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Research Article

Evaluation of steroid hormone-induced changes in blood biochemical parameters in white mice

Thuan Thi Minh Nguyen[1*], Nhi Tu Duong[1], Nhi Diem Nguyen[1], Anh Thi Lan Nguyen[2]

[1] Department of Biochemistry, School of Pharmacy, University of Medicine and Pharmacy at Ho Chi Minh City, Viet Nam
[2] Institute of Public Health Ho Chi Minh City, Viet Nam

ABSTRACT

Exogenous hormones from food may accumulate in edible tissues, potentially increasing the health hazard to consumers. Their effects on metabolic disorders at low concentrations are poorly known. The aim of this study was to evaluate the effects of testosterone (TE), 17β-estradiol (E2) and melengestrol acetate (MGA) residues on some blood parameters to elucidate metabolic disorders in white mice. 112 Swiss Albino mice were divided into 4 groups including control group, TE-group, E2-group and MGA-group. Mice in test groups received oral TE 100 µg/kg, E2 100 µg/kg and MGA 50 µg/kg once daily, respectively. After 5 weeks, half of the mice were sacrificed, and the other half stopped receiving exogenous hormones for a week. Blood samples were collected at weeks 5 and 6 for biochemical parameters' assessment. Results showed that after oral hormone administration for 5 weeks, plasma AST, cholesterol, triglycerides, total protein increased, while HDL-cholesterol decreased in TE-group compared to the control group ($p<0.05$). Cholesterol and triglycerides of TE-female group were different from the TE-male group $p<0.05$). After stopping hormone for 1 week, cholesterol, triglycerides, and total protein continued to increase, while HDL-cholesterol decreased insignificantly in the TE-group compared to the control group. AST activity of E2-group and glucose of the MGA-group continued to increased compared to the control group ($p<0.05$). The HDL-cholesterol of the MGA-female group was different from the MGA-male group ($p<0.05$). In conclusion, this study's findings serve as a warning that prolonged exposure to low concentrations of oral steroid hormones can cause changes in blood biochemical parameters.

Keywords:
Melengestrol acetate; 17β-estradiol; Testosterone; Biochemical parameters; White mice

1. INTRODUCTION

Currently, the use of steroid hormones to promote growth and increase meat and milk production in livestock is very common. Veterinarians and livestock producers have used steroid hormones to improve animal performance due to their anabolic activity [1]. However, the overuse is becoming increasingly serious and leads to many health risks for humans. The impact of growth hormone residues on public health through accumulation in human tissues and environmental effects has been demonstrated [1,2]. Natural hormones such as testosterone (TE), 17β-estradiol (E2), or synthetic hormones such as melengestrol acetate (MGA) are widely used as growth stimulants in animals and have been detected in milk and meat [3]. Recent evidence suggests that hormone residues in meat products from hormone-treated animals may pose a danger to consumers. The harmful effects of hormones include developmental and neurobiological impacts, genotoxicity, and carcinogenicity [4]. TE is a steroid hormone that plays an important role in sexual development, fertility as well as the functioning of the musculoskeletal, nervous and cardiovascular systems. TE is used in livestock because it promotes protein assimilation,

***Corresponding author:**
* Thuan Thi Minh Nguyen Email: ntmthuan@ump.edu.vn

increases muscle mass, and increases meat and milk production. E2 and MGA are natural estrogens and synthetic progestins, respectively, commonly used in cattle for growth promotion purposes [5]. The Food and Drug Administration (FDA) has approved the use of these steroid hormones in beef cattle and sheep due to their effectiveness in increasing animal growth [6]. However, the use of hormonal growth promoters in livestock has not been allowed in Europe [7]. The long-term effects of TE, E2 and MGA in animals induce some reproductive disorders or hormone-related cancers [8]. Studies on the long-term effects of TE, E2 and MGA in animals were typically conducted at high concentrations [9-11]. However, studies on the metabolic potential of these hormones at low doses over the long term in animals are limited [12]. Therefore, there are concerns that humans may be at risk of exposure to exogenous hormones in food because the impact of these residue levels on metabolism is not yet fully understood. The aim of this study was to investigate the effects of low doses of TE, E2, and MGA hormones on morphological changes and blood biochemical parameters in white mice in order to better understand metabolic disorders.

