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Plasma Lipid Variations and Fatty Acid Composition in Normal Male and Female Adult Rabbits.* (30042)

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Rabbits have been used extensively in studies on lipid metabolism and experimental atherosclerosis. Many reports in the literature present data on the plasma lipids of rabbits under a variety of experimental conditions. Few data, however, have been reported on the plasma lipid values of normal rabbits, variations in plasma lipid between male and female rabbits, or the fatty acid composition of each plasma lipid fraction(1-4). Fillios and Mann(5) reported cholesterol values to be higher in female than male rabbits, but data on other lipid fractions were not available. The data presented here deal with values of plasma lipids in normal rabbits and variations of the plasma lipids between the sexes. An analysis of the fatty acid composition of all the plasma lipid fractions in rabbits, as determined by gas liquid chromatography, is also presented.

Experimental. This study was performed on adult albino New Zealand rabbits weighing between 3.6 and 4.8 kg. Twenty-five male and twenty-five female rabbits were used. The animals were maintained on Purina Rabbit Chow for at least 7 days before each experiment. Food was restricted for 12 hours prior to blood-letting; water was available to the animals at all times. Blood for plasma lipid analysis was obtained from the central ear artery in a 15 ml vacutainer tube containing 0.2 ml of 5% sodium disequestrin. Blood for plasma lipid fractionation and total fatty acid analysis was obtained by exsanguinating the animals by cardiac puncture. The plasma was separated immediately from the formed elements of the blood, regardless of method of collection, by centrifugation at 4°C and 800 × *g*.

All chemicals used were of reagent grade and all solvents were redistilled prior to use. Plasma lipoprotein lipase activity (LPL) was determined by the method of Korn(6) immediately following plasma separation. Total fatty acid (TFA) analyses were carried out on chloroform:methanol (2:1) extracts of plasma as described by Albrink(7). Plasma unesterified fatty acid (UFA) concentrations were determined by the method of Dole(8). Total lipid (TL), cholesterol (C), and phospholipid (PL) concentrations were determined on aliquots obtained from Bloor's extraction of proteins and lipids precipitated by the method described by Somogyi(9). Cholesterol levels were determined by the method of Sperry and Webb(10), and phospholipid levels by the method of Stewart and Hendry (11). Total lipid concentrations were determined gravimetrically on the same extracts. Glyceride concentrations were calculated by difference from the above results.

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TABLE I. Mean Lipid Values for Normal Male and Female Adult New Zealand Albino Rabbits.

Fraction (Male N—Female N)	Mg %: mean values		Statistical probability*
	Male	Female	
Total lipid (TL) (25-25)	283.0	346.0	.02
Phospholipid (PL) (25-25)	95.0	121.0	.01
Total cholesterol (TC) (25-25)	35.0	53.0	.01
Free " (FC) (25-25)	10.5	14.0	.05
Cholesterol esters (CE) (25-25)	48.0	67.0	.05
Glycerides (G) (9-14)	124.0	156.0	.05
Unesterified fatty acids (UFA) (9-14)	3.8	2.7	.1
Lipoprotein lipase (LPL) (9-14)	5.4	4.7	.1
Total fatty acids (TFA) (9-14)	165.0	175.0	.1

* P-Wilcox rank sum test.
(N = No. of animals reported.)

Plasma for fatty acid analysis was extracted directly with Bloor's solution(12) taken to dryness, and purified according to the method described by Folch *et al*(13). The phospholipid fraction was separated from the other lipids on silicic acid columns following elution scheme B as described by Hirsch and Ahrens(14). The solvents containing the remaining lipid fractions were pooled, taken to dryness, and applied to florisil columns and eluted following the method described by Carroll into 6 fractions: cholesterol esters (CE), triglycerides (TG), free cholesterol (FC), diglycerides (DG), and monoglycerides (MG), and unesterified fatty acids (UFA)(15).

