Dietary and gonadal hormone effects on lipid metabolism in the rat

LILLA AFTERGOOD and R. B. ALFIN-SLATER

Division of Nutritional Science, School of Public Health, University of California, Los Angeles, California

SUMMARY Hepatic cholesterol, triglyceride, and phospholipid concentrations, plasma cholesterol levels, cholesterol biosynthesis, and fatty acid patterns in plasma lipids and liver lipid fractions, have been studied in intact, gonadectomized, and hormone-treated gonadectomized male and female rats fed a fat-free diet, a control diet containing fat, and a diet containing cholesterol, to determine relationships between diet, sex hormones, and lipid metabolism.

Both estradiol benzoate and testosterone propionate affected lipid metabolism; in general, the estrogenic influence was more pronounced and more predictable. The greatest effects were found in animals fed the essential fatty acid-deficient diet (a sex difference in susceptibility to essential fatty acid deficiency has previously been reported). It is concluded that the effect of estrogenic deficiency on lipid metabolism includes: (*a*) decreased hepatic cholesterol biosynthesis; (*b*) increased hepatic sterol ester and decreased phospholipid concentration; (*c*) increased depletion of unsaturated fatty acid in plasma and liver during essential fatty acid deficiency; and (*d*) increase in severity of essential fatty acid deficiency symptoms, using as criteria the ratios of trienoic to tetraenoic fatty acids in plasma and liver lipids.

KEY WORDS gonadectomy · sex hormones · diet · lipid metabolism · rat · liver · plasma · fatty acid composition · cholesterol biosynthesis · sex differences · estradiol benzoate · testosterone propionate

THERE HAVE BEEN NUMEROUS REPORTS in the literature concerned with sex differences in lipid metabolism of the rat. For example, it has been observed that female rats have higher plasma cholesterol but lower liver cholesterol levels than do male rats (1, 2). Methyltestosterone has been reported to exert a hypocholesterolemic effect in male rats (3). Female animals have higher rates of hepatic cholesterol biosynthesis; the administration of estradiol to males causes an increase in biosynthesis (4). In agreement with this observation is the report of Coleman et al. (5) that hepatic cholesterol biosynthesis is lower after gonadectomy of the female rat. Noble and Boucek (6) found that the administration of estrogen stimulated cholesterol biosynthesis in male rats. Boyd (7) reported that estrogen administration to male rats resulted in a shortening of the biological half-life of plasma cholesterol.

Other studies have also shown effects of sex hormones on lipid metabolism. Mitochondria from intact female rats oxidized cholesterol to a greater extent than those from intact males (8). Furthermore, some studies with the chick have suggested that estrogens protect the animal against atherosclerosis (9–11), although similar studies with rabbits gave inconclusive results (12).

The sex of the animal also affects fatty acid composition of liver lipids (13, 14) and plasma lipids (15–17). It was found that, in plasma phospholipids, females had a higher level of stearic acid than did males, whereas males consistently had more palmitic acid than did females. In intact animals, estradiol benzoate facilitates the removal of unsaturated fatty acid from hepatic cholesterol esters and also increases the level of plasma cholesterol arachidonate (18). When rats were maintained on an essential fatty acid-deficient diet, females retained more arachidonate for a longer time in liver phospholipids and sterols esters than did the males (19).

Reports in the literature on the relationship of sex hormones and lipid metabolism continue to be contradictory. This is not very surprising in view of the complex interrelationships among pituitary, gonadal, and adrenal secretions. This investigation was undertaken to provide a fuller understanding of the relationships among sex hormones, diet, and lipid metabolism in the rat.

MATERIALS AND METHODS

Weanling male and female albino rats of our colony (the former USC strain) were placed on three different diets:

TABLE 1 AVERAGE GAIN IN WEIGHT (IN GRAMS) AFTER 22 WEEKS ON EXPERIMENTAL DIETS

	Control Diet		Fat-Free Diet		Cholesterol Diet	
	M	F	M	F	M	F
Intact	260	225	204	170	336	215
X	287	250	230	157	342	270
X + H	232	180	220	150	244	190

8–13 animals per group.
X, gonadectomized.
X + H, hormone-treated gonadectomized.

fat-free, 15% cottonseed oil (control), and 15% cottonseed oil plus 1% cholesterol.[1] After 6 weeks, one-third of the animals in each group were continued as controls while the rest were gonadectomized under light ether anesthesia. After they had recovered, one-half of the castrated rats received three injections per week of one of the following hormones dissolved in methyl oleate: testosterone propionate (1.66 mg/100 g male rat) or estradiol benzoate (0.16 mg/100 g female rat).[2] This treatment was continued for 12 weeks. At this time the animals were killed by removal of blood from the heart under nembutal anesthesia; the liver was immediately excised, trimmed, and frozen.

