Effects of the diet consumed during the pregnancy on the lipids content in maternal and fetal rat lungs

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This study was designed to determine if the quantity of lipids in the diet fed to pregnant rats would affect the deposition of fat in the fetal lung. Wistar rats were fed with two different diets during pregnancy: Standard Diet (StD; 4.000 cal/g) and High Fat Carbohydrate Free Diet (HFCFD; 6.000 cal/g). The rats consumed daily the same amount of calories from these different diets. The concentrations of triglycerides (TG), phospholipids (PL), total, esterified and free cholesterol (TC, EC and FC, respectively) were determined in serum and lung from pregnant rats as well as from their 19 day old fetuses. In the serum of rats fed with HFCFD, the cholesterol concentration increased in relation to that of rats fed with StD. In pregnant rat lung, the PL concentrations decreased and the TC, EC and FC concentrations increased with HFCFD in relation to StD. The triglycerides were not modified in any case. The lipidic composition of the sera and fetal lung were not changed by the two diets consumed by pregnant rats. This may be a biological protective mechanism to assure an adequate synthesis of alveolar surfactant.

INTRODUCTION

The lung maturation has considerable importance for the neonate health (20). However, there are several reports on the effects of the maternal diet composition fed during pregnancy on the lipidic composition of the maternal and fetal lungs. Thus, it has been observed that the ingestion of fish oils during pregnancy improves fetal lung maturation (6). On the contrary, starvation (24), hypocaloric diets (4) and essential fatty acids (21) alter the lipidic composition of the lungs, but their functional capacity is unaffected. Otherwise, there is resistant lung fatty acid synthesis to inhibition by dietary fat in the meal (7).

In the present study we searched for the changes in triglycerides, cholesterol, phospholipids and protein concentrations in maternal and fetal lungs when the pregnant rats consumed a high fat carbohydrate free diet (HFCFD) instead of a standard diet (StD), in order to determine if the quantity of fat in the diet could affect the lipidic composition of the maternal and fetal lungs.

MATERIAL AND METHODS

Chemicals. Standards for thin layer chromatography and bovine serum albumin were obtained from Sigma (St. Louis, Mo). All chemicals were of the highest grade of purity available.

Diets: Two different diets were used: standard diet and high fat diet. Their compositions are shown in Table I.

Animals and feeding procedures: Adult Wistar female rats weighing 200-220 g were used. They were housed in an animal room with 10:14 h dark: light periods and controlled temperature (22-24° C). Rats were mated overnight and the presence of sperm in vaginal smears was designated as day 1 of pregnancy. The rats were separated into two lots and fed with the different diets. The rats were housed in individual cages from day 1 throughout 19th day of pregnancy. The rats consumed approximately about 12 g of standard diet and 9 g of high fat diet daily.

Tissue collection and analysis. Rats were killed under light anesthesia with diethyl ether between 9 and 11 hours of the 19th day of

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TABLE I

Diet composition consumed by rats

Ingredients	StD	HFCFD
Caseine	25.4	34.72
Dextrine	47.2	-
Lard	19.0	49.4
Corn Oil	1.9	8.12
Methionine	0.63	0.75
Mineral mix*	5.1	5.63
Vitamin premix**	0.75	0.75
Vitamins A, D, E (mg)	7.70	9.32
Choline Chloride	0.13	0.15
Calories/kg	4.840	6.670

Values expressed as %, except when indicated.
Both diets prepared in our laboratory.
* Gimenez *et al.* (11).
** Draper *et al.* (9).

pregnancy. Lungs were excised, trimmed free of all connective and tracheal tissues, washed with Na Cl 0.9%, dried with paper, weighed and placed into an ice-cold saline solution. Fetuses were quickly removed from uterus and weighed. Fetal lungs were separated, weighed and placed into an ice saline solution. The pregnant rat lungs were pooled and chopped; 250 mg samples were taken and lipids were extracted according to Folch *et al.* (10). The lungs from pairs of fetuses were pooled and processed in the same way. Dry lipidic extracts were resuspended in chloroform: methanol (2:1 v/v). One aliquot was used for total cholesterol and phospholipids determinations and another one was applied to silica gel G (Merck, Darmstadt, Germany) and developed in hexane: diethyl ether: acetic acid (80:20:1 v:v) as solvent for development. Lipids were detected by exposing the plate to iodine vapors. They were scraped off and used directly for determinations of PL (3), EC (31), TC (1) and TG (27). The recovery of TC and PL from thin layer chromatography was above 95%. After lipid extraction from the tissue, pellets were redissolved in NaOH 3 N at 56° C overnight and proteins were determined by Biuret methods (19) using fraction V of bovine serum albumin as standard. Serum lipids were determined using the assay kit from Wiener Laboratory (Rosario, Argentina).

Statistical methods. Results are presented as means ± SD. Significance of data was determined by using unpaired Student's "t" tests.

RESULTS

The results obtained showed that the HFCFD increased the cholesterol concentration in the serum of pregnant rats. No changes were observed in the lipidic composition of fetal serum (Table 2).

