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Effects of Sex, Age and Dietary Modification on Plasma Lipids and Lipoproteins of *Macaca nemestrina* (40819)[1]

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Hypercholesterolemia has been shown to be one of the major risk factors of atherosclerosis. Elevation of plasma cholesterol concentrations by increasing dietary intake of cholesterol increases the severity and extent of atherosclerotic lesions in all animals with naturally occurring atherosclerosis and induces the formation of lesions in some animals that do not normally develop the disease (1). With dietary cholesterol, the increase in total plasma cholesterol concentration of these animals is usually due to an increase in plasma low density lipoproteins (2). Animals that have an increase in plasma high density lipoproteins and that transport the major portion of their plasma cholesterol in this fraction develop little or no atherosclerosis (1, 3). Recent epidemiologic studies on human beings support these findings in animal models, showing a direct relationship between plasma low density lipoproteins and coronary heart disease, while an inverse relationship exists with high density lipoproteins (4, 5). An understanding of the mechanisms that afford protection against, or an increase in, the risks of atherosclerosis would be facilitated by identification of animal models with variance in high and low density lipoprotein cholesterol levels similar to those found within the human population.

In recent years nonhuman primates have been used increasingly as animal models for research on atherosclerosis because of the similarities of the experimentally induced lesions to atherosclerosis in man (6). However, only a few of the approximately 200 species of nonhuman primates have been examined for suitability for atherosclerosis research and plasma lipoprotein cholesterol distributions have been studied in even fewer species (2). Most of the studies which have examined plasma lipoprotein cholesterol concentrations in large numbers of nonhuman primates have been conducted while the animals consumed natural diets or diets devoid of cholesterol (e.g., Monkey Chow). The vast majority of the human population consume diets containing some cholesterol. In order to approximate the human dietary situation as nearly as possible, nonhuman primates should be examined while consuming a diet similar in cholesterol and fat concentrations to that consumed by the average North American human being. These studies were designed to examine the variability due to sex and age in plasma lipids and lipoprotein concentrations within a large population of nonhuman primates and to determine the influence of a fat enriched, cholesterol-containing diet on these measurements. The results for male and nulliparous female animals are presented in this report. Companion studies on the effects of pregnancy on plasma lipids will be reported in a separate communication.

Materials and methods. Colony-born and feral *Macaca nemestrina* from the breeding colony at the University of Washington Regional Primate Research Center, Medical Lake, Washington, were used in this study. The animals were housed indoors in rooms measuring approximately 2.2 × 3.4 × 2.8 m, with a diurnal cycle of 11 hr of light and 13 hr without light. All animals were maintained either in breeding harems of one male with six to eight females and their offspring or in juvenile groups of 10 to 20 animals (7).

Infants, juveniles, and fully mature

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[2] Deceased.

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breeder monkeys were included for study. The age of colony-born animals was determined to the nearest whole year using the date of birth. Two methods, developed at the University of Washington Regional Primate Research Center, were used to determine age in feral animals. The first was dentition. Standards are available for tooth eruption and wear. The second method utilizes body weights after the animals leave quarantine. The body weights were compared with a growth curve of age-known animals. This gives a conservative estimate of age and is reasonably accurate to within six months for animals up to six years of age.

The monkeys consumed a control diet of Purina Monkey Chow, the regular diet of the breeding colony. Blood samples were obtained at two consecutive weekly intervals after the animals had been fasted for at least 12 hr. Following acquisition of the second blood sample, the monkeys were fed the test diet consisting of Monkey Chow evenly coated with 10% lard (by weight) to which crystalline cholesterol had been added so that the final diet contained 0.3 mg cholesterol/kcal. This level of dietary cholesterol is similar to that consumed by the average North American human being (8). The percentage of total calories from fat was approximately 20%. This could be considered a very prudent level of intake since the average fat consumption by North Americans is 30–40% of total calories. After the animals had been fed the test diet for approximately 4.5 months, blood samples were drawn from fasted animals, again at two consecutive weekly intervals.