Plasma was extracted for cholesterol analysis with alcohol–acetone 1:1 (v/v), and for total lipids with chloroform–methanol 2:1, using a modification of the Sperry-Brand method (20). Cholesterol analyses were performed by a modified Sperry-Schoenheimer method as reported by Nieft and Deuel (21). Total lipid was fractionated into triglycerides, sterol esters, free sterols, and phospholipids by silicic acid chromatography, and the resulting fractions were processed as reported by Morin et al. (22). Methyl esters of fatty acids of each fraction were analyzed on a gas–liquid chromatograph (Barber-Colman Model 20 with ionization detector, radium source). Stationary phase was 15% ethylene glycol succinate polyester on 80–100 mesh Gas Chrom P, at 190° and argon inlet pressure of 30 psi. Chromatographic peaks were identified either by comparison of retention times with those of standards or from a graph representing the relationship between log retention time and the number of carbon atoms. Only chromatographs of comparable peak size were included in the calculations.

Cholesterol biosynthesis in liver slices was determined using the method of Mukherjee and Alfin-Slater (23) with the following change: Krebs-Ringer bicarbonate buffer was used as an incubation medium in an atmosphere of 5% CO_2–95% O_2 rather than phosphate buffer. Approximately 1 μc of sodium acetate-1-C^{14} was employed as a substrate. The radioactivity of the synthesized cholesterol (converted to digitonide) was measured with a probable percentage error of 0.93%. All counts were corrected for background and self-absorption.

RESULTS AND DISCUSSION

Throughout the experimental period, all animals fed the control and cholesterol-containing diets were in good health and gained in weight (Table 1). Animals fed the fat-free diet showed the poor weight gain associated with EFA deficiency. In most cases gonadectomy resulted in elevated weight gains, whereas hormone administration to castrated animals had the opposite effect. Since food consumption was not recorded, it is possible that the weight changes were a reflection of dietary intake as well as the result of the hormonal changes.

Plasma cholesterol levels are shown in Table 2. On all three diets males had lower plasma cholesterol levels than did the females. These differences were smaller on the fat-free diet and larger on the cholesterol-containing diet than on the control diet. Plasma cholesterol levels of the females were not significantly affected by either gonadectomy or gonadectomy followed by hormone administration, but males other than those on the fat-free diet reacted to the lack of testosterone by increases in plasma cholesterol levels; androgen administration resulted in lowering of the plasma cholesterol level. This effect was most evident in animals fed the cholesterol-containing diet.

TABLE 2 PLASMA CHOLESTEROL LEVELS OF INTACT, GONADECTOMIZED, AND HORMONE-TREATED GONADECTOMIZED RATS

	Males		Females	
	Total	Free	Total	Free
	mg/100 ml	*%*	*mg/100 ml*	*%*
		Control Diet		
Intact	44.3 ± 4.4*	42.6	61.5 ± 2.0	41.6
X	76.1 ± 1.2	37.1	74.7 ± 9.1	44.0
X + H	49.2 ± 1.3	41.5	69.3 ± 3.2	37.1
		Fat-Free Diet		
Intact	37.6 ± 1.4	27.1	47.5 ± 3.0	30.9
X	31.9 ± 3.3	32.6	48.6 ± 2.9	31.9
X + H	47.0 ± 4.3	29.7	55.6 ± 2.7	33.9
		Cholesterol Diet		
Intact	72.8 ± 4.8	31.3	108.7 ± 8.8	29.1
X	123.0 ± 5.7	30.2	145.2 ± 19.5	32.6
X + H	62.0 ± 2.9	35.2	132.0 ± 11.1	45.0

6–9 animals per group.
* Standard error of the mean.

[1] All diets contained 23% protein (as casein), inorganic salts and vitamin mixtures, cellulose, and sucrose; fat was added to the diet at the expense of sucrose. The cholesterol-containing diet also contained 0.25% of bile salts to facilitate absorption.

[2] The gift of hormones from the Schering Corp., Bloomfield, N.J., is gratefully acknowledged.

TABLE 3 LIVER CHOLESTEROL IN INTACT, GONADECTOMIZED, AND HORMONE-TREATED GONADECTOMIZED RATS ON 3 DIETS

	Males		Females	
	Total	Free	Total	Free
	mg/g	*%*	*mg/g*	*%*
	Control Diet			
Intact	2.25 ± 0.15*	81.3	2.06 ± 0.06	85.9
X	2.27 ± 0.10	81.4	1.84 ± 0.06	80.4
X + H	2.06 ± 0.10	88.8	2.34 ± 0.05	83.3
	Fat-Free Diet			
Intact	3.42 ± 0.17	57.4	2.75 ± 0.10	64.4
X	3.61 ± 0.10	55.4	3.25 ± 0.17	64.0
X + H	3.32 ± 0.04	61.7	2.92 ± 0.15	70.2
	Cholesterol Diet			
Intact	24.7 ± 1.6	14.5	33.7 ± 2.9	13.4
X	54.3 ± 3.7	12.3	54.9 ± 3.7	11.3
X + H	24.2 ± 2.4	17.4	30.0 ± 2.6	15.9

8–13 animals per group.
* Standard error of the mean.

