Oral Contraceptive Steroids: Effects on Various Nutrient Balances and Body Composition in Adult Female Rats[1] (34562)

K. MANOHARAN[2], M. G. YANG, AND O. MICKELSEN

Foods and Nutrition Department, Michigan State University, East Lansing, Michigan 48823

Recently, certain oral contraceptive preparations have been found to affect the pituitary and adrenal cortical secretions (1–3). Some oral contraceptive pills have been reported to cause changes in carbohydrate (4–6), fat (7, 8), and protein (9) metabolism. Progesterone was found to increase the secretion of mineralocorticoids from the adrenal (10, 11). This change in secretion rates of mineralocorticoids might be expected to change the mineral metabolism of those taking oral contraceptive pills. The present studies were designed to determine whether the digestibility and retention of various nutrients were affected by feeding female rats for short and long periods, norethynodrel and mestranol, the synthetic progestational and estrogenic compounds used in the preparation of several oral contraceptives. The present experiments were also undertaken to study whether these oral steroids would alter body composition and to determine whether the alterations were reversible upon withdrawal of the steroids.

Methods. Ninety 3-week-old female Sprague-Dawley rats were fed a basal grain ration[3] until they were 11 weeks old and weighed, on an average, 256 g. At this time, steroids were fed to 45 of these rats. The other 45 remained on the basal diet and served as controls. Ten rats each of the control and treated rats were used for measuring digestibility and balance of several nutrients. The remaining rats, 35 each in the control and treated groups were used for the determination of body compositional changes during and after withdrawal of the steroid therapy.

The treated rats were fed the steroids at a level comparable to that used by women on a body weight basis (0.1 mg norethynodrel and 0.0015 mg mestranol per kg per day). The steroids were fed to the rats by dissolving them in 70 % ethyl alcohol and then mixing thoroughly with the basal diet by means of a food mixer. The concentration of the steroids in the diet for the entire group of treated rats was adjusted weekly according to changes in body weights and food intake of the 10 treated rats used in the digestibility and balance studies. Both treated and control rats were fed the diet and water on an *ad libitum* basis. All rats were housed in individual suspended wire cages and were maintained in a room at a constant temperature of 27° and 12 hr each of light and darkness. Data were analyzed by analysis of variance (13).

Digestibility and balance studies. Two collections of urine and feces were made for the purpose of calculating digestibility and net retention of nutrients. The first collection was made after 22 days of steroid treatment, whereas the second collection was made after 173 days. The collection periods were 4 days on both occasions.

Feces were dried in a forced-air oven at 90° and ground in a Wiley Mill to a fine powder. Urine was made up to known volumes. The feces, urine, and diets were then analyzed for nitrogen, fat, sodium, and potassium.

Body composition. The 35-treated rats were sacrificed after 28 or 178 days of feed-

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[2] Present address: 9614 Nadar West Street, Aruppukottai, Tamil Nadu, India. Requests for reprints should be addressed to M. G. Yang, Foods and Nutrition Department, Michigan State University, East Lansing, Michigan 48823.

[3] Basal diet composition: Previously described (12).

ing steroids or at the end of 28 and 180 days plus 42 days each of feeding the control diet only. The number of rats were 10, 10, 8, and 7, respectively, for the four intervals. At each interval the same number of control rats were sacrificed.

The gastrointestinal tracts of the rats were removed and their contents washed out. The carcasses and the gastrointestinal tracts were autoclaved and homogenized (14).

Aliquots of carcass homogenates were analyzed for moisture by drying to constant weight. Nitrogen in the urine, feces, diets, and carcasses was determined by the Kjeldahl method and then converted to protein by multiplying by 6.25. Fat in feces, diets, and carcasses was extracted by diethyl ether in a Goldfisch extractor. Samples of feces, diets, and carcasses were ashed, whereas urine was diluted with deionized water for the determination of minerals. Sodium and potassium in the ashed samples and urine were determined by atomic adsorption and flame emission spectroscopic methods, respectively.