The blood, obtained by venipuncture, was immediately mixed with ethylenediaminetetraacetic acid (EDTA, 1.0 mg/ml). Cellular components were separated by centrifugation and the plasma was transferred to screw-cap tubes. The tubes were then packed in wet ice and sent by air freight to the Bowman Gray School of Medicine. Samples were received within 4 days of the time they were drawn and remained refrigerated throughout transit.

Plasma lipids and lipoproteins were assayed while the animals consumed the cholesterol-free diet as well as the diet enriched with cholesterol and fat. Plasma cholesterol and triglyceride concentrations were determined simultaneously by the AutoAnalyzer II method of Rush *et al.* (9). High-density lipoprotein cholesterol (HDL-chol) concentrations were determined on the supernatant fluid after heparin-manganese precipitation of the low- (LDL) and very low-density (VLDL) lipoproteins (10). Since the animals were fasted, there were no chylomicra present. The LDL + VLDL cholesterol (LDL + VLDL-chol) concentration was calculated as the difference between total plasma cholesterol (TPC) and HDL-chol concentrations. All plasma lipids were determined in our lipid analytic laboratory that is in complete complicance with the cooperative lipid standardization program of the U.S. Department of Health, Education and Welfare.

Plasma lipoprotein electrophoresis was done by a modified method of Noble (11), using a 0.5% agarose solution, with no agar added. Lipids were stained at 40°C for 2–4 hr in 60% ethanol saturated with Oil Red 0 and Fat Red 7B. The electrophoresis strips were scanned by a densitometer (Densicord Model 552), which simultaneously provides chart recordings of the lipoprotein peaks and integration of the area under each peak by digital printout representing the percentage distribution of each lipoprotein. The relative distribution of the beta (β), pre-beta (Pβ), and alpha (α) migrating lipoproteins was estimated in terms of the percentage of the lipid stain taken up by these lipoprotein fractions. Comparison of the data for lipoproteins separated by electrophoresis, centrifugation, and agarose column chromatography (unpublished data) showed that the relative staining intensity among individual lipoprotein fractions closely paralleled the cholesterol distribution among the lipoprotein fractions. Thus the quantification by scanning of lipoprotein electrophoretograms is valuable in that it gives a second independent measure of relative lipoprotein distribution.

In the present study, 151 nulliparous females and 127 males were examined during the control period, while 135 females and 105 males were examined during the test period. The reduction in number during the study was due to pregnancy, death and reassignment of animals to other projects. No statistically significant difference for any variable was found between the two control period determinations. Therefore, data from these two blood samples were averaged to give a single control value. The same was true for the data from the two blood samples obtained at the end of the test period. The means of the control and test period data for each animal were used in all subsequent analyses. Each variable was examined statistically under three different conditions: during the control period, during the test period, and the difference between the test and control periods (test − control = Δ). Data analyses providing means, standard error of the mean, *t* tests, analyses of variance and covariance, and regression analyses were calculated using the standard statistical methods available through the Biomedical Computer Programs, BMDP package (12). *P* values less than or equal to 0.05 were considered significant.

Results. Male animals included in this study were older than the females; 5 ± 0.3 and 3 ± 0.1 (years, mean ± SEM), respectively. In addition, they were significantly heavier while consuming both the control and test diets than were females (Table I). Both sexes gained weight during the test period with the magnitude of the weight gain being significantly greater in the males.

Sex. Plasma lipid and lipoprotein concentrations for these animals during the control and test periods are also given in Table I. While consuming the control diet, females had higher concentrations of plasma triglycerides, TPC, and LDL + VLDL-chol than did the male animals, and there were no sex-related differences in HDL-chol concentrations. During the test period, triglyceride concentrations decreased, but females continued to have higher concentrations than males. There were no statistically significant male–female differences in TPC, HDL-chol, or LDL + VLDL-chol concentrations, although the increase in response to the test diet (Δ value) was larger in males for TPC and LDL + VLDL-chol.

