Effect of pregnancy and lactation on lipoprotein and cholesterol metabolism in the rat

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Abstract **Origins of hyperlipidemia and cholestasis that occur during pregnancy were investigated by examining expression of key elements related to plasma and hepatic cholesterol metabolism during pregnancy, lactation, and post-lactation in the rat model. Among major findings were: during pregnancy, the activities of hepatic 3-hydroxy-3-methylglutaryl coenzyme A reductase, acyl coenzyme A:cholesterol acyltransferase, acyl coenzyme A:diacylglycerol acyltransferase, cholesterol 7α-hydroxylase, cholesterol ester hydrolases, low density lipoprotein receptors, LRP, and mdr2 were significantly lower or similar to non-pregnant controls while SR-B1 was elevated. Once lactation began, reductase, cholesterol acyltransferase, 7α-hydroxylase activities, low density lipoprotein receptors, and mdr2 increased while SR-B1 decreased. In later stages of lactation most hepatic elements returned to near control levels. Plasma cholesterol levels were higher than control at birth and during lactation with increase in LDL-size particles. By 24 h post-lactation, plasma triglycerides were 3.7-fold higher while cholesterol remained unchanged. Very large lipoproteins were present while LDL-size particles were now absent. Hepatic cholesterol acyltransferase had decreased to 27% of control while diacylglycerol acyltransferase increased 3-fold and low density lipoprotein receptors doubled. Most elements were normalized 3 weeks after weaning except for LRP and low density lipoprotein receptors which were elevated. These studies provide an integrated picture of expression of key elements of hepatic and plasma cholesterol metabolism during pregnancy and lactation and advance understanding of hyperlipidemia and cholestasis during these states.—**Smith, J. L., S. R. Lear, T. M. Forte, W. Ko, M. Massimi, and S. K. Erickson. **Effect of pregnancy and lactation on lipoprotein and cholesterol metabolism in the rat. *J. Lipid Res.* 1998.** 39: **2237–2249.**

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Hyperlipidemia of pregnancy in humans was first described over 150 years ago (1) and has been studied extensively since (2–7). It occurs in virtually all pregnant women and resolves in most in the first months after delivery. A positive association of this hyperlipidemia with later development of cardiovascular disease has been suggested (8); and a supraphysiologic increase has been proposed as a marker for development of non-pregnancy-associated hyperlipidemia in later life (9). A low density lipoprotein (LDL) pattern B profile, i.e., one predisposing towards the development of atherosclerosis (10), has been described in pregnant women which tended to revert towards a more favorable LDL pattern A in most individuals by 4–5 weeks after delivery (11). Cholesterol and lipoprotein concentrations tend to normalize in the year after pregnancy (12, 13). Epidemiological studies of the relationship(s) between pregnancy and later risk for cardiovascular disease have shown mixed results (14 and references therein).

An increased propensity for cholesterol gallstone formation in human pregnancy also is well known (15, 16) and is a predictor for development of cholesterol gallstone disease in later life (17). Intrahepatic cholestasis of pregnancy also is a well-described entity in humans which generally also resolves after delivery. It was first noted over 100 years ago (18) and described in detail in 1954 (19). It is associated with higher than normal serum lipid levels during pregnancy (20, 21), suggesting a link between cholestasis and hyperlipidemia. In one study, serum cholyl-

Abbreviations: HMG-CoA, 3-hydroxy-3-methylglutaryl coenzyme A; ACAT, acyl coenzyme A:cholesterol acyltransferase; CEH cholesterol ester hydrolase; DGAT, acyl coenzyme A:diacylglycerol acyltransferase; LRP, low density lipoprotein receptor-related protein; LDL, low density lipoproteins; VLDL, very low density lipoproteins; HDL, high density lipoproteins; apo, apolipoprotein; MVA, mevalonate; FA, fatty acid.

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glycine levels doubled during pregnancy in all individuals studied (22) suggesting that some degree of cholestasis is normal in all pregnancies. Although the incidence of clinically apparent cholestasis is less than 0.1–2% in most countries, native American/Hispanic and Asian populations are at higher risk with a 10–20% incidence in some subgroups (21–23).

Thus, in humans, pregnancy induces changes in cholesterol metabolism that result in phenotypes associated with the development of pathologies such as atherosclerosis and cholesterol gallstone disease in the general population. However, the origins of these changes remain obscure.

The liver plays a central role in the maintenance of whole body cholesterol homeostasis by integrating regulation of a group of hepatic enzymes, receptors, and other proteins important for cholesterol, lipoprotein, and biliary metabolism. The interactions and regulation of these pathways to maintain cholesterol homeostasis during pregnancy and lactation have not been studied systematically or in an integrated fashion. We hypothesized that hepatic regulatory elements and their integration for maintainence of cholesterol homeostasis undergo major changes during pregnancy and lactation to ensure that cholesterol homeostasis is maintained optimally for both the mother and the developing offspring and further, that these changes are related to the etiology(ies) of hyperlipidemia and cholestasis of pregnancy.

The rat was chosen as a model for these studies because, like the human but unlike a number of other species (24, 25), the rat develops hyperlipidemia during pregnancy (26–28) and, like humans, the rat also develops cholestasis (29) as does the hamster (30). Thus, the rat is a suitable model for studying the origins of changes in both lipoprotein and biliary metabolism that are observed during pregnancy and lactation in humans.

To gain further and more detailed insight into the regulation of cholesterol, lipoprotein, and biliary metabolism during pregnancy and lactation, the hepatic expression of a number of enzymes, receptors, and proteins important in hepatic cholesterol metabolism was investigated. A scheme showing key elements involved in the regulation of hepatic cholesterol, lipoprotein and biliary metabolism is shown in **Fig. 1**.

METHODS

Animals

Adult female (264–305 g) and timed pregnant (250–395 g) Sprague-Dawley rats (Bantin and Kingman, Newark, CA, or Simonsen, Gilroy, CA) were housed under reverse illumination (lights on 3 pm; lights off 3 am) for at least 1 week prior to use. They were allowed free access to food (Purina Rat Chow) and water at all times. Animals were anesthetized with isoflurane, blood was drawn into tubes containing gentamycin, 1 mg/ml, and EDTA for lipoprotein profiles and/or into separate tubes for serum, and the livers were removed. In pregnant animals the fetuses were removed prior to removal of blood samples followed by removal of the liver. All liver and blood samples were collected at or about 9:00 am (D6), except for two immediate post-delivery samples (2 h) which were collected at 3:40 pm and 6:15 pm. All lactating dams had 9–14 suckling pups. Suckling pups were removed from lactating dams at 21 days of age. All protocols were approved by the Animal Studies Subcommittee at the VA Medical Center, San Francisco.

Preparation of liver fractions

Homogenates, microsomal membranes, and cytosolic fractions were prepared as previously described (31). A sinusoidal plasma membrane-enriched fraction also was prepared as previously described (32). All preparations were from freshly isolated livers.

Plasma and serum cholesterol and triglyceride concentrations

Plasma and serum cholesterol and triglyceride concentrations were determined as described previously (32).

