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Studies on human fetal tissues-II. Lipid composition of human fetal tissues in relation to gestational age, fetal size and maternal nutritional status

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When lipids of different tissues were compared for the period 16-24 weeks of gestation, the liver has the highest concentration of all the lipid components studied. The concentrations in the small intestine were close to that of the liver in the case of the neutral lipids. The lung and heart were found to have comparable concentrations of various lipids. Fetal growth retardation seems to be associated with a higher concentration of cholesterol in the liver, the lung and small intestine. Lower values for phospholipid concentration and phospholipid to cholesterol ratio were observed in the lung, heart and small intestine with such retardation. These studies suggest the delayed maturation of these tissues, as these lipids play an important role in the maintenance of cellular integrity, structure and function of plasma membrane as well as subcellular membranes.

Key words : Human fetal tissues; lipid composition.

During embryogenesis the predominant processes are cell differentiation and organogenesis. This is followed by increase in cell number and cell size in that order.[1] The increase in cell size is associated with changes in morphological features as well as chemical composition. The integrity of cells and tissues as well as their normal functioning depend on a normal course of biochemical and morphological maturation. One of the critical factors in the maintenance of cellular integrity and function is the structure and function of the plasma membrane as well as subcellular membranes in which lipids, especially, phospholipids, and cholesterol play an important role. Besides these, lipids play a vital role in various functions and one of them is as lung surfactants.

Previous studies in this laboratory on experimental animals have demonstrated that the biochemical maturation with regard to lipids of tissues such as the lung,[2] small intestine[3] and the brain[4] is affected by maternal nutritional deprivation. It is also found that the fetally growth retarded infant exhibits features such as poor pulmonary function.[5,6]

These observations prompted the present investigations which were designed

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to understand the pattern of maturation in selected tissues with regard to lipids.

Materials and Methods

The subjects hailed from both low (99) and high income groups (31). Further description of the low income and high income group families is given in detail elsewhere.[30] Differences in the dietary intake and biochemical status of pregnant women between the two groups have been documented earlier.[7]

The method of collection of fetuses, and assessment of gestational age are given in a previous paper.[30]

The fetuses were kept on ice as soon as they were aborted, transported to the laboratory and kept in the frozen state before dissection and analysis. The tissues studied were the liver, lung, heart and small intestine. The lipids studied were total lipids, phospholipids and cholesterol. Glycerides were calculated as the difference between total lipids and the sum of the other two components as they form quantitatively the major constituents other than phospholipids.

Lipids from the tissues were extracted by the method of Folch et al.[8] Total lipid was estimated gravimetrically according to the method given by AOAC.[9] Phospholipids was estimated by the method of Bartlett[10] and cholesterol was determined by the method of Bowman and Wolf.[11]

All the methods were checked for reliability, using varying concentrations of standards. The reproducibility of the method was ascertained initially by determinations on multiple aliquots of the same sample.

For assessment of mean differences between groups, student's 't' test was used.[12]

Results

As no social class differences were found with regard to lipid concentrations at different stages of gestation, the values for the two groups were combined accordingly.

The lipid composition (mg/g) of different tissues is shown in Tables I to IV. In the case of the liver (Table I), the data suggest a tendency for phospholipid concentration to rise in midpregnancy and show a decline thereafter. Cholesterol concentration remained more or less unchanged throughout the period of gestation. Similarly, no change was found in phospholipid to cholesterol ratio. In the case of total lipids and glycerides, the data suggest a tendency to decline with the progress of gestation, although these differences are not significant.

In the case of the lung (Table II), a significant increase in total lipids towards term is associated with an increase in phospholipids as well as cholesterol. However, a further rise in cholesterol was not seen after midpregnancy. Glycerides registered a gradual decline with the progress of gestation. Phospholipid to cholesterol ratio was found to be maximum towards term after registering a slight decline around midpregnancy.

In the case of heart (Table III), a significant increase in total lipids towards term is associated with an increase in glycerides as well as cholesterol. Phospholipid to cholesterol ratio was relatively high up to 24 weeks and registered a decline thereafter.

