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Changes in Plasma Lipids and Lipoproteins in *Macaca nemestrina* during Pregnancy and the Postpartum Period[1] (40848)

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During pregnancy, decreases in plasma cholesterol concentrations have been reported for rabbits (1–3), *Macaca mulatta* (4–6), *Macaca nemestrina* (7), and the baboon (8). Zilversmit and co-workers (3) found this decrease to affect all plasma lipoproteins, very low-density lipoproteins (VLDL), low-density lipoproteins, (LDL), and high-density proteins HDL in rabbits. In our preliminary report (7), we found that only the HDL cholesterol fraction in *M. nemestrina* was affected. In human females, plasma cholesterol concentrations decrease early in the pregnancy and then progressively increase, with a maximum reached by the third trimester (9–12). Plasma LDL concentrations have been found to increase, while reports containing HDL concentrations are inconsistent (9, 11–15).

In the present study we have measured the plasma lipid and lipoprotein concentrations in a large population of nonhuman primates (the University of Washington Regional Primate Research Center breeding colony of *M. nemestrina*) during pregnancy and during the postpartum period in hopes of providing a better understanding of factors which may influence plasma lipid concentrations and perhaps ultimately the development of atherosclerosis. Nonhuman primates were chosen for this study because their lipoprotein metabolism and atherosclerotic lesions are similar to those observed in man (16, 17). We measured plasma lipid and lipoprotein concentrations while the animals consumed their regular low-fat, cholesterol-deficient diet and while they consumed a diet containing cholesterol plus added fat, to more closely approximate the diet of the average North American. Thus, the effects of pregnancy and the postpartum period upon plasma lipids and lipoproteins were examined during dietary conditions which would be expected to modify plasma lipid levels. This situation gave us the chance to more closely compare the response in this species to that in humans, and to evaluate the effect of pregnancy on plasma lipid responses to dietary modification.

Materials and methods. Two hundred and thirty-eight *M. nemestrina* from the breeding colony at the University of Washington Regional Primate Research Center were either pregnant or in the postpartum period at the time of this study. Housing of the animals and methods of age determination have been described previously (18). The mean age of the animals included in this study was 6.7 ± 0.1 years.

The reproductive status of each female monkey was noted each time blood samples were drawn for lipid determinations. Pregnancy was determined and the stage estimated by palpation. The estimated age of the pregnancy was corrected retrospectively after parturition. The number of days postpartum was determined using the date of parturition for each female that had given birth prior to or during the study. Lactation was determined by the presence of an infant of suckling age or by the presence of milk in the breast.

All animals consumed a control diet of Purina Monkey Chow, the regular diet of the breeding colony. Two venous blood samples were obtained, at weekly intervals, from animals that had been fasted at least 12 hr. After obtaining the second blood sample from the control phase of the experiment, the animals were started on the test diet consisting of Monkey Chow evenly coated with 10% lard (by weight) containing

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0.3 mg of dissolved crystalline cholesterol/kcal. The animals consumed the test diet for approximately 4½-months, then two more blood samples were obtained after fasting, again at weekly intervals.

Total plasma cholesterol (TPC) and triglyceride (TG) concentrations were determined simultaneously by the AutoAnalyzer II method of Rush *et al.* (19). The heparin-manganese precipitation method of Burstein *et al.* (20) was used for HDL cholesterol (HDL-chol) determinations. Low-density lipoprotein plus very low-density lipoprotein cholesterol (LDL + VLDL-chol) concentrations were calculated as the difference between TPC and HDL-chol. Electrophoresis of the lipoproteins was done by the modified method of Noble (21). Sample acquisition and methods were described in more detail previously (18).

In the present study, 100 pregnant females and 106 in the postpartum state were studied during the control diet period. During the test diet period, 121 pregnant and 117 postpartum females were studied. Data management and the methods of statistical analyses have been described previously (18). Probability values less than or equal to 0.05 were considered significant.

