PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE 182, 386–392 (1986)

Parallel Changes in Plasma Cholesterol and Lipid Transfer Activity in Pregnant Rabbits[1] (42356)

DAVID W. QUIG AND DONALD B. ZILVERSMIT[2,3]

Division of Nutritional Sciences, Section of Biochemistry, Molecular and Cell Biology, Division of Biological Sciences, Cornell University, Ithaca, New York 14853

Abstract. The relationship between the concentration of plasma cholesterol and the lipid transfer activity (LTA) of lipoprotein-deficient plasma ($d > 1.21$) was studied in two models of pregnancy in the rabbit. Plasma cholesterol and the protein-mediated transfer of cholesteryl ester and triglyceride were monitored throughout gestation, 48 hr after parturition, and during lactation in New Zealand white (NZW) and heterozygous WHHL rabbits. Lipoprotein cholesterol was determined prior to and 48 hr after parturition. For both NZW and heterozygous WHHL rabbits, the progressive hypocholesterolemia of gestation was associated with parallel changes in LTA. Similarly, the rapid postpartum increase in plasma cholesterol was paralleled by increased LTA for both strains. In relation to basal values, the relative changes in plasma cholesterol and LTA were virtually identical. These data provide further evidence that in the rabbit plasma cholesterol and LTA are closely related.

The plasma of man (1) and the rabbit (2) contain a lipid transfer protein which facilitates the exchange and net transfer of esterified cholesterol and triglyceride among plasma lipoproteins, *in vitro*. Little is known about the metabolic regulation of lipid transfer activity (LTA) but, a study (3) of plasma lipids and LTA across numerous animal species showed that LTA is not correlated with plasma total cholesterol or the rate of plasma cholesterol esterification and that it is only weakly ($r = 0.35$) correlated with the concentration of very low-density lipoprotein cholesterol. In contrast, it has recently been reported that plasma LTA is highly correlated with plasma total cholesterol and very low- and low-density lipoprotein cholesterol in three models of hypercholesterolemia in the rabbit (4).

The purpose of the current research was to study the relationship between LTA and plasma cholesterol within a single species, the rabbit. The pregnant rabbit was chosen as the experimental model due to the drastic changes in plasma cholesterol which occur during gestation (5, 6) and upon parturition (6) in this species. The data demonstrate that LTA and plasma cholesterol change in unison with pregnancy in the rabbit and that the relative changes, both increases and decreases, were of surprisingly similar magnitude.

Materials and Methods. *Animal handling and care.* Virgin female New Zealand white rabbits (Beckens Research Animal Farm, Sanborn, N.Y.) and heterozygous Watanabe rabbits weighing 2.5 to 3.5 kg were used in these studies. Rabbits were individually housed and had free access to tap water. During gestation animals were provided with 100 to 200 g of commercial rabbit chow (Purina 5321, Ralston Purina, St. Louis, Mo.) per day (6); during lactation chow was provided *ad libitum*.

All rabbits used in these studies were artificially inseminated. The semen was collected from homozygous Watanabe heritable hyperlipidemic (WHHL) bucks as described by Bredderman *et al.* (7). WHHL semen was used for the purpose of generating WHHL heterozygotes and ultimately WHHL homozygotes for further studies. Fresh semen was extended (1/6 v/v) in buffer containing glycerol and 4.5% dimethyl sulfoxide and stored in liquid nitrogen according to the procedure described by Von Weitze *et al.* (8). To induce ovulation, does were given 5 mg of pituitary luteinizing

[1] These studies were supported by Research Training Grants HL 07245 and HL 10933 from the National Heart Lung and Blood Institute of the U.S. Public Health Service.

[2] Career Investigator of the American Heart Association.

[3] To whom reprint requests should be addressed at the Division of Nutritional Sciences, 200 Savage Hall, Cornell University, Ithaca, N.Y. 14853.

0037-9727/86 $1.50

hormone (Burns-Biotec Laboratories, Inc., Omaha, Nebr.) as an iv injection. The experimental protocols were in accordance with university guidelines.