Results and Discussion. Food consumption and body weight gain. Food consumption and body weight gains were significantly less for the steroid-treated rats than the controls throughout the entire experimental period ($p<0.01$). The treated rats consumed on an average 2.0 to 2.5 g of feed less per day than the control rats throughout the experiment. At 1, 5, 10, 15, 20, and 25 weeks after the initiation of the feeding, the rats fed steroids throughout this time averaged 248, 266, 276, 269, 305, and 302 g in body weight, respectively, whereas the controls for the same periods were 268, 296, 307, 296, 333, and 347 g. For the first week the steroid treated rats lost weight and then gradually regained their lost weight during the next 3 weeks. The results of the present study are in agreement with those reported for rats treated with estrogen alone (15, 16) or with estrogen and progesterone (17). It has been shown that the growth inhibiting effect of estrogen was due mainly to its ability to depress appetite (15, 16). But another study showed that the daily injection of stilbestrol resulted in a marked loss of body weight which exceeded that of the pair-fed controls (18). The author claimed that the difference was sufficient to suggest some direct effect of the hormone on body weight. Other mechanisms by which these hormones depress body weight apart from the restriction of food intake have been discussed (19).

TABLE I. Percent Digestibilities of Various Nutrients Based on 4 Days Collection of Urine and Feces from 10 Rats Each Time.[a]

Nutrients	Treated	Control
After 22 days of steroid treatment		
Nitrogen	82.4 ± 1.3	81.0 ± 1.8
Fat	85.4 ± 8.0	87.0 ± 2.2
Sodium	96.2 ±11.6	95.0 ± 2.5
Potassium	93.4 ± 3.2	91.9 ± 3.7
After 173 days of steroid treatment		
Nitrogen	83.7 ± 2.3	82.2 ± 1.0
Fat	80.0 ±10.0	78.5 ± 6.0
Sodium	93.7 ± 5.9	93.2 ± 6.6
Potassium	94.1 ± 4.3	93.9 ± 4.5

[a] Values are means ± standard deviations.

Digestibility and nutrient balance. The digestibilities of protein, fat, sodium, and potassium were not significantly different between the steroid treated and control rats either after 22 or after 173 days of steroid treatment (Table I). However, after 22 days of feeding, the control rats retained significantly more dietary nitrogen in their body ($p<0.05$) than the treated rats. The increased percentage of nitrogen retention of the control rats was associated with a lesser quantity of urinary nitrogen expressed as a percent of what was absorbed. Average urinary nitrogen excreted in the 4-day period for the treated and control rats were 1.53 and 1.57 g, respectively, even though the treated rats consumed less feed. In the second balance study (after 173 days of feeding) the treated rats retained significantly ($p < 0.01$) more dietary nitrogen than the control rats (Table II). Thus, it seems that there is a possibility that the treated rats had adapted to the steroids and had begun to conserve nitrogen.

The percent retention of dietary sodium was significantly higher ($p < 0.05$) for the treated rats than for the control rats after 22

TABLE II. Percent Retentions of Various Nutrients Based on 4 Days Collection of Urine and Feces from 10 Rats Each Time.[a]

Nutrients	Treated	Control
After 22 days of steroid treatment		
Nitrogen[b]	8.2 ± 2.4	11.5 ± 4.4
Sodium[c]	32.1 ± 7.6	25.1 ± 4.9
Potassium	25.7 ± 7.2	24.2 ± 5.3
After 173 days of steroid treatment		
Nitrogen[d]	15.0 ± 4.8	7.8 ± 5.0
Sodium	41.4 ±34.8	32.1 ±19.7
Potassium	28.0 ±13.0	25.1 ± 7.3

[a] Values are means ± standard deviations.

[b] The control rats had significantly more nitrogen retention than the treated rats ($p < 0.05$).

[c] The treated rats mean retention of 32.1 was significantly more than that of 25.1 for the control rats ($p < 0.05$).

[d] The treated rats had higher nitrogen retention than the control rats ($p < 0.01$).

days of feeding. After 173 days of feeding the average retention was no longer statistically higher for the treated rats ($p > 0.05$). Potassium retention averaged slightly higher for the treated rats in both occasions of the balance trial. Unfortunately, it was not practical to measure retention of either sodium or potassium throughout the 173-day study. However, indirect evidences suggest that treated rats retained more of these minerals than control rats, because the treated rats consumed a lesser quantity of diet than the control rats but had about equal concentration of sodium and potassium in their lean body masses (Table V).

Body composition. On an absolute basis, the control rats had more dry matter, water, protein, and fat in their carcasses than the treated rats even when the latter had been refed the basal diet (Table III). Subsequent to 28 days of steroid treatment, feeding only basal diet to the treated rats for 42 days increased the body weight gain of these rats when compared to the controls (24.0 vs. 18.7 g). In addition, after 180 days of treatment, the feeding of the basal diet only for 42 days increased body weight by about 17 g compared to 24.4 g for the same period of time for the controls (Table IV). These data indicate that 42 days of refeeding the basal diet to the treated rats were not sufficient for full recovery of weight-gaining capacities.