Plasma lipoprotein profiles

Rat plasma collected as above was adjusted to density 1.21 g/ml by the addition of solid NaBr and then subjected to ultracen-

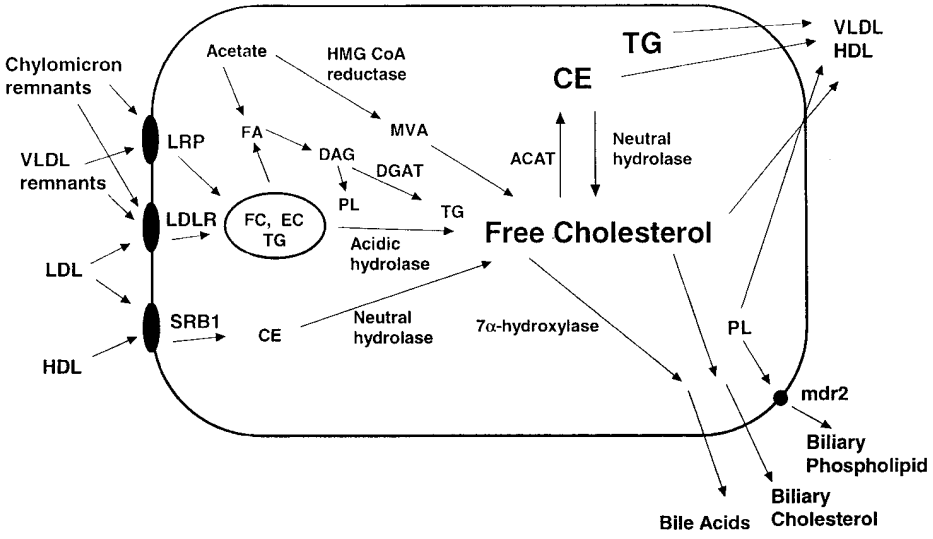

Fig. 1. Scheme for hepatic cholesterol, lipoprotein, and biliary metabolism.

trifugation at 40,000 rpm in a Beckman 40.3 rotor, for 24 h at 4°C. The d < 1.21 g/ml fraction (top 1 ml) containing the lipoproteins was isolated by aspiration. The size distribution of VLDL and LDL was determined on 2–16% non-denaturing polyacrylamide gradient gels, and of HDL, on 4–30% gels according to the method of Nichols, Krauss, and Musliner (33).

Serum apolipoprotein profiles

Rat serum was fractionated by SDS gel electrophoresis and immunoblotted for apolipoproteins (apo) A-I, E, A-IV, C-III, and for albumin using specific antisera provided by Dr. Karl Weisgraber of the Gladstone Foundation, UCSF, and for apoB-100 and apoB-48 using a specific antiserum provided by Dr. Robert Hamilton of the Department of Anatomy, UCSF followed by densitometry. All were assayed in the linear ranges of detection as determined in preliminary experiments. The values are expressed in units defined relative to a standard pooled rat plasma set as 100, an aliquot of which was run simultaneously on each gel.

Enzyme assays

Enzyme activities were measured in freshly prepared microsomes or cytosol. The activities of 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase (31), acyl coenzyme A:cholesterol acyltransferase (ACAT) (34), the cholesterol ester hydrolases (CEH) (34), cholesterol-7α-hydroxylase (34), and acyl coenzyme A:diacylglycerol acyltransferase (DGAT) (35) were assayed by radiochemical assays as previously described.

Hepatic receptor concentrations

Low density lipoprotein (LDL) receptor protein content was estimated in membrane fractions enriched in sinusoidal plasma membranes by SDS gel electrophoresis under reducing conditions followed by transfer and immunoblotting as described previously (32) using an LDL receptor-specific antiserum provided by Dr. Janet Boyles of the Gladstone Foundation, UCSF. LDL receptor-related protein (LRP; α_2-macroglobulin receptor) content was estimated by $^{45}Ca^{++}$ blotting based on the method of Maruyama, Mikawa, and Ebashi (36) as described previously (34). Scavenger recepter, Class B (SR-B1) content was estimated by immunoblotting as described above using whole liver homogenates prepared as described above and an SR-B1 specific antiserum provided by Dr. Monty Krieger, Department of Biology, MIT.

Hepatic mdr2 concentrations

mdr2 protein was estimated by immunoblotting as described above using whole liver homogenates prepared as described above and an mdr2-specific antiserum provided by Dr. Irwin Arias, Departments of Physiology and Medicine, Tufts University.

Other assays

Protein was estimated by the biuret method (37) using bovine serum albumin as reference standard.

RESULTS

Serum cholesterol and triglyceride concentrations

As reported previously by others (26–28), pregnant rats at term were hypercholesterolemic (**Fig. 2A**). This condition lasted throughout the lactation period. In contrast, triglyceride concentrations were in the normal range in near term rats. This may reflect the time of day at which they were killed (D6); other studies used basal (L6) rats. Triglycerides decreased to about one-third to one-half of control, $P < 0.001$, after first suckling and remained low throughout lactation (Fig. 2B). The day after weaning of the pups, cholesterol concentrations were variably elevated and remained significantly elevated thereafter until 21 days post weaning (42 days postpartum) when they were similar to controls (Fig. 2A). Plasma triglyceride concentrations were elevated 4.4-fold after weaning of the pups, ($P < 0.002$, compared with controls); however, by 7 days post weaning, in contrast to cholesterol, triglyceride levels were similar to controls and remained at this level thereafter (Fig. 2B).

Plasma lipoprotein particle distribution

The distribution of VLDL, LDL, and HDL from lactating and control rats was determined by non-denaturing polyacrylamide gel electrophoresis. Non-denaturing gradient gel (2–16%) analyses, which profile VLDL and LDL, showed that plasma from control rats possessed a small

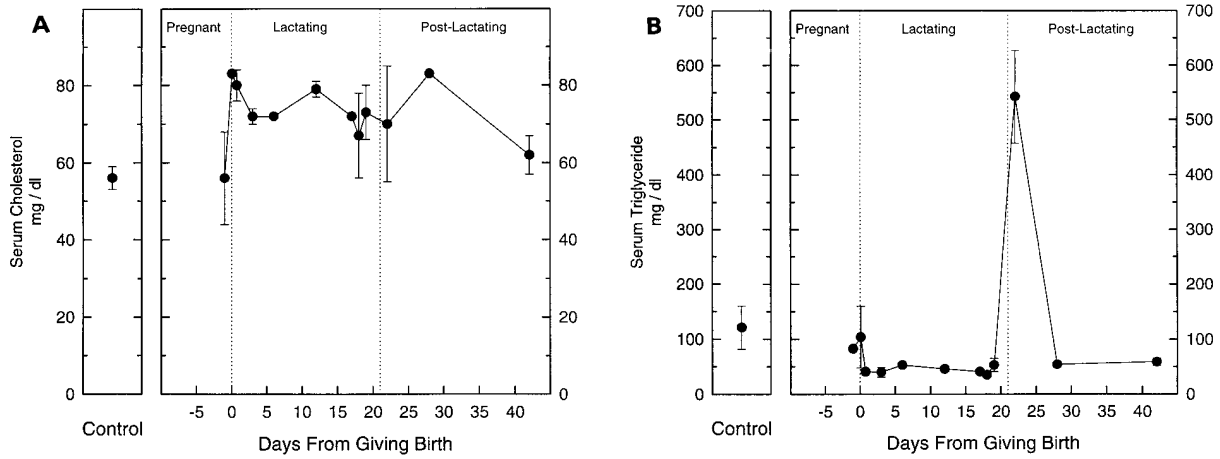

Fig. 2. Plasma cholesterol and triglyceride concentrations during pregnancy, lactation, and post-lactation. The number of animals in each group were: at day −1, 5; +0.08, 3, +0.75, 4, +3, 5; day +6, 2; day +12, 5; day +17, 2; day +18, 3; day +19, 7; day +22, 5; day +28, 4, day +42, 3; controls, 18 adult females. Pups were delivered at day 0. Negative days refer to days of pregnancy prior to birth. Pups were weaned at day 21. Panel A, cholesterol; panel B, triglycerides.

