REVISTA ESPAÑOLA DE FISIOLOGIA, 38, 201-208. 1982

Lipid Metabolism in Pregnancy. Perfusion of Liver in Pregnant Rats

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(Received on October 13, 1981)

R. M. ARAHUETES, A. FRAILE and A. SUAREZ. *Lipid Metabolism in Pregnancy. Perfusion of Liver in Pregnant Rats.* Rev. esp. Fisiol., 38, 201-208. 1982.

Livers of pregnant or nonpregnant rats were perfused, and incorporation of ^{14}C-acetate into lipids was studied.

Total lipids were extracted from samples of perfusion medium, taken at the entrance and exit from the liver, at different time intervals, and from the liver homogenates at the end of the experiment.

Time-course of the incorporation of ^{14}C-acetate into different lipid classes from perfusion medium and liver tissue was compared for pregnant and nonpregnant rats.

Radioactivity incorporation into circulant FFA and TG was higher in the control rats than in the pregnant animals. Contrariwise, there was a high incorporation of labelled fatty acids into phosphoglycerides in pregnant rats.

There was an increase of unsaturated fatty acids of large carbon chain (24 : 1 and 22 : 6) in the perfusion media of pregnant liver. Moreover, the percentages of labelled fatty acids 16:1, 18:1 and 18:0 were higher in pregnant rats than in controls.

Pregnancy is characterized by notable changes of the lipid metabolism; however, the variation of the plasma lipid levels and the mechanisms whereby it is achieved will be different according to the animal species. Third trimester human pregnancy is characterized by a 2-3 fold increase in plasma triacylglycerols and lesser increases in cholesterol and phosphoglycerides (16, 20) in contrast to other species including rabbit (14). Hypertriglyceridemia of late gestation occurs in humans after an overnight fast (22, 34) and in rats in the fed (10, 21, 26, 29, 30) and fasted (5, 29) states. These changes originate a pathological situation in human massive hypertriglyceridemia in pregnancy (25).

On the other hand, high lipid levels have been found in newborns of different species including man (37). The origin of these lipids is not yet clear; some fetal tissues, as liver and adipose tissue, exhibit a high lipogenic activity to fulfill the needs of the fetus during some periods of fetal development (1, 8, 17-19, 27, 28,

31-33). Also, during the second half of the pregnancy, the maternal increase of the plasma lipids might be expected to account for the storage of feto-placental lipids (4, 6, 9, 12, 15, 23, 29); the placenta had to carry out the metabolic interconversions to supply metabolites to its own component cells and on behalf of the fetus. Different authors (2, 7, 13) have attempted to evaluate this metabolic contribution.

This study was undertaken to obtain some new data on the lipid metabolism during gestation, by perfusion of liver in pregnant rats.

Materials and Methods

Materials. Sodium (1-^{14}C) acetate (sp. act. 60 mCi/mmol) was purchased from The Radiochemical Centre (Amersham). Bovine serum albumin, fraction V, Triton X-100 and scintillation reagents were supplied by Sigma. All solvents and reagents were from analytical grade.

Animals. Wistar female rats weighing 150-200 g at mating were used. Day 1 of gestation refers to the day after the night of breeding. Positive pregnancy was tested through vaginal frotis.

Rats of a similar weight range (± 10 g) were used as nonpregnant controls. Rats were allowed *ad libitum* access to food and water until the time of sacrifice. Food consisting of Purina rat chow contained 5% fat (w/w).

Rats were anesthetized by intraperitoneal injection of nembutal (0.15 ml of 6% solution per 100 g body weight). Then 100 units of heparin were injected into the saphenous vein.

Perfusion of the liver. Livers of either pregnant or nonpregnant rats were perfused *in situ* according to the method and design of HEMS *et al* (11). The perfusion medium passes the liver through the portal vein and leaves it through the vena cava, and drops into a collecting vessel; from here the medium is pumped to the top of a multibulb oxygenator (water saturated 95% O_2 plus 5% CO_2) and then returned to the reservoir. All operations were performed in a perfusion cabinet able to carry out two simultaneous liver perfusions and thermostatized at 35 ± 1° C. The perfusion medium consisted of aged human red cells (stored 30 days at 4° C), bovine serum albumin (Fraction V) and physiological saline of Krebs and Henseleit (pH 7.4, haemoglobin 2.5 g/100 ml and albumin 2.6 g/100 ml). Perfusion pressure was 20-30 cm H_2O and flow 15-20 ml/min for a total volume of 150 ml. Once the liver preparation was connected to the perfusion apparatus, the circulation was maintained during 40 min, and at the 38 min the labelled sodium acetate was added to the collecting vessel according to the experiment. 5 ml perfusion medium samples were taken at the entrance (E) into the liver and at the exit (S) from the liver at different time intervals (5, 10, 15, 20 and 50 min). At the end of the perfusion experiments, livers were homogenized with cold 8.5 sucrose solution and the homogenates were used for analytical lipid determinations.