Results. Examination of the data indicated that most of the changes in lipid concentrations appeared to occur between the 35th and 65th day of pregnancy. For this reason, the data were analyzed in more detail after the animals were assigned to groups composed of animals 1–35 days pregnant (control n = 29, test n = 32), 36 to 65 days pregnant (control n = 25, test n = 22), and 66 days pregnant to parturition (control n = 46, test n = 67) rather than the traditional trimester divisions.

The changes in TPC, LDL + VLDL-chol, and HDL-chol concentrations during pregnancy are shown graphically in Fig. 1 for animals consuming the control diet. The means and standard error of the means have been placed on the graph for each time period. The regression lines have been added for the time periods where statistically significant changes with time occurred. A pattern of change in HDL-chol

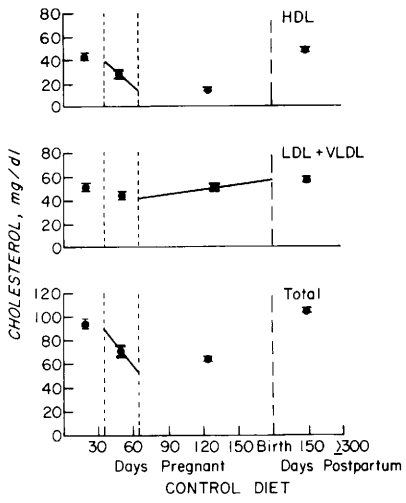

FIG. 1. Total plasma cholesterol, LDL + VLDL cholesterol, and HDL cholesterol concentrations during pregnancy and the postpartum period in *M. nemestrina* fed the control diet. The number of observations for each time period are: 1–35 days, n = 29; 36–65 days, n = 25; 66 days to parturition, n = 46; postpartum, n = 105.

concentration with time of pregnancy was found (Fig. 1). The values did not decrease significantly during the first 35 days of pregnancy and remained at 43 mg/dl (Table I), but decreased linearly with time during the period from 36 to 65 days of pregnancy, to less than 20 mg/dl HDL-chol (mean for this period 25 ± 2.4, see Table I). For the remainder of the time during pregnancy, HDL-chol concentrations remained low, mean ± SEM, 15 ± 1.1. After parturition, HDL-chol values returned to prepregnancy levels of 48 ± 1.3 mg/dl (Table II).

A very similar pattern of change during pregnancy was seen for TPC concentrations and is also shown in Fig. 1. In contrast, the LDL + VLDL-chol concentrations remained relatively constant although a decrease to 44 mg/dl occurred in the period of 36–65 days which was followed by a return to higher levels during the period of 66 days to parturition (42 mg/dl on Day 66 to 57 mg/dl just prior to delivery).

In animals fed the test diet, the same trends were apparent in the data as were found in control diet fed animals (Fig. 2), even though the absolute values for cholesterol concentratons were higher