quantity of VLDL and LDL-size material (**Fig. 3A**). Control rat VLDL were of relatively small size with a mean particle diameter of 36–37 nm. The entire lactation period was unique in that there was no evidence of VLDL particles (Fig. 3A). The control LDL were small in diameter (23 nm). However, by the mid and late lactation period (days 12–19, Fig. 3A) there was a clear shift to larger-sized LDL particles (25–26 nm). Upon cessation of lactation the VLDL–LDL pattern underwent major changes (Fig. 3B). The 1-day post-lactation stage was distinguished by the absence of LDL particles and the presence of a substantial quantity of very large lipoproteins which remained at the top of the gel (Fig. 3B). These particles probably represent chylomicrons and/or large VLDL and/or remnants in agreement with the high plasma triglycerides observed at this time point. Seven days after cessation of lactation, no VLDL were found although large LDL (24.1 nm) again were seen (Fig. 3B). By 21 days post-lactation, the VLDL–LDL profile was comparable to that of controls (compare Fig. 3B with Fig. 3A).

HDL from control rats, as assessed by 4–30% non-denaturing gradient gel electrophoresis, consisted of a symmetrical peak with particles of large diameter (12 nm, Fig. 3A). During lactation, rather than becoming larger, the particles were skewed towards smaller size. At 3 days, an asymmetrical peak at 10.5 nm with a shoulder at 11.2 nm was evident (Fig. 3A). With increasing time of lactation the HDL particles became even smaller (9.7 nm at 19 days, Fig. 3A). After the pups were weaned, the HDL pattern began to revert to larger-sized particles and the pattern was normalized by 21 days post-lactation (compare Fig. 3B with Fig. 3A).

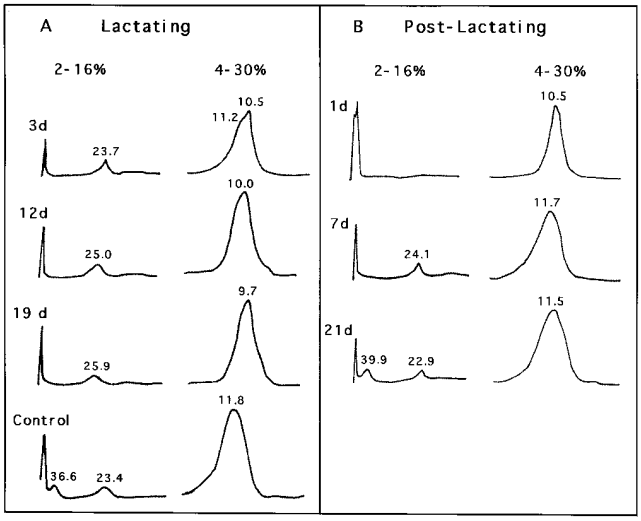

Fig. 3. Lipoprotein size distribution patterns in plasma from lactating and postlactating rats determined by non-denaturing gradient gel electrophoresis. Gels were stained with Coomassie R250 and scanned for profiles. In all cases 2–16% gels provide profiles for VLDL and LDL while 4–30% gels provide profiles for HDL. Panel A: 3 days lactation; 12 days lactation; 19 days lactation; control. Panel B: 1 day after removal of pups; 7 days after cessation of lactation; and 21 days after cessation of lactation. Numbers over the peaks correspond to particle diameters in nm. Representative profiles are shown.

Serum apolipoproteins

Relative to control, little statistically significant change in apoB-100, which is mostly of liver origin, was observed across the entire spectrum of pregnancy, lactation, and post-lactation stages examined except for day 12 of lactation when it was increased about 2-fold, $P < 0.05$ (**Fig. 4A**). This corresponds to a time when the pups may begin to ingest solid food as well as milk. ApoB-48, which is of both liver and intestinal origin in the rat, was more variable; in the late stages of pregnancy, levels were about 30–40% higher than those in control (Fig. 4A). After first suckling, apoB-48 was at control levels and remained similar to this until day 6 when levels had begun to increase. By days 12 and 17 of lactation, apoB-48 had increased significantly to about 2.5- to 3-fold greater than controls respectively, $P < 0.01$. At this time point, serum apoB-48 may be enriched with that from the intestine because the intestine has hypertrophied 2-fold by the midsuckling period (38–40) potentially leading to increased secretion of lipoproteins. The lack of VLDL-size particles and presence of large LDL-size particles at this time period suggest that most of the apoB-48 may be in small remnants. Just prior to weaning of the pups, apoB-48 levels decreased about 50%, but nevertheless, they remained significantly above control values, $P < 0.01$. Immediately after weaning, apoB-48 increased to levels about 3-fold higher than control, $P < 0.005$. This reflects the presence of the very large lipoproteins found at this time period (see above) which likely reflects an overshoot phenomenon due to coupling of normal or enhanced intestinal chylomicron production with a decrease in mammary gland production of remnants. By 7 days post-lactation, apoB-48 levels were similar to those in controls.

ApoE was decreased to 10–30% of the levels present in controls in late pregnancy, at birth, throughout lactation, and immediately after weaning (Fig. 4B; $P < 0.05$ at all time points). Because most of the plasma apoE is thought to be of liver origin, this suggests that liver production has been down-regulated, perhaps to ensure longer residence of lipoproteins in the circulation in order to fulfill placental and mammary gland requirements for lipids in late pregnancy and for mammary gland milk production during lactation. Alternatively, apoE levels are low because it was removed very rapidly from the circulation, in particular, by the liver in late pregnancy and by other organs during lactation. However, by 7 days after cessation of lactation, levels of apoE had begun to increase and by 21 days post-lactation, they were statistically similar to those of controls.

ApoA-IV also was markedly decreased during late pregnancy, about 15% of control levels, $P < 0.001$ (Fig. 4C). However, after birth, levels increased to about 50% of control ($P < 0.02$) and this level was maintained throughout the early stages of lactation. By day 12 of lactation, apoA-IV had increased to levels similar to control and remained at this level until the pups were weaned when it dropped significantly to levels about half those of control, $P < 0.02$. By 21 days post-lactation, apoA-IV had returned to control levels.

ApoA-I also was decreased in late pregnancy, to about 40% of control values, $P < 0.01$ (Fig. 4D). Levels dropped

