.51 ± 0.26	0.46 ± 0.08	0.48 ± 0.16
18:1	36.75 ± 0.59	25.25 ± 0.98	15.02 ± 1.57
18:2	23.27 ± 1.57	22.87 ± 2.07	67.20 ± 0.27
18:3	—	20.31 ± 1.20	—
20:1	Tr.	—	—
20:2	0.97 ± 1.07	0.06	2.45 ± 0.29
20:3	5.30 ± 0.76	2.08 ± 0.15	2.18 ± 0.72
20:4	2.25 ± 0.53	4.86 ± 0.73	2.51 ± 1.43
20:5	—	5.51 ± 1.28	—
?	—	1.61 ± 0.63	—

Values in Tables 3–8 are the average ± SD of four rats.
* Number of carbon atoms:number of double bonds.

The methyl esters were analyzed with a Shimadzu GC-1B gas chromatograph equipped with a hydrogen flame ionization detector. The analysis was carried out on a 6 ft × 4 mm column of ethylene glycol succinate polyester on Chromosorb W, at a flow rate of 100 ml/min N_2 and at a temperature between 220 and 225°. The inlet heater was kept at 280°. Methyl esters were identified by the linear relationship between the logarithm of the retention time and chain length, and comparison of retention times with those of standards obtained from NIH, and Applied Science Laboratories, State College, Pa. The fatty acid composition is expressed by area per cent. The identification of 22:4 was only tentative. Rahm and Holman (23) recently reported that a fatty acid tentatively identified as 22:4 from the retention time data was determined to be 22:5ω6 by a method using reductive ozonolysis. In our present experiments procedures for the identification of this acid were not carried out.

RESULTS

The growth of animals in the experimental period was as shown in Table 2. The groups fed linoleate and linolenate showed almost the same rate of growth. Compared to these two groups, the rats fed a fat-free diet showed some retardation of growth.

In what follows, group C rats are regarded as the control animals.

Fatty Acid Composition of Cardiolipins

This fraction constitutes about 4% of the total phospholipids in the normal rat liver. It is contained almost

exclusively in mitochondria. The cardiolipin fraction in group C (linoleate fed) mitochondria was rich in 18:2 (Table 3); other major components were 18:1 and palmitoleic acid (16:1). In group A (fat-free), 18:1 and 16:1 were markedly increased at the expense of 18:2. Eicosatrienoic acid (20:3) also increased, but to a small extent. The decrease of 18:2 was almost equal in groups A and B. In the latter group, the percentage decrease of 18:2 was mainly compensated for by an increase in 18:3, so that comparatively small increases of 18:1 and 16:1 were found; the highly unsaturated acids 20:4 and 20:5 increased considerably.

Fatty Acid Composition of Major Phospholipid Fractions in Group C

The "cephalins" of both mitochondria and microsomes (Table 4) were rich in the more highly unsaturated fatty acids 22:4 and 22:6 after linoleate feeding. Arachidonic acid (20:4) constituted about 30% of total fatty acids in cephalins and also in lecithin.

The percentage compositions of fatty acids in both of the major phospholipid fractions were almost the same in the two subcellular fractions, except that slightly larger amounts of palmitic acid (16:0) and stearic acid (18:0) were detected in microsomes than in mitochondria.

Fatty Acid Compositions of Major Phospholipid Fractions in Groups A and B

In both of these groups, the decrease of 18:2 compared with group C in the major phospholipid fractions ("cephalins" and lecithin) was much more striking than in the cardiolipin fraction. In group B (Table 5), 18:3 itself was incorporated into the major fractions to only a small extent. However, 20:5, 22:5, and 22:6 attained a total of 25–40% at the expense of the linoleic acid series 18:2, 20:4, and 22:4. There was no significant increase of 16:1.

