Skeletal muscle lipids. III. Changes in fatty acid composition of individual phosphoglycerides in man from fetal to middle age

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Abstract The fatty acids in cardiolipin, choline phosphoglyceride, ethanolamine phosphoglyceride, inositol phosphoglyceride, and serine phosphoglyceride in the human gastrocnemius muscle were studied for changes between early fetal age to middle age. In cardiolipin, choline phosphoglyceride, and ethanolamine phosphoglyceride, the relative total amount of the linoleic acid series increased considerably during the prenatal period and reached maximum values during the first year of life. In inositol phosphoglyceride and serine phosphoglyceride, only slight changes in the sum of fatty acids of the linoleic acid series were found. An increase in the concentration of fatty acids of the linolenic acid series was found in ethanolamine phosphoglyceride and serine phosphoglyceride, while only minor amounts of these fatty acids were present in choline phosphoglyceride and inositol phosphoglyceride and not at all in cardiolipin. During the period studied, the amount of plasmalogen in ethanolamine phosphoglyceride decreased slightly during the first year of life and then remained at 40%. The amount of plasmalogen increased in choline phosphoglyceride from 8% at the middle of the gestational period to 15% 1 yr after birth. The individual phospholipids were found to have characteristic fatty acid patterns.

Supplementary key words thin-layer chromatography · gas–liquid chromatography · essential fatty acids · plasmalogens

In the study of physiological factors affecting the phosphoglycerides of skeletal muscles, it was found that age had an important influence on the fatty acid composition of lecithin in human muscles (1). A continuous increase in the percentage of fatty acids of the linoleic acid series was found, from 10% at the beginning of the second trimester of gestation to almost 50% at the age of 1 yr. Such a profound increase in the percentage of essential fatty acids had not been found in any phosphoglycerides of human organs before. As muscle is one of the major tissues of the body, the lecithin in the skeletal muscles requires a substantial part of the linoleic acid available. Detailed elucidation of these requirements necessitates knowledge of the variation in the fatty acid composition of the other phosphoglycerides.

MATERIALS AND METHODS

Material

The source of the material and the procedure used for collection have been described earlier (1). The material in this study consisted of muscle samples from 10 fetuses, 10 infants, 7 children aged 1 to 10 yr, 5 males aged 10 to 20, 2 adults between 20 and 30 yr of age, and 3 adults between 50 and 60 yr of age. Most of the samples were obtained from males.

The chemicals and solvents used were of analytical grade. The solvents were redistilled before use.

Silica gels G and H were obtained from Fluka A.G., Buchs, Switzerland, and 14% BF_3 in methanol in sealed ampoules was purchased from Applied Science Laboratories, Inc., State College, Pa.

Methods

Total lipid extracts were obtained from the muscle samples after homogenization, extraction with chloroform–methanol, and partitioning of the lipid extracts (1). Phosphorus was determined in a portion of the lipid extract, equivalent to 8–10 mg wet weight, with a modified Fiske-SubbaRow method (2).

Isolation of individual phospholipids. Lipid extracts, equivalent to 10–15 μg of phosphorus, were applied to thin-layer chromatography plates as bands 3–4 cm broad. For the separation of cardiolipin and EPG, silica gel H was used with the solvent chloroform–methanol–water 80:20:2 (v/v). The plate was developed for 10 cm and then air-dried, after which it was developed in light petroleum–diethyl ether–acetic acid 87:13:1 (v/v) up to the

Abbreviations: fatty acids are designated by chain length:number of double bonds; n − 6 denotes that the first double bond from the methyl group occurs after the sixth carbon atom, the methyl group being counted as no. 1. CPG, choline phosphoglyceride, lecithin; EPG, ethanolamine phosphoglyceride; IPG, inositol phosphoglyceride; SPG, serine phosphoglyceride; TLC, thin-layer chromatography; DEGS, diethylene glycol succinate polyester.