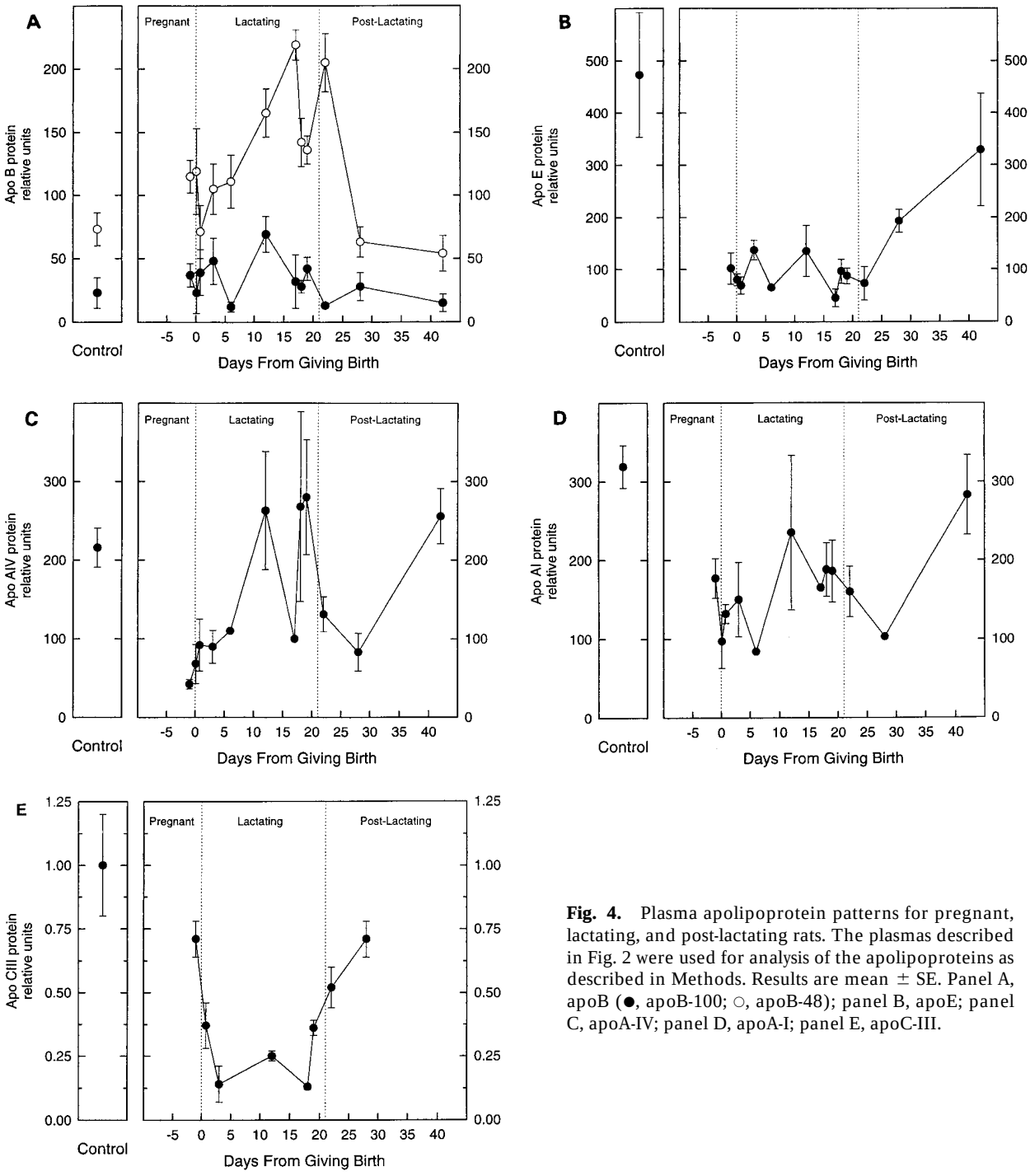

Fig. 4. Plasma apolipoprotein patterns for pregnant, lactating, and post-lactating rats. The plasmas described in Fig. 2 were used for analysis of the apolipoproteins as described in Methods. Results are mean ± SE. Panel A, apoB (●, apoB-100; ○, apoB-48); panel B, apoE; panel C, apoA-IV; panel D, apoA-I; panel E, apoC-III.

further at birth but increased again to 50% of control by day 3 of lactation, $P < 0.02$. At day 12 of lactation, values were highly variable and not statistically significantly different from control. Thereafter, apoA-1 levels were maintained at about 50% of control, $P < 0.02$, until 21 days post-lactation when they had returned to control levels.

ApoC-III levels also were significantly decreased during the early to mid-lactating period (Fig. 4E), about 10–25% of control values, $P < 0.01$, which may be coordinated with the low apoE levels. Late pregnancy and post-lactation apoC-III values tended to be in the control range.

Taken together, the changes in apolipoproteins E, A-IV, A-I, and C-III probably contribute to the changes in HDL species described above.

Hepatic enzymes important in cholesterol, lipoprotein, and biliary metabolism

HMG-CoA reductase. This is a rate-limiting enzyme for mevalonate and cholesterol synthesis. Its activity was suppressed about 60% ($P < 0.01$) in pregnant animals relative to control (**Fig. 5A**). This reflects data on cholesterol synthesis (41, 42) and HMG-CoA reductase (43) reported by others and underlines the fact that the fetus makes most of its own cholesterol rather than deriving it from

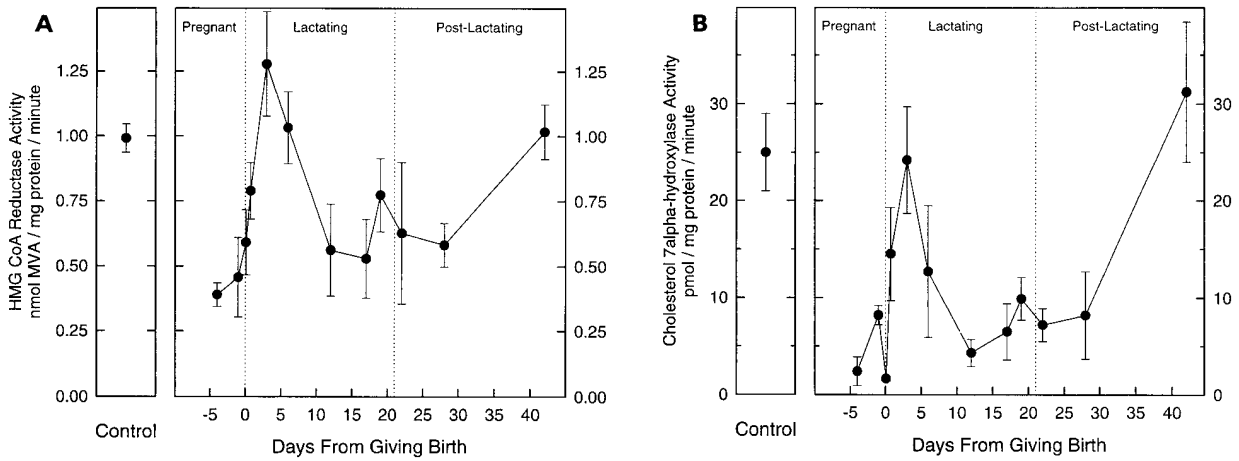

Fig. 5. Hepatic HMG-CoA reductase and cholesterol 7α-hydroxylase activities during pregnancy, lactation, and post-lactation. The numbers of animals in each group were for reductase: at day −4, 5; day −1, 6; day +0.08, 3; day +0.75, 6; day +3, 5; day +6, 6; day +12, 5; day +17, 2; day +19, 6; day +22, 4; day +28, 4; day +42, 3; controls were adult females, n = 3. For 7α-hydroxylase, they were: at day −4, 4; day −1, 4; day +0.08, 3; +0.75; day +3, 4; day +6, 4; day +12, 4; day +17, 2; day +19, 5; day +22, 5; day +28, 3; day +42, 3; controls were adult females, n = 9. Results are mean ± SE. Panel A, reductase, panel B, 7α-hydroxylase.

maternal lipoproteins (42, 44). Reductase activity increased after delivery of the pups, reaching levels above control by 3 days after birth ($P < 0.05$) suggesting increased need for de novo synthesis of cholesterol and/or other mevalonate-derived products in this time period. Reductase then began to decrease, falling to about 50% below control level ($P < 0.02$) by mid-lactation in agreement with others (45). It did not return to control values until 21 days post-lactation, suggesting a decreased requirement for de novo synthesized sterols in the late lactation and post lactation periods.