TABLE I. MEAN BODY WEIGHT AND PLASMA LIPIDS OF PREGNANT *M. nemestrina* FEMALES FED THE CONTROL AND TEST DIETS

	Days pregnant								
	1–35			36–65			> 66		
	Control $N = 29$	Test $N = 32$	$p \leq$	Control $N = 25$	Test $N = 22$	$p \leq$	Control $N = 46$	Test $N = 67$	$p \leq$
Body weight, kg	5.70 ± 0.21[a]	5.70 ± 0.21	NS	6.18 ± 0.35	6.35 ± 0.42	NS	6.90 ± 0.22	7.35 ± 0.20	NS
Triglycerides, mg/dl	42.0 ± 3.6	32.0 ± 4.8	NS	27.0 ± 2.0	26.0 ± 7.7	NS	39.0 ± 3.6	40.0 ± 3.6	NS
Total cholesterol, mg/dl	95.0 ± 3.7	142.0 ± 12.1	0.01	69.0 ± 4.5	124.0 ± 13.1	0.01	64.0 ± 2.1	118.0 ± 7.5	0.01
HDL cholesterol, mg/dl	43.0 ± 2.6	46.0 ± 3.0	NS	25.0 ± 2.4	34.0 ± 4.2	NS	15.0 ± 1.1	18.0 ± 1.1	0.05
LDL + VLDL cholesterol, mg/dl	52.0 ± 2.5	97.0 ± 10.8	0.01	44.0 ± 2.9	90.0 ± 13.0	0.01	49.0 ± 1.9	100.0 ± 7.6	0.01
β-Lipoprotein, %	39.0 ± 1.8	48.0 ± 2.3	0.01	46.0 ± 1.7	51.0 ± 3.0	NS	60.0 ± 2.2	59.0 ± 2.0	NS
pβ-Lipoprotein, %	17.0 ± 1.4	14.0 ± 1.3	NS	21.0 ± 1.7	17.0 ± 1.8	NS	19.0 ± 1.8	19.0 ± 1.4	NS
α-Lipoprotein, %	44.0 ± 1.7	39.0 ± 1.8	0.05	34.0 ± 1.4	33.0 ± 2.4	NS	22.0 ± 1.2	22.0 ± 1.2	NS

[a] All results are the mean ± SEM.

TABLE II. MEAN BODY WEIGHT AND PLASMA LIPIDS OF POSTPARTUM FEMALE *M. nemestrina* FED THE CONTROL AND TEST DIETS

	Postpartum		
	Control $N = 105$	Test $N = 117$	$p \leq$
Body weight, kg	6.47 ± 0.16[a]	6.78 ± 0.17	NS
Triglycerides, mg/dl	42.0 ± 2.9	30.0 ± 2.0	0.01
Total cholesterol, mg/dl	105.0 ± 2.3	165.0 ± 4.6	0.01
HDL cholesterol, mg/dl	48.0 ± 1.3	61.0 ± 1.9	0.01
LDL + VLDL cholesterol, mg/dl	57.0 ± 1.7	104.0 ± 4.9	0.01
β-Lipoprotein, %	39.0 ± 0.7	45.0 ± 1.1	0.01
Pβ-Lipoprotein, %	16.0 ± 0.6	13.0 ± 0.7	0.01
α-Lipoprotein, %	46.0 ± 0.6	42.0 ± 0.9	0.01

[a] All results are the mean ± SEM.

(Table I). This was true for HDL-chol concentrations at all intervals except the 65 days to parturition interval, during which HDL values were nearly equivalent to control diet values. Thus, the decrease in HDL-chol concentration in response to pregnancy in animals fed the test diet was more pronounced than in control diet-fed animals. The decrease in TPC during pregnancy in the test diet group was due almost entirely to the decrease in HDL-chol concentration.

Plasma triglyceride concentrations also varied with the stage of pregnancy (Fig. 3, Table I). In control diet animals, the trend was to decrease up to 65 days of pregnancy, after which a significant positive correlation ($r = 0.69$, $P \leq 0.01$) was found between pregnancy and triglyceride concentration. A similar trend was apparent in the animals fed the test diet, although a significant trend toward increasing TG concentrations with

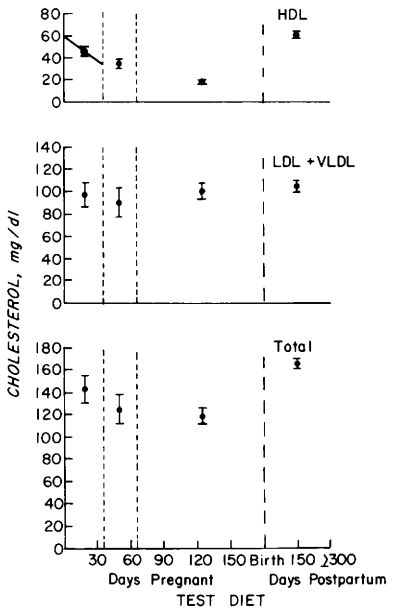

FIG. 2. Total plasma cholesterol, LDL + VLDL cholesterol, and HDL cholesterol concentrations during pregnancy and the postpartum period in *M. nemestrina* fed the test diet. The number of observations for each time period are: 1–35 days, $n = 32$; 36–65 days, $n = 22$; 66 days to parturition, $n = 67$; postpartum, $n = 117$.