In the case of the small intestine (Table IV), a decrease in phospholipid values after mid pregnancy is associated with an increase in cholesterol. Glycerides

Table I. Lipid composition of fetal liver in relation to gestational age. Values expressed as mg/g liver; Mean±SE and range in parantheses

Parameter	Weeks of gestation				
	<16	16—20	20—24	24—32	>32
No. of subjects	28	50	25	20	5
Total lipids	26·3±0·93 (18·5—35·0)	26·5±0·63 (19·1—38·9)	26·4±0·74 (20·6—32·8)	25·4±1·04 (19·6—33·8)	25·0±1·54 (22·4—30·7)
Phospholipids (P)	11·8±0·58 (6·6—18·4)	12·6±0·59 (8·2—18·1)	12·3±0·43 (8·1—16·6)	12·0±0·51 (7·7—16·5)	11·8±0·43 (11·0—13·4)
Cholesterol (C)	4·2±0·21 (1·9—7·0)	4·4±0·21 (2·2—8·9)	4·4±0·28 (2·3—8·4)	4·2±0·18 (2·9—5·8)	4·3±0·33 (3·3—5·2)
Glycerides*	10·4±0·45 (6·7—14·0)	9·8±0·44 (4·0—18·3)	9·7±0·40 (7·3—14·0)	9·2±0·58 (4·6—16·3)	8·8±1·21 (5·6—12·5)
P/C ratio	2·9±0·20 (1·1—4·8)	3·1±0·21 (1·4—5·8)	2·9±0·15 (1·6—4·7)	2·9±0·14 (1·6—3·7)	2·8±0·23 (2·2—3·6)

*Calculated as difference between total lipids and the sum of other two lipids

Table II. Lipid composition of fetal lung in relation to gestational age. Values expressed as mg/g lung. Mean±SE and range in parantheses

Parameter	Weeks of gestation				
	<16	16—20	20—24	24—32	>32
No. of subjects	25	45	22	15	3
Total lipids	18·5±0·43 (14·6—23·5)	19·3±0·47 (14·4—26·7)	19·8±0·47 (16·8—24·2)	21·4±0·49** (17·7—24·8)	22·5±0·44** (21·6—23·0)
Phospholipids (P)	8·2±0·30 (5·8—12·2)	9·3±0·25 (5·4—13·4)	9·5±0·51 (5·4—13·9)	11·6±0·61** (8·1—16·5)	13·2±0·42** (12·6—14·0)
Cholesterol (C)	2·6±0·20 (1·3—6·0)	3·0±0·16 (1·4—6·3)	3·3±0·24* (1·9—6·5)	3·2±0·17* (1·8—5·0)	3·2±0·49 (2·3—4·0)
Glycerides*	7·4±0·35 (4·3—11·9)	6·9±0·32 (2·7—13·5)	7·0±0·38 (4·5—11·5)	6·6±0·58 (4·0—11·7)	6·1±0·96 (4·6—7·9)
P/C ratio	3·5±0·23 (1·2—5·3)	3·4±0·17 (1·2—6·5)	3·2±0·30 (1·1—5·8)	3·7±0·27 (2·1—5·9)	4·4±0·66 (3·2—5·5)

*Calculated as difference between total lipids and the sum of other two lipids; Values significantly different from <16 weeks of gestation, *$P<0·05$ and **$P<0·001$

TableIII. Lipid composition of fetal heart in relation to gestational age. Values expressed as mg/g heart. Mean±SE and range in parantheses

Parameters	Weeks of gestation				
	<16	16—20	20—24	24—32	>32
No. of subjects	23	40	19	13	2
Total lipids	18·4±0·55 (15·0—24·3)	19·6±0·51 (15·0—28·1)	19·6±0·68 (13·6—24·1)	20·2±0·42* (17·7—23·0)	20·8±0·85* (20·0, 21·7)
Phospholipids (P)	10·0±0·43 (7·0—14·8)	10·7±0·38 (7·4—15·0)	10·3±0·48 (5·1—14·8)	9·7±0·45 (7·3—13·0)	8·9±1·30 (7·6, 10·2)
Cholesterol (C)	2·7±0·20 (1·5—6·3)	2·7±0·15 (1·4—6·1)	2·7±0·15 (1·9—3·8)	3·1±0·21 (1·8—4·3)	3·4±0·55 (2·9, 4·0)
Glycerides*	5·6±0·35 (3·0—8·5)	6·2±0·34 (3·0—11·9)	6·5±0·40 (3·2—9·3)	7·5±0·29** (5·8—9·0)	8·5±1·00* (7·5, 9·5)
P/C ratio	3·9±0·26 (1·5—6·9)	3·9±0·17 (1·6—7·0)	3·9±0·22 (2·1—5·9)	3·2±0·20* (2·3—4·4)	2·7±0·10** (2·6, 2·8)

*Calculated as difference between total lipids and the sum of other two lipids. Values significantly different from <16 weeks of gestation values, *$P<0·05$ and **$P<0·001$