Sample collection and analysis. Rabbits were bled (4 ml) from the marginal ear vein 1 week and 24 hr prior to artificial insemination and at weekly intervals thereafter during gestation and lactation. In the later studies, animals were bled only on Day 28 of gestation and 48 hr after parturition. With exception for some of the 48-hr postpartum samples, all blood samples were obtained between 10 and 12 AM. Blood was collected in tubes containing 0.01 ml of 0.4 *M* EDTA/4% NaN_3/ml blood and held on ice no longer than 1 hr before separation of plasma by low-speed centrifugation (4°C). Plasma total cholesterol was determined after saponification (9) and extraction with hexane (10) by the method of Zak *et al.* (11). For determination of LTA, 1 ml of lipoprotein-deficient plasma was prepared by ultracentrifugation (d = 1.21) in a Beckman 50.3 rotor at 4°C for $3 \times 10^8 g \times$ min. The d > 1.21 infranatant was recovered from the bottom two-thirds of the tube by tube slicing and dialyzed against 0.15 *M* NaCl/0.02% NaN_3 at 4°C overnight. As previously reported (4), >95% of the LTA was recovered from rabbit plasma by this procedure.

Transfer assay substrate preparation. Glycerol tri[9,10(n)-^{3}H]oleate and [4-^{14}C]cholesterol were purchased from Amersham Corporation (Arlington Heights, Ill.). [^{14}C]Cholesteryl ester was synthesized from [4-^{14}C]-cholesterol and oleoyl chloride (Nucheck Prep, Inc., Elysian, Minn.) as described by Goodman (12). The radiolabeled lipids were purified at >98% purity by thin-layer chromatography on precoated silica gel plates (Silica Gel 60, E. Merck, Darmstadt, F.R.G.) with hexane/diethyl ether/acetic acid (80/20/1 v/v) as the developing solvent and eluted in chloroform. The radiolabeled lipids were incorporated into phosphatidylcholine (Lipid Products, Nutfield, England) unilamellar vesicles which were incubated with fresh human plasma, as previously described (13). The labeled LDL were isolated from whole plasma by ultracentrifugation (1.019 < d < 1.063) and washed by recentrifugation (d = 1.063, $2.76 \times 10^8 g \times$ min). Human HDL were isolated from fresh plasma by ultracentrifugation (d > 1.063 mg/ml, $3.99 \times 10^8 g \times$ min). The lipoprotein substrates were dialyzed against 0.15 *M* NaCl/0.02% NaN_3 and bovine serum albumin was added (0.5% wt/v) prior to storage at 5°C.

Plasma lipid transfer assay. The LTA of lipoprotein-deficient plasma was determined as previously described (13). Briefly, the assay measures transfer of radioactive cholesteryl ester and triglyceride from human LDL to human HDL. After incubation, donor particles (LDL) were precipitated with sodium phosphate and manganese chloride (14) and the radioactivity in the supernatant was counted in aqueous scintillation cocktail (ACS; Amersham Corp.). Catalyzed transfer activity was calculated as described by Pattnaik *et al.* (1) using a two-pool closed model (15). LTA is expressed as $\%kt$ per milliliter plasma where k is the fraction of lipid transferred per unit time and t is the assay time. Over the course of the studies, four different preparations of substrates were utilized in the transfer assay. To eliminate intraassay variability introduced by the use of different or aging substrates, an external standard of pooled (d > 1.21) rabbit plasma kept at −20°C was routinely analyzed in duplicate. The lipid transfer activity of this rabbit standard was unaffected by storage at −20°C for at least 6 months; the coefficient of variation for the LTA of this rabbit standard was 8.4% when assayed with four different, freshly prepared substrate preparations over a period of 6 months.

Lipoprotein cholesterol determination. The esterified and free cholesterol concentrations of plasma lipoproteins were determined for some samples on Day 28 of gestation and 48 hr postpartum. Lipoproteins were isolated by sequential ultracentrifugation at densities 1.019 and 1.063, as described for LTA substrate preparation. Lipids in each density class were extracted with hexane and free cholesterol and cholesteryl esters were separated on precoated TLC plates (Silica Gel 60) with hexane/diethyl ether/acetic acid (80/20/1 v/v). Lipids were eluted from silica gel with chloroform/methanol (9/1 v/v), saponified (9), and again extracted with hexane. Recovery of cholesterol by this protocol was at least 90%, as determined with a porcine plasma standard of known cholesterol content. Lipoprotein cholesterol was quantified by gas–liquid chromatography on a 60-cm glass column with