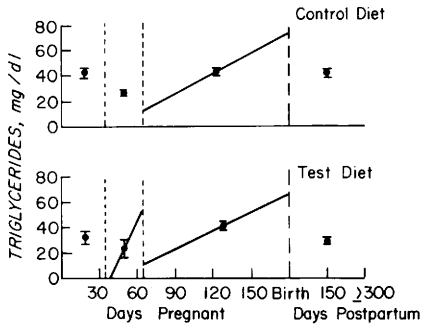

FIG. 3. Plasma triglyceride concentrations during pregnancy and the postpartum period in *M. nemestrina* fed control and test diets. The number of observations for each time period are: 1–35 days, control $n = 29$ and test $n = 32$; 36–65 days, control $n = 25$ and test $n = 22$; 66 days to parturition, control $n = 46$ and test $n = 67$; postpartum, control $n = 105$ and test $n = 117$.

advancing time of pregnancy appeared earlier, during the time interval of 35–65 days, and continued throughout the remainder of the time of pregnancy ($r = 0.50$, $P \leq 0.01$).

The electrophoretic data provided an independent method of evaluating the changes in plasma lipoproteins by giving indications of the distribution of lipids among the lipoprotein classes. In fact, electrophoretic screening of the plasma of these animals provided us with the first indication that pregnancy was influencing the α-lipoprotein fraction. Examples of the lipoprotein electrophoretograms and densitometric scans are shown in Figs. 4 and 5 for animals fed the control and test diets, respectively. Males and nulliparous females have been included for comparison with females in various stages of pregnancy. As can be seen, the relative percentage of α-lipoprotein progressively decreases during pregnancy to a minimum near parturition. For the other lipoprotein fractions, the beta (β) and pre-beta (Pβ)-lipoproteins, considerable individual animal variability makes any consistent pattern hard to detect. The electrophoresis data were quantitated and in Table I are expressed as the relative percentage of stainable lipid within each lipoprotein band. The trends seen by this procedure are similar to those seen for HDL- and LDL + VLDL-chol. The relative percentage in the α-lipoproteins is lower in each stage of pregnancy, following the trend seen for HDL-chol. The fact that an increase in the β-lipoproteins occurred while no change occurred in the Pβ-lipoproteins, reflects a change in the distribution of lipids between these two regions rather than a concentration change.

Significant effects of the test diet on lipoprotein distribution were only found during the earliest time interval when percentage in β-lipoproteins was higher and the percentage in the α-lipoproteins was lower. The relative percentages of β- and α-lipoproteins after the 36th day of pregnancy were similar in the control and test animals.

Immediately following parturition, HDL-chol concentrations increased dramatically and remained constant for the remainder of the postpartum period ob-

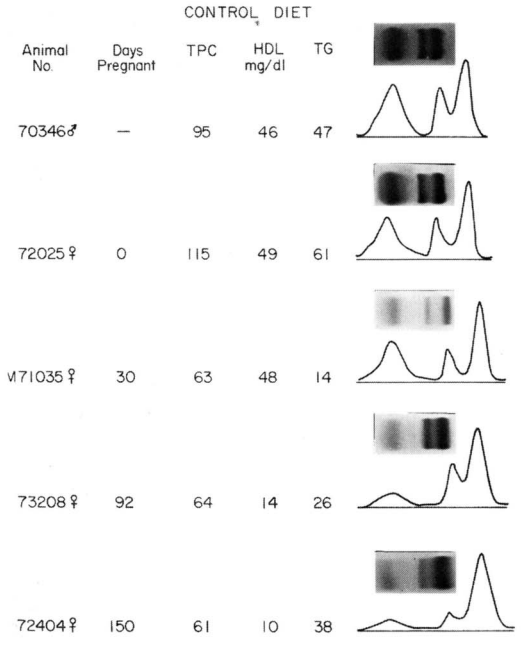

FIG. 4. Plasma lipoprotein electrophoretograms and densitometric scans for *M. nemestrina* fed the control diet. Lipoprotein fractions are (from left to right) α, Pβ, and β.