Table IV. Lipid composition of fetal small intestine in relation to gestational age. Values expressed as mg/g small intestine; Mean±SE and range in parantheses

Parameter	Weeks of gestation				
	<16	16—20	20—24	24—32	>32
No. of subjects	28	50	25	20	5
Total lipids	22·3±0·94 (16·1—36·7)	21·8±0·42 (15·7—27·0)	21·4±0·56 (15·2—26·0)	21·7±0·75 (16·5—29·3)	22·0±1·40 (18·5—27·1)
Phospholipids (P)	8·7±0·40 (5·0—15·5)	8·7±0·31 (4·0—13·8)	8·3±0·41 (5·2—12·2)	8·1±0·34 (6·4—12·7)	8·0±0·44 (6·8—9·2)
Cholesterol (C)	3·6±0·25 (2·0—7·5)	3·6±0·18 (2·0—7·5)	3·8±0·24 (2·2—6·9)	3·8±0·23 (2·5—6·2)	4·0±0·59 (2·9—6·2)
Glycerides*	9·9±0·61 (4·4—14·8)	9·4±0·30 (4·6—14·3)	9·4±0·43 (5·9—13·1)	9·7±0·43 (5·3—13·2)	10·0±0·84 (8·2—13·0)
P/C ratio	2·6±0·17 (0·8—5·1)	2·6±0·13 (0·7—4·4)	2·4±0·20 (0·8—4·8)	2·3±0·12 (1·3—3·1)	2·2±0·23 (1·3—2·7)

*Calculated as difference between total lipids and the sum of other two lipids

registered a gradual decline upto 20-24 weeks and increased thereafter. A similar pattern was also observed with regard to total lipids. The ratio of phospholipid to cholesterol showed a decline after mid-pregnancy.

As fetal growth is found to be associated with tissue growth with the progress of gestation, lipid components in various tissues were also expressed per whole tissue (mg/tissue) (Fig. 1). As expected, in all the tissues, the lipid components were found to increase with the progress of gestation. However, a sharp increase in all the lipids was seen first after 16 weeks of gestation, with a continuous increase till 24-32 weeks and showing a sudden rise thereafter.

Tissues differing in structure and function not only differ in the concentration of lipids but also in the distribution of different lipids. The composition of lipids in different tissues is compared for the period 16-24 weeks of gestation (Fig. 2). The liver has the highest concentration of all the lipid components studied. Glyceride concentration in the small intestine was close to that in the liver. The lung and heart seem to have comparable concentrations of various lipids.

Fetal weights were calculated as per cent of expected weight for gestational age according to Widdowson's norms[13], using a cut off point of 60%, the fetuses were classified as 'growth-normal' and 'growth-retarded'. No social class differences in 'growth-normal' fetuses were found with regard to lipid concentrations in different tissues. The overall lipid pattern in 'growth normal' fetuses, in the low and high income groups were comparable. The concentrations of these lipids in 'growth-retarded' fetuses as per cent of 'growth-normal' values (LIG+HIG) are shown in Fig. 3. In the case of the liver, higher values for cholesterol, lower values for

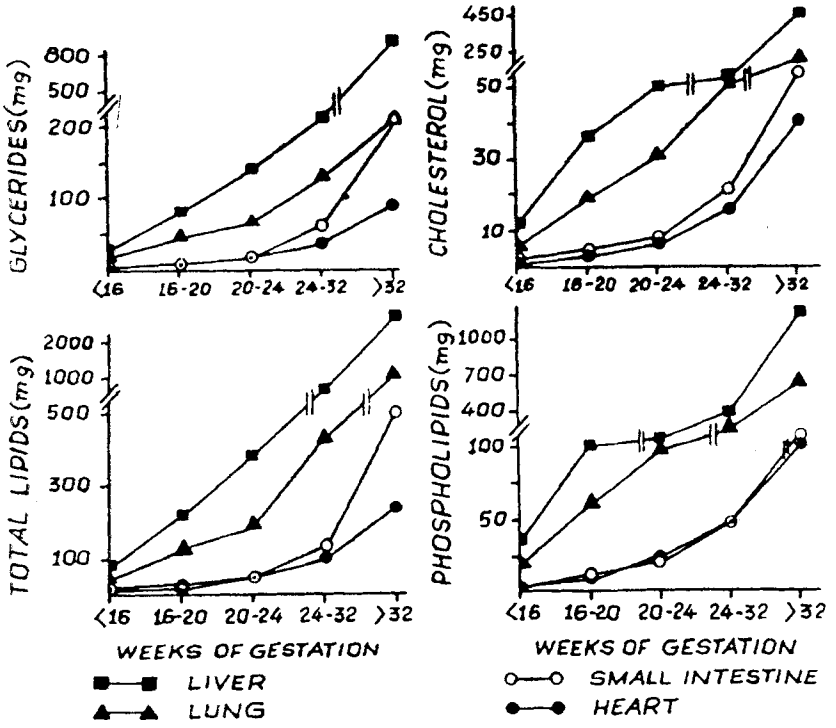

Fig. 1. Lipid content of different tissues in relation to gestational age.