In group A (Table 6), the decrease of 20:4 was not marked in the "cephalin" fraction. Characteristic features of this group were quite significant differences in the fatty acid composition between "cephalin" and lecithin fractions other than the difference commonly shown in the content of highly unsaturated C_{22} fatty acids. The decrease of 20:4 was striking in the lecithin fractions; 18:1 and 20:3 correspondingly increased. The increase of 18:1 was slightly more marked in microsomes than in mitochondria. Palmitoleic acid increased slightly but significantly in this group.

The percentages of the major saturated fatty acids, 16:0 and 18:0, were almost equal in corresponding phospholipid classes among all three groups.

Inositol-Containing Fraction

In the inositol-containing fraction of microsomes (Table 7), the linoleate-fed group C was characterized by the high percentages of 18:0 and 20:4. In group B the decrease of 20:4 and the increase of 20:5 in this fraction were much less than in the "cephalin" and lecithin fractions (compare Table 5). In group A, 20:3 increased more markedly than in any other phospholipid class (Tables 3 and 6). The mitochondrial fractions (Table 8) did not show these characteristic distribution patterns.

DISCUSSION

The present work was carried out as one of the steps to make clear the relationships between the specific distri-

TABLE 4 FATTY ACID COMPOSITION OF LIVER PHOSPHOLIPIDS OF RATS FED LINOLEATE (GROUP C)

	Cephalins of		Lecithin of	
	Mitochondria	Microsomes	Mitochondria	Microsomes
	%	%	%	%
14:0	0.12 ± 0.09	0.11 ± 0.05	0.15 ± 0.05	0.24 ± 0.10
14:1	0.03	0.02	0.04	Tr.
15:0	0.05	0.06	0.15 ± 0.07	0.26 ± 0.04
16:0	11.86 ± 2.00	13.50 ± 1.24	18.47 ± 0.93	18.15 ± 1.47
16:1	2.09 ± 0.21	1.68 ± 0.82	2.86 ± 0.58	2.20 ± 0.36
17:0	0.58 ± 0.20	0.56 ± 0.18	0.49 ± 0.32	0.39 ± 0.13
?	0.28 ± 0.16	0.28 ± 0.11	0.18 ± 0.09	0.30 ± 0.15
18:0	17.27 ± 0.39	19.12 ± 0.16	15.66 ± 1.00	16.42 ± 0.59
18:1	11.19 ± 1.20	11.01 ± 0.75	11.49 ± 1.28	11.10 ± 0.67
18:2	10.82 ± 0.94	10.29 ± 0.46	13.11 ± 1.10	13.12 ± 0.80
20:1	0.27 ± 0.12	0.34 ± 0.15	0.46 ± 0.15	0.22 ± 0.11
20:2	0.83 ± 0.26	0.72 ± 0.32	0.62 ± 0.18	0.62 ± 0.21
20:3	0.53 ± 0.09	0.38 ± 0.20	1.12 ± 0.64	0.90 ± 0.00
20:4	31.77 ± 1.10	28.81 ± 2.22	31.14 ± 1.17	30.69 ± 2.17
22:3	0.65 ± 0.38	1.33 ± 0.50	Tr.	0.33 ± 0.20
22:4	5.85 ± 1.95	6.24 ± 1.63	2.19 ± 0.72	3.16 ± 0.74
22:6	5.81 ± 1.87	5.55 ± 0.86	1.87 ± 1.03	1.90 ± 0.45

TABLE 5 FATTY ACID COMPOSITION OF LIVER PHOSPHOLIPIDS OF RATS FED LINOLENATE (GROUP B)