an internal diameter of 4 mm. The column was packed with 3% SP2401 on 100–120 mesh Supelcofort (Supelco Inc., Bellefonte, Pa.). Analyses were performed on a Hewlett–Packard Model 5790A gas chromatograph equipped with a flame ionization detector. Nitrogen was used as carrier gas at a flow rate of 40 ml/min. The injection port, column, and detector temperatures were kept at 235°C. Cholesteryl methyl ether (Sigma, St. Louis, Mo.) was used as the internal standard and had a retention time 0.7 times that of cholesterol. The internal standard method was used to calculate cholesterol content. The mean standard deviation for duplicate analysis was 2.18%, as calculated from 75 samples.

Results. In these studies, 6 New Zealand white rabbits were artificially inseminated with semen collected from WHHL homozygous bucks. The conception rate was 55% (15 pregnancies/27 inseminations); 2 does apparently aborted between Days 21 and 28 of gestation. The litter size was 5.2 ± 0.8 (mean ± SE) live young.

The changes in plasma total cholesterol concentration and LTA during gestation and lactation for a typical pregnant rabbit and nonpregnant control are illustrated in Fig. 1. Week-to-week variability in both plasma cholesterol and LTA are evident for the control animal, with no apparent systematic relationship between the two variables. In contrast, changes in plasma cholesterol and LTA occurred in unison for the pregnant rabbit; the two curves were parallel throughout gestation

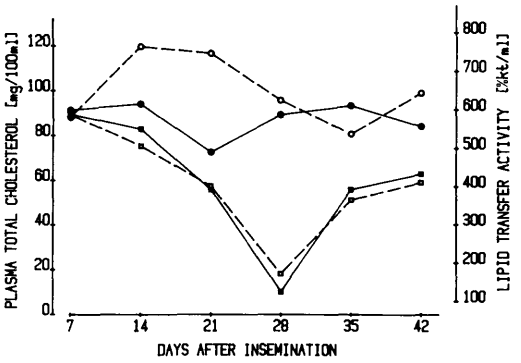

FIG. 1. Plasma total cholesterol and lipid transfer activities for a typical pregnant and control rabbit. Plasma cholesterol: control (●), pregnant (■); plasma lipid transfer activity: control (○), pregnant (□).

TABLE I. RELATIVE PLASMA TOTAL CHOLESTEROL LEVELS AND LIPID TRANSFER ACTIVITIES DURING GESTATION AND LACTATION[a]

Days after insemination	Total cholesterol[b]	Transfer activity[b] Cholesteryl ester	Triglyceride
Gestation			
7 (6)	99.2 ± 5.1	101.0 ± 7.5	98.6 ± 6.3
14 (5)	89.1 ± 8.9	80.0 ± 4.0	82.9 ± 3.6
21 (6)	50.2 ± 7.3	49.5 ± 4.3	51.9 ± 4.9
28 (6)	17.2 ± 2.0	27.3 ± 4.5	28.7 ± 4.7
Lactation			
35 (4)	58.4 ± 12.8	58.9 ± 14.7	62.2 ± 18.1
42 (6)	61.0 ± 8.5	71.3 ± 5.1	70.4 ± 6.1

[a] Group mean ± SE; number in parentheses represents number of observations.

[b] % Basal values were calculated for each animal as (value at designated day postinsemination/basal value) × 100. Basal values (mean ± SE) were plasma cholesterol, 72.6 ± 5.8 mg/dl; cholesteryl ester and triglyceride transfer activities, 594 ± 28 and 489 ± 29% kg/ml, respectively.

and lactation. Although not shown, changes in triglyceride transfer activity were parallel to those of cholesteryl ester transfer activity.

To facilitate comparison of weekly changes in plasma cholesterol and LTA for the group of pregnant rabbits, the weekly values for plasma cholesterol and LTA for each animal were expressed as percentages of the respective preinsemination (basal) values. The group mean ± SE for basal plasma total cholesterol was 72.6 ± 5.8 mg/100 ml. The cholesteryl ester and triglyceride transfer activities were 594 ± 28 and 489 ± 29 %*kt*/ml plasma, respectively. The group mean ± SE for these variables at weekly intervals during gestation and lactation, expressed relative to basal values, are presented in Table I. Both plasma cholesterol and cholesteryl ester or triglyceride transfer activities decreased progressively during gestation and reached minimum values by Day 28. After parturition (Day 35), plasma cholesterol and LTA rebounded in a parallel manner to about 60% of basal values.

