J. Lipid Research, January, 1962
Volume 3, Number 1

Hypocholesterolemic effect of methyltestosterone in rats*

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[Received for publication May 23, 1961]

SUMMARY

In rats fed a stock diet, the oral administration of 17α-methyltestosterone (0.05% of the diet) produced a 46% average reduction of serum total cholesterol concentration. The ratio between the cholesterol content of the α- and β-lipoprotein fractions remained unchanged. In rats made mildly hypercholesterolemic by the addition of 1% cholesterol to the stock diet, the oral administration of 0.1% methyltestosterone prevented the rise in serum cholesterol concentration. This effect resulted primarily from a reduction of the cholesterol content of the α-lipoprotein fraction. The production of a more intense hypercholesterolemia by the administration of 1% cholesterol plus 0.5% cholic acid abolished the hypocholesterolemic action of 0.1% methyltestosterone. Analyses of the cholesterol content of the liver and intestinal wall indicated, in animals on both low and high cholesterol diets, that methyltestosterone did not exert its effect by producing a redistribution of the cholesterol between the serum and the tissues examined.

Furman *et al.* (1) have reported that large doses of the synthetic androgen, 17α-methyltestosterone, consistently produce a lowering of the serum cholesterol concentration in normal and hypercholesterolemic dogs. This finding has been confirmed in our laboratory (2). In the present paper, data are presented showing that 17α-methyltestosterone also exerts its hypocholesterolemic effect in rats with normal cholesterol levels and in rats with hypercholesterolemia produced by adding 1% cholesterol to the basic control diet. This small laboratory animal thus becomes available for studies on the mechanism of action of methyltestosterone.

METHODS

Male Carworth Strain (CFN Wistar) rats weighing 250 to 300 g were housed in individual cages, were supplied food and water *ad libitum*, and were weighed weekly. The stock diet consisted of ground Purina laboratory chow. Cholesterol and methyltestosterone in the indicated amounts were dissolved in ether and the solution was poured over the appropriate amount of ground chow. The chow was then well mixed and the ether allowed to evaporate. Cholic acid was dissolved in acetone and mixed with the ration in the same manner. The rats were bled from the tail vein under light ether anesthesia at the beginning of the experiments, when the animals were maintained on the stock diet, and 4, 8, and 12 weeks after the experimental diets were started. At the end of the experiment, the rats were exsanguinated by cardiac puncture; after sacrifice, the liver and the small intestine were removed. Serum total cholesterol concentrations were determined by a modification of the method of Abell *et al.* (3), using the Zlatkis and Zak (4) color reaction instead of the Liebermann-Burchard reagent to increase the sensitivity of the method. For the lipoprotein analyses, 1 to 3 ml of serum, pooled from serum of 2 or 3 rats, was diluted with KBr solution to give a final protein-free specific gravity of 1.063 and fractionated in the preparative Spinco Ultracentrifuge Model L at 40,000 rpm for 20 hours (5). The tubes were sliced in the middle; the upper (low-density; β-lipoprotein) and lower (high-density; α-lipoprotein) fractions were transferred to volumetric cylinders. The tubes were washed with 0.9% NaCl solution and the washings added to the appropriate fractions. Aliquots were analyzed for

* Supported in part by Grants H-52 and A-597, National Institutes of Health, Bethesda, Maryland; and by grants from the Nutrition Foundation, Inc., and the Ames Company, Inc.

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TABLE 1. SERUM CHOLESTEROL PATTERNS OF RATS

	Stock Diet		Stock + 1% Cholesterol Diet		Stock + 1% Cholesterol + 0.5% Cholic Acid Diet	
	Group 1		Group 2		Group 3	
	−	+ Methyltestosterone 0.05%	−	+ Methyltestosterone 0.1%	−	+ Methyltestosterone 0.1%
No. of rats	44*	12	8	8	16	8
Average serum cholesterol, mg/100 ml	74 ± 1.7† (58)‡	43 ± 1.1 (16)	81 ± 3.2 (24)	52 ± 2.1 (23)	186 ± 9.8 (44)	167 ± 16.4 (25)
	$p < 0.01$		$p < 0.01$		$p < 0.01$	
Cholesterol in α-lipoprotein, mg/100 ml	53 ± 2.1 (24)	30 ± 2.3 (4)	37 ± 2.1 (12)	22 ± 1.1 (12)	28 ± 1.0 (11)	20 ± 2.3 (7)
	$p < 0.01$		$p < 0.01$		$p < 0.01$	
Cholesterol in β-lipoprotein, mg/100 ml	22 ± 1.1 (24)	15 ± 1.2 (4)	38 ± 3.9 (12)	26 ± 2.4 (12)	127 ± 11.3 (11)	119 ± 25.5 (7)
	$p < 0.01$		$p < 0.02$			

* The data in this column represent the average values of all rats while on stock diet.
† Standard error of the mean.
‡ Numbers in parentheses indicate the number of determinations made.

total cholesterol by the method of Abell *et al.* (3).