The fatty acid composition of each lipid fraction was determined by gas liquid chromatography (GLC) following methyl interesterification as described by Farquhar *et al* (16). Analysis was carried out on a F & M model 609 gas-liquid chromatography (GLC) unit equipped with 5 foot by 1.25 mm aluminum columns. Ethylene glycol adipate and apiezon "L" were employed as the stationary phases. The columns were maintained at 197°C with an inlet pressure of helium of 20 lb per square inch. The GLC was equipped with a flame ionization detector. Calculations of the area under the curves were made by triangulation, and the results reported are as per cent of the total free fatty acid present.

Results and discussion. Table I shows the mean plasma lipid concentrations for adult rabbits along with the probability of t for the differences between sexes. Total lipid (TL), phospholipid (PL), total cholesterol (TC), free cholesterol (FC), cholesterol ester (CE), and glyceride (G) concentrations were found to be consistently higher for female animals, while values for the unesterified fatty acid (UFA), lipoprotein lipase (LPL), and total fatty acid (TFA) were not significantly different for the 2 sexes.

The following 20 fatty acids were detected by GLC in rabbit plasma: 8:0, 10:0, 12:0, 13:0, 14:0, 15:0, iso 15:0, 16:0, 16:1, 17:0, iso 17:0, 18:0, 18:1, 18:2, 18:3, 20:0, 20:1, 20:2, 20:3, 20:4. Palmitic (16:0), palmitoleic (16:1), stearic (18:0), oleic (18:1), and linoleic (18:2) acids composed more than 90% of the total fatty acid of the plasma. All other fatty acids were present in trace amounts only.

Table II shows the per cent fatty acid composition of the lipid fractions for those fatty acids present in concentration of at least one per cent of total fatty acids. This Table also shows the comparison between the plasma fatty acid composition of male and female rabbits. There is no significant difference between the fatty acid concentrations in male and female rabbits.

Although no difference was found in fatty acid compositions between the sexes, statistically significant differences between the per cent fatty acid composition among the various plasma lipid fractions were found. As it is shown in Table II, palmitic acid comprises 19.5% of the PL fatty acids, while it makes up 30.4% of the fatty acids of the TG fraction. Stearic acid is responsible for 17.5% of the fatty acids in the PL fraction, but comprises only 4.4% in the TG fraction. Oleic acid makes up 24.8 and 25.1% of the TG and DG fatty acids, but comprises 37%

TABLE II. Fatty Acid Composition of Total Lipids and Lipid Fractions and Comparison of Male to Female Rabbits.

		% TL	% PL	% CE	% TG	% DG	% MG	% UFA
Myristic acid 14:0	No differentiation between sexes	1.2	1.0	1.0	2.3	2.7	4.2	3.1
Palmitic acid 16:0	Male	23.7	19.5	22.2	30.4	26.9	25.0	29.7
	Female	25.2	20.9	16.2	24.3	24.7	21.4	27.5
Palmitoleic acid 16:1	Male	—	—	3.4	2.2	5.2	7.0	4.0
	Female	—	—	2.6	2.6	4.8	6.0	3.8
Stearic acid 18:0	Male	11.5	17.5	3.1	4.4	6.4	8.6	15.3
	Female	12.4	20.5	2.7	3.8	8.2	8.0	19.7
Oleic acid 18:1	Male	23.7	14.1	26.7	24.8	25.1	37.0	26.0
	Female	23.2	13.5	23.1	27.0	27.9	43.8	23.2
Linoleic acid 18:2	Male	33.0	40.4	36.7	29.0	25.4	12.0	15.3
	Female	30.6	34.6	45.6	32.5	27.9	11.8	15.0
Linolenic acid 18:3	No differentiation between sexes	1.5	—	2.7	2.6	2.6	—	1.1
Arachidonic acid 20:4	*Idem*	1.1	6.0	1.9	.6	—	—	—

of the MG fatty acid composition and only 17.5% of the Pl fraction. Linoleic acid makes up 12.0% of the MG fatty acids, but the per cent composition doubles to 25.4 and 29.0% in the DG and TG and is 40.4% for the PL fraction.