The average length of gestation for rabbits in this study was 31 days (range, 30–33 days), Day 0 being the day of insemination. The first postpartum sample was collected 35 days after insemination. Therefore, the Day-35 samples for the animals were not uniform with respect to time after parturition. To characterize the

TABLE II. RELATIVE CHANGES IN PLASMA CHOLESTEROL AND LIPID TRANSFER ACTIVITY IN PREGNANT RABBITS 48 hr AFTER PARTURITION[a]

	Total cholesterol[b]	Transfer activity[b]	
		Cholesteryl ester	Triglyceride
Day 28 of gestation	16.2 ± 3.0	23.2 ± 4.6	22.6 ± 3.1
48 hr postpartum	47.5 ± 5.5	43.2 ± 1.8	39.0 ± 2.8

[a] Group mean ± SE, six animals.
[b] % Basal values were calculated for each animal as (values at designated day postinsemination or hour postpartum/basal value) × 100. Basal values (mean ± SE) were plasma cholesterol, 99.5 ± 15.6 mg/dl; cholesteryl ester and triglyceride transfer activities, 587 ± 28 and 480 ± 36% *kt*/ml, respectively.

drastic postpartum increases in plasma cholesterol and LTA more accurately, the rabbits were reinseminated and blood samples were collected on Day 28 and 48 hr after parturition. Samples were not collected closer to parturition to avoid jeopardizing the first generation WHHL young, which were to be further studied. The group mean ± SE for pre- and postpartum plasma cholesterol and LTA values, expressed relative to basal values, are shown in Table II. The relative values for the variables on Day 28 of gestation in this study were not different from those observed in the first study (Table I). By 48 hr postpartum, plasma cholesterol and transfer activities returned to greater than 43% of basal values. Relative to the values of Day 28, plasma cholesterol and LTA had increased 3.6 ± 1.0-fold and 2.2 ± 0.7-fold, respectively, within 48 hr of parturition.

To determine if the observed changes in the LTA of lipoprotein-deficient plasma were associated with changes in lipid distributions among circulating lipoproteins, the cholesterol concentrations of the plasma lipoproteins of some pregnant rabbits were measured. Presented in Table III are the group means ± SE for lipoprotein total and esterified cholesterol concentrations for pregnant rabbits on Day 28 of gestation and 48 hr after parturition. Compared to nonpregnant controls, the total and esterified cholesterol concentrations of all lipoprotein density classes of pregnant rabbits were reduced near parturition. Large changes were noted for both LDL and HDL. In addition, there appeared to be reductions in the relative cholesteryl ester content of the $d < 1.019$ lipoproteins. By 48 hr after parturition LDL and HDL cholesterol levels had risen to about 50% of basal values.

The relationship between plasma cholesterol and LTA was also studied in heterozygous WHHL rabbits during pregnancy. Heterozygous WHHL does were acquired as a result of inseminating New Zealand white females with semen collected from WHHL homozygous bucks. The six heterozygotes were inseminated twice over a period of 8 weeks and the same two animals conceived on both occasions (4 pregnancies/12 inseminations). Blood samples were collected prior to and 28 days after insemination and 48 hr after parturition. Presented in Table IV are the absolute and relative values for plasma cholesterol and LTA for the heterozygous

TABLE III. PLASMA LIPOPROTEIN CHOLESTEROL AND CHOLESTERYL ESTER CONCENTRATIONS IN PREGNANT RABBITS[a]

Group	VIDL (mg/dl)	LDL (mg/dl)	HDL (mg/dl)
Nonpregnant controls	5.0 ± 1.5[b] (71 ± 2)[c]	64.3 ± 16.8 (67 ± 2)	27.1 ± 4.8 (68 ± 3)
Pregnant, Day 28	2.1 ± 0.4 (58 ± 2)	2.6 ± 0.5 (63 ± 2)	4.7 ± 1.3 (70 ±1)
48 hr postpartum	3.9 ± 2.4 (69 ± 2)	33.4 ± 9.7 (64 ± 5)	12.8 ± 2.1 (67 ± 1)

[a] VIDL (very low- and intermediate-density lipoproteins), $d < 1.019$; LDL (low-density lipoproteins), $1.019 < d < 1.063$; HDL (high-density lipoproteins), $d > 1.063$.
[b] Group mean ± SE. Nonpregnant controls, $n = 3$; 48 hr postpartum, $n = 4$; pregnant, Day 28, $n = 4$, except that LDL and HDL cholesterol values were derived from three observations.
[c] Numbers in parentheses represent the cholesteryl ester as percentage of cholesterol.