Cholesterol-7α-hydroxylase. Biliary metabolism in rats is known to be altered in pregnancy and lactation (29, 46–48). Cholesterol-7α-hydroxylase is a rate-limiting enzyme for cholesterol catabolism and bile acid synthesis. Its activity was very low in late pregnancy, about 10% of control, $P < 0.002$, (Fig. 5B) consistent with reports of cholestasis at this time point (29). 7α-Hydroxylase activity increased about 4-fold just before the animals gave birth although it remained below control values. 7α-Hydroxylase activity fell again after birth, but increased after first suckling, reaching control values by day 3. This suggests that the cholestasis of pregnancy had been reversed significantly. However, by day 6 of lactation, levels of 7α-hydroxylase activity had decreased significantly ($P < 0.05$), and by day 12, had dropped to 10% of control, $P < 0.001$. Activity then increased modestly to reach about 30% of control by day 19 of lactation, $P < 0.002$, and remained low until returning to values similar to control by 21 days post-lactation. The data taken together suggest that some level of intrahepatic cholestasis may have been present during much of the lactation period or alternatively, an increased enterohepatic circulation is present.

ACAT. This activity is rate-limiting for intracellular cholesterol esterification. ACAT levels had been determined previously in near-term pregnant rats assayed using the endogenous cholesterol pool as substrate (43). In the present work, ACAT activity in pregnant rats was 32–64% lower than controls, $P < 0.02$, (**Fig. 6A**) when assayed using endogenous cholesterol as substrate. ACAT was somewhat higher in early lactation, reaching about 2-fold ($P < 0.05$) on day 6, but then fell below control, about 50% ($P < 0.02$) by day 12 and remained at this level until 21 days post-lactation when it had returned to control levels. These changes likely reflect, at least in part, the availability of free cholesterol substrate from various metabolic pools in the liver. In contrast, when ACAT activity was assayed with exogenous cholesterol, which measures the activity under apparent V_{max} conditions, and thus, approximates the amount of active ACAT enzyme present, activities were lower than control throughout pregnancy and lactation at most time points assayed (Fig. 6A). In near-term pregnant animals, activity was 40% of control ($P < 0.001$), but it increased after delivery, reaching values in the control range by day 6 of lactation. By day 12 of lactation, activity had declined and by day 17, was 37% of control ($P < 0.02$). By day 19, activity again increased to 58% ($P < 0.02$) of control. After weaning of the pups, activity dropped to 27% of control ($P < 0.001$); however, as the length of post-lactation time increased, activity began to return to control values and by 21 days post-lactation, it was similar to control values. These data taken together suggest that the amount of active ACAT present may be regulated by factors other than substrate availability per se.

DGAT. This activity is the rate-limiting enzyme for triglyceride synthesis. It was about 2-fold higher than control at day 19 of pregnancy ($P < 0.05$) (Fig. 6B) suggesting an increased requirement for synthesis and secretion of lipoprotein triglycerides, likely for use by the placenta and mammary glands. VLDL secretion is reported to be increased at this time point (46). Thereafter, values were below control ($P < 0.05$) at all time points until day 22, 1 day after weaning. This suggests that the liver may not se-

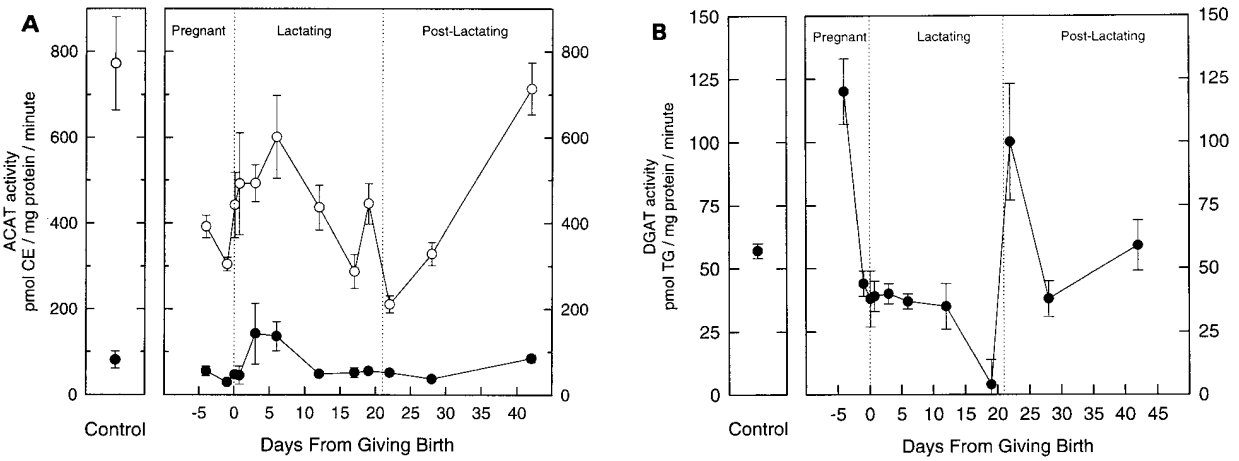

Fig. 6. Hepatic ACAT and DGAT activities during pregnancy, lactation, and post-lactation. Experimental details are the same as for reductase in Fig. 5. Results are mean ± SE. Panel A, ACAT, ●, activity determined in the absence of exogenous cholesterol; ○, activity determined in the presence of exogenous cholesterol; panel B, DGAT.

crete large amounts of triglyceride-rich lipoproteins after parturition, and supports the notion that much of the apoB-48 in plasma is of intestinal origin during the lactation period (see above). At day 22, DGAT was about 3 times higher than previously ($P < 0.05$) and almost twice that of control. This suggests a response by the liver to an influx of free fatty acids and of triglyceride-enriched lipoproteins due to cessation of lactation, beginning of involution of the mammary gland, and down-regulation of mammary gland lipoprotein lipase. By 21 days post-lactation, values for DGAT were indistinguishable from control.

Neutral cholesterol ester hydrolase. These activities are both cytosolic and membrane-associated. They are responsible for cellular hydrolysis of cholesteryl esters. These enzymes may play roles in regulating the amount of free cholesterol in the cell and in facilitating selective uptake of cholesteryl esters from lipoproteins. The specific activity of neutral CEH was higher in hepatic microsomes compared with hepatic cytosol at all time points studied with the exception of livers from 19-day pregnant animals in which the microsomal specific activity was similar to that in cytosol (**Fig. 7A**). CEH in both compartments was decreased to 60% of the control value ($P < 0.001$) in these animals suggesting decreased intracellular free cholesterol needs from these sources. The microsomal activity increased through the remainder of pregnancy and after delivery of the pups, reaching values 40% higher than control by day 3 of lactation ($P < 0.05$). It then decreased to levels similar to control by day 6. From the mid- to late-lactation stages, activity again increased, reaching 140% ($P < 0.005$) and 130% ($P < 0.02$) of control by days 17 and 19 of lactation, respectively, suggesting increased needs for free cholesterol. Activity returned to control levels after weaning of the pups, but it was at a higher level at 21 days after cessation of lactation ($P < 0.05$). The pattern of neutral CEH activity in cytosol was different from that in microsomes (Fig. 7A). Activity in near term dams was 72% of that in controls, $P < 0.02$. After birth, levels similar to control were maintained until day 12. The activity then dropped to about 70% of control, $P < 0.02$. After weaning of the pups, activity had declined further to 47% of control ($P < 0.05$), the lowest level of all the time points examined. This was in marked contrast to the microsomal activity which had increased. CEH cytosolic activity was similar to control values at 21 days post-lactation. The data taken together suggest that these two enzyme activities are different proteins subject to different regulatory mechanisms.