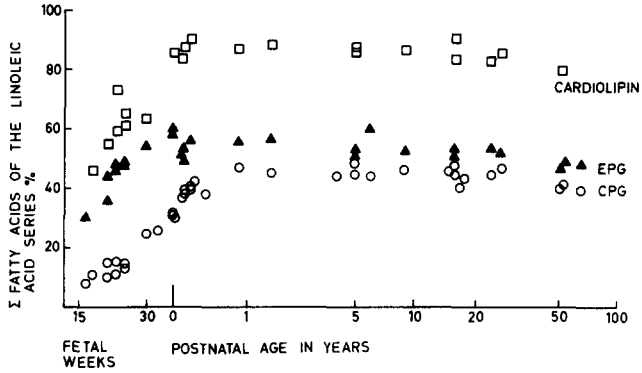

Fig. 1. Concentration of the sum of fatty acids belonging to the linoleic acid series in cardiolipin, EPG, and CPG of human gastrocnemius muscles at different ages. The concentration is expressed as percentage by weight of total fatty acid methyl esters.

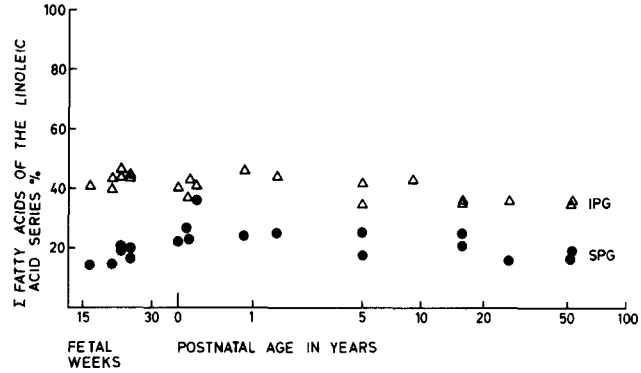

Fig. 2. Concentration of the sum of fatty acids belonging to the linoleic acid series in IPG and SPG of human gastrocnemius muscles at different ages. The concentration is expressed as percentage by weight of total fatty acid methyl esters.

edge of the plate. CPG was separated on silica gel G plates developed in chloroform–methanol–water 65:25:4 (v/v). IPG and SPG were separated on silica gel G plates developed in chloroform–methanol–12 N ammonia 70:30:5 (v/v). The advantages of the different solvent systems have been described previously (3).

Methanolysis of fatty acids and aldehydes. The areas containing lipids were indicated by spraying with bromothymol blue. In those areas containing the appropriate phosphoglyceride, the layers were scraped off, and the scrapings were transferred to open tubes and dried overnight over P_2O_5 in vacuo. 2 ml of 0.1 M sodium methoxide in dry methanol was added to the tubes, and methanolysis was performed at room temperature for 1 hr. The methyl esters were extracted with light petroleum (4).

In order to obtain dimethyl acetals of the aldehydes, lipids equivalent to 75 μg of phosphorus were separated by TLC, as described above, and the areas containing EPG and CPG were scraped off. The phosphoglycerides were extracted from the gel. Lipids containing 8–12 μg of lipid phosphorus were methylated with 14% BF_3 in methanol (5). Dimethyl acetals and methyl esters were recovered by extraction with light petroleum. They were then separated on, and recovered from, TLC plates of silica gel G, developed with benzene (5) in order to enable identification of any minor dimethyl acetals.

Gas–liquid chromatography of methyl esters and dimethyl acetals. The fatty acid methyl esters were analyzed on a Hewlett-Packard gas chromatograph, model 402 (glass column with 15% DEGS on Diatoport S, 80–100 mesh, at 190°C, carrier gas argon, 25 ml/min) equipped with a Hewlett-Packard electronic integrator 3370A. The peaks of the methyl esters were identified according to Svennerholm (4). Samples containing methyl esters and dimethyl acetals or dimethyl acetals only were analyzed in the same manner, but at a temperature of 173°C. With this column and at this temperature, the dimethyl acetals 16:0, 18:0, and 18:1 were resolved from the fatty acid methyl esters 16:0, 16:1, and 18:0.

RESULTS

Age variation of the linoleic and linolenic acid series

Figs. 1 and 2 show the variation with age of the sum of the linoleic acid series of the different phosphoglycerides. The values are expressed as percentages by weight of the total fatty acids. The phosphoglycerides shown in Fig. 1 are cardiolipin, CPG, and EPG. In these three lipids, the variation with age showed the same pattern. There was a considerable increase in the amount of the linoleic acid series during the fetal period. The highest percentages of these fatty acids were found for EPG at term, for cardiolipin at 2 months after birth, and for CPG at the age of 1 yr. In cardiolipin the percentage of these fatty acids increased from 45% at the gestational age of 16 wk to 90% 2 months after birth, while it increased from 8% to 45% 1 yr after birth in CPG and from 30% to 60% at term in EPG.