TABLE IV. THE EFFECTS OF PREGNANCY ON PLASMA CHOLESTEROL AND LIPID TRANSFER ACTIVITIES IN HETEROZYGOUS WHHL RABBITS[a]

Condition	Animal	Pregnancy:[a]	Plasma cholesterol (mg/100 ml)		Lipid transfer activity (%kt/ml)			
					Cholesteryl Ester		Triglyceride	
			1	2	1	2	1	2
Basal	A		176	172	532	522	345	509
	B		317	360	715	945	672	658
Gestation, Day 28	A		26 (14.7)[b]	17 (9.8)	78 (14.7)	114 (21.8)	77 (22.0)	117 (23.0)
	B		23 (7.3)	20 (5.6)	135 (18.9)	—	94 (14.0)	—
48 hr after parturition	A		108 (61.4)	99 (57.5)	359 (67.5)	337 (65.0)	303 (87.8)	299 (58.7)
	B		84 (26.5)	77 (21.3)	223 (31.2)	395 (41.8)	150 (22.0)	264 (40.1)

[a] WHHL does were inseminated twice over an 8-week period; pregnancies are indicated by 1 and 2.
[b] Percentage of basal values shown in parentheses.

WHHL does during both pregnancies. As evident from the basal values, there were large differences in plasma cholesterol and LTA between the two rabbits. Despite these differences, plasma cholesterol and LTA were reduced to low levels for both animals on Day 28 of gestation. Similar to the response in New Zealand white rabbits, 48-hr postpartum rebounds in plasma cholesterol and transfer activities were observed for the two WHHL heterozygotes. Presented in Table V are the lipoprotein cholesterol and cholesteryl ester concentrations for the two WHHL heterozygotes on Day 28 and 48 hr after parturition (second pregnancy). It is not possible to determine clearly the extent to which the cholesterol concentrations of the lipoprotein fractions were affected on Day 28 because basal values were not determined. However, postpartum increases in plasma cholesterol and LTA were associated with 4-fold increases in LDL and HDL cholesterol for one animal and 20- and 2-fold increases in VLDL + IDL and LDL cholesterol, respectively, for the other animal.

Discussion. The present studies demonstrate that the drastic changes in plasma cholesterol observed in the pregnant rabbit are associated with similar changes in plasma LTA. Furthermore, the association between plasma cholesterol and LTA during pregnancy was observed for relatively hypercholesterolemic WHHL heterozygotes as well as for normolipidemic New Zealand white rabbits. In a previous report (4) it was demonstrated that three different models of hypercholesterolemia in rabbits were associated with increased plasma LTA. While the mechanistic nature of this association is not known, it is apparent that plasma LTA changes concomitantly with large changes in plasma cholesterol levels in the rabbit.

In the current studies, pregnant rabbits were utilized to study the relationship between plasma cholesterol and LTA. This model was particularly useful because it afforded the opportunity to monitor LTA with decreasing and increasing plasma cholesterol concentrations. The relative decreases for the two variables were virtually identical during gestation. Even more striking was the rapid increase in LTA with increased plasma cholesterol shortly after parturition.

TABLE V. LIPOPROTEIN CHOLESTEROL AND RELATIVE CHOLESTERYL ESTER CONCENTRATIONS IN HETEROZYGOUS WHHL RABBITS DURING THE SECOND PREGNANCY[a]

Sample	Animal	VIDL (mg/100 ml)	LDL (mg/100 ml)	HDL (mg/100 ml)
Gestation, Day 28	A	2.4 (65)[b]	17.2 (66)	5.1 (61)
	B	2.0 (55)	13.6 (54)	5.2 (46)
48 hr after parturition	A	5.2 (50)	69.7 (60)	20.5 (58)
	B	40.0 (65)	27.3 (69)	6.8 (63)

[a] VIDL (very low- and intermediate-density lipoproteins), $d < 1.019$; LDL (low-density lipoproteins), $1.019 < d < 1.063$; HDL (high-density lipoproteins), $d > 1.063$.
[b] Numbers in parentheses represent the cholesteryl ester as percentage of cholesterol.

