Serum lipoproteinemia in pregnant and lactating rats

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ABSTRACT Serum lipoproteins of pregnant and puerperal rats were studied by preparative and analytical ultracentrifugation. The concentration for the fraction with density less than 1.019 was markedly elevated in rats during the 3rd wk of gestation and in lactating rats. This fraction showed similar triglyceride fatty acid composition and immunoelectrophoretic behavior whether it was derived from pregnant or nonpregnant rats, and when partially delipidized, the lipoproteins from both groups showed similar immunoelectrophoretic characteristics and sedimentation rates.

When lactation was interrupted during puerperium, serum lipoproteins returned to control levels; but in lactating rats, high levels of serum very low density lipoprotein persisted up to 3 wk post partum.

KEY WORDS rat · pregnant · lactating · serum · very low density lipoproteins · milk production · partial delipidation

It has been well established that during gestation and lactation marked changes of lipid pattern occur in mammals (1–3). McKay and Kaunitz (3) observed an elevation of serum lipids in rats studied on the 21st day of pregnancy, and although this animal is most suitable for experimental work, to our knowledge no other studies have been reported in which this species was utilized for investigating the effects of gestation on serum lipoproteins. Post-partum changes in serum lipids were reported by Dannenburg, Burt, and Leake (4), who showed that the hyperlipemia of pregnancy disappears rapidly in puerperal rats, but this work was done in nonlactating rats. Thus, the possible effect of lactation on post-partum serum lipids is not known. We report here a study of lipoproteins obtained by ultracentrifugation of sera of pregnant, lactating, and puerperal nonlactating rats.

Abbreviation: TLC, thin-layer chromatography.

METHODS

Animals

Sprague-Dawley female rats were used in all experiments. They were selected at random from our colonies and thereafter kept in individual cages. The rats were mated for 24 hr with one male each. At least five pregnant rats at 1, 2, or 3 wk after the mating date and a control group of 13 nonpregnant animals were studied, as well as two other groups of rats: one group of nine rats suckling their young during three consecutive weeks after parturition, and another of five rats in which we interfered with lactation by separating the litters from their mothers. Since it was observed that 2-wk old rats ate rat chow in addition to mother's milk, we replaced them with new-born rats in order to insure milk production in the mothers during the third week. Unless otherwise indicated, all animals were allowed to feed ad lib. on rat chow. Food was withdrawn 18–20 hr before blood collection. Drinking water was unrestricted.

Extraction of Serum Lipoproteins and Lipids

The abdominal aorta was cannulated with polyethylene tubes under ether anesthesia. Serum was separated by centrifugation at 1500 *g* for 10 min at 5°C after the blood had been allowed to clot for 3–5 hr at 5°C.

Lipoprotein fractions were obtained according to the method of Havel, Eder, and Bragdon (5) in a Spinco Model L ultracentrifuge with the use of a 40.2 rotor at about 20°C. Background densities of the samples to be fractionated were adjusted by addition of sodium bromide solutions. Densities of the salt solutions were measured to the fourth decimal place with a Christian Bekker specific gravity balance.

TABLE 1 CONCENTRATIONS AND FLOTATION COEFFICIENTS OF SERUM LIPOPROTEIN FRACTIONS FROM PREGNANT AND NONPREGNANT RATS DETERMINED BY ANALYTICAL ULTRACENTRIFUGATION

Group of Rats	S_f(1.21) Ranges (0–12) Concn	(0–12) S_f(1.21) Midarea Ordinate	(12–60) Concn	(12–60) S_f(1.21) Mid-Area Ordinate	(60–700) Concn	(60–700) S_f(1.21) Mid-Area Ordinate
	mg/100 ml		*mg/100 ml*		*mg/100 ml*	
Fasting, 3 wk pregnant (4)*	251† ± 16	3.7 ± 0.5	66 ± 32	27 ± 2	373 ± 256	293 ± 44
Fasting nonpregnant (3)	245 ± 57	3.0 ± 0.0	69 ± 16	25 ± 1.5	5	‡
Not fasting, nonpregnant (4)	179 ± 45	4.0 ± 0.8	76 ± 56	23 ± 3	149 ± 118	220 ± 18

* Number of animals per group.
† Means ± SD.
‡ Too low a concentration for the evaluation of flotation coefficients.

Lipids were extracted and washed according to the method of Folch, Ascoli, Lees, Meath, and Le Baron (6). All solvents used were analytical grade reagents (E. Merck A.G., Darmstadt, Germany).

Analytical Methods

Determinations of total cholesterol (7), glycerides (8), phospholipids (9), and proteins (10) were made in all lipoprotein fractions, which were not purified by re-centrifugation since we have found that further manipulation causes extensive phospholipid breakdown.