The electrophoretic data provide an independent means of evaluating the changes in plasma lipoproteins by giving indications of the percentage distribution of lipids among the lipoprotein fractions. Table I shows these data expressed as the relative percentage of lipid stain detected densitometrically within each lipoprotein band. The trends seen by this procedure were similar to those observed for HDL- and LDL + VLDL-chol. During the control period, no sex-related differences in the electrophoretic distribution of lipoproteins were observed. During the test period, however, females had proportionately more α-lipoproteins, while males had more β-lipoproteins. The percentage of lipid in the Pβ-lipoproteins did not differ between the sexes. In response to the test diet (Δ value), Pβ-lipoproteins decreased in both males and females, but the proportion of lipid stain in the β-lipoprotein fraction increased significantly. The proportion in the α-lipoprotein fraction decreased significantly in males. These findings reflect a sex difference in the distribution of the lipids among the lipoprotein classes.

Age. Although the average age of the males studied was greater than for the females, a larger problem in the interpretation of age-related differences between males and females was the lack of sufficient numbers of females in the older age groups and the limited numbers of males available for study. Thus, the data are presented as a starting point for further study. In general, TPC appeared to decline slightly with age in males fed the control diet (Fig. 1). This was due primarily to a decrease in LDL + VLDL-chol concentrations. However, this trend appeared to be reversed in males fed the test diet, that is, the older animals generally had higher TPC and LDL + VLDL-chol values than young animals (Fig. 2). Females fed the control diet showed no

TABLE I. PLASMA LIPID AND LIPOPROTEIN DATA FOR MALE AND FEMALE *Macaca nemestrina* FED CONTROL AND TEST DIETS

	Females				Males				Females vs males		
	Control (n = 151)	Test (n = 135)	Control vs test ($P<$)	Δ[a] (n = 135)	Control (n = 127)	Test (n = 105)	Control vs test ($P<$)	Δ (n = 105)	Control ($P<$)	Test ($P<$)	Δ ($P<$)
Body weight (kg)	3.5 ± 0.09[b]	3.9 ± 0.10	.05	0.4 ± 0.03	6.7 ± 0.34	7.2 ± 0.38	NS	0.6 ± 0.07	.001	.001	.001
Triglycerides (mg/dl)	37 ± 1.4	29 ± 1.6	.01	−6 ± 1.5	29 ± 1.0	19 ± 1.0	.01	−8 ± 1.0	.001	.001	NS
Total cholesterol (mg/dl)	103 ± 1.6	153 ± 3.4	.01	52 ± 3.0	96 ± 1.5	160 ± 5.0	.01	64 ± 4.7	.005	NS	.05
HDL cholesterol (mg/dl)	52 ± 1.1	72 ± 1.6	.01	20 ± 1.1	51 ± 0.9	69 ± 1.6	.01	18 ± 1.5	NS	NS	NS
LDL + VLDL cholesterol (mg/dl)	50 ± 1.2	81 ± 3.1	.01	32 ± 2.9	45 ± 1.1	91 ± 4.9	.01	46 ± 4.8	.005	NS	.01
Beta lipoprotein (%)	35.3 ± 0.63	39.3 ± 0.84	.01	4.2 ± 0.86	35.8 ± 0.49	42.5 ± 1.23	.01	6.7 ± 1.19	NS	.05	NS
Pβ lipoprotein (%)	13.7 ± 0.54	10.9 ± 0.53	.01	−2.8 ± 0.36	13.6 ± 0.50	11.3 ± 0.59	.01	−2.3 ± 0.46	NS	NS	NS
α lipoprotein (%)	51.0 ± 0.61	50.2 ± 0.72	NS	−0.7 ± 0.76	51.1 ± 0.56	46.8 ± 0.99	.01	−4.2 ± 1.05	NS	.005	.01

[a] Δ = test concentration minus control concentration.