Lipid extraction and analyses. Extraction of lipids was carried out by the method of BLIGH and DYER (3) with chloroform-methanol. A chloroform solution (10 mg/0.1 ml) was prepared with the total lipids previously obtained and 50 µl of the solution was used for separating classes of lipids by thin-layer chromatography. Separation was done on Silica Gel G plates, 300 µm, in hexane-ether-acetic acid (70:30:1, v/v/v) as solvent system. The bands were detected by a brief exposure to iodine vapour. Iodine was allowed to evaporate before removal of the bands. Thin-layer bands were scraped off the plate and either directly transferred to scintillation vials for coun-

ting or submitted to methanolysis. Fatty acid methyl esters were obtained from either total lipids or Silica Gel bands after separating lipid classes. Boron trifluoride (24) in methanol (25 %, w/w) was used as methanolysis reagent in sealed tubes at 100-110° C for 90 min. Fatty acid methyl esters were extracted with pentane, washed with water and dried over sodium sulphate. Chromatographic separation of methyl esters was carried out with a Perkin-Elmer model F20FE flame ionization instrument equipped with a stainless steel column (6 feed 1/8 inch) packed with 10 % EGS on Chromosorb W (60-80 mesh) and a carrier gas (He) flow of 18.3 ml/min at 180° C. Methyl ester radioactivity was determined by means of a Perkin-Elmer model RGC 170 flow-through reactor/proportional counter connected to the above gas-liquid chromatographic unit through a stream splitting device (split ratio 16:1). The main effluent stream was driven into the flow-through reactor where oxidising degradation of methyl esters to CO_2 and water took place; the latter was removed with the aid of a drying-tube at the end of which methane was continuously fed through a T-piece to give a He/CH_4 ration of 1/3. The counting tube had an active volume of 10 ml and was operated at 2,700 V. Working temperatures were: proportional counting tube, 100° C; reactor furnace, 620° C; connection tube between stream-splitter and furnace, 200° C. Relative activities were determined by dividing the number of counts under a given peak by the average residence time of components within the active volume of the proportional detector and then correcting for background. Specific activities are conveniently expressed as counts/min per unit area under each mass peak.

Results

The mean values of animal and liver weights, as well as of the total lipids either per whole liver or per gram of tissue from control and pregnant rats, were compared as shown in table I. The amount of total lipids per whole pregnant liver was about 50 per cent greater than that of whole control liver.

Following ^{14}C-acetate addition to the perfusion medium, the time-course of the lipid concentration in the medium was measured simultaneously at both the entrance (E) into the liver and at the exit (S) from the organ in series of experiments carried out with pregnant and control animals (table II). Lipid concentration values in the perfusion media did not change with time in pregnant rats, although they were slightly higher than those obtained in the time-course experiments using control animals.

The time-course of the ^{14}C-acetate incorporation into the most important lipid classes of the perfusion media of the liver, from either control or pregnant rats, is given in tables III and IV, respectively. The isotope incorporation was measured with time in the free fatty acids, diacyl-

Table I. *Mean values ± SD of rat and liver weights for control (n = 15) and pregnant (n = 11) animals. Total lipids of the liver are given (mg) as well as the lipids (mg) per gram of hepatic tissue.*

Animal	Rat weight	Liver weight	Lipids/liver	Lipids/tissue g
Control	236 ± 22	9.0 ± 1.6	285.1 ± 45.2	32.1 ± 5.6
Pregnant	344 ± 41	11.7 ± 1.6	432.5 ± 53.0	37.2 ± 3.4

Table II. *Changes in the concentration of total lipids (mg of total lipids/5 ml of perfusion medium ± SD) in the perfusion media at the entrance (E) and the exit (S) from the liver.*