2. MATERIALS AND METHODS

2.1. Reagents

Testosterone (TE), 17β-estradiol (E2) and MGA (MGA) were purchased from Sigma-Aldrich. Solutions of standard steroids were separately diluted with double distilled water to obtain the solutions of 10 ppm testosterone, 10 ppm E2, and 5ppm MGA. These steroid solutions were stored at 4 ℃. Reagent kits for the determination of plasma aspartate transaminase (AST), alanine transaminase (ALT), plasma creatinine, and plasma glucose were obtained from ELITechGroup. Reagent kits for the determination of total protein, total cholesterol, triglycerides and HDL-cholesterol were purchased from AMS Alliance.

2.2. Animal care

One hundred and twelve Swiss Albino white mice (48 males and 48 females) weighing approximately 18 – 23g and animal food was purchased from Pasteur Institute Ho Chi Minh City (Vietnam). Mice were housed at room temperature of 25 ^{0}C – 27 ^{0}C with relative humidity of 50 – 60%, and were given free access to food and water daily. All the procedures were carried out under the Animal Research Advisory Committee Guidelines. This study was performed with permission from University of Medicine and Pharmacy at Ho Chi Minh City, Vietnam.

2.3. Study design

The Ministry of Agriculture and Rural Development of Vietnam has issued maximum residue limits for MGA in food, but no MRL for TE and E2 [13]. Furthermore, previous studies on the long-term effects (4–12 weeks) of TE, E2 and MGA on animals were often conducted at high concentrations of 0.5–50 mg/kg/day [9–11]. This study aimed to evaluate the impact of the hormones TE, E2, and MGA on changes in biochemical parameters and the recovery of biological parameters after hormone cessation in mice. Therefore, the low doses of TE and E2 for mice were designed to be higher than the dose of MGA, at 100 µg/kg body weight (b.w)/day for TE and E2 and 50 µg/kg b.w/day for MGA.

Mice were steadily fed for 7 days prior to the start of the experiment. 112 mice were randomly divided into 4 groups (14 female and 14 male mice/groups) including control groups, TE-groups, E2-groups and MGA-groups. Mice in the TE, E2 and MGA groups received orally 100 µg/kg of TE, 100 µg/kg of E2 and 50 µg/kg of MGA once daily, respectively. The average body weights between 4 groups were not significantly different at the beginning of the experiment. All mice were weighed every week during the experimental period. The weight gain of the mice was monitored weekly by calculating the difference in body weight each week compared to the first week (week 0).

The control group received an equivalent volume of water in the same manner. After 5 weeks, half of the mice in each group were sacrificed. The other half in the experimental groups stopped receiving these hormones for a week to assess the recovery of biological parameters.

2.4. Sampling

Sampling from mice was conducted at the end of week 5 and week 6 of the experiment. Mice were anesthetized with CO_2, then whole blood samples from the heart were collected into EDTA blood tubes and centrifuged at 3000 *x g* for 10 min at room temperature. The plasma layers were separated immediately for biochemical analysis or stored at -5 ^{0}C until analysis (within 7 days). Liver and kidney samples from mice were carefully removed and washed with 0.9% NaCl solution. After drying with filter paper, the liver and kidneys were weighed and their macroscopic morphology, including shape and color, was assessed visually.

2.5. Analytical method

Plasma creatinine concentrations were determined by the rate-blanked Jaffe assay. AST and

ALT activities were determined by the IFCC method (International Federation for Clinical Chemistry). Plasma total protein concentrations were determined by the Biuret method. Plasma triglyceride, glucose and total cholesterol concentrations were determined by the enzymatic-colorimetric method. HDL-cholesterol (HDL-C) concentrations were determined by the precipitation method using phosphotungstic acid and Mg^{2+}. All these colorimetric methods were performed on Polker PPC 110 (Italia). LDL-cholesterol (LDL-C) concentrations were calculated indirectly by using Friedewald formula [14].

Normal values of biochemical parameters in mouse plasma included plasma glucose 97 – 239 mg/dL, triglycerides 75.6 – 198.5 mg/dL, total cholesterol 57.7 – 123.8 mg/dL, total protein 4.9 – 7.3 g/L, aspartate transaminase (AST) 35 – 137.7 IU/L, alanine transaminase (ALT) 0 – 222.2 IU/L, creatinine 0.2 – 0.7 mg/dL [15].

2.6. Statistical analysis

Statistical analyses were performed using R software (R Development Core Team, New Zealand, version 4.2.0). Data were expressed as mean ± standard error of the mean (SEM) and verified for normality (Shapiro test). One-way ANOVA test was used for pairwise comparison. The Dunnett test is used as a post-hoc test for the ANOVA to adjust for multiple comparisons. Unpaired Student's t-test was used to compare differences between male and female groups. All tests were considered statistically significant if p-values were < 0.05.