Liver cholesterol levels (Table 3) did not follow the same pattern. On the fat-free diet, males had a higher hepatic cholesterol concentration than the females, whereas on the cholesterol-containing diet the situation was reversed. On these atherogenic diets, gonadectomy resulted in elevations in liver cholesterol levels in both sexes; hormone administration counteracted this effect.

Table 4 presents the results obtained in studies on cholesterol biosynthesis using liver slices. It may be observed that on the fat-free diet the biosynthesis of cholesterol in females was more than four times greater than that in males ($P < 0.05$). In the male, biosynthesis was not significantly affected by the removal or subsequent replacement of testosterone. On the other hand, females showed a significant reduction in cholesterol biosynthesis following gonadectomy; estradiol treatment restored it to the original rate. The decreased biosynthesis was probably due in part to the inhibitory effect of endogenously accumulated cholesterol in liver on biosynthesis (24).

On the control diet, although biosynthesis proceeded at a greater rate than in animals fed the fat-free diet, gonadectomy and estrogen administration to gonadectomized female animals had effects similar to those which occurred with the fat-free diet. These effects, however, seemed to be independent of the influence of the hepatic cholesterol concentration. As expected, in the group fed the cholesterol-containing diet, the effect of the exogenous cholesterol (24) was of such magnitude that it masked any possible hormonal influences.

Tables 5, 6, and 7 show the percentage composition of major fatty acids of total plasma lipids of male and female rats. On the fat-free diet (Table 5) intact males had a higher percentage of palmitic (16:0), palmitoleic (16:1), and oleic (18:1) acids whereas females had more stearic (18:0), linoleic (18:2), and arachidonic (20:4) acids. The effect of the fat-free diet is reflected in the appearance of the 5,8,11-eicosatrienoic (20:3) acid which is characteristic of the EFA deficiency state. This acid has been recognized by Fulco and Mead (25) as originating from oleic acid and may reflect an attempt of the organism to increase polyunsaturation in its fatty acid composition.

It is interesting to note that despite the differences in the diets, the amount of palmitic acid in the plasma of intact males was essentially the same in all three cases. In animals on the fat-free diet, the levels of 16:1 and 18:1 acids were considerably higher in both males and females than in the groups fed either the control diet or the cholesterol-containing diet. In these latter diets, linoleic acid is abundant. Again, these higher levels of monounsaturated fatty acids may represent an attempt toward maintaining a constant ratio of saturated to unsaturated fatty acids, possibly to maintain a constant physical state of body lipids (26). The presence of dietary cholesterol resulted in the elevation of the level (compared with that on the control diet) of the 18:2 acid and a lowering of the 20:4 acid at the expense of the 18:1 acid in both sexes, which possibly reflects an interference with the conversion of the 18:2 acid to the 20:4

TABLE 4 BIOSYNTHESIS OF LIVER CHOLESTEROL IN INTACT, GONADECTOMIZED, AND HORMONE-TREATED GONADECTOMIZED RATS ON 3 DIETS

	Control Diet	Fat-Free Diet		Cholesterol Diet
	Females	Males	Females	Females
	cpm/g	*cpm/g*		*cpm/g*
Intact	10,980 ± 5,350*	1,005 ± 455	4,766 ± 228	350 ± 181
	$P < 0.05$		$P < 0.01$	
X	876 ± 389	1,327 ± 320	580 ± 267	278 ± 134
	$P < 0.05$		$P < 0.01$	
X + H	8,374 ± 4,000	2,260 ± 813	3,695 ± 571	260 ± 14

Incubation time, 3 hr.
* Standard deviation. Values represent averages of duplicate counts from duplicate sets of liver slices from 3 animals per group.

TABLE 5 MAJOR FATTY ACIDS OF PLASMA LIPIDS FROM INTACT, GONADECTOMIZED, AND HORMONE-TREATED GONADECTOMIZED RATS ON FAT-FREE DIET