On a percentage or proportional basis, the treated rats contained a higher concentration of moisture in the body than the control rats after 28 days of feeding ($p < 0.05$; Table III). Refeeding of the basal diet to the treated rats or feeding the steroids for a longer period (178 days) seemed to cause the treated rats to recover from the initial water accumulation (Table III). The increased water retention for the treated rats which had been fed the steroids for 28 days could partially be explained on the basis of an increased sodium retention measured after 22 days of feeding (Table II). Another explanation for this could be the lesser proportion of fat in the treated than the control rats, thus increasing the proportion of water in the treated rats. Expressing moisture as a percentage of lean body mass showed that the treated rats had essentially equal body water concentration as the control rats treated either for 28 or 178 days ($p > 0.05$, Table V).

There was significantly more body fat as a percentage of the carcass in the control than in the treated rats ($p < 0.05$, Table III). The lesser food intake of the treated rats is probably the major reason for their decrease in body fat. Many investigators found that women treated with contraceptive steroids had decreased glucose tolerances (4–6). It has also been shown that plasma nonesterified fatty acid was elevated in patients taking oral contraceptive pills (5). This increased release of fatty acids has been suggested to be caused by an impairment in glucose utilization which might lead to a greater mobilization of body fat for energy purposes. If this holds true for rats, both of these abnormalities could also decrease the amount of body fat observed in the treated rats of the present trial.

Feeding 28 days of steroids plus 48 days of feeding only the basal diet to the treated rats caused an average increase of 4.1 g of fat compared to 3.6 g for controls. Fat deposition in those rats refed the basal diet after feeding on the diet containing steroids for

TABLE III. Body Composition.[a]

	No. of rats	Wet carcass[b] (g)	Dry carcass (g)	Body water (g)	Body water (%)	Body protein (g)	Body protein (%)	Body fat (g)	Body fat (%)
				After 28 days steroid treatment					
Treated	10	263.2 ± 26.4	101.0 ± 13.0	162.2 ± 14.5	61.6 ± 2.8[c]	51.0 ± 5.5	19.4 ± 1.9	34.1 ± 8.7	13.0 ± 2.5
Control	10	276.1 ± 21.6	112.1 ± 10.0[d]	164.0 ± 11.7	59.4 ± 1.1	54.9 ± 4.5	19.9 ± 0.7	43.0 ± 6.3[e]	15.5 ± 1.7[f]
				After 28 days steroid treatment + 42 days of feeding the control diet only					
Treated	10	257.4 ± 13.8	101.8 ± 9.8	155.5 ± 9.4	60.4 ± 2.7	51.2 ± 2.6	19.9 ± 0.8	38.2 ± 8.7	14.8 ± 3.1
Control	10	290.0 ± 35.7	118.3 ± 18.9[d]	171.7 ± 16.3[g]	59.2 ± 2.1	55.5 ± 6.9	19.1 ± 2.4	46.6 ± 12.4	16.1 ± 2.9
				After 178 days steroid treatment					
Treated	8	279.8 ± 16.7	110.3 ± 12.1	169.5 ± 7.9	60.6 ± 2.4	57.1 ± 2.5	20.4 ± 1.1	36.2 ± 12.7	12.9 ± 4.0
Control	8	313.5 ± 23.6	129.6 ± 12.9[h]	183.9 ± 16.0[g]	58.7 ± 2.7	62.1 ± 4.2[i]	19.8 ± 0.8	51.5 ± 14.7[e]	16.4 ± 4.4
				After 180 days steroid treatment + 42 days of feeding the control diet only					
Treated	7	293.4 ± 30.5	118.9 ± 19.2	174.5 ± 15.1	59.5 ± 3.3	58.8 ± 4.5	20.0 ± 1.1	43.8 ± 16.4	14.9 ± 4.8
Control	7	333.4 ± 30.2	143.1 ± 21.6[d]	190.3 ± 13.1	57.1 ± 3.2	65.2 ± 4.1[i]	19.6 ± 1.0	59.7 ± 19.8	17.9 ± 4.6

[a] Values are means ± standard deviations.
[b] Weights of the rats without gastrointestinal contents.
[c] Treated rats had a higher mean % water ($p < 0.05$).
[d] Control rats had significantly more dry weight ($p < 0.05$).
[e] Control rats had more absolute fat ($p < 0.05$).
[f] Control rats had a higher mean % of fat ($p < 0.05$).
[g] Control rats had significantly more water ($p < 0.05$).
[h] Control rats had significantly more dry weight ($p < 0.01$).
[i] Control rats had more protein ($p < 0.05$).