	Cephalins of		Lecithin of	
	Mitochondria	Microsomes	Mitochondria	Microsomes
	%	%	%	%
14:0	0.16 ± 0.08	0.07	0.14 ± 0.03	0.18 ± 0.03
14:1	0.01	0.07	Tr.	—
15:0	0.04	0.02	0.18 ± 0.05	0.21 ± 0.04
16:0	11.41 ± 1.34	13.54 ± 0.74	18.36 ± 0.72	19.23 ± 1.00
16:1	2.98 ± 0.47	1.67 ± 0.37	3.87 ± 0.85	3.41 ± 0.60
17:0	0.68 ± 0.19	0.50 ± 0.44	0.53 ± 0.15	0.41 ± 0.06
?	0.14 ± 0.06	0.05	0.22 ± 0.11	0.18 ± 0.10
18:0	17.89 ± 0.70	19.14 ± 1.09	15.48 ± 0.93	17.06 ± 0.51
18:1	9.20 ± 0.97	9.94 ± 1.50	13.35 ± 0.61	14.43 ± 0.78
18:2	3.45 ± 1.08	3.94 ± 1.15	6.47 ± 0.58	7.79 ± 2.13
18:3	3.57 ± 0.47	3.87 ± 1.10	3.77 ± 0.58	3.87 ± 1.49
20:1	0.12 ± 0.16	0.12 ± 0.08	0.26 ± 0.07	0.24 ± 0.07
20:2	—	—	—	—
20:3	Tr.	—	0.36 ± 0.16	0.39 ± 0.21
20:4	8.62 ± 1.04	9.18 ± 1.33	8.19 ± 1.15	8.08 ± 1.06
20:5	20.46 ± 1.30	19.52 ± 1.36	18.63 ± 2.18	15.86 ± 3.62
22:3	—	—	—	—
22:4	—	—	—	—
22:5	4.49 ± 1.32	4.14 ± 0.80	2.02 ± 0.57	1.55 ± 0.46
22:6	16.78 ± 0.55	14.23 ± 1.48	8.17 ± 0.63	7.11 ± 0.33

bution of fatty acids among phospholipid classes, their functions, and metabolic pathways. Although the simple comparison of the content of each fatty acid seems not to be sufficient for the elucidation of the problem, it is considered that changes in the fatty acid composition brought about by dietary supplementation of fatty acids might reflect the selectivity for particular fatty acids exerted by the biosynthetic mechanism for each lipid fraction.

The fatty acid distribution pattern obtained in our present experiments agrees well with that reported by Macfarlane et al. (12) and Getz et al. (13) for normal rat liver, if differences in the dietary conditions are taken into consideration. The most characteristic features are shown in minor fractions.

Cardiolipin is a specific lipid class that is located only in mitochondria. It has been concluded that cardiolipin is an important factor in the respiratory chain of the mitochondrion (24, 25). Rose (15) showed that ox heart cardiolipin contained a larger amount of 18:2 than rat liver cardiolipin, and connected this difference with the different amounts of linoleate consumed by each organ.

TABLE 6 FATTY ACID COMPOSITION OF LIVER PHOSPHOLIPIDS OF GROUP A RATS (FAT-FREE DIET)

	Cephalins of		Lecithin of	
	Mitochondria	Microsomes	Mitochondria	Microsomes
	%	%	%	%
14:0	0.21 ± 0.08	0.11 ± 0.06	0.19 ± 0.06	0.20 ± 0.03
14:1	0.05	0.05	Tr.	Tr.
15:0	Tr.	0.02	0.09	0.07
16:0	11.51 ± 1.20	13.63 ± 1.17	16.36 ± 0.40	17.39 ± 1.31
16:1	4.96 ± 0.23	2.89 ± 0.52	5.75 ± 0.28	4.80 ± 0.73
17:0	0.43 ± 0.29	0.27 ± 0.08	0.31 ± 0.11	0.23 ± 0.03
?	0.27 ± 0.16	0.13 ± 0.10	0.20 ± 0.09	0.21 ± 0.06
18:0	17.48 ± 1.32	19.08 ± 2.82	17.52 ± 1.98	17.03 ± 1.46
18:1	14.36 ± 0.80	13.53 ± 2.18	20.92 ± 2.61	22.14 ± 2.09
18:2	3.37 ± 0.46	3.34 ± 0.33	5.53 ± 1.13	5.74 ± 1.44
20:1	0.53 ± 0.16	0.52 ± 0.24	0.44 ± 0.24	0.62 ± 0.11
20:2	—	Tr.	0.13 ± 0.13	Tr.
20:3	7.73 ± 1.87	9.04 ± 2.92	12.24 ± 2.51	11.89 ± 3.11
20:4	23.62 ± 1.78	24.11 ± 1.20	13.68 ± 2.18	14.14 ± 3.81
22:3	—	—	—	—
22:4	2.55 ± 0.40	1.61 ± 0.51	1.29 ± 0.24	0.99 ± 0.27
22:6	12.93 ± 1.81	11.67 ± 1.82	5.35 ± 1.10	4.55 ± 1.10