The relative amounts of the linoleic acid series in IPG and SPG (Fig. 2) showed only minor age variations. There was a slight increase in the concentration of these fatty acids in SPG but a small decrease in IPG during the prenatal period.

Appreciable amounts of the sum of the linolenic acid series were found only in EPG and SPG. In EPG there occurred an increase from 5% at the middle of the fetal stage to the highest percentage, 14%, 6 wk after birth. After this period a slight decrease was noted. The same increase with age was found in SPG, but the maximum value was not reached until the age of about 5 yr.

Age variation of the individual fatty acids

Cardiolipin. The increase in the sum of the linoleic acid series was due only to an increase in the amount of 18:2, from 40% at the 16th fetal week to almost 90% 2 months after birth. At the same time, there was a slight decrease in the amount of 20:4(n − 6). 18:1 and 16:1 decreased, from 35% and 10% respectively, to 8% and 1%,

respectively, 2 months after birth. A decrease was noted for 16:0, while 18:0 remained constant.

CPG. The percentage of 18:2 increased continuously from the beginning of the second trimester (2%) to the age of 1 yr (36%). 20:4(n − 6) increased from 5% to 12% at term, after which it decreased to 7% at 2 months after birth and then remained at this level. 18:1 varied inversely with the linoleic acid, from 36% at the beginning of the second trimester to 14% at the age of 1 yr.

EPG. During the latter part of the fetal stage, 20:4(n − 6) was the dominating fatty acid and increased from 20% at the 16th fetal week to almost 40% at birth, after which it decreased to about 30% 2 months after birth. 18:2 remained constant (less than 5%) until birth, but when 20:4(n − 6) decreased 18:2 increased, with the result that the sum of these two fatty acids remained constant. After the age of 2 months, 18:2 remained almost constant. The major fatty acid of the linolenic acid series was 22:6(n − 3), which increased from 3% at the middle of the fetal period to about 10% at birth. 18:1 was the fatty acid that decreased most: from 30% at the middle of the fetal stage to 10% at birth. During the same period a slight decrease was noted for 16:0, while 18:0 increased reciprocally.

IPG. While the sum of the linoleic acid series remained constant, there was an increase in the concentration of 18:2 and 20:3(n − 6) and a concomitant decrease in the concentration of 20:4(n − 6). 18:2 increased from 1% in the middle of the fetal period to 10% 2 months after birth, and during the same period the percentage of 20:3(n − 6) increased from 2% to 9%. 20:4(n − 6) decreased from 35% to 25% during this period.

SPG. 20:4(n − 6) remained almost constant, while 18:2 increased from 1% to almost 10% 2 months after birth. A concomitant decrease was noted for 18:1 from 30% to 15% during the same period.

Fatty acid composition of individual phosphoglycerides

After the age of 1 yr, the variations in the percentage amounts of the different fatty acids in the individual phosphoglycerides were rather small. The values from the analyses of individuals above 5 yr of age are therefore given as means and standard deviations in Table 1. The highest percentage of the linoleic acid series was found in cardiolipin, while SPG showed the lowest. The opposite was found for the linolenic acid series; in SPG these acids constituted about 15% of total fatty acids, while there were no fatty acids belonging to this series in cardiolipin. EPG also contained rather large amounts (about 10%) of these fatty acids.

In cardiolipin and CPG, the major fatty acid of the linoleic acid series was linoleic acid, while arachidonic acid was the dominating fatty acid of this series in EPG and IPG. SPG contained almost the same amount of both acids.

In the linolenic acid series, the dominating fatty acids were 22:5(n − 3) and 22:6(n − 3). In all phosphoglycerides, the amount of linolenic acid was very small, and it was often difficult to separate this fatty acid from impurities and other fatty acids, mainly 20:1.