TABLE IV. Cumulative Body Weight Gain of Rats Used for the Determination of Body Composition.

Feeding schedule	No. of rats/group	Body weight gain (g)	
		Treated	Control
28 days' steroid treatment[a]	10	11.7 ± 6.2[b]	31.7 ± 9.4
28 days' steroid treatment plus 42 days' refeeding of control diet[c]	10	35.7 ± 8.9	50.4 ± 17.5
178 days' steroid treatment[a]	8	39.3 ± 17.3	72.9 ± 18.9
180 days' steroid treatment plus 42 days' refeeding of control diet[c]	7	57.7 ± 26.3	97.3 ± 27.5

[a] Control rats gained more than the treated rats ($p < 0.01$).

[b] Means ± standard deviations.

[c] Control rats gained more than the treated rats ($p < 0.05$).

180 days amounted to about 7.6 g. For the control rats it was 8.2 g (Table III). The increase of fat from the 28th day to the 178th day of steroid feeding was only 2.1 g compared to 8.5 g for the controls (Table III, 36.2–34.1 and 51.5–43.0). The lesser quantity of fat in the body could be due to decreased fat deposition or increased fat mobilization. The latter has already been suggested for women taking pills. Interestingly, one report claimed that there was a shift of body fatty tissue from one area to another. This study was made with the use of photographic techniques and no direct measurements were made (20).

Summary. Norethynodrel and mestranol fed to 11-week-old female rats on a physiological level reduced food consumption and body weight gain. The withdrawal of the oral steroids for 42 days did not accelerate the growth rates of the treated rats when they had been previously fed the steroids for 28 or 180 days. Treatment with steroids for a short or long period did not affect digestibility of protein, fat, sodium, or potassium. However, retention of dietary nitrogen was higher for control rats than for treated rats after 22 days of steroid treatment ($p < 0.05$). At this time the treated rats retained significantly more dietary sodium than the control

TABLE V. Composition of Lean Body Mass.[a]

	Lean body mass[b] (g)	Moisture (%)	Sodium (mg/100 g)	Potassium (mg/100 g)
After 28 days' steroid treatment				
Treated	229.1 ± 19.8	70.8 ± 2.7	127.0 ± 6.0	375.4 ± 29.7
Control	233.1 ± 18.3	70.4 ± 0.5	128.6 ± 4.0	367.5 ± 25.4
After 28 days' steroid treatment + 42 days of feeding the control diet only				
Treated	219.2 ± 12.4	71.0 ± 0.9	124.4 ± 6.8	377.2 ± 16.6
Control	243.4 ± 24.1	70.5 ± 1.6	134.3 ± 9.9	372.2 ± 11.0
After 178 days' steroid treatment				
Treated	243.6 ± 9.8	69.6 ± 0.7	146.1 ±15.2	418.0 ± 42.5
Control	262.0 ± 21.3	70.2 ± 1.4	141.4 ± 6.7	417.0 ± 8.8
After 180 days' steroid treatment + 42 days of feeding the control diet only				
Treated	249.6 ± 20.3	69.9 ± 0.6	140.2 ± 3.4	415.5 ± 13.0
Control	273.7 ± 18.5	69.5 ± 0.4	140.3 ±13.6	404.2 ± 35.6

[a] Values are means ± standard deviations.

[b] Lean body mass = wet carcass—body fat.

rats ($p < 0.05$). After feeding the oral steroids for 178 days, the treated rats retained more dietary nitrogen than did the control rats ($p < 0.01$). No statistically significant effect on sodium retention was observed at this time. Thus, the effects caused by the oral steroids on the retention of this mineral as well as nitrogen was temporary in nature. The oral steroids did not significantly alter the retention of dietary potassium whether the treatment was for short or long time. Concomitant with the increased retention of sodium measured after 22 days of treatment was an increased proportion of water in the carcasses of treated rats compared to the control rats. Again, this effect disappeared after 178 days of treatment or after refeeding of control diet subsequent to 22 or 180 days of treatment. Furthermore, there was no difference in body moisture concentration when it was expressed as a percentage of lean body masses.

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