The intestines were opened, washed carefully with water, blotted on filter paper, and weighed. The entire small intestine and 5-g samples of liver of each rat were hydrolyzed with 100 ml of 10% alcoholic KOH for 3 hours under reflux. After cooling, an equal volume of water was added and the alkaline solution was shaken twice with 200-ml portions of *n*-hexane to extract the nonsaponifiable lipids. The solvent was removed by evaporation under nitrogen. The residue was dried, weighed, and analyzed for cholesterol by the Schoenheimer-Sperry method (6).

Three groups of rats were studied. Group 1 consisted of 24 rats. After being maintained for 3 weeks on the stock diet, the animals were divided into 2 subgroups; 12 rats were continued on the stock diet throughout the experiment, and 12 rats received the experimental diet, consisting of stock diet plus 0.05% methyltestosterone, for 6 weeks. At the start of the experiment, the average weight of the rats in both groups was identical (299 g). At the end of the experimental period, the average weight of the control group had increased by 8%, whereas the average weight of the experimental group had remained unchanged.

Group 2 consisted of 16 rats; 8 received a diet containing 1% cholesterol throughout a 12-week experimental period, and 8 received the 1% cholesterol diet supplemented with 0.1% methyltestosterone.[1] At the start of the experiment, the weight of the control group averaged 279 g and the experimental group 270 g. At the end of the 12-week feeding period, the average weights were 408 g for the controls and 353 g for the drug-treated animals ($0.02 > p > 0.01$).

Group 3 consisted of 24 rats; 16 received a diet containing 1% cholesterol plus 0.5% cholic acid, and 8 received the same diet supplemented with 0.1% methyltestosterone. The cholic acid was added to achieve a higher degree of hypercholesterolemia than was encountered in the animals of group 2. The average starting weights of the rats were 260 g for the control group and 266 g for the experimental group. The weights at sacrifice, at the end of 12 weeks, were 383 g and 332 g, respectively ($p = 0.02$). It should be noted that in all experiments the drug-treated rats gained less weight than the controls.

[1] This dose of methyltestosterone was chosen because in preliminary experiments a level of 0.05% methyltestosterone did not produce hypocholesterolemia in cholesterol-fed rats.

RESULTS

Table 1 presents the average serum cholesterol concentrations of the rats on the control and experimental diets, together with the distribution of the cholesterol between the high- and low-density lipoprotein fractions (α- and β-lipoproteins). All analyses performed during each experimental regimen were combined, as the typical lipoprotein pattern was already established when the first blood sample was taken after 3 weeks on the regimens and no further change was observed.

The addition of methyltestosterone to the stock diet (group 1) caused a highly significant fall in the serum cholesterol concentration of these rats, from an average of 74 mg per 100 ml to an average of 43 mg per 100 ml. In each instance, 70% of the cholesterol was present in the α-lipoprotein fraction.

TABLE 2. CHOLESTEROL CONCENTRATIONS OF TISSUES*

	Stock Diet		Stock + 1% Cholesterol Diet		Stock + 1% Cholesterol + 0.5% Cholic Acid Diet	
	Group 1		Group 2		Group 3	
	−	+ Methyltestosterone 0.05%	−	+ Methyltestosterone 0.1%	−	+ Methyltestosterone 0.1%
Liver	2.13 ± 0.09† (6)‡	2.21 ± 0.05 (6)	11.42 ± 1.41 (8)	14.49 ± 1.77 (8)	80.8 ± 4.7 (16)	84.3 ± 4.5 (7)
Intestines	1.98 ± 0.10 (6)	1.94 ± 0.04 (6)	2.85 ± 0.08 (8)	2.63 ± 0.07 (8)	2.72 ± 0.09 (16)	2 01 ± 0.08 (7)
					$p < 0.01$	

* Mg/g wet weight.
† Standard error of the mean.
‡ Numbers in parentheses indicate the number of determinations made.

The effect of methyltestosterone was next tested in animals maintained on a diet containing 1% cholesterol (group 2). The addition of cholesterol to the stock diet caused only a slight rise in serum cholesterol concentration, from an average of 74 mg per 100 ml to an average of 81 mg per 100 ml. This increase was not statistically significant. The distribution of cholesterol between the α- and β-lipoprotein fractions differed, however, from that seen in the rats on the stock diet. The cholesterol in the α-fraction was reduced and that in the β-fraction was increased so that only 50% of the circulating cholesterol was found in the α-lipoprotein fraction. The addition of methyltestosterone to the 1% cholesterol diet was associated with a lowering of the serum cholesterol levels to below control values without, however, affecting the abnormal distribution of cholesterol between the lipoprotein fractions.