Plasmalogens and aldehydes

The amount of plasmalogen and the aldehyde compositions of CPG and EPG were determined in eight individuals of different ages. The results are given in Table 2. After the age of 5 yr, the variation with age was rather small, and therefore the values for individuals older than 5 yr are given as means and standard deviations.

In EPG, the amount of plasmalogen seemed to reach a maximum value of 55% at birth, whereafter it decreased during the first year of life. In CPG there was an increase from 9% to 15% during the same period.

In both phosphoglycerides, 16:0 was the dominating aldehyde. The percentage amounts of the aldehydes 18:0 and 18:1 were much lower, particularly in CPG. Some additional aldehydes might have been present, but as the amounts were small, they were neither identified nor estimated.

DISCUSSION

Very few studies on the variation with age of the fatty acid composition of individual phosphoglycerides in human organs are available. Brain (2, 6), and now muscles, seem to be the only organs thoroughly studied. Less comprehensive investigations have been performed on some phosphoglycerides in erythrocytes and plasma (7, 8).

It has been shown by Svennerholm and Vanier (2) that the fatty acid composition of individual human brain phosphoglycerides varies with age. A general feature was that the percentage amount of the sum of the linoleic acid series decreased with increasing age in both cerebral cortex and white matter, while the sum of the linolenic acid series increased in cortex and decreased in white matter. These changes with age were not confined to the period before and soon after birth, but continued throughout life. There are large differences between the fatty acid pattern of the individual phosphoglycerides in adult brain and muscles, which makes direct comparison of the age variations less meaningful. Moreover, the changes in the fatty acid patterns of brain phosphoglycerides are in part related to the myelination, while no such morphological changes occur in muscles during the late prenatal or postnatal development.

Olegård and Svennerholm (7, 8) compared the fatty acid compositions of lecithin and cephalins in erythrocytes and of total phosphoglycerides in plasma from newborn infants and their mothers and from older infants. The mothers and their infants had identical concentrations of the total polyunsaturated fatty acids. The infants' fatty

TABLE 1. Fatty acid compositions of individual phosphoglycerides in skeletal muscles of human males aged 5–55 yr

Fatty Acid	Cardiolipin	CPG	EPG	IPG	SPG
			% (by wt) of total fatty acids[a]		
16:0	1.9 (0.9)	27.3 (1.4)	2.0 (0.6)	5.1 (1.8)	8.0 (3.5)
16:1	0.9 (0.4)	2.0 (0.7)	0.4 (0.4)	0.7 (0.5)	1.3 (1.2)
18:0	0.9 (0.5)	9.5 (1.7)	27.3 (1.8)	46.3 (2.9)	37.3 (1.4)
18:1	8.7 (2.2)	14.0 (2.3)	6.8 (1.4)	6.0 (1.8)	16.3 (1.8)
18:2(n − 6)	84.4 (3.4)	37.7 (2.8)	17.7 (2.5)	6.4 (2.5)	7.9 (1.6)
20:3(n − 6)	0.3 (0.2)	1.2 (0.3)	1.7 (0.3)	3.6 (0.7)	2.0 (0.6)
20:4(n − 6)	1.2 (0.4)	5.2 (1.0)	30.7 (2.5)	26.8 (3.6)	7.8 (1.6)
22:6(n − 3)		1.0 (0.5)	7.3 (2.0)	1.6 (0.8)	11.1 (3.0)
Σ18–22(n − 6)	85.8 (3.3)	44.2 (2.8)	51.4 (2.3)	37.7 (3.4)	20.9 (4.0)
Σ18–22(n − 3)		2.6 (1.0)	10.2 (2.2)	3.1 (1.5)	15.0 (3.6)
No. of individuals	8	12	9	8	6

[a] Mean (SD).

acid patterns differed from the mothers' by having much lower concentrations of 18:2 and 18:3 and correspondingly increased concentrations of the more unsaturated fatty acids of the two series. At the age of 3 months, the phosphoglycerides had already assumed a fatty acid pattern of adult type. In conformity with the phosphoglycerides in erythrocytes, at the age of 2–3 months the fatty acid pattern of the phosphoglycerides in muscles had assumed an adult pattern.