	Males			Females		
	Intact	X	X + H	Intact	X	X + H
		%			%	
16:0	25.7 ± 3.1*	23.0 ± 3.1	22.8 ± 5.8	18.8 ± 3.6	24.7 ± 2.1	21.9 ± 4.5
16:1	17.1 ± 1.5	11.4 ± 1.4	12.7 ± 1.6	12.0 ± 3.4	16.6 ± 5.4	11.8 ± 3.0
18:0	8.6 ± 0.9	10.5 ± 1.7	9.7 ± 1.0	12.2 ± 0.5	12.1 ± 2.1	11.2 ± 1.9
18:1	33.3 ± 3.2	25.7 ± 4.2	31.4 ± 0.8	25.9 ± 3.6	27.2 ± 3.8	30.6 ± 4.8
18:2	1.0 ± 1.3	4.5 ± 0.8	5.1 ± 1.2	3.6 ± 1.1	1.3 ± 2.2	4.2 ± 1.2
20:3†	10.4 ± 1.8	11.5 ± 3.3	8.6 ± 2.0	12.6 ± 1.9	6.3 ± 3.3	8.1 ± 2.4
20:4	0.9 ± 1.4	3.2 ± 3.5	6.4 ± 1.9	3.2 ± 1.6	2.0 ± 3.3	7.2 ± 2.1
Triene/tetraene	10	4	1.5	4	3.2	1

6–8 animals per group.
* Standard deviation.
† 5,8,11-Eicosatrienoic acid.

acid. The highest level of the 20:4 acid was found in the female rat on the control diet (Table 6).

Gonadectomy resulted in the elevation of linoleic acid in male rats on the fat-free diet ($P < 0.05$) (Table 5). The addition of the male hormone to the castrated animals had no effect on the elevated linoleic acid concentration and, further, caused an increase in arachidonic acid content over that observed in intact animals.

The triene:tetraene ratio has been used as a measure of dietary linoleate adequacy. According to Holman (27) a value of less than 0.4 indicates that the minimum dietary requirement for linoleic acid has been met. In Table 5 it can be seen that the triene:tetraene ratio is well above 0.4 in all cases, indicating a linoleate deficiency. In the castrated male animal, there is a decrease in this value from 10 to 4, and hormone administration results in a further lowering of the ratio to 1.5. In the female, the triene:tetraene ratio of 4 in the intact animal is not significantly changed after gonadectomy but is decreased to 1 by administration of estrogen.

On the control diet (Table 6), a sex difference in plasma fatty acid composition was apparent in the intact animals, with males having higher 16:0, 16:1, and 18:1 acid levels and females having the higher 18:0 and 20:4 acid levels. In males, the most significant effect of gonadectomy was the elevation of the 20:4 acid and its return after hormone administration to the level observed in the intact animal. Oleic acid, on the other hand, decreased as a result of gonadectomy and increased after hormone administration. In the female no significant changes were apparent. Similarly, as a result of gonadectomy or subsequent hormone administration (Table 7), no significant changes in fatty acid composition in the plasma were observed in animals fed the cholesterol-containing diet.

TABLE 6 MAJOR FATTY ACIDS OF PLASMA LIPIDS FROM INTACT, GONADECTOMIZED, AND HORMONE-TREATED GONADECTOMIZED RATS ON CONTROL DIET

	Males			Females		
	Intact	X	X + H	Intact	X	X + H
		%			%	
16:0	27.8	20.9	20.9	18.4	20.0	21.4
16:1	3.8	1.4	4.8	tr	1.9	2.5
18:0	13.2	14.0	9.2	18.1	16.0	17.5
18:1	15.9	7.9	23.3	7.2	7.8	9.7
18:2	24.5	27.8	27.8	25.1	22.8	21.5
20:4	14.3	25.8	10.9	25.8	24.3	22.3

Pooled samples from 3–5 rats per group.

Lipid distribution in the liver of male and female rats on different diets is presented in Table 8. In general, atherogenic diets produced increased levels of lipids in both sexes: in males, $P < 0.05$ for the difference between fat-free and control diets, $P < 0.01$ for the cholesterol diet, and in females $P < 0.01$ for the effect of the cholesterol diet. There is also a change in the distribution of the lipid fractions. The percentage of sterol esters in liver lipids increased in males on the fat-free diet ($P < 0.01$) and markedly increased in both sexes when cholesterol was present in the diet ($P < 0.01$). Hepatic triglyceride levels were not affected, except in females on the fat-free diet, but marked decreases in the ratio of phospholipids to sterol esters occurred in animals fed the atherogenic diets. It is postulated that these decreased ratios in the fat-free and cholesterol-fed animals may result from a competition between cholesterol and the precursor of the phospholipid molecule for the unsaturated fatty acids.

The effects of gonadectomy and hormone adminstration on hepatic lipid distribution are also shown in Table 8. On the fat-free diet, estrogen increased the proportion of phospholipids from 40.6% in gonadectomized females to 50.4% ($P < 0.01$). Since the changes in the liver concentration of total lipids were negligible, this increase

TABLE 7 MAJOR FATTY ACIDS OF PLASMA LIPIDS FROM INTACT, GONADECTOMIZED, AND HORMONE-TREATED GONADECTOMIZED RATS ON CHOLESTEROL-CONTAINING DIET