The mechanism(s) for decreased plasma cholesterol and LTA during gestation and the rapid postpartum rebounds for these variables are not known. The hypocholesterolemic effects of exogenous estrogen have been demonstrated in the rat (16, 17) and postmenopausal women (18, 19) and are characterized by decreased LDL cholesterol. In rats, the estrogen-induced reduction in LDL cholesterol is associated with an increased number of hepatic LDL receptors (16) and subsequent stimulation of hepatic LDL uptake (20). Plasma estrogen increases during gestation in the rhesus monkey (21) and in women (22). However, plasma cholesterol decreases in the monkey (23) and increases in women (24) during gestation. Furthermore, postpartum increases in plasma cholesterol in the rabbit are not associated with a change in plasma estrogen (25), and plasma cholesterol (24) and estrogen (22) both decrease at term in women. Thus, it is unlikely that plasma estrogen is the sole determinant of plasma cholesterol during pregnancy.

To date, little is known about the regulation of plasma LTA. Partial lipolysis of lipoprotein substrates has been shown to enhance transfer activity *in vitro* (26). In the current studies we have focused on changes in the LTA of lipoprotein deficient plasma. Therefore the observed changes in LTA could represent changes in the mass or specific activity of lipid transfer protein or of an endogenous inhibitor. Although inhibitor activity has been found in the lipoprotein deficient plasmas of humans, rats, and pigs, inhibitory activity was not detected in lipoprotein deficient rabbit plasma (13).

The mechanism(s) for the apparent relationship between changes in plasma cholesterol and LTA in the rabbit are not known. Low levels of LTA have been detected in recirculating, hepatic perfusates of rabbits (27) and it has been demonstrated that plasma LTA binds to VLDL (26, 28), LDL (28), and HDL (27, 28) *in vitro*. In the current studies of pregnant rabbits, changes in LTA were associated with large changes in plasma cholesterol primarily in the LDL and HDL fractions. In a previous study (4), the increased LTA for two different models of diet-induced hypercholesterolemia was highly correlated with increased VLDL and LDL cholesterol. The common features between these studies appear to be the parallelism between plasma LTA and both LDL and total plasma cholesterol. These findings raise the possibility that plasma LTA is regulated not only by a balance between its secretion into and removal from plasma but also by the availability of lipoprotein particles to which the transfer protein can bind.

The technical assistance of Kenneth Cranker, Eduardo Erhenwald, and Brian Wilcox, is gratefully acknowledged. We also thank Joan Roberts for preparation of this manuscript.

1. Pattnaik NM, Montes A, Hughes LB, Zilversmit DB. Cholesteryl ester exchange protein in human plasma: Isolation and characterization. Biochim Biophys Acta **530**:428–438, 1978.
2. Zilversmit DB, Hughes LB, Balmer J. Stimulation of cholesteryl ester exchange by lipoprotein-free rabbit plasma. Biochim Biophys Acta **409**:393–398, 1975.
3. Ha YC, Barter PJ. Differences in plasma cholesteryl ester transfer activity in sixteen vertebrate species. Comp Biochem Physiol B **71**:265–269, 1982.
4. Son YSC, Zilversmit DB. Increased lipid transfer activities in hyperlipidemic rabbit plasma, in press.
5. Baumann EJ, Holly OM. Cholesterol and phosphatide