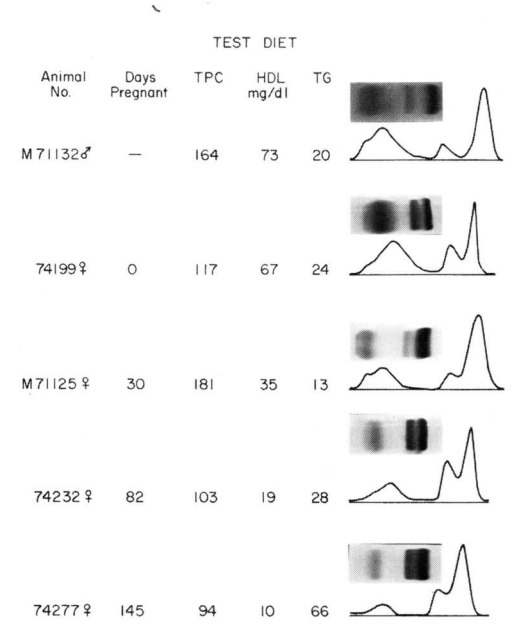

FIG. 5. Plasma lipoprotein electrophoretograms and densitometric scans for *M. nemestrina* fed the test diet. Lipoprotein fractions are (from left to right) α, Pβ, and β.

served, 300 days (Figs. 1 and 2, Table II). There was also an increase in TPC concentrations following parturition reflecting the increase in HDL-chol. Comparisons of the data for animals fed the control and test diets during the postpartum period can be made in Table II. Plasma triglyceride concentrations were significantly lower in test animals, while all plasma cholesterol concentrations were higher. A diet-induced change in the distribution of plasma lipids was reflected in the relative percentage of lipid staining material carried by each lipoprotein fraction. The animals consuming the test diet had higher concentrations of cholesterol in the LDL + VLDL-chol fraction and this was consistent with the greater percentage of lipid staining area found after electrophoresis in the β-lipoprotein fraction. As a result, the percentage of material carried by the Pβ- and α-lipoproteins was lower in the test animals than in controls.

Plasma lipid concentrations were examined for possible effects of the number of conceptions the females had experienced, whether the pregnancy ended with a viable offspring or prenatal death, and whether the female was lactating. None of these parameters showed association with any of the plasma lipids: TPC, LDL + VLDL-chol, HDL-chol, or TG concentrations.

Discussion. The primary finding of this study was the change in plasma HDL-chol concentrations during pregnancy and at parturition. Plasma HDL-chol concentrations decreased rapidly during the first 65 days of pregnancy, remained at a low level for the remainder of the pregnancy and then increased sharply after parturition. The same trend of HDL-chol decrease with pregnancy and increase at parturition was confirmed using quantitative agarose electrophoresis as an independent method of analysis. For the most part, the change in TPC paralleled that of HDL and was due to the changing HDL concentrations. The LDL + VLDL-chol concentrations were relatively more constant throughout the periods of pregnancy. The agarose electrophoresis data indicated that the β-lipoproteins (presumably mostly LDL) are the fraction which becomes the predominant plasma lipoprotein during the latter stages of pregnancy. The Pβ-lipoproteins [which represent VLDL and Lp(a) (22)] do not appear to fluctuate much with time of pregnancy. In a separate study using a small number of animals, VLDL and LDL have been isolated and quantified. The conclusions from that study are consistent with the data in this paper.[2] Essentially the same trend in lipoprotein patterns was found in pregnant animals fed a diet enriched in cholesterol and saturated fat compared to monkey chow fed animals, indicating that factors controlling the HDL-chol decrease override the dietary influence, which was to increase HDL concentrations. The increase in HDL-chol concentration with this diet modification has been found in males and nulliparous females of the same colony (18).