	Males			Females		
	Intact	X	X + H	Intact	X	X + H
	%			%		
16:0	25.9 ± 3.6*	20.0 ± 4.2	21.8 ± 3.0	20.3 ± 2.1	18.1 ± 4.2	17.3 ± 1.4
16:1	0.9 ± 2.1	2.6 ± 1.8	1.0 ± 2.1	2.4 ± 1.2	3.2 ± 1.3	2.9 ± 0.3
18:0	11.1 ± 0.7	11.8 ± 1.4	11.1 ± 1.5	13.5 ± 2.4	12.4 ± 1.8	13.6 ± 2.6
18:1	16.1 ± 3.9	13.6 ± 1.0	16.2 ± 0.9	15.8 ± 2.1	15.4 ± 1.6	14.5 ± 1.2
18:2	31.2 ± 4.7	32.2 ± 1.8	35.8 ± 4.3	30.7 ± 1.5	28.7 ± 3.0	29.6 ± 1.8
20:4	10.6 ± 5.7	12.8 ± 2.8	8.1 ± 1.3	13.2 ± 2.1	15.2 ± 1.4	17.5 ± 2.7

6 animals per group.
* Standard deviation.

also holds for absolute (milligrams per gram liver) concentrations. However, animals on the control diet responded to gonadectomy with a slight increase of total lipids and as a result, the percentage increase in phospholipids observed following hormone administration is probably a relative, rather than an absolute, increase.

Gonadectomy of animals fed the cholesterol-containing diet caused a marked increase in liver total lipids. Sterol ester percentages increased and the phospholipid percentage decreased in the gonadectomized animals; following hormone treatment, values returned to those of intact controls on this diet.

The fatty acid patterns of liver sterol esters were similar in the intact male and female animals, and they remained unchanged following gonadectomy with or without hormone administration.

When the fatty acid patterns of liver triglycerides of intact males and females fed the various diets were compared, only small differences were observed. However, on the fat-deficient diet, the gonadectomized females had a decreased concentration of the 20:4 acid, which was increased after hormone administration (Table 9). Gonadectomy also caused changes in the triene:tetraene ratio. A value of 4.5 in the intact male was reduced to 2.8 after gonadectomy and further reduced to 0.7 after hormone administration. In contrast, gonadectomy of the female increased the ratio; hormone administration again reduced it to a low value (0.5). This indicates that the absence of female hormone aggravated this effect of EFA deficiency whereas absence of male hormone counteracted it.

In the triglycerides of the animals fed the cholesterol-containing diet (Table 10) an eicosatrienoic fatty acid appeared, although in small amounts. This 20:3 acid is an isomer (8,11,14-eicosatrienoic acid) of the 20:3 acid (5,8,11-eicosatrienoic acid) which appears in EFA deficiency and is probably an intermediate in the conversion of linoleic to arachidonic acid (22). No significant

TABLE 8 LIVER LIPID DISTRIBUTION OF INTACT, GONADECTOMIZED, AND HORMONE-TREATED GONADECTOMIZED RATS ON 3 DIETS

	Males				Females			
	Total Lipids	SE	TG	PL	Total Lipids	SE	TG	PL
	mg/g	%	%	%	*mg/g*	%	%	%
				Control				
Intact	28.0 ± 4.8*	5.8 ± 2.7	24.9 ± 5.7	61.2 ± 7.2	33.6 ± 6.0	9.3 ± 2.8	23.7 ± 3.8	57.1 ± 5.0
X	37.4 ± 11.6	9.3 ± 3.2	26.5 ± 3.6	54.4 ± 2.2	41.6 ± 9.4	6.8 ± 2.2	27.4 ± 2.4	50.5 ± 3
X + H	29.2 ± 8.2	4.6 ± 1.5	21.0 ± 4.0	66.2 ± 2.4	27.3 ± 13.1	4.1 ± 1.4	26.2 ± 4.3	60.9 ± 2.2
				Fat-Free Diet				
Intact	49.4 ± 9.4	16.6 ± 3.1	27.2 ± 3.6	46.5 ± 2.4	41.4 ± 6.9	12.3 ± 3.1	37.1 ± 4.2	42.8 ± 3.7
X	57.6 ± 8.1	14.8 ± 4.6	30.7 ± 6.0	43.0 ± 2.8	43.4 ± 4.3	9.5 ± 0.7	31.8 ± 3.3	40.6 ± 2.2
X + H	54.3 ± 6.4	12.3 ± 5.0	34.5 ± 2.0	39.9 ± 5.1	40.8 ± 7.0	9.6 ± 3.8	26.3 ± 4.0	50.4 ± 1.2
				Cholesterol Diet				
Intact	69.7 ± 10.0	31.7 ± 0.7	32.9 ± 0.7	25.5 ± 2.4	78.8 ± 18.5	39.4 ± 7.0	27.9 ± 4.6	16.7 ± 2.2
X	130.0 ± 12.0	41.6 ± 2.2	34.2 ± 5.0	13.1 ± 2.2	126.1 ± 28.0	45.4 ± 7.5	31.8 ± 3.1	13.0 ± 2.8
X + H	83.1 ± 11.3	33.1 ± 5.8	34.2 ± 8.4	21.8 ± 1.6	64.7 ± 13.3	37.8 ± 9.7	33.9 ± 7.4	17.9 ± 1.7

5 animals per group. SE = sterol ester, TG = triglyceride, PL = phospholipid. The amounts of free sterols, mono- and diglycerides, and hydrocarbons, were determined but are not included in this table.
* Standard deviation.