TABLE 7 FATTY ACID COMPOSITION OF THE MICROSOMAL INOSITOL-CONTAINING FRACTION

	Group of Rats		
	A Fat-free	B Linolenate	C Linoleate
	%	%	%
14:0	0.11 ± 0.03	0.06	0.08
14:1	—	0.01	0.04
15:0	0.11 ± 0.07	0.03	0.06
16:0	8.36 ± 0.63	8.36 ± 0.66	10.03 ± 1.76
16:1	1.95 ± 0.36	1.06 ± 0.41	0.94 ± 0.26
17:0	0.09	0.35 ± 0.17	0.46 ± 0.13
18:0	33.56 ± 1.32	32.11 ± 0.98	32.85 ± 2.06
18:1	8.33 ± 1.18	7.95 ± 1.82	6.99 ± 1.13
18:2	2.26 ± 0.59	1.99 ± 0.57	8.16 ± 1.60
18:3	—	1.38 ± 0.56	—
20:1	1.39 ± 0.53	0.45 ± 0.11	—
20:2	1.92 ± 0.28	1.53 ± 0.18	0.78 ± 0.15
20:3	19.83 ± 1.77	1.64 ± 0.54	2.37 ± 1.07
20:4	16.42 ± 1.68	15.05 ± 1.22	32.21 ± 1.88
20:5	—	7.43 ± 0.99	—
?	1.78 ± 1.54	—	—
22:3	—	—	1.21 ± 0.34
22:4	1.29 ± 0.27	—	2.47 ± 0.41
22:5	—	9.14 ± 0.15	—
22:6	2.60 ± 1.10	11.46 ± 1.20	1.35 ± 0.60

Linoleate, then, may be centrally concerned in the function of this lipid. Linoleate in group C, 18:3 in group B, and 16:1 and 18:1 in group A are the major fatty acids of cardiolipins. Although rather high percentages (about 20%) of 18:2 are maintained in this fraction in groups A and B, the rate of decrease of this acid in these groups compared to group C was almost of the same order as in the major phospholipid fractions. If 18:2 were obligatory for the function of cardiolipin, there might be some control mechanism working to maintain 18:2 at a much higher level than other phospholipid fractions. Rapid changes in the fatty acid composition and the incorporation of the first-step members of unsaturated fatty acids make us consider a possibility that this phospholipid is concerned in some way with double bond formation or dehydrogenation of fatty acids. The presence of highly unsaturated fatty acids such as 20:4 in the cardiolipin fraction in our present experiments may be due to contamination with other lipids. Similarly, increases of highly unsaturated fatty acids in the linolenate fed group might possibly be due to changes in the degree of contamination. Further fractionation procedures would be necessary to elucidate this point.

"Cephalins" seem to incorporate preferentially more highly unsaturated fatty acids. The content of 22:4 and 22:6 in the groups fed linoleate and linolenate were much larger in "cephalins" than in the lecithin fraction (Tables 4 and 5), and in the fat-free group the decrease of 20:4 was smaller in the "cephalin" fraction than in the lecithin fraction (Table 6 compared with Table 4).