Of significance also is the finding that there is considerable difference in degree of saturation between fatty acids that form the pool of the UFA, and fatty acids of all other plasma lipid fractions.

Thus while 43% of the UFA fraction was comprised of unsaturated fatty acid, unsaturated fatty acids accounted for 59% of the PL, 73% of the CE, and 61, 64, and 67% for the TG, DG, and MG respectively.

This is significant especially in view of the major importance that is attributed to the UFA, PL, and CE fractions in development of atherosclerosis. It is also known that the degree of saturation of the fatty acids present in the phospholipid molecule plays an important role in the coagulation mechanism.

No concrete explanation can at present be offered for the differences found in concentration of the various lipid fractions between male and female rabbits. The influence of sex hormones on the plasma lipids could be cited as a major factor contributing to the differences in rabbit plasma lipids reported here. Many reports(17-21) pertain to the effect of hormones on plasma lipids in a variety of animals and in humans.

Summary. Plasma lipid concentration was determined for adult male and female New Zealand rabbits. Comparison was made of the various lipid fractions between the sexes, and the female rabbit was found to have significantly higher concentrations of total lipids, glycerides, phospholipids, free and esterified cholesterol. The fatty acid composition of various lipid fractions was also determined. Statistically significant differences were found to exist in the fatty acid composition of the above fractions. On the other hand, sex did not affect significantly the fatty acid composition of the same lipid fractions.

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Radioactive Distribution of Tritium Labeled Acetophenone Derivative of 16*a*, 17*a*-Dihydroxyprogesterone. (30043)

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A new class of progesterone derivatives has been reported by Fried and his associates(1). One compound of this class, the acetophenone derivative of 16*a*, 17*a*-dihydroxyprogesterone (D),* was shown to be biologically active in the Clauberg assay. This derivative is twice as potent as progesterone parenterally and twice as active as norethisterone orally (2). D maintains pregnancy in ovariectomized rats(3) and does not virilize the internal or external sex structure of the newborn rat(4). The labeling of this steroid with tritium at the 6,7 positions enabled us to investigate the distribution of D in the Sprague-Dawley rat after intravenous, intramuscular, and oral administration.

Materials and methods. Tritiated D was prepared by reduction of $\Delta^{4,6}$-3-keto-16,17-dihydroxyacetophenonide with tritium and 5% palladium-charcoal mixture in toluene under mild conditions. The resultant steroid had a specific activity of approximately 1.5 millicuries per mg.

A 20% ethanol solution containing .25 mg of D was injected intravenously into one female rat. Another female rat was given 25 mg of D in a mixture of 60% corn oil—40% benzyl benzoate orally. Each animal received a total of 1.3×10^7 dpm. Twenty-four hours after administration of the compound, the animals were killed. Organs, blood, feces, and urine were analyzed for total radioactivity.

In a second study D was administered intramuscularly. Five 250 g female rats were used. Each animal received 1 mg of D dissolved in 70% corn oil—30% benzyl benzoate. The amount of radioactivity given to each animal was 6.7×10^8 dpm. Blood samples were taken from 2 of the animals at various time intervals up to 24 hours, at which time one of the animals was killed. The 4 remaining animals were killed at weekly intervals. During this period all urine and feces were collected daily. Blood samples were taken from 2 of the animals once a day for one week. The same animal was never bled on 2 successive days. After the first week, blood was sampled from the remaining animals twice weekly. At time of sacrifice, each animal was dissected and various tissues were frozen for subsequent determination of radioactivity.

The method used to determine radioactivity consisted of a modification of the closed oxygen flask combustion technic developed by Kelly *et al*(5). Approximately 50 mg portions of wet tissue were wrapped in Schoniger sample holder papers and enclosed in platinum sample holders. These holders were imbedded in Corning 6710 ground glass joints

* Droxone® Squibb Dihydroxyprogesterone Acetophenide.