Animals		Time-course (min) 5	10	15	20	50
Control	E	1.16 ± 0.33	1.20 ± 0.20	1.20 ± 0.20	1.42 ± 0.27	1.55 ± 0.25
	S	1.35 ± 0.48	1.12 ± 0.10	1.14 ± 0.15	1.20 ± 0.14	1.77 ± 0.36
Pregnant	E	1.77 ± 0.38	1.70 ± 0.42	1.65 ± 0.20	1.70 ± 0.42	1.80 ± 0.15
	S	1.77 ± 0.18	1.95 ± 0.07	1.45 ± 0.49	1.70 ± 0.14	1.71 ± 0.33

glycerols, triacylglycerols, total phosphoglycerides and cholesterol, isolated from the total lipids extracted of the perfusion media at the entrance (E) into the liver and at the exit (S) from the organ. The isotope incorporation was also determined in the lipid classes isolated from the hepatic tissue at the end of the experiments (tables III and IV).

Figure 1 shows the patterns of the fatty acid composition of total lipids from the perfusion media of the liver at the 50 min interval of both control and pregnant rats.

At last, labelling of fatty acids from ^{14}C-acetate in the total lipids from perfusion media of control and pregnant animals was examined by radiogaschromatography; both patterns are given in figure 2.

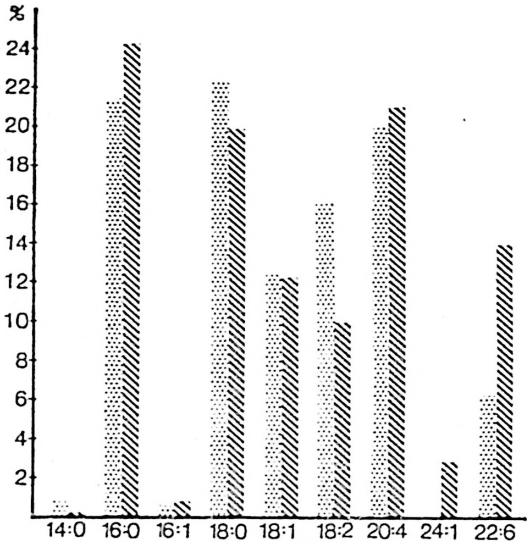

Fig. 1. *Fatty acid composition of total lipids from the perfusion media of control rat liver (pointed bars) and pregnant rat liver (striped bars).*

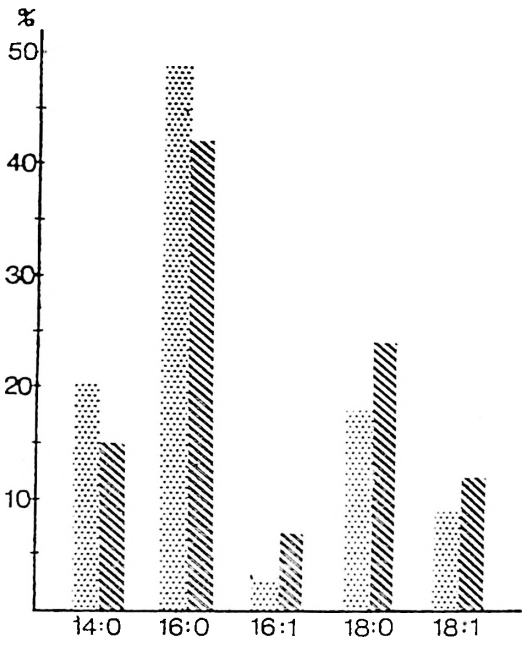

Fig. 2. *Fatty acid patterns, synthesized from (^{14}C) acetate, obtained by radio gas-liquid chromatography from lipids of the perfusion media of control rat liver (pointed bars) and pregnant rat liver (striped bars).*

Table. III. *Time-course of the incorporation of (^{14}C) acetate into different lipid classes (dpm/0.5 mg of total lipids ± SD) from liver perfusion media and liver tissue of control rats (n = 15). Samples were taken at the entrance (E) into the liver and at the exit (S) from the liver.*

FFA, free fatty acids; DG, diacyltriglycerols; TG, triacylglycerols; PG, phosphoglycerides; Ch, cholesterol; ChE, cholesterol esters.