Triglycerides from d<1.019 lipoproteins were isolated by TLC according to Mangold (11) with petroleum ether–ethyl ether–acetic acid 90:10:1. Triglycerides were identified by means of a TLC standard mixture (Applied Science Laboratories Inc., State College, Pa.). The triglyceride areas were scraped off the TLC plate and methanolyzed (without elution) with 2.5% HCl in methanol (12). Fatty acid methyl esters were analyzed by gas–liquid chromatography in a Barber-Colman 5000 gas chromatograph with hydrogen flame detector and a 180 cm × 4 mm I.D. column containing 10% EGSS-X (an ethylene glycol succinate polyester combined with a silicone) on Gas-Chrom P (Applied Science) at 180°C. Nitrogen was used as the carrier gas. Mixtures of fatty acid methyl esters (Applied Science) were used for identification of chromatographic peaks and as standards for the quantitative determinations.

Analysis of the standard "KD" agreed with the stated composition to within 5% for major components and 8% for minor ones. Areas under the peaks were determined with the aid of a Barber-Colman disc integrator.

Serum lipoprotein patterns were examined in the Spinco Model E analytical ultracentrifuge by the method of Del Gatto, Lingren, and Nichols (13). Calculations of lipoprotein concentrations and their ranges of S_f at density 1.21 were performed as described by DeLalla and Gofman (14). Centrifugations were performed at 20° ± 0.2°C. No extrapolation to zero concentration was made.

Immunoelectrophoretic analyses were carried out according to Scheidegger (15). The serum of one male rabbit immunized against rat total serum was used as the precipitating antiserum.

RESULTS

Pregnant Rats

Chemical analyses of serum lipoprotein fractions from rats at the end of 1, 2, and 3 wk of pregnancy and from nonpregnant controls are shown in Fig. 1. The concentration of very low density lipoproteins (d<1.019) increased sharply during the 3rd wk of pregnancy. None of the other lipoprotein levels showed a notable change during gestation. Serum lipoprotein concentrations and S_f of midarea ordinate measured by analytical ultracentrifugation in fasting 3-wk pregnant rats and in nonpregnant controls are shown in Table 1. The results are in agreement with the chemical analyses presented in Fig. 1, in that the fraction with S_f (1.21) 60–700 is elevated in fasting, 3-wk pregnant rats, to about the same extent as in nonfasting, nonpregnant controls. Since, however, the value of the midarea ordinate for S_f (1.21) 60–700 lipoproteins was different for these two groups of rats, we studied further the characteristics of the particles present in the very low density fraction from both groups. The fatty acid compositions of the triglycerides of the two fractions (Table 2) were not significantly different. The fractions were partially delipidized according to Gustafson (16). The final apolipoprotein could be solubilized in 1% NaCl and was found to contain less than 5% neutral fats. The protein:phospholipid ratio by weight was 1.0; moving boundary sedimentation analysis indicated a single component with a sedimentation rate of 4.6 in both samples. Recovery of soluble protein in this experiment was better than 96%.

The immunoelectrophoretic behavior of the d<1.019 fraction was also studied. Partially delipidized fractions