[b] All results are the mean ± standard error of the mean.

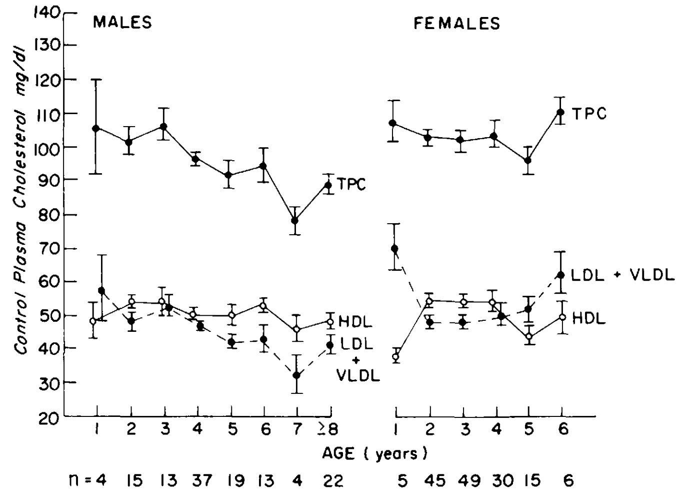

FIG. 1. Relationship of age to concentrations of TPC, LDL + VLDL- and HDL-chol concentrations in male and female *M. nemestrina* during the control period.

trend throughout the small age range studied (Fig. 1). However, at all ages observed, the concentrations of TPC were higher than those observed in males, due principally to the higher LDL + VLDL-chol levels. In the females fed the test diet, an increase in TPC with age appeared to occur, due primarily to an increase in LDL + VLDL-chol concentrations (Fig. 2). Although this trend may have been similar to the overall trend in males fed the test diet, a difference in the proportion of LDL + VLDL- and HDL-chol concentrations between males and females of comparable ages was apparent. This was particularly true in animals 2–5 years of age, the groups with the largest number of observations, where males had significantly more cholesterol in the LDL + VLDL fraction than did the females.

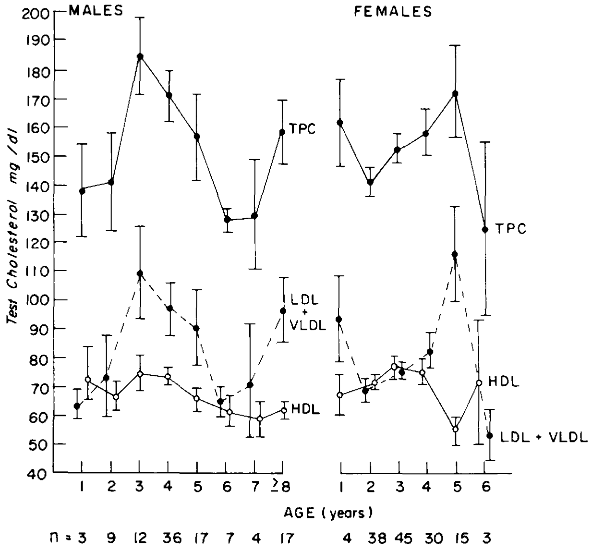

FIG. 2. Relationship of age to concentrations of TPC, LDL + VLDL- and HDL-chol concentrations in male and female *M. nemestrina* during the test period.

Diet. All plasma lipid concentrations were significantly altered by the change from the control diet to the test diet (Table I). Plasma triglyceride concentrations decreased during the test period in both females and males (17 and 32%, respectively), with females having higher concentrations than males. Consumption of the test diet caused an increase in cholesterol concentrations in all lipoprotein fractions in both males and females. However, males had a greater increase in TPC than did females, due primarily to the increase in LDL + VLDL-chol concentrations.