3. RESULTS

3.1. Evaluation of macroscopic morphology of mice

During the first 5 weeks of oral steroid hormone administration, no mice died in both the experimental and control groups. All mice in the control group were healthy, eating and acting normally. Male and female mice drinking testosterone (TE) 100 µg/kg/day ate and acted normally, while male and female mice in the group taking E2 100 µg/kg/day ate less than the control group. Some mice taking MGA 100 µg/kg/day experienced mild diarrhea. Results of morphological evaluation of mouse liver and kidney showed that the control mice had no internal abnormalities. In groups of mice receiving oral steroid hormone administration, the results showed that oral exogenous steroid hormones did not change the color, shape, and size of the liver at the investigated dose, but increased the proliferation of perirenal adipose tissue around the kidneys in both male and female mice (see Figure 1A).

After stopping oral steroid hormones for 1 week, male and female mice in the TE, E2 and MGA groups initially had similar activities to the control group and similar to the previous 5 weeks. When dissecting mice in the experimental group, fatty tissues around the kidneys in both male and female mouse groups were still observed (see Figure 1B).

3.2. Evaluation of mouse body weight

At the beginning of the experiment, there was no difference in average body weight between the control male mouse group and the experimental male mouse groups, and between the control female mouse group and the experimental female mouse groups (p = 0.74 and p = 0.471, respectively) (see table 1).

During 5 weeks of exposure to TE, the average body weight of TE mice increased rapidly after the first 2 weeks, then increased slowly over the next 3 weeks, while the weight of mice in the control group increased

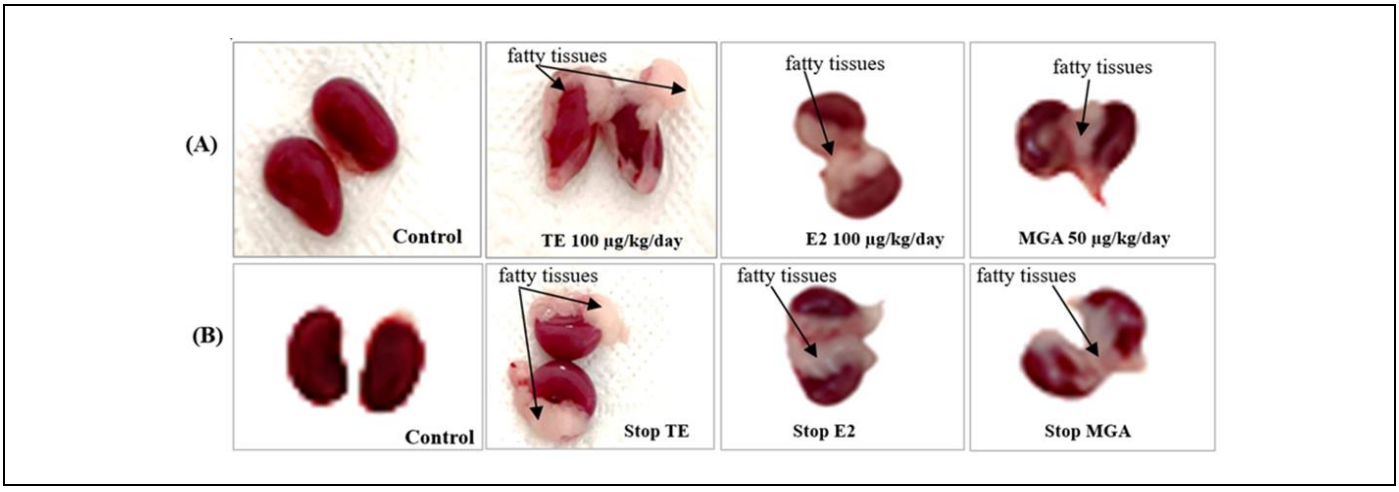

Figure 1. Macroscopic morphology of mouse kidneys in control and experimental groups after 5 weeks of receiving (A) and 1 week of stopping (B) steroid hormones.