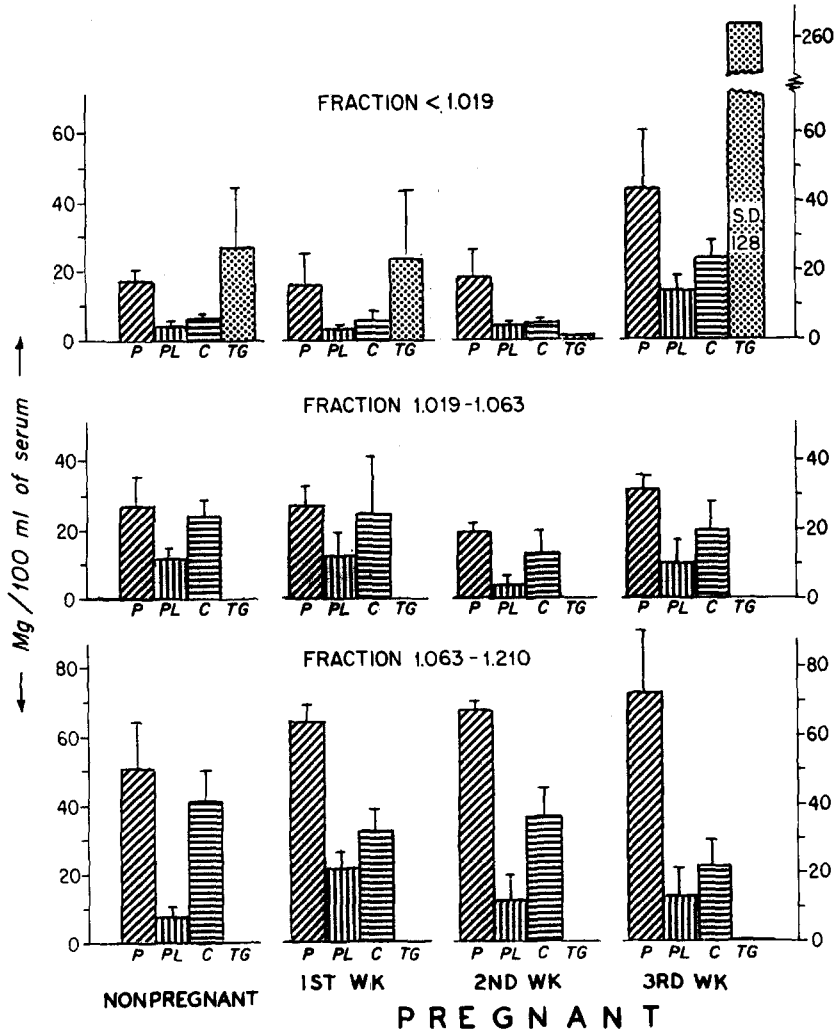

FIG. 1. Serum lipoprotein fractions d<1.019, d 1.019–1.063, and d 1.063–1.121 in fasting rats at the end of 1, 2, and 3 wk of gestation and the same fractions in nonpregnant controls. *P*, protein; *PL*, phospholipids; *C*, total cholesterol; and *TG*, triglycerides. Values are means ± SD.

obtained either from fasting 3-wk pregnant rats or from nonfasting controls, and nondelipidized fractions from fasting and nonfasting animals had very similar patterns.

Lactating and Nonlactating Rats after Parturition

The results of analyses of d<1.019 lipoproteins in lactating and nonlactating rats are given in Table 3. Whereas the concentration of serum very low density lipoproteins in the lactating group was much higher ($P<0.01$) than in the nonpregnant controls shown in Fig. 1, in the nonlactating rats the concentration of this fraction was equal to that of the controls. Lipoprotein fractions d 1.019–1.063 and d>1.063 were found to be similar in the lactating, nonlactating, and control rats.

DISCUSSION

The numerous studies related to the biosynthesis and the physiological role of d<1.019 lipoprotein (17–19) suggest that a considerable part of the triglyceride moiety of d<1.019 lipoprotein is synthesized in the liver from free fatty acids derived from plasma. Since McKay and Kaunitz (3) have shown that serum free fatty acids are increased in rats during the 3rd wk of pregnancy, it is conceivable that the high levels of serum very low density lipoprotein encountered in the present work in the pregnant rats may be the results of an increased transport of free fatty acids from depot fats to the site(s) where the d<1.019 lipoprotein is formed. On the other hand, dissimilarities in the physicochemical characteristics of the d<1.019 lipoproteins derived from pregnant and nonpregnant rats may lead to differences in the rate of utilization of these entities, which could result in different serum concentrations. With the exception of a small difference in the S_f values of the midarea ordinate of the two nondelipidized fractions, all other properties studied were the same. The sedimentation rate, the immuno-

TABLE 2 FATTY ACID COMPOSITION OF TRIGLYCERIDES ISOLATED FROM SERUM LIPOPROTEIN FRACTION d<1.019 OF PREGNANT AND NONPREGNANT RATS*

Group of Rats	16:0	16:1	18:0	18:1	18:2
Fasting, 3 wk pregnant (3)†	39.9 ± 0.7	1.9 ± 0.7	6.9 ± 0.5	33.1 ± 1.7	18.5 ± 0.3
Fasting, nonpregnant (7)	36.4 ± 2.0	2.9 ± 0.5	6.9 ± 0.9	36.6 ± 2.2	18.6 ± 2.6
Not fasting, nonpregnant (3)	36.3 ± 1.3	2.9 ± 0.2	6.1 ± 6.1	38.4 ± 1.5	17.3 ± 1.4

* Fatty acids designated by chain length: no. of double bonds. Amount of each fatty acid expressed as per cent of the total area under the 16:0 to 18:2 peaks. Values are means ±SD.
† Number of animals per group.

TABLE 3 TRIGLYCERIDES AND TOTAL CHOLESTEROL CONCENTRATIONS IN d<1.019 LIPOPROTEINS IN LACTATING AND NONLACTATING RATS 3 WK POST PARTUM

Experimental Group	Triglycerides	Cholesterol
	mg/100 ml Serum	
Lactating rats (9)*	63.6 ± 26.0	6.5 ± 4.0
Nonlactating rats (5)	24.2 ± 7.6	2.3 ± 1.3

Values are means ± SD.
* Number of animals.

electrophoretic behavior, and the phospholipid:protein ratios of the partially delipidized lipoproteins of the two groups were also identical.

During the first 2 wk of pregnancy, the serum lipoprotein concentrations were not significantly different from control values. Only during the last week of pregnancy an abrupt elevation in the d<1.019 lipoprotein occurred and was maintained up to 3 wk postpartum in the lactating rats, while in the nonlactating animals it declined to control levels. Dannenburg et al. (4) showed that in nonlactating rats a decline in serum lipids could be observed as early as 2 days after parturition.

Our findings lead us to speculate that there exists an interrelationship in female rats between serum very low density lipoprotein and the production of milk. It is interesting in this connection that Glascock et al. (20) reported that in the cow 35–48% by weight of milk fat was derived from serum β-lipoprotein triglycerides.

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