TABLE 9 TRIENOIC AND TETRAENOIC FATTY ACIDS OF LIVER TRIGLYCERIDES FROM INTACT, GONADECTOMIZED, AND HORMONE-TREATED GONADECTOMIZED RATS ON FAT-FREE DIET

	Males			Females		
	Intact	X	X + H	Intact	X	X + H
		%			%	
20:3*	3.6 ± 1.3†	2.8 ± 2.8	1.7 ± 1.6	5.5 ± 3.3	1.8 ± 1.2	2.1 ± 1.6
20:4	0.8 ± 0.8	1.0 ± 1.4	2.5 ± 2.3	2.3 ± 1.8	0.4 ± 0.4	4.0 ± 2.8
Triene/tetraene	4.5	2.8	0.7	2.4	4.5	0.5

6–8 animals per group.
* 5,8,11-Eicosatrienoic acid.
† Standard deviation.

changes were apparent as a result of the surgical and hormonal manipulations in the animals fed either the control or cholesterol-containing diets.

Major fatty acids of liver phospholipids are shown in Tables 11 and 12. The fatty acid patterns in phospholipids differ from those of the other lipid fractions in that longer-chain unsaturated fatty acids are present, and the proportions of the C_{16} and C_{18} fatty acids are lower than those found in cholesterol esters or triglycerides.

In animals fed the fat-free diet (Table 11) males had a higher percentage of 16:0 acid than did females ($P < 0.05$). Large quantities of 5,8,11-eiosatrienoic acid were present in liver phospholipids of all fat-deficient animals; this acid was not present in the phospholipids of the control animals.

On the fat-free diet (Table 11), although gonadectomy had no effect on the phospholipid fatty acid pattern, the administration of testosterone to the castrated male decreased the concentration of the 20:3 acid, increased the 20:4 acid levels, and therefore decreased the triene:tetraene ratio from 4 to 2 in the direction of alleviating the EFA deficiency. In the female, both gonadectomy and hormone administration produced decreases in the level of 20:3, increases in 20:4, and therefore decreases in the triene:tetraene ratio.

Phospholipids in animals on the control diet (Table 12) contained no 16:1, a reduced amount of 18:1, but large amounts of 18:2 and 20:4 when compared with animals fed the fat-free diet. The females in this group had less 16:0 and more 22:6, which was reduced to control levels by hormone administration.

The fatty acid pattern of hepatic phospholipids of animals fed the cholesterol-containing diet was not affected by either gonadectomy or hormone administration.

In general, the sex differences in fatty acids are most apparent in the liver phospholipid fraction. Unsaturated fatty acids are less rapidly depleted when estrogens are present, as in the intact females or in gonadectomized females given estrogens.

CONCLUSIONS

Whereas estrogens have no appreciable effect on the plasma cholesterol levels of female rats they do affect liver cholesterol values. On the other hand, the influence of the androgen, testosterone, on male rats is seen predominantly in plasma cholesterol values. In intact animals, regardless of diet, lower plasma cholesterol levels in male rats seem to be associated with low concentrations of 20:4 in plasma lipids. On the control diet, males have an increased plasma cholesterol level after gonadectomy and, at the same time, the concentration of 20:4 is increased. Similarly in the animals on the fat-free diet, when estrogen administration causes an increase in the content of 20:4 in the female rats, their cholesterol level is also increased. In female rats on the control and cholesterol-containing diets, hormonal

TABLE 10 TRIENOIC AND TETRAENOIC FATTY ACIDS OF LIVER TRIGLYCERIDES FROM INTACT, GONADECTOMIZED, AND HORMONE-TREATED GONADECTOMIZED RATS ON CHOLESTEROL-CONTAINING DIET

	Males			Females		
	Intact	X	X + H	Intact	X	X + H
20:3*	1.3 ± 0.8†	2.0 ± 1.8	1.8 ± 0.9	0.6 ± 0.5	0.7 ± 0.6	1.4 ± 1.0
20:4	6.1 ± 3.2	4.3 ± 2.3	7.9 ± 3.1	9.1 ± 3.6	4.5 ± 2.5	9.1 ± 4.1

9–10 animals per group.
* 8,11,14-Eicosatrienoic acid (retention time compared with standard sample kindly provided by Dr. James Mead).
† Standard deviation.