The relationship of the Δ value to the control values was examined to determine whether the control concentrations would be useful in predicting the degree of the response of the animals to the test diet. Figure 3 shows that the distribution of Δ values for TPC concentrations was completely independent of the control TPC for both females and males. Among animals with low control TPC and among animals with high control TPC concentrations were animals that responded minimally to the test diet, while others responded with large increases. The same situation was true for LDL + VLDL-chol concentrations. The Δ values of LDL + VLDL-chol were controlled independently of the baseline concentrations (data not shown). Thus, the control concentrations of TPC and LDL + VLDL-chol did not predict the response to the test diet in either males or females. The same independence was observed for HDL-chol concentrations in females, but in males there was a tendency for animals with higher control HDL-chol concentrations to have a smaller increase in response to the test diet than males with low control HDL-chol concentrations.

The changes in the distribution of lipids carried by the various lipoprotein fractions during the control and test periods, as determined by electrophoresis, are also shown in Table I. In both males and females fed the test diet, the percentages of lipids in the β-lipoproteins increased significantly while the proportion of lipids present in the Pβ-lipoproteins decreased significantly. A concurrent decrease in the percentage of lipids carried by the α-lipoproteins was significant only in the males and reflects the redistribution of lipids among the lipoprotein classes. Again, the trends seen by this method were similar to those observed for LDL + VLDL- and HDL-chol. That is,

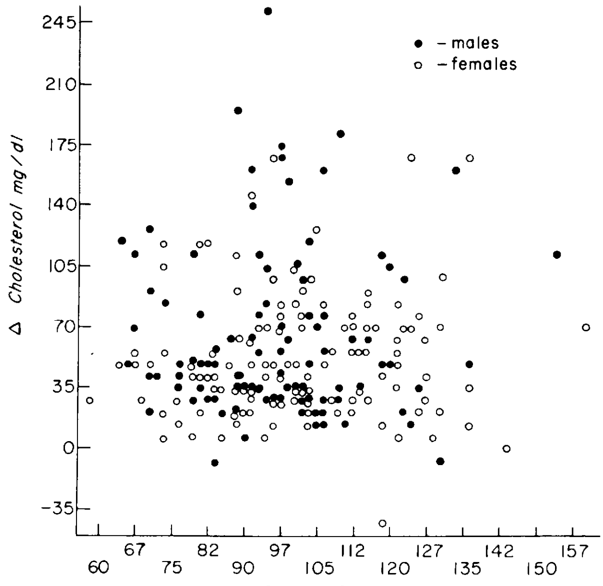

FIG. 3. Relationship of control TPC concentrations to Δ values in male and female *M. nemestrina*.

during the test period, male animals had a greater percentage of lipid in the β-lipoprotein class and less in the α-lipoprotein class than did the females.

Discussion. Several sex-related differences were found in lipid concentrations of *M. nemestrina*. When fed a Monkey Chow diet, females had higher TPC and LDL + VLDL-chol levels than males. Similar sex differences in TPC concentrations were observed by Blakley *et al.* (13) in Malaysian and Sumatran *M. nemestrina* fed commercial chow, although these authors reported higher absolute concentrations than those observed in this study. When the animals in our study were fed the diet enriched with fat and cholesterol, there was an increase in TPC, LDL + VLDL-, and HDL-chol concentrations in both males and females. However, the increase in TPC and LDL + VLDL-chol was greater in males than in females. The HDL/TPC ratio was lower in males than in females showing that males actually had more cholesterol in the LDL + VLDL fraction than females. This was confirmed by the measurements of lipoprotein electrophoresis which showed that males had more lipids in the β-lipoprotein class and less in the α-lipoprotein class than females. These findings are consistent with studies in other nonhuman primates (2, 14) and may suggest the potential for male–female differences in atherosclerosis in *M. nemestrina*.