The HFCFD consumed during pregnancy modified the lipidic composition of the maternal lungs (Table 3).

The HFCFD increased the cholesterol and decreased the phospholipid concentrations in the pregnant rat lungs, without associated changes in triglycerides and protein contents. No changes in the lipidic concentration in the lungs of the fetuses were observed.

DISCUSSION

We observed that the lipidic content of the diet modified the amount of lipids in the pregnant lungs, but no effect was found on the lipidic concentrations in fetal lungs.

Although the amount of diets with which the rats were fed were similar in energy quantity, the different diet compositions were reflected in the serum lipidic concentrations. The hypercholesterolemic serum observed in

TABLE II

Effects of different diets fed during pregnancy on the lipids in maternal and fetal sera.

	StD	HFCFD
Maternal serum:		
Triglycerides (10)	1.52 ± 0.5	1.52 ± 0.3
Cholesterol (13)	1.00 ± 0.1	2.11 ± 0.5*
Fetal serum:		
Triglycerides (7)	0.71 ± 0.1	0.68 ± 0.1
Cholesterol (6)	0.82 ± 0.1	0.80 ± 0.1

Values expressed in mg/dl.
Means ± SD. Numbers in parentheses, pregnant rats and pools of 5 fetuses in each diet group.
* $P < 0.001$.

TABLE III

Effect of the different diets fed during pregnancy on the lipidic composition of pregnant and fetal rat lungs

	StD	HFCFD
Maternal lung:		
Triglycerides	281 ± 80	276 ± 14
Phospholipids	13 ± 20	5 ± 20*
Total Cholesterol	695 ± 86	1266 ± 32*
Esterified Cholesterol	62 ± 19	351 ± 48*
Free Cholesterol	600 ± 82	851 ± 23*
Proteins	94 ± 30	98 ± 30
Fetal lung:		
Triglycerides	253 ± 52	214 ± 48
Phospholipids	2 ± 0.1	2 ± 0.1
Total Cholesterol	205 ± 68	216 ± 89
Esterified Cholesterol	23 ± 6	22 ± 41
Free Cholesterol	159 ± 40	170 ± 46
Proteins	33 ± 9	24 ± 6

Values expressed in μg/g tissue, except for phospholipids (μmol P/g tissue).
Means ±SD. Number of pregnant rats = 14 for each diet. Number of fetuses = 14 pooled samples of two fetuses each, for each diet.
* $P < 0.001$.

rat fed with HFCFD could be a consequence of the greater amount of fat content in the diet. On the other hand, the HFCFD is hyperproteic in relation to the StD. It is known that the high casein diet has hypercholesterolemic effects (18). It is also known that the fatty acid fat diets provoke changes in the lipidic composition of different tissues (16, 22). The high cholesterol present in the lungs of pregnant rats fed with the HFCFD could be consequence of two factors: i) most of the cholesterol is taken up from circulating low-density lipoproteins or high-density lipoproteins (14); ii) increased synthesis in lung from precursor substrates. The lung is capable of endogenous cholesterol synthesis and can use palmitate as substrate (23). Cholesterol represents over 50% of the neutral lipids of both total surfactant and lamellar body fractions (13). Cholesterol and proteins may play an important role in the dispersion of the surfactant over the alveolar air-liquid interface and enhance the surfactant stability with time (28).

The lower phospholipid content in the lungs of pregnant rats fed with HFCFD cannot be easily explained. It could be consequence of the decreased availability of precursors for their synthesis. The HFCFD lacks carbohydrates and the fatty acids are the major energy source. This diet probably decreases the amount of glycerol-3-phosphate necessary for phospholipid synthesis (26), although there is availability of CDP-choline-cytidyltransferase activated by fatty acids (2, 5). Otherwise, the lower phospholipid content in homogenate lungs from pregnant rats fed with HFCFD does not imply necessarily a decrease in the dipalmitoylphosphatidylcholine synthesis in alveolar type II cells.

The decrease of the phospholipids may affect approximately 40 different types of cells of the lung (29). For this reason these results could not reflect what happens with the synthesis of dipalmitoylphosphatidylcholine (DPPC), the major component of the synthesized surfactant in alveolar type II cells. Besides, only 30% of DPPC is synthesized in alveolar type II cells (30). The lipidic composition of the lungs and sera of fetuses was not modified by the different diets consumed by the pregnant rats. Similar results have been observed in the fetal liver after feeding pregnant rats with diets containing different amounts of fatty acids (12). However, a high polyunsaturated diet increases their HMGCoA reductase in maternal and fetal livers (8). It is known that maternal fatty acids can cross the placenta and may contribute up to 50% of fetal fatty acids during late gestation in the rat (17).

The fetuses may take the necessary fatty acids needed and return all excesses to the maternal circulation (25). Otherwise, the continuous passage of glucose from mother to fetus makes unnecessary the gluconeogenesis in the fetal liver. In this organ, there is an extremely low activity of certain gluconeogenic enzymes and the metabolic control mechanism in the fetus may be different from that of the maternal tissues (15).

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