Perfusion time (min)		Lipid classes					
		FFA	DG	TG	PG	Ch	ChE
5	E	6 428 ± 344	250 ± 58	407 ± 123	966 ± 92	531 ± 116	
	S	9 862 ± 245	345 ± 95	437 ± 115	1 901 ± 108	1 294 ± 112	
10	E	8 386 ± 287	321 ± 89	3 340 ± 250	1 186 ± 105	2 024 ± 97	
	S	12 121 ± 180	550 ± 89	1 110 ± 125	1 116 ± 106	4 016 ± 128	
15	E	16 356 ± 260	1 092 ± 90	1 081 ± 148	4 239 ± 150	5 610 ± 120	
	S	36 134 ± 177	1 052 ± 122	10 385 ± 170	3 560 ± 120	11 236 ± 193	
20	E	39 821 ± 99	971 ± 86	11 980 ± 162	2 883 ± 135	10 950 ± 118	
	S	66 042 ± 149	1 222 ± 100	29 044 ± 132	3 966 ± 142	17 267 ± 145	
50	E	173 462 ± 592	2 967 ± 93	160 000 ± 565	16 377 ± 341	69 961 ± 245	
	S	189 114 ± 186	4 455 ± 143	272 000 ± 389	13 320 ± 241	82 512 ± 124	
Liver		2 040 ± 52	10 966 ± 98	63 615 ± 550	58 066 ± 139	88 129 ± 329	13 807 ± 309

Table. IV. *Time-course of the incorporation of (^{14}C) acetate into different lipid classes (dpm/0.5 mg of total lipids ± SD) from liver perfusion media and liver tissue of pregnant rats (n = 14). Samples were taken at the entrance (E) into the liver and at the exit (S) from the liver.*

FFA, free fatty acids; DG, diacylglycerols; TG, triacylglycerols; PG, phosphoglycerides; Ch, cholesterol; ChE, cholesterol esters.

Perfusion time (min)		Lipid classes					
		FFA	DG	TG	PG	Ch	ChE
5	E	485 ± 30	1 097 ± 112	647 ± 86	6 452 ± 373	273 ± 97	
	S	975 ± 57	1 185 ± 101	805 ± 114	4 399 ± 334	244 ± 62	
10	E	672 ± 54	910 ± 110	598 ± 120	4 115 ± 122	248 ± 102	
	S	1 462 ± 47	1 447 ± 135	766 ± 130	5 806 ± 192	507 ± 102	
15	E	1 347 ± 47	1 182 ± 125	924 ± 127	7 143 ± 266	734 ± 98	
	S	4 014 ± 85	3 615 ± 173	2 818 ± 186	8 307 ± 569	1 963 ± 105	
20	E	2 798 ± 181	1 936 ± 130	1 157 ± 141	9 931 ± 136	1 539 ± 105	
	S	4 018 ± 126	2 855 ± 207	3 981 ± 118	8 882 ± 150	2 019 ± 174	
50	E	4 907 ± 158	7 988 ± 119	9 244 ± 194	33 800 ± 198	5 978 ± 117	
	S	7 025 ± 89	6 282 ± 233	42 978 ± 141	9 481 ± 347	10 184 ± 153	
Liver		3 414 ± 190	5 688 ± 110	33 378 ± 235	26 928 ± 315	38 395 ± 114	1 556 ± 39

Discussion

Although the total lipids in the liver from pregnant rats were higher, total lipids/tissue gram ratios from control and pregnant rats were not significantly different.

The higher lipid concentration values in the perfusion media of the pregnant rats may be due, also, to the higher liver weight in these animals as compared with controls.

^{14}C-acetate was efficiently incorporated into fatty acids by the liver throughout the formation of acetyl-CoA and the activity of the fatty acid synthetase; free fatty acids were washed out the liver and circulated in the perfusion medium, from which they were determined. Radioactivity incorporation into free fatty acids present in the perfusion medium was notably higher in the control rats than in the pregnant animals; thus, the physiological lipid modification of pregnancy has important implications for fetal growth. The lower fatty acid incorporation into triacylglycerols from pregnant liver can be due to an increase in the combustion of free fatty acids by the mother organ in order to spare other energy sources for fetal use. However, in both control and pregnant experiments the ^{14}C-acetate incorporation into fatty acids at the exit from the liver was notably higher than that at the entrance into the organ; this increase (tables III and IV) can be attributed to the fatty acid synthesis during the residence time of ^{14}C-acetate in the liver, from which the labelled fatty acids were easily removed by the perfusion medium. Thus, the radioactivity present in the lipids of the liver tissue as free fatty acids was very scarce.