TABLE 11 MAJOR FATTY ACIDS OF LIVER PHOSPHOLIPIDS FROM INTACT, GONADECTOMIZED, AND HORMONE-TREATED GONADECTOMIZED RATS ON FAT-FREE DIET

	Males			Females		
	Intact	X	X + H	Intact	X	X + H
	%			%		
16:0	19.1 ± 1.8*	17.7 ± 1.6	17.3 ± 1.8	15.5 ± 1.7	15.7 ± 2.5	15.5 ± 1.1
16:1	7.2 ± 0.3	7.0 ± 1.9	6.9 ± 1.4	6.1 ± 0.7	7.8 ± 1.7	6.4 ± 1.8
18:0	20.7 ± 2.1	22.4 ± 1.9	22.4 ± 1.8	24.1 ± 1.4	23.2 ± 1.5	23.3 ± 4.0
18:1	22.6 ± 1.8	21.7 ± 2.6	23.4 ± 0.5	22.5 ± 1.0	22.3 ± 1.6	23.0 ± 3.1
18:2	1.8 ± 0.8	2.7 ± 1.5	2.8 ± 0.5	2.6 ± 1.4	2.3 ± 1.2	2.9 ± 0.8
20:3†	21.3 ± 2.7	21.5 ± 4.1	15.4 ± 1.7	23.2 ± 4.8	17.3 ± 3.0	15.2 ± 1.3
20:4	5.2 ± 2.5	5.0 ± 3.0	8.8 ± 2.8	4.6 ± 2.6	8.3 ± 1.9	9.6 ± 2.2
22:5	tr	tr	1.0 ± 0.7	1.0 ± 0.7	0.5 ± 0.9	1.2 ± 2.0
22:6	0.5 ± 1.6	tr	0.7 ± 1.4	tr	—	1.3 ± 1.2
Triene/tetraene	4.1	4.3	1.8	5.0	2.0	1.6

8–9 animals per group.
† 5,8,11-Eicosatrienoic acid.
* Standard deviation.

manipulation is without effect on the fatty acid composition of plasma lipids; the plasma cholesterol levels of these animals are also unaffected.

It appears, then, that both androgens and estrogens exert an influence on lipid metabolism. The role of testosterone propionate is less clear than that of estradiol benzoate. On occasion its action parallels that of the estrogen, at other times it is opposite in activity, while in other cases no activity is apparent. The estrogen influence on lipid metabolism can be summarized as follows: (*a*) it increases cholesterol biosynthesis in the liver; (*b*) it affects liver lipid distribution by proportionally decreasing sterol ester and increasing phospholipid concentrations; (*c*) it prevents the rapid loss of unsaturated fatty acids from plasma and liver during essential fatty acid depletion; (*d*) in general, it decreases the triene:tetraene ratio in essential fatty acid deficiency.

Variations in weight gain were observed after hormonal manipulation. These different weight increments may have been caused by changes in food consumption, and subsequent studies must include pair-fed controls.

The metabolism of phospholipids is related to that of cholesterol in the involvement of the transfer of fatty acids (28). Enzymatic mechanisms for synthesis and turnover of phospholipids containing the essential fatty acids are probably different from those for the more saturated fatty acids (29). At the same time the rate of metabolism of a cholesterol molecule may be a function of the fatty acid to which it is esterified (30, 31). If it is assumed that in the liver a high concentration of unsaturated fatty acid gives rise to increased phospholipid or increased unsaturated cholesterol esters, and if in turn it is assumed that unsaturated cholesterol esters are more easily transported and subsequently metabolized, then estrogens definitely enhance cholesterol transport and metabolism, as has been shown in the data presented here. It has been reported that phospholipid biosynthesis is enhanced by the presence of polyunsaturated fatty acids; also estrogens can act as a stimulant in phospholipid synthesis in rat uterus (32).

The activity of other enzyme systems has been shown to be enhanced by estrogen, e.g., 16-hydroxysteroid dehydrogenase (33). It is possible that estrogens affect

TABLE 12 MAJOR FATTY ACIDS OF LIVER PHOSPHOLIPIDS FROM INTACT, GONADECTOMIZED, AND HORMONE-TREATED GONADECTOMIZED RATS ON CONTROL DIET

	Males			Females		
	Intact	X	X + H	Intact	X	X + H
	%			%		
16:0	17.5 ± 0.5*	15.9 ± 2.0	18.3 ± 1.9	13.2 ± 1.0	16.6 ± 2.1	15.0 ± 1.2
18:0	26.5 ± 2.6	29.4 ± 1.4	28.4 ± 2.4	30.9 ± 1.0	30.4 ± 0.9	27.6 ± 3.0
18:1	5.3 ± 0.7	3.9 ± 0.9	4.9 ± 1.2	3.7 ± 0.6	4.7 ± 1.0	4.1 ± 2.4
18:2	15.1 ± 1.1	12.5 ± 1.6	14.9 ± 1.5	11.0 ± 0.7	11.9 ± 1.0	12.9 ± 0.9
20:4	31.8 ± 0.9	30.6 ± 1.4	29.0 ± 2.3	28.4 ± 2.0	29.1 ± 3.1	29.2 ± 1.3
22:6	2.5 ± 1.5	7.0 ± 1.2	3.7 ± 2.3	11.3 ± 1.6	6.3 ± 3.8	9.2 ± 0.7

5 animals per group.
* Standard deviation.

enzyme systems which aid in cholesterol esterification and cholesterol degradation as well. Studies on these systems are now in progress.