As noted previously, identification of age related differences in plasma lipids between males and females has been hampered by inequality of age between males and females and by the insufficient numbers of females in older age groups and of males over all ages. However, trends are suggested by the data which can provide the basis for future study. The increases in TPC and LDL + VLDL-chol concentrations with advancing age in both males and females consuming the diet enriched with fat and cholesterol and the higher concentrations of TPC and LDL + VLDL-chol in males 2–5 years of age support the possibility that male–female differences in atherosclerosis may occur in this species, particularly in older animals.

The wide distribution of Δ values observed for *M. nemestrina* (Fig. 3) corresponds with the individuality in response to dietary cholesterol reported for other species of nonhuman primates including *Saimiri sciureus*, *M. mulatta*, *M. arctoides*, *M. fascicularis*, *Cercopithecus aethiops*, and *Erythrocebus patas* (15). Animals having large increases in TPC concentrations in response to dietary cholesterol challenge, hyperresponders, have been shown to have more extensive and severe atherosclerosis than animals with a small response, the hyporesponders (16). The ability to predict the TPC response of an animal to dietary cholesterol by using the basal (control) TPC concentrations would be advantageous for atherosclerosis research by eliminating the time-consuming process of dietary challenge. However, the data from this study suggest that the response of an animal to a fat and cholesterol-enriched diet cannot be predicted on the basis of either basal TPC or LDL + VLDL-chol concentrations.

The lack of correlation between the basal concentrations of TPC and LDL + VLDL-chol and the concentrations observed in response to dietary challenge also suggest that the mechanisms controlling the basal concentrations and those controlling the response to dietary cholesterol are different. For example, under control conditions cholesterol synthesis must be the major source of cholesterol, while in animals consuming cholesterol, the diet can contribute significantly to plasma cholesterol concentrations (17). Thus, it is not unreasonable to speculate that the factors controlling TPC concentrations may be different depending upon the source of cholesterol entering body pools each day. Heritability studies tend to reinforce this conclusion. Studies in mice and rabbits have shown that 30 to 50% of the variability in basal TPC concentrations is due to genetic factors (18–20), while 50 to 60% of the individuality of response of TPC concentrations has been attributed to genetic factors after dietary challenge in rabbits and squirrel monkeys (16, 21). It is difficult to know the extent to which the higher heritability estimates for TPC levels in cholesterol-fed animals may be due to the use of different animals species. However, preliminary data from ongoing studies in this laboratory have also suggested higher heritability estimates for TPC concentrations in *M. arctoides* fed a cholesterol-containing diet than in animals of the same species fed the same diet without added cholesterol. Thus, the differences in heritability estimates for basal TPC concentrations compared with the response of TPC levels to dietary cholesterol and the lack of correlation between these two parameters are consistent with the conclusion that different physiological mechanisms control TPC concentrations in the presence or absence of dietary cholesterol.

Summary. Female *M. nemestrina* fed a Monkey Chow diet had higher TPC and LDL + VLDL-chol concentrations than males. When the animals were fed a fat and cholesterol-enriched Monkey Chow diet, there were increases in TPC, LDL + VLDL-chol, and HDL-chol concentrations in both sexes. However, the increase in TPC and LDL + VLDL-chol was greater in males than in females. Older males fed the Monkey Chow diet had lower TPC and LDL + VLDL-chol levels than younger males, while no trend was observed in females fed the same diet. When fed the enriched diet, TPC and LDL + VLDL-chol concentrations increased with advancing age in both sexes, although males had a greater proportion of LDL−VLDL-chol than females. These data suggest the potential for male-female differences in atherosclerosis in this species. No correlation was found between basal TPC concentrations and the magnitude of response of TPC levels to the fat and cholesterol enriched diet, indicating that the response of individual animals cannot be predicted on the basis of basal concentrations. The lack of correlation between these two parameters also suggests that different physiological mechanisms control TPC concentrations in the presence or absence of dietary cholesterol.

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