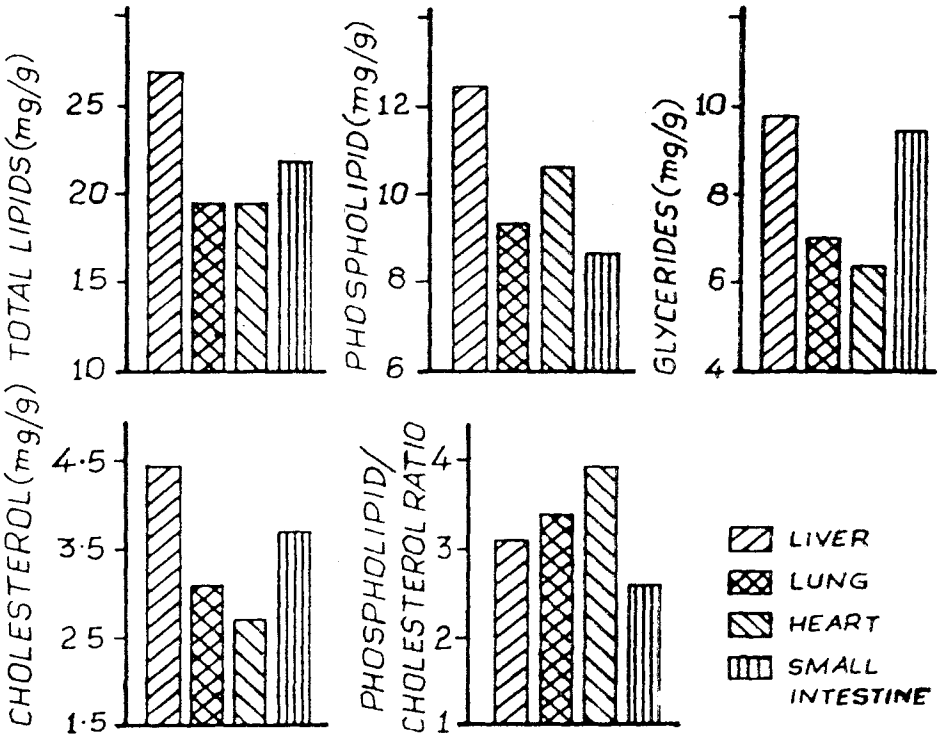

Fig. 2. Comparative data on the lipid composition of fetal tissues during 16-24 weeks of gestation