This work was supported by PHS Research Grant HE 04918 from the National Institutes of Health, U.S. Public Health Service.

Manuscript received June 8, 1964; accepted December 9, 1964.

References

1. Aftergood, L., H. J. Deuel, Jr., and R. B. Alfin-Slater. *J. Nutr.* **62:** 129, 1957.
2. Fillios, L. C. *Endocrinology* **60:** 22, 1957.
3. Abell, L. L., and E. H. Mosbach. *J. Lipid Res.* **3:** 88, 1962.
4. Fillios, L. C., R. Kaplan, R. S. Martin, and F. J. Stare. *Am. J. Physiol.* **193:** 47, 1958.
5. Coleman, R. D., Y. M. Chen, and R. B. Alfin-Slater. *Circulation Res.* **6:** 172, 1958.
6. Noble, N. L., and R. J. Boucek. *Circulation Res.* **5:** 573, 1957.
7. Boyd, G. S. *Federation Proc.* **21** Supp.: 86, 1962.
8. Kritchevsky, D., S. A. Tepper, E. Staple, and M. W. Whitehouse. *J. Lipid Res.* **4:** 188, 1963.
9. Pick, R., J. Stamler, S. Rodbard, and L. N. Katz. *Circulation* **6:** 276, 1952.
10. Pick, R., J. Stamler, S. Rodbard, and L. N. Katz. *Circulation* **6:** 858, 1952.
11. Pick, R., J. Stamler, and L. N. Katz. *Circulation Res.* **5:** 515, 1957.
12. Stamler, J., R. Pick, and L. N. Katz. *Ann. N. Y. Acad. Sci.* **64:** 596, 1956.
13. Okey, R., A. Shannon, J. Tinoco, R. Ostwald, and P. Miljanich. *J. Nutr.* **75:** 51, 1961.
14. Okey, R., R. Ostwald, A. Shannon, and J. Tinoco. *J. Nutr.* **76:** 353, 1962.
15. Lyman, R. L., A. Shannon, and R. Ostwald. *Federation Proc.* **21:** 391, 1962.
16. Lyman, R. L., A. Shannon, and R. Ostwald. *Federation Proc.* **23:** 502, 1964.
17. Monsen, E. R., R. Okey, and R. L. Lyman. *Metab. Clin. Exptl.* **11:** 1113, 1962.
18. Lyman, R. L., A. Shannon, and R. Ostwald. *Federation Proc.* **22:** 502, 1963.
19. Ostwald, R., P. Bouchard, and R. L. Lyman. *Federation Proc.* **23:** 502, 1964.
20. Sperry, W. M., and F. C. Brand. *J. Biol. Chem.* **213:** 69, 1955.
21. Nieft, M. L., and H. J. Deuel, Jr. *J. Biol. Chem.* **177:** 143, 1949.
22. Morin, R. J., S. Bernick, J. F. Mead, and R. B. Alfin-Slater. *J. Lipid Res.* **3:** 432, 1962.
23. Mukherjee, S., and R. B. Alfin-Slater. *Arch. Biochem. Biophys.* **73:** 359, 1958.
24. Gould, R. G., and C. B. Taylor. *Circulation* **2:** 467, 1950.
25. Fulco, A. J., and J. F. Mead. *J. Biol. Chem.* **234:** 1411, 1959.
26. Rahm, J. J., and R. T. Holman. *J. Lipid Res.* **5:** 169, 1964.
27. Holman, R. T. *J. Nutr.* **70:** 405, 1960.
28. Glomset, J. A. *Biochim. Biophys. Acta* **65:** 128, 1962.
29. Morin, R. J., and R. B. Alfin-Slater. *Metab. Clin. Exptl.* **13:** 69, 1964.
30. Klein, P. D., and R. A. Martin. *J. Biol. Chem.* **234:** 1685, 1959.
31. Gelb, A. M., and J. I. Kessler. *Am. J. Physiol.* **204:** 821, 1963.
32. Gorski, J., and J. A. Nicolette. *Arch. Biochem. Biophys.* **103:** 418, 1963.
33. Ryan, K. J., R. A. Meigs, Z. Petro, and G. Morrison. *Science* **142:** 243, 1963.