Free fatty acids in the liver were afterwards efficiently incorporated into acylglycerols and phosphoglycerides. Triacylglycerols were the lipid class more enriched in labelled fatty acids; the fatty acid esterification into triacylglycerols was notably higher in the liver of control rats than in the organ of pregnant animals. Nevertheless, the sharp increase of the isotope incorporation into triacylglycerols that can be observed at the 50 min of perfusion was clearly shown at the start out of the medium in both control and pregnant livers. This high presence of labelled triaclyglycerols in the perfusion medium of liver from control rats accounted for the high levels of labelled triacylglycerols in the hepatic tissue at the end of the perfusion experiments and also for the higher yield (6.8 $\pm$ 0.5 %) of incorporation of ^{14}C-acetate into the tissue lipids of the control animals in relation to the isotope incorporation yield (3.7 $\pm$ 0.3 %) into the lipids of the liver of pregnant rats.

The low isotope incorporation into triacylglycerols of the liver of pregnant rats at the first minutes of the time-course of the perfusion is counterbalanced by the high labelled fatty acid incorporation into total phosphoglycerides; this was not observed in the control animals. Thus, *de novo* biosynthesis of triacylglycerols in the liver was reduced during pregnancy, whereas acylation reactions in the synthesis of phosphoglycerides were clearly increased. This fact can be ascribed to a hormonal regulation of the acyltransferases that support the increased needs for phosphoglycerides during fetal development and histogenesis.

Another contrasting effect of rat pregnancy is seen in the relative isotope incorporation values of triacylglycerols and phosphoglycerides at the 50 min perfusion. The diminution of the labelled phosphoglycerides at this time of perfusion was accompanied by a remarkable increase of the synthesis of triacylglycerols (table IV); it appears that labelled phosphoglycerides could be degraded in the liver and reused for the triacylglycerol synthesis. This utilization of phosphoglycerides was more significant in the liver of pregnant rats than in that of control animals. WASFI *et al.*

(35, 36) attained similar results working with oleate.

The main alteration caused for pregnancy in the pattern of the fatty acid composition of total lipids from the perfusion media of the liver at the 50 min interval was the increase of the percentage of unsaturated fatty acids of large carbon chain (24:1 and 22:6).

The main characteristic of the labelled fatty acid pattern from lipids of the perfusion medium of pregnant liver was the relative increase of the unsaturated fatty acids (16:1 and 18:1) as well as the saturated 18:0. This observation is consistent with an increase during pregnancy of the elongation and desaturation systems of the liver; palmitic acid would be elongated to stearic acid and both fatty acids would be desaturated to the corresponding monounsaturated fatty acids 16:1 and 18:1. This increase in the levels of unsaturation of the fatty acids from pregnant rats would be in agreement with the high isotope incorporation into phosphoglycerides (table IV) during the whole perfusion experiment, taking into account that this lipid class is involved in the increase of the unsaturation degree in fatty acids during pregnancy in other animal species and also the participation of the unsaturated phosphoglycerides in the organization of the new fetal membranes.

Resumen

Se estudia en hígados perfundidos de ratas gestantes y no gestantes la incorporación de C^{14}-acetato a lípidos.

Se extraen los lípidos totales en muestras del líquido de perfusión, tomadas a la entrada y a la salida del órgano, en diferentes intervalos de tiempo; al final del experimento se extraen también los lípidos del tejido hepático.

Se compara el proceso de incorporación de C^{14} a las distintas fracciones lipídicas del líquido de perfusión y del tejido hepático en animales gestantes y no gestantes.

La incorporación de radiactividad a los ácidos grasos libres y a los triglicéridos circulantes es mayor en las ratas normales que en las gestantes, en las que, por el contrario, se observa una mayor incorporación de ácidos grasos marcados a los fosfolípidos.

En el medio de perfusión de los hígados de ratas gestantes hay una mayor cantidad de ácidos grasos insaturados de cadena larga (24:1 y 22:6) y son mayores los porcentajes de 16:1, 18:1 y 18:0 marcados.

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