Acidic cholesterol ester hydrolases. These activities primarily represent lysosomal and endosomal activities and likely play a key role in making cholesterol and fatty acids available from lipoproteins that have been internalized by receptor-mediated endocytosis. The specific activity of acidic CEH was greater in microsomes than in the corresponding cytosolic fractions at all time points examined (Fig. 7B). Microsomal acidic CEH activity was similar to control from just after birth through late suckling. At weaning, it dropped to 65% that of control, $P < 0.05$. However, by 21 days post-lactation, activity was 1.7-fold greater than control, $P < 0.04$, suggesting that the endocytic pathway may be more active at this time. Cytosolic acidic CEH activity in near term and early lactating dams was similar to controls (Fig. 7B). However, at 6 days of lactation it was about half that of control, $P < 0.02$, and remained at this level until 1 week post-lactation. By 21 days post-lactation, activity was similar to control values.

Hepatic cell surface receptors and proteins important in cholesterol, lipoprotein, and biliary metabolism

LDL receptors. These receptors recognize both apoB-100 and apoE. The liver LDL receptors are responsible for much of the clearance of LDL and apoE-containing lipoproteins from the plasma (for review, cf. ref. 49). In late pregnant animals LDL receptor protein level was similar to that of controls (**Fig. 8A**); it increased after delivery,

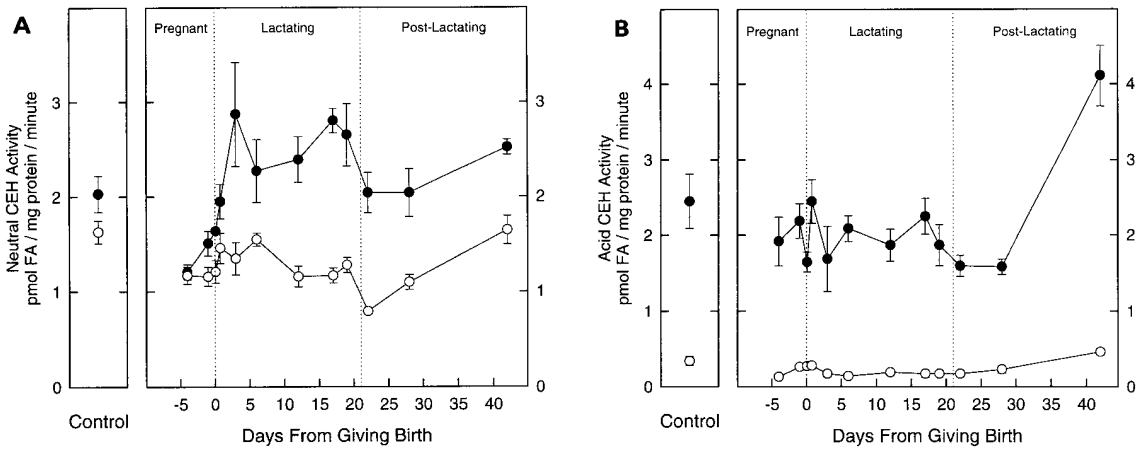

Fig. 7. Neutral CEH and acidic CEH activities in hepatic cytosol and microsomes during pregnancy, lactation, and post-lactation. Experimental details are the same as for reductase in Fig. 5. Results are mean ± SE. ○, cytosol; ●, microsomes. Panel A, neutral CEH; panel B, acidic CEH.

$P < 0.02$, and reached a value 2-fold higher ($P < 0.001$) by 18 h post-partum (after first lactation). Over the next 6 days of the lactating period, levels fell to control levels. However, by day 12 (mid-lactation period) LDL receptors had increased to about 150% of control ($P < 0.004$) and remained at this level through 21 days post-lactation, suggesting a need for increased lipoprotein clearance via the endocytic pathway in this time period.

LRP or α2-macroglobulin receptors. This receptor recognizes apoE and other ligands (50, 51). Thus, its levels reflect requirements both for lipid metabolism and for plasma protein clearance. The LRP level was similar to control in 19-day pregnant dams; however, just prior to delivery, LRP increased about 2-fold ($P < 0.001$) (Fig. 8B). It then fell to levels somewhat higher than control at post parturition followed by a 1.6-fold ($P < 0.02$) increase at day 3 before dropping to about 50% ($P < 0.02$) of control by day 6. LRP then rose to reach a value similar to control by day 12 followed by a further increase (about 1.6-fold, $P < 0.05$) at day 18. Just prior to weaning, LRP levels fell 30% to near control. After weaning of the pups, LRP increased 1.5- to 1.7-fold higher than control, $P < 0.02$, and remained elevated at 7 and 21 days post-lactation, suggesting increased need for clearance of this receptor's ligands from the circulation in this time period.

The patterns of both LDL receptors and LRP likely reflect not only requirements for nurture of the fetuses and pups, but also tissue remodeling processes necessary for pregnancy, parturition, lactation, and return to a control state. These receptors also do not regulate in parallel at all time points, suggesting differential control mechanisms.

SR-B1. This receptor has been proposed to be involved in selective uptake (52) of cholesteryl esters both from HDL and LDL (53) and in regulation of biliary cholesterol levels (54). Its levels were increased in late pregnancy and at birth, about 2-fold ($P < 0.05$) (**Fig. 9A**) sug-

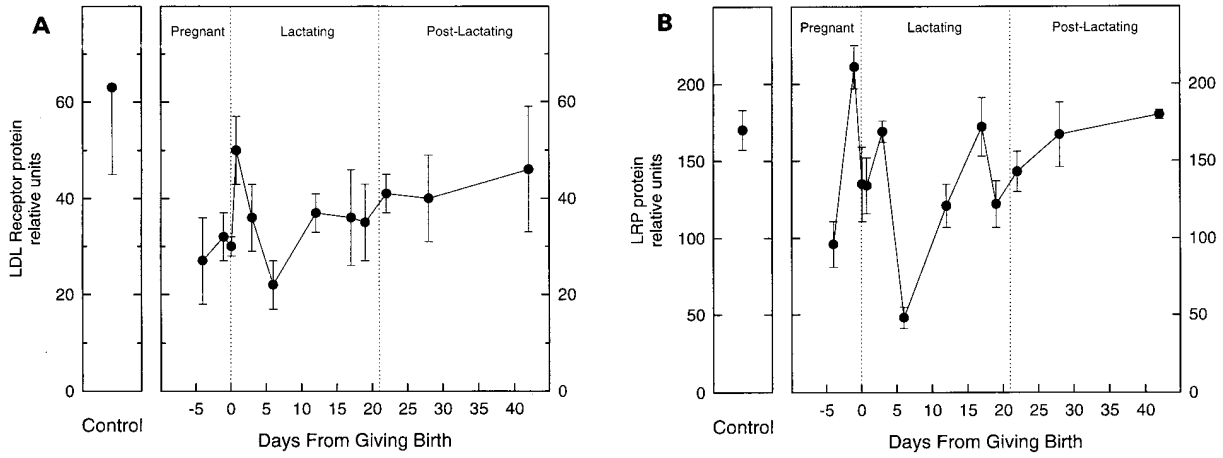

Fig. 8. Hepatic LDL receptor and LRP (α_2-macroglobulin receptor) protein concentrations during pregnancy, lactation, and post-lactation. A sinusoidal membrane-enriched fraction of rat liver was prepared and assayed as described in Methods. Experimental details are the same as for reductase in Fig. 5 except that the control represents the results from 29 individual adult females for LDL receptors and the results from 25 adult females for LRP. Results are mean ± SE. Panel A, LDL receptor; panel B, LRP.