A significantly larger amount of 16:0 and 18:2 in the lecithin fraction might be connected with the function of this phospholipid in the transport of lipids from the liver into the blood stream or bile. Our previous results on the choline deficient fatty liver (26) showed that the synthesis of lecithin in the liver through Kennedy's pathway (27) was selectively connected to diglycerides containing palmitic, oleic, and linoleic acids, and that lecithin containing stearic, arachidonic, and more highly unsaturated fatty acids was derived via the methylation of phosphatidyl ethanolamine (28). Marked increases of 20:3 and 18:1 in the lecithin fraction in the fat-free group might be explained by this consideration, for fatty livers are induced by fat-free diets even in the presence of a full supplementation with choline. It is of special interest that in the linolenate-fed group, in which fatty liver was not detected, the incorporation of 18:3 into lecithin was not greater than in the incorporation into "cephalins."

There were no marked differences in the fatty acid composition of lecithin between mitochondrial and microsomal fractions in the fat-free group, except for some differences in the content of saturated fatty acids that were detected also in the linoleate-fed group. Our previous data in similar experiments, in which linoleate as well as choline were depleted, showed marked differences in the fatty acid composition of lecithin between mitochondria and microsomes. Further experiments are

TABLE 8 FATTY ACID COMPOSITION OF THE MITOCHONDRIAL INOSITOL-CONTAINING FRACTION

	Group of Rats		
	A Fat-free	B Linolenate	C Linoleate
	%	%	%
14:0	0.11 ± 0.04	0.09	0.11 ± 0.02
14:1	0.07	0.04	0.02
15:0	—	0.04	0.08
16:0	12.97 ± 1.33	13.10 ± 1.15	14.77 ± 0.77
16:1	7.36 ± 1.57	3.99 ± 0.35	4.01 ± 0.51
17:0	0.52 ± 0.18	0.45 ± 0.09	0.40 ± 0.10
?	0.14 ± 0.08	0.09	0.09
18:0	22.64 ± 1.27	24.03 ± 1.51	20.06 ± 1.22
18:1	16.69 ± 1.05	11.77 ± 0.88	11.55 ± 0.62
18:2	5.07 ± 1.57	4.62 ± 1.24	14.32 ± 2.47
18:3	—	3.65 ± 1.05	—
20:1	0.65 ± 0.17	0.14 ± 0.03	0.09
20:2	0.68 ± 0.29	0.39 ± 0.05	0.69 ± 0.60
20:3	12.93 ± 1.22	1.74 ± 1.05	1.27 ± 0.83
20:4	15.83 ± 2.15	11.47 ± 0.65	26.39 ± 0.18
20:5	—	11.03 ± 1.19	—
22:3	—	—	0.75 ± 0.33
22:4	0.94 ± 0.56	—	2.97 ± 0.79
22:5	—	4.89 ± 1.40	—
22:6	3.40 ± 0.46	8.47 ± 0.79	2.43 ± 0.86

now underway to find the explanation of this difference.

The inositol-containing fraction showed a marked increase of 20:3 in the fat-free group. Even in the group fed linolenate, the incorporation of 20:5 was smaller, and the decrease of 20:4 was also less marked in this fraction than in the other major fractions. Although the methods applied in our present experiments do not yield a pure fraction of inositol phosphatides, it is reasonable to conjecture from the results that this fraction selectively incorporates 20:4 as well as 20:3. The above data agree with the results reported by Tischer et al. (29).

The especially large amounts of 20:5, 22:5, and 22:6 in the liver phospholipids of rats fed linolenic acid is in agreement with reports of other authors (9, 30–32). It cannot be deduced, however, whether these high percentages are due to selective incorporation or to slow breakdown of phospholipids containing these acids. The same doubt exists concerning the high percentages of 22:6 in the fat-free group.

Manuscript received September 10, 1964; accepted December 1, 1964.

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