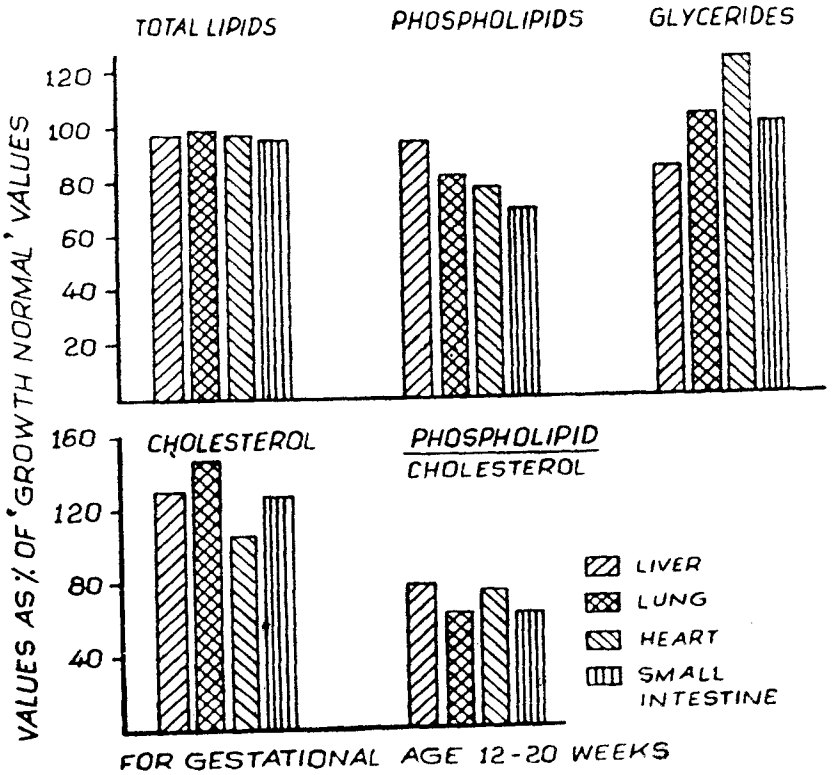

Fig. 3. Concentration of tissue lipids in 'growth retarded' fetuses as per cent of 'growth normal' fetuses.

glycerides and phospholipid to cholesterol ratio were found in 'growth retarded' fetuses. In the case of the lung, with such retardation, higher values or cholesterol and lower values for phospholipids, and phospholipid to cholesterol ratio were found, whereas in the heart, phospholipids and phospholipid to cholesterol ratio were found to decrease. In the case of small intestine, a significant decrease in total lipids, phospholipids and phospholipid to cholesterol ratio were found. However, here also, a significant increase in cholesterol was noted.

Discussion

Very few studies are available in the literature with regard to lipid composition in human fetal tissues, specially the heart and small intestine. The few reports available on other tissues are not concerned with changes in tissue lipid concentration with the progress of gestation. The values for total lipid concentration in the liver, lung and heart are in the range of those reported by other.[14-16] Similarly, concentration of phospholipids in the liver and lung are also in the range of those values reported by Roux and Yoshioka,[17] Roux et al[18] and Coltart.[16] Watanabe[19] reported a significant increase in triglyceride content after 32 weeks of gestation in the case of liver.

The comparatively higher values for glycerides in the liver and small intestine are consistent with observations on experimental animals.[3,20] In both the liver and small intestine, glycerides serve as precursors of lipoproteins, specially chylomicrons[21] and may also serve as metabolic precursors for the synthesis of other lipids.[22] The liver also shares this function with regard to phospholipids and cholesterol.

Among the tissues studied, the small intestine had the least phospholipid to cholesterol ratio. This low ratio is believed to be important to withstand the higher osmotic pressure to which the microvilli are subjected from the external environment in the intestinal lumen.[23]

The decreased levels of phospholipid concentration in the lung, heart and small intestine found in 'growth retarded' fetuses may reflect changes in membrane phospholipids. Increased levels of cholesterol in the liver, lung and small intestine suggest either an increase in the synthesis of cholesterol or a slower rate of its removal.

The finding that phospholipid concentration in the lung of 'growth-retarded' fetuses is low is consistent with observations on the ratio of lecithin to sphingomyelin (L/S) on amniotic fluid which is found to correlate with gestational age and maturation of the fetal lung so that this ratio has been considered as an index of maturation of this organ.[24,25] It may be pointed out that changes in lecithin would be reflected in total phospholipids. Scarpelli[26] demonstrated that amniotic fluid had surface tension similar to that of the lung which is related to phospholipid content. Biezenski[27] reported that phospholipid levels in amniotic fluid were found to be lower in toxemias of pregnancies. In this connection, Nelson[28] reported a similar decrease in abnormal pregnancies associated with respiratory distress syndrome and encephaly with polyhydraminos.

Previous studies in this laboratory on the rat small intestine suggest that maternal protein deficiency during gestation and lactation as well as postweaning protein deficiency result in decreased concentrations of total lipids, phospholipids and

glycerides whereas no change was found in the case of cholesterol. However, an increase in the concentration of cholesterol and decrease in the concentration of glycerides was found during prenatal and neonatal vitamin A deficiency.[3]

Southgate and Hey[16] found 'growth retarded' fetuses to contain less deep body fat and subcutaneous fat with a greater deficit in the former. Similarly, poor stores of glycogen in the liver, heart and skeletal muscle were found in infants dying from respiratory distress syndrome.[29]

In conclusion, these findings suggest that fetal growth retardation seems to be associated with a higher concentration of cholesterol in the liver, the lung and small intestine. Lower values for phospholipid concentration and phospholipid to cholesterol ratio were observed in the lung, heart and small intestine with such retardation. These studies suggest the delayed maturation of these tissues, as these lipids play an important role in the maintenance of cellular integrity, structure and function of the plasma membrane as well as subcellular membranes.

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