This appears to be the first in-depth description of the pattern of plasma lipoprotein changes with pregnancy in a nonhuman primate species. Similar results have not been previously reported in any species. The lipoprotein pattern observed is distinct from that reported for the rabbit (3) in which the concentration of all lipoprotein classes decreased in pregnant females. The published reports on the decrease in serum lipid concentrations in pregnant rhesus monkeys did not include plasma lipoprotein measurements, although a similar pattern of change to that described herein was observed for both plasma cholesterol and triglycerides (5, 6). The pattern of change in plasma lipoproteins that occurs with increasing time of pregnancy in women was reviewed by Barclay (14). Concentrations of VLDL and HDL increase, HDL_2 decreases, and HDL_3 increases during pregnancy, so that the lipemia which has been consistently observed in the second and third trimester of pregnancy in human females (9–12, 14, 15) has, as one of its key aspects, an increased LDL to HDL ratio. Thus, this end result of pregnancy on plasma lipoprotein distribution is similar in humans to that we have found in *M. nemestrina*, although the specific changes among the lipoprotein classes may not be

[2] L. L. Rudel, M.. R. McMahan and R. S. Shah, Pregnancy effects on nonhuman primate high density lipoproteins. Submitted for publication.

the same, i.e., more of an HDL decrease in monkeys compared to humans.

Although we did not measure hormone levels in the present study, two lines of evidence suggest that progesterone may be associated with the decrease in HDL-chol concentrations in pregnant *M. nemestrina* and possibly in the subsequent abrupt increase following parturition. First, the changes in HDL-chol concentrations parallel the reported changes in progesterone concentrations during pregnancy and following parturition (23–27). And second, progesterone has been shown to cause a decrease in HDL, while estrogens cause an increase in HDL concentrations. In both cases, the primary subfraction affected is HDL_2 (28–31).

The effects of adding cholesterol and fat to the diet during the test period were to increase the variability among individuals and to increase the concentrations of TPC and LDL + VLDL-chol during pregnancy and the postpartum period over those observed in the control period. However, the effects of diet on HDL-chol concentrations were very different. During pregnancy, HDL-chol declined with little individual variability and with no difference in absolute concentrations between the control and test periods. Whereas, during the postpartum period, HDL-chol was significantly higher in animals fed the test diet. Thus, the effects of pregnancy on HDL-chol concentrations were stronger and overrode the effects of diet.

A high degree of current interest exists in factors which control HDL levels, brought about by the epidemiological observations of the inverse relationship between HDL-chol concentration and risk for premature coronary heart disease (32, 33). The observation that some factor in pregnancy may overcome dietary effects on HDL may eventually provide an important clue for those seeking to identify mechanisms of control of plasma HDL concentrations.

Summary. Plasma lipid concentrations and the relative distribution of lipoprotein fractions were examined in pregnant and postpartum female *M. nemestrina* while the animals consumed a control diet of Monkey Chow and again after the animals had consumed a diet of Monkey Chow–cholesterol–lard for 4½ months. Plasma HDL-chol concentrations decreased dramatically during the first 65 days of pregnancy and remained at a low level until parturition when there was a rapid increase to the level maintained throughout the postpartum period. Plasma LDL + VLDL-chol concentrations increased slightly only in the latter days of pregnancy and did not change after parturition. Dietary challenge caused an increase in TPC and LDL + VLDL-chol concentrations, but did not override the changes in HDL-chol concentrations caused by pregnancy. Plasma TG concentrations increased during the latter stages of pregnancy and were affected by diet only during the postpartum period. The net result of the lipoprotein changes during pregnancy was for the LDL to HDL ratio to increase significantly as a result of the decrease in HDL concentrations.

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