When methyltestosterone was administered to the animals of group 3, which received 1% cholesterol plus 0.5% cholic acid, no effect of the drug could be observed upon the serum total cholesterol concentrations. The decreases in serum total cholesterol concentration (from 186 mg per 100 ml to 167 mg per 100 ml) and in the cholesterol content of the β-lipoprotein fraction (from 127 mg per 100 ml to 119 mg per 100 ml) were not statistically significant. The decrease in cholesterol content of the α-lipoprotein fraction (from 28 mg per 100 ml to 20 mg per 100 ml) was significant; however, the absolute change in this fraction was too small to be reflected in the serum total cholesterol concentration.

Table 2 presents the results of cholesterol analyses of the livers and intestinal walls. In groups 1 and 2 there was no difference between the control and the methyltestosterone-treated animals in the cholesterol content of these tissues. The liver cholesterol concentrations of the rats of group 3 were the same in the control and in the experimental animals. There was, however, a significant difference in the cholesterol concentration of the intestinal walls.

The average weights of livers and intestines of the

TABLE 3. WET WEIGHT OF TISSUES

	Stock Diet		Stock + 1% Cholesterol Diet		Stock + 1% Cholesterol + 0.5% Cholic Acid Diet	
	Group 1		Group 2		Group 3	
	−	+ Methyltestosterone 0.05%	−	+ Methyltestosterone 0.1%	−	+ Methyltestosterone 0.1%
No. of animals	10	11	8	8	16	7
Liver (g)	11.52 ± 0.61*	10.69 ± 0.29	13.98 ± 0.69	14.08 ± 0.42	19.27 ± 0.72	14.75 ± 1.50
					$p < 0.01$	
Small intestine (g)	7.64 ± 0.21	7.10 ± 0.21	6.44 ± 0.28	6.31 ± 0.15	8.75 ± 0.40	6.39 ±0.22
					$p < 0.01$	

* Standard error of the mean.

rats of all groups are shown in Table 3. Significant differences in total organ weights between methyltestosterone-treated and untreated animals were found only in group 3.

DISCUSSION

The observations reported here are in general agreement with our earlier studies carried out in dogs (2). In those experiments it was found that the oral administration of 50 mg per kg of methyltestosterone reduced the serum cholesterol concentrations of dogs kept on high and low cholesterol diets to approximately one-half of the control values. The cholesterol content of both the α- and β-lipoprotein fractions of the serum fell.

In the present study, the effect of methyltestosterone in rats maintained on the low cholesterol stock diet was similar. This finding is of interest as the rat has usually proved resistant to attempts to reduce the serum cholesterol concentration by pharmacologic or dietary means. It could not be demonstrated that the hypocholesterolemic action of methyltestosterone was caused by a redistribution of cholesterol between serum and the tissues examined at autopsy.

The feeding of a diet containing 1% cholesterol resulted in a very slight degree of hypercholesterolemia. The elevated cholesterol content of livers and intestinal walls demonstrates that the dietary cholesterol had been absorbed. The administration of methyltestosterone prevented the hypercholesterolemia throughout the experimental period. As observed previously in cholesterol-fed dogs, the lowering of the serum cholesterol levels in rats of group 2 resulted primarily from a decrease in the cholesterol content of the α-lipoprotein fraction.

In rats receiving the cholesterol-cholic acid diet, a hypocholesterolemic effect of methyltestosterone could not be demonstrated, even though the dosage of the drug was large enough to prevent normal weight gain. It is possible that the increased absorption of cholesterol caused by the administration of bile acid (as demonstrated by the accumulation of cholesterol in the tissues) may have masked the hypocholesterolemic effect of the drug. It is difficult to understand why in this group there was a significant difference in the cholesterol concentration of the intestinal wall between the control and the experimental animals. If this finding implies an effect of methyltestosterone on cholesterol absorption, this is certainly not reflected in the liver or serum cholesterol concentrations.

It has been shown that under suitable experimental conditions methyltestosterone can exert hypocholesterolemic effects in the dog (2, 7), the rat, the rabbit, and in man (8). The mechanism of action of methyltestosterone cannot be deduced from the data presently available. Its site of action may be at the intestinal wall, or it may act upon lipoprotein synthesis in the tissues. On the basis of Furman's findings (8) that in man the hypocholesterolemic effect of methyltestosterone can be demonstrated only on diets low in protein, it is possible that the action of the drug may be related to its anabolic protein-sparing action.

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