metabolism in pregnancy. Amer J Physiol **75**:618–632, 1925–26.
6. Zilversmit DB, Hughes LB, Remington M. Hypolipidemic effect of pregnancy in the rabbit. J Lipid Res **13**:750–756, 1972.
7. Bredderman PJ, Foote RH, Yassen AM. An improved artificial vagina for collecting rabbit semen. J Reprod Fert **7**:401–403, 1964.
8. Von Weitze KF, Scharnholz A, Helleman C. Tiefgefrierkonservierung von kaninchensperma. iv. Einflub von glycerin auf das befruchtungsvermogen. Zuchthygiene **18**:67–70, 1983.
9. Abell LL, Levy BB, Brodie BB, Kendall FE. A simplified method for the estimation of total cholesterol in serum and demonstration of its specificity. J Biol Chem **195**:357–366, 1952.
10. Thompson JN, Erdody P, Brien R, Murray TK. Fluorometric determination of vitamin A in human blood and liver. Biochem Med **5**:67–88, 1971.
11. Zak B, Moss N, Boyle AJ, Zlatkis A. Reactions of certain unsaturated steroids with acid iron reagent. Anal Chem **26**:776–777, 1954.
12. Goodman DS. Hydrolysis and formation of cholesterol esters in rat liver. Methods Enzymol **15**:522–537, 1969.
13. Morton RE, Zilversmit DB. A plasma inhibitor of triglyceride and cholesteryl ester transfer activities. J Biol Chem **256**:11992–11995, 1981.
14. Morton RE, Zilversmit DB. The separation of apolipoprotein D from cholesteryl ester transfer protein. Biochim Biophys Acta **663**:350–355, 1981.
15. Shipley RA, Clark RE. Tracer Methods for *in Vivo* Kinetics: Theory and Applications. New York, Academic Press, p132, 1972.
16. Kovanen PT, Brown MS, Goldstein JL. Increased binding of low density lipoprotein to liver membranes from rats treated with 17 α ethinyl estradiol. J Biol Chem **254**:11367–11373, 1979.
17. Handley DA, Arbeeny CM, Eder HA, Chien S. Hepatic binding and internalization of low density lipoprotein-gold conjugates in rats treated with 17α-ethinyl estradiol. J Cell Biol **90**:778–787, 1981.
18. Hazzard WR, Haffner SM, Kushwaha RS, Applebaum-Bowden D, Foster DM. Preliminary report: Kinetic studies on the modulation of high-density lipoprotein, apolipoprotein, and subfraction metabolism by sex steroids in a postmenopausal women. Metabolism **33**:779–784, 1984.
19. Wallace RB, Hoover J, Barret-Conner E, Rifkind BM, Hunning-Hake DB, Mackenthur A, Heiss G. Altered plasma lipid and lipoprotein levels associated with oral contraceptive and estrogen use. Lancet **2**:211–214, 1979.
20. Chao YS, Windler EE, Chen GC, Havel RJ. Hepatic catabolism of rat and human lipoproteins in rats treated with 17α-ethinyl estradiol. J Biol Chem **254**: 11360–11366, 1979.
21. Liskowski L, Wolf RC, Chandler S, Meyer RK. Uninary estrogen excretion in pregnant rhesus monkeys. Biol Reprod **3**:55–60, 1970.
22. Turner CD, Bagnara JT. General Endocrinology. Philadelphia, Saunders, p501, 1976.
23. Martin DE, Wolf RC, Meyer RK. Plasma lipid levels during pregnancy in the rhesus monkey. Proc Soc Exp Biol Med **138**:638–641, 1971.
24. Oliver MF, Boyd GS. Plasma lipid and serum lipoprotein patterns during pregnancy and puerperium. Clin Sci **14**:15–23, 1955.
25. Lau IF, Saksena SK, Salmonson R. The concentration of progesterone, 20 -dihydroprogesterone, testosterone, oestrone and oestradiol-17 in serum, amnionic fluid and placental tissue of pregnant rabbits. Acta Endocrinol **99**:605–611, 1982.
26. Sammett D, Tall AR. Mechanisms of enhancement of cholesteryl ester transfer protein activity by lipolysis. J Biol Chem **260**:6687–6697, 1985.
27. Pattnaik NM, Zilversmit DB. Interaction of cholesteryl ester exchange protein with human plasma lipoproteins and phospholipid vesicles. J Biol Chem **254**: 2782–2786, 1979.
28. Morton RE. Binding of plasma-derived lipid transfer protein to lipoprotein substrates. The role of binding in the lipid transfer process. J Biol Chem **260**:12593–12599, 1985.

Received January 20, 1986. P.S.E.B.M. 1986, Vol. 182.
Accepted April 11, 1986.