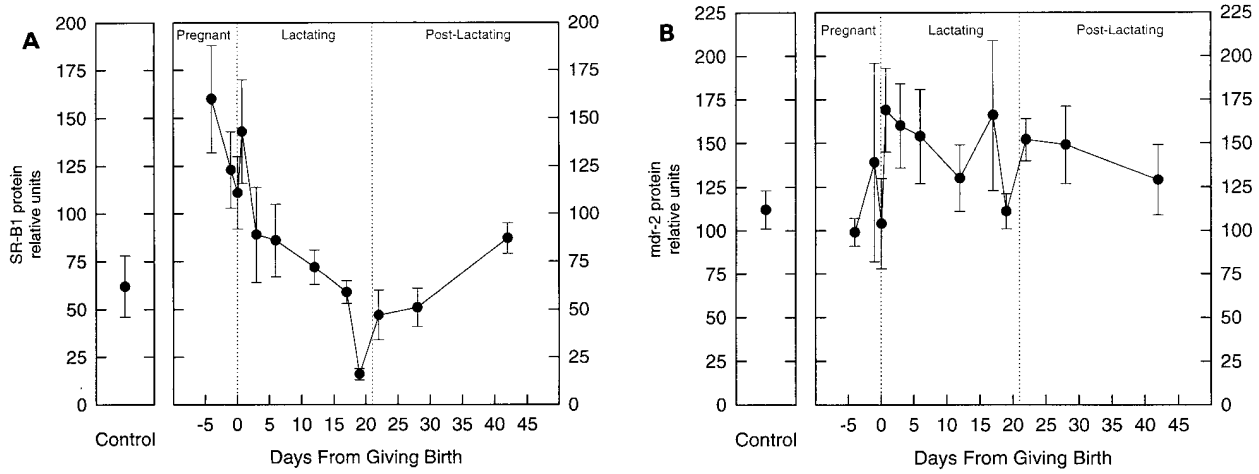

Fig. 9. Hepatic SR-B1 and mdr2 concentrations during pregnancy, lactation, and post-lactation. Rat liver homogenates were prepared and analyzed as described in Methods. Each point is the mean ± SE for at least 3 animals. Panel A, SR-BI; panel B, mdr2.

gesting that selective uptake was a significant source of exogenous cholesterol in this time period. By day 3 postpartum, values were similar to control. They remained in this range until day 19 of lactation when they had dropped about 75% ($P < 0.01$). The day after weaning of the pups, SR-B1 levels had risen to the control range and remained within the control range thereafter. This pattern is quite different from that for the LDL receptor at most time points and suggests that cholesterol derived from selective uptake may be compartmentally and metabolically different from that derived from receptor-mediated endocytosis. The data also suggest different regulatory mechanisms are operational for these receptors.

mdr2. This protein is a member of the multidrug resistant gene family. It is involved in lipid secretion into bile (55). Mice deficient in mdr2 develop intrahepatic cholestasis and impaired biliary excretion of both phospholipids and cholesterol (55). mdr2 levels were in the control range during pregnancy, but they increased to about 50% above control ($P < 0.05$) after birth and first suckling of the pups (Fig. 9B) suggesting increased biliary secretion of phospholipid and cholesterol could occur at this time point. mdr2 remained elevated until day 19 when it returned to control values, suggesting increased demand for biliary lipid secretion throughout the lactation period. At day 22, after weaning of the pups, mdr2 was significantly elevated relative to day 19 ($P < 0.05$) suggesting an even greater requirement for increased biliary lipid secretion. mdr2 levels decreased thereafter to within the control range. The data suggest that these changes reflect liver lipid flux as well as biliary phospholipid and cholesterol secretion requirements per se.

DISCUSSION

Based on the marked changes in plasma and biliary metabolism reported during pregnancy and/or lactation in both humans and rats (this work and references 1–9, 11–13, 16–23, 26–30), it is clear that major changes in hepatic lipid metabolism must occur in order to maintain cholesterol homeostasis for both the mother and offspring simultaneously. Despite these observations, few of the putative regulatory elements involved had been studied previously and then usually only at limited time points, making it difficult to understand how the various elements of cholesterol metabolism are integrated in these metabolic states. **Table 1** summarizes the findings at the different time points studied. Of additional interest is the fact that liver total, free, and esterified cholesterol levels are reported to be unchanged during pregnancy and lactation (42, 43, 56) as are liver triglycerides (56). This suggests that a gross cellular balance in these lipids is maintained.

Effects of pregnancy. Although LRP (α_2-macroglobulin receptor) and LDL receptors, both of which utilize the endocytic pathway to deliver their ligands, were in the control range in late pregnancy, they increased at parturition. SR-B1 was high both in late pregnancy and at parturition. In contrast, HMG-CoA reductase was low. These data suggest that the liver was preferentially utilizing lipoprotein-derived cholesterol, rather than de novo synthesized cholesterol as a substrate for VLDL secretion, known to be higher in late pregnancy (46). This increased VLDL secretion would explain the increase in DGAT activity at this time point and suggests diversion of diacylglycerols from biliary phospholipid synthesis to triglyceride synthesis. Consistent with the high DGAT activity, ACAT activity, which competes with DGAT for fatty acyl CoA, was low during pregnancy.

mdr2 was lower in late pregnancy than after birth suggesting that the relative decrease in mdr2 resulted in decreased biliary phospholipid secretion capacity and cholestasis as reflected also by the low cholesterol 7α-hydroxylase activity. This decreased biliary phospholipid secretion also would make more phospholipid available for VLDL assembly and secretion.

Taken together, the data suggest that in late pregnancy, liver lipid metabolism may be directed towards VLDL se-

TABLE 1. Summary of changes in elements related to cholesterol and lipoprotein metabolism during pregnancy and lactation

	Late Preg	Delivery	1st Suckling	Early Lact.	Mid Lact.	Late Lact.	Weaning	1wk Post	3 wks Post
Choles	↑	↑	↑	↑	↑	↑	↑→	↑	→
TG	→	→	↓	↓	↓	↓	↑↑↑	→	→
VLDL	↑			↓	↓	↓	⇑	↓	→
LDL				↑	↑	↑	↓	↑	→
HDL				small HDL	←	→	HDL size increasing		
ApoB-100	↑→	→	↑→	→	↑	→	→	→	→
ApoB-48	↑	→	→	↑→	↑	↑↑	↑↑↑	→	→
ApoE	↓	↓	↓	↓	↓	↓	↓	↓	→
ApoA-IV	↓↓	↓	↓	↓	→	→	↓	↓	→
ApoA-I	↓	↓	↓	↓	↓→	↓	↓	↓	→
ApoC-III	↓→	↓	↓	↓	↓	↓	↓	↓→	ND
Reductase	↓↓	↓	↓	↑→	↓	↓	↓	↓	→
ACAT endo	↓	↓↓	↓	↑	↓	↓	↓	↓	→
ACAT exo	↓	↓	↓	↓→	↓	↓	↓	↓	→
DGAT	↑↑	↓	↓	↓	↓	↓↓	↑↑	↓	→
NCEH m	↓	↓	→	↑	↑→	↑	↓	→	↑
NCEH cy	↓	↓	↓→	→	↓→	↓→	↓	↓	→
LDLR	→	↑	↑	→	↑	↑	↑	↑	↑
LRP	→	↑	→	↓→	↑	↑	↑	↑	↑
ACEH m	↓→	↓	→	↓→	→	→	↓	↓	↑
ACEH cy	↓	→	→	↓	↓	↓	↓	↓	→
7α-H	↓↓	↓	↓	→	↓	↓	↓	↓	→
SRB1	↑↑	↑	↑↑	↑→	↑→	↓	→	→	↑→
mdr2	→	↑	↑	↑	↑	→	↑	→	→

cretion at the expense of biliary secretion. This would supply lipids, especially triglycerides, to the placenta for fetal energy requirements and to the mammary gland for milk lipids in anticipation of the onset of suckling (57–60).