In a recent study, Roux, Takeda, and Grigorian (9) analyzed the fatty acid pattern of total phosphoglycerides, triglycerides, and cholesterol esters from some fetal organs of different ages during gestation. They concluded that fetal brain, liver, and lung show no evidence that the fatty acid composition of the phosphoglycerides varies markedly between the 16th and 32nd week of gestation. Nor did they find any differences in the fatty acid composition of the fetal adipose tissue and that of newborns. They therefore assumed that no fatty acid alterations occur in any fetal tissue during gestation. Observations made in the present investigation, however, refute this conclusion in that considerable alterations were found to occur in one of the largest pools of phosphoglycerides in the body, the skeletal muscles.

The fatty acid compositions of the individual phosphoglycerides other than CPG have never before been determined in intact adult human skeletal muscle. Takagi et al. (10, 11) determined the fatty acid composition of CPG in leg muscles and the fatty acid compositions of CPG, EPG, and IPG in sarcoplasmic reticulum. The fatty acid patterns of CPG, EPG, and IPG in the present study were in good agreement with the patterns of the same phosphoglycerides from sarcoplasmic reticulum.

The present study on the plasmalogen content of CPG and EPG and similar studies (12, 13) have shown that the percentage of plasmalogen in CPG increases during the fetal stage and first year of life, while the percentage in EPG changes only little with age. The variations in the amount of plasmalogen were not sufficient to explain the variation in the fatty acid composition of CPG or EPG.

While no major increases in the percentage amount of the sum of the linoleic acid series in human phospholipids

TABLE 2. Aldehyde composition and plasmalogen content of CPG and EPG of human skeletal muscles

	Age					
					5–60 yr, n = 4	
Aldehyde	Fetus, 24 wk	Full Term	6 wk	9 mo	Mean	SD
			Choline phosphoglyceride			
16:0[a]	76.2	90.7	88.2	79.7	84.7	1.4
18:0[a]	8.1	4.2	8.5	9.2	7.1	0.7
18:1[a]	15.7	5.2	3.1	11.2	8.2	0.9
% Plasmalogen[b]	7.7	9.2	8.6	14.4	14.8	1.7
			Ethanolamine phosphoglyceride			
16:0[a]	57.4	68.3	45.1	60.7	52.2	4.3
18:0[a]	22.4	19.6	36.0	25.9	29.7	3.9
18:1[a]	20.2	12.2	18.9	13.3	18.2	0.6
% Plasmalogen[b]	49.8	54.5	52.4	33.7	40.9	0.9

[a] Values are percentages (by wt) of total aldehydes.
[b] Percentage of plasmalogen in total CPG or EPG.

previously seem to have been found, such alterations in different phosphoglycerides of rat liver (14, 15) and different ovine organs (16) have been shown during development.

Jahn et al. (14) studied the fatty acid compositions of CPG, EPG, and IPG + SPG in livers from rat fetuses and their mothers. In all phosphoglycerides there was a slightly higher concentration of the sum of linoleic fatty acids in the maternal than in the fetal lipids. In a similar study, Winkler, Buanga, and Goetze (15) showed that in fetal livers linoleic acid constituted over 40% of the fatty acids in cardiolipin compared with almost 60% in the adult. In a comparison between the fatty acid compositions of total phosphoglycerides of some organs in fetal lambs and sheep, Scott, Setchell, and Bassett (16) found the greatest differences in the amount of the sum of linoleic acids in heart; the increase was slight in liver, and adult brain contained less linoleic acids than fetal brain.

One major implication of the studies in this series is that the CPG of skeletal muscles should be considered when fatty acid alterations are to be studied in humans. The lecithin in this tissue is one of the largest compartments of phosphoglycerides in the human body and thus contains one of the largest pools of essential fatty acids. There is a considerable increase in the magnitude of this pool during the fetal stage and first year of life. This is due to the increase in the percentage amount of linoleic acid but also to the growth of the muscles. During this period the fatty acid composition of skeletal muscle lecithin ought to be particularly sensitive to essential fatty acid deficiency. We have performed studies on rats fed diets with two different levels of essential fatty acids (17). It was shown that the fatty acid pattern of CPG from muscles was affected more than that from liver and serum. Further studies are now in progress to evaluate the vulnerability of rat skeletal muscles to essential fatty acid deficient diets.

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