Effects of birth and first suckling. Both LDL receptors and SR-BI increased after first lactation, suggesting an increased hepatic requirement for cholesterol, likely for bile acid synthesis and secretion and biliary secretion of cholesterol as suggested by the abrupt rise at this time in cholesterol 7α-hydroxylase activity relative to its level during pregnancy and the increase in mdr2. This latter change also could result in reversal of any pregnancy-induced hepatic cholestasis. ACAT also increased after birth, concomitant with the drop in DGAT and the increase in LDL receptors, indicating that significant numbers of apoE- and/or apoB-containing plasma lipoproteins are being cleared from the circulation and that this lipoprotein cholesterol enters the ACAT substrate pool. In addition to the increase in lipoprotein receptors, HMG-CoA reductase also increased, likely in part to provide de novo synthesized cholesterol and bile acids to the biliary pool, cholesterol to membranes for liver hypertrophy known to occur during the lactation period (38–40), and for lipoprotein secretion to provide cholesterol and triglycerides for milk production (57–59). The increase in reductase also may have been to satisfy increased needs for nonsterol mevalonate products necessary for liver hypertrophy.

Taken together, the changes at this time point suggest that requirements both for de novo synthesized mevalonate and cholesterol and for exogenous (lipoprotein) cholesterol via the LDL receptor-mediated endocytotic pathway had increased substantially and were important for maintenance of both hepatic and whole body cholesterol homeostasis.

Effects in the lactation period. Little has been reported on lipoprotein profiles during lactation in any species. In the present work plasma triglyceride levels of lactating rats were low, while plasma cholesterol levels were elevated. As a reflection of this, plasma VLDL particles were absent during lactation. Nevertheless, apoB levels were generally increased as a consequence of the increase in LDL-size particles, whereas apos E, A-I, A-IV, and C-III were generally decreased, reflecting the changes in HDL profile. The data, taken together, suggest that triglyceride-rich particles are rapidly cleared from the circulation during the lactating phase, likely by conversion to IDL/LDL-size particles through the action of mammary gland lipoprotein lipase to supply lipids for milk production (57, 58). Milk triglycerides and cholesterol are known to be derived from lipoproteins (59) as well as from de novo synthesis in the mammary gland (59, 60).

In early lactation, HMG-CoA reductase, ACAT, neutral CEH, LDL receptors, LRP, and SR-B1 were all elevated, suggesting that requirements for increased hepatic cholesterol availability are great in the initial period of lactation. These changes coincide with the known increased concentration of cholesterol in rat's milk in the early stages of lactation (61, 62), and beginnings of evident hypertrophy of the liver and proliferation of the intestine (38–40), both of which require increased cholesterol availability for membrane synthesis. Thus, the increases in hepatic activities likely reflect increased requirements for lipoprotein cholesterol to satisfy needs of the mammary gland and probably also the intestine. Increased bile acid synthesis and biliary secretion as shown by the increases in 7α-hydroxylase and mdr2 likely are to promote increased lipid absorption from the intestine to maintain both the mother and offspring optimally.

Changes in hepatic cholesterol metabolism are evident again at day 6 of lactation. HMG-CoA reductase and ACAT are still elevated; however, the other elements have decreased, including all of the lipoprotein receptors. This suggests that decreased amounts of the plasma lipoproteins are being cycled back to the liver, allowing sequestration of lipoprotein lipid by the mammary gland to support growth of the offspring via milk production and also by the intestine to support its needs for increased growth and absorption.

Around the mid-lactation point (about day 12) hepatic LRP and LDL receptors increased. This coincides with a decrease in cholesterol content of the milk (61) suggesting the liver has begun to increase clearance of plasma lipoproteins to compensate for declining needs of the mammary gland. The decreases in reductase and 7α-hydroxylase also likely reflect this decreased need.

HDL metabolism also was altered in the mid-lactation and post-lactation periods. A shift from large particles to smaller ones was associated with preferential decrement of a specific subpopulation of HDL because although a major peak was present at 10.5 nm, the shoulder at 11.2 nm, still in evidence after 3 days of suckling, disappeared as the lactation period proceeded. This was consonant with the decreased concentrations of plasma apoE, apoA-I, A-IV, and apoC-III found during lactation. Recently, strong evidence has been published that HDL cholesterol is preferentially secreted in bile (63) and other reports suggest it can be a precursor for bile acids (64, 65). Further, evidence of coordinate genetic control of HDL levels and bile acid metabolism was published recently (66). Thus, the changes in HDL species may reflect changes in hepatic biliary metabolism during this period.

Effects at weaning and 3 weeks later. The next point of major change was around the time of weaning. Just prior to this both LRP and SR-B1 decreased dramatically, perhaps in response to weaning-related regulatory processes. The striking increase in dam's plasma triglyceride concentrations and apoB-48 immediately after weaning of the pups mirrored the presence of very large lipoprotein particles in the plasma, suggesting a rebound effect wherein mammary gland lipoprotein lipase activity is essentially down-regulated, but the intestine still produces large amounts of triglyceride-rich lipoproteins. As the intestine is still almost 2-fold larger in mass at this time point (38–40) the proportion of intestinally derived lipoproteins in the plasma likely is high. The persistence of large triglyceride-rich particles during early post-weaning continues to reflect this state and is further evidence that triglyceride contained in these particles represented a significant source of lipid for secretion into milk.

Liver DGAT also increased at weaning. This likely reflects, in part, increased availability of cellular free fatty acids due to decreased uptake of triglycerides by mammary gland and consequently, return of increasing amounts of both lipoprotein triglycerides and probably, free fatty acids, to the liver as the mammary gland begins to involute. LDL receptors, LRP, and mdr2 also all increased after weaning, suggesting a response to the tissue alterations and remodeling which were beginning to occur, in particular, return of the mammary gland, liver, intestine, and circulatory elements to a control state.

Most of the hepatic elements were at control levels by 21 days post-lactation. However, LDL receptors, LRP, and acidic CEH remained markedly elevated at this time point, suggesting that the liver endocytic pathway was up-regulated specifically, perhaps to continue to deal with the extensive physiological tissue remodeling that occurs after weaning. The membrane-associated neutral CEH also was up-regulated with variable increases in SR-B1, suggesting they too were playing a role. The data also demonstrate that the metabolic response time to normalize the different hepatic and plasma elements involved is variable and suggest that coordinate regulation of the different elements occurs by different mechanisms dependent on the physiological state of the mother.

Taken together, the above data provide for the first time an integrated picture of hepatic cholesterol and lipoprotein metabolism during pregnancy, lactation, and post-lactation. Clearly, major compensatory changes in the regulatory elements of hepatic cholesterol metabolism occur during these important physiological states. Further, the data taken together provide a biochemical rationale at the level of the liver both for the hyperlipidemia of pregnancy and for the cholestasis of pregnancy and for their resolution. In addition, these results emphasize the complexity of the degree and type of integration of regulation of the various key elements involved in cholesterol metabolism observed under these conditions.

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