Table 1. Comparison of average body weight of female and male mice in experimental groups treated with TE, E2, and MGA with the control group

Week	Groupe	Control (Mean ± SEM, gam)	TE (Mean ± SEM, gam)	E2 (Mean ± SEM, gam)	MGA (Mean ± SEM, gam)
0	F	18.60 ± 0.28	19.46 ± 0.28	18.60 ± 0.18	18.50 ± 0.16
	M	18.20 ± 0.47	18.69 ± 0.39	17.30 ± 0.80	18.49 ± 0.16
1	F	21.20 ± 0.41	**22.53 ± 0.24***	20.70 ± 0.74	21.70 ± 0.24
	M	22.30 ± 0.60	**23.58 ± 0.17***	21.00 ± 0.80	22.52 ± 0.51
2	F	24.20 ± 0.47	**24.21 ± 0.18***	23.00 ± 0.81	24.40 ± 0.37
	M	27.13 ± 0.61	**28.53 ± 0.21***	**23.40 ± 0.69****	27.18 ± 0.51
3	F	25.60 ± 0.59	25.28 ± 0.19	24.30 ± 0.84	26.50 ± 0.38
	M	29.40 ± 0.74	30.03 ± 0.26	**25.70 ± 0.69***	30.78 ± 0.40
4	F	26.60 ± 0.63	26.27 ± 0.16	25.70 ± 0.86	28.00 ± 0.36
	M	30.80 ± 0.73	31.16 ± 0.28	**26.60 ± 0.60****	32.26 ± 0.35
5	F	27.30 ± 0.69	27.32 ± 0.18	27.00 ± 0.93	**30.40 ± 0.32***
	M	31.40 ± 0.88	32.22 ± 0.28	**26.70 ± 0.59****	**34.15 ± 0.31***
		Control (Mean ± SEM, gam)	**Stopped-TE (Mean ± SEM, gam)**	**Stopped-E2 (Mean ± SEM, gam)**	**Stopped-MGA (Mean ± SEM, gam)**
6	F	29.53 ± 1.30	28.16 ± 0.26	28.09 ± 1.47	33.13 ± 0.57
	M	32.33 ± 1.00	33.67 ± 0.39	29.80 ± 0.11	35.30 ± 0.33

*, **: significant difference between the groups receiving oral TE, E2 and MGA with control groups ($p < 0.05$ and < 0.001, respectively, t-test).

steadily throughout the 5 weeks. After stopping receiving TE for 1 week, the average body weight of mice in the stopped-TE group increased without statistical significance compared to the control group ($p > 0.05$, t-test) (see table 1).

The average body weight of male mice in the E2 group decreased significantly in the first 3 weeks and continued to decrease in the next 2 weeks, while the weight loss in female mice in the E2 group was insignificant compared to control was not significant ($p>0.05$) during first 5 weeks of exposure to E2. After 1 week of stopping using E2, the body weight of male mice in the stopped-E2 group was still significantly lower than that of control mice ($p < 0.05$, t-test) (see table 1).

During 5 weeks of exposure to MGA, the average body weight of female and male mice in the MGA groups increased significantly compared to the female and male control. After 1 week of stopping exposure to MGA, the average body weight of mice in the stopped-MGA group continued to increase more than the control group of mice, but it was not significant ($p > 0.05$) (see table 1).

The mouse body weight gain was determined by the difference in mouse body weight in the next weeks with the first week (week 0). From week 1 to week 6 of the experiment, the body weight growth rate of male and female mice in the MGA group was higher than the control group, while the body weight growth rate of male and female mice in the E2 group was lower than the control group. The body weight growth rate of female mice in the TE group was lower than that of the control group, while the body weight growth rate of male mice in the TE group was equal to the control group (see Figures 2 A and 2B).

3.3. Evaluation of changes in biochemical parameters after 5 weeks of receiving oral steroid hormones

For the group of mice receiving TE 100 µg/kg/day for 5 weeks, ALT activity, cholesterol and blood triglyceride levels of female TE mice were statistically different from those of male TE mice ($^{i}p_{ALT} = 0.03$; $^{i}p_{cholesterol}$, $^{i}p_{triglycerides} = 0.018$). Biochemical parameters in plasma including total protein, glucose, HDL-C, LDL-C, AST, creatinine was not statistically different between the two groups of male and female TE mice ($p > 0.05$). AST activity, cholesterol and blood triglyceride levels increased in the TE group with statistical significance compared to the control group ($^{*}p_{AST} = 0.002$; $^{*}p_{cholesterol} = 0.003$; $^{*}p_{triglycerides} = 0.025$). Biochemical parameters in plasma including total protein, glucose, HDL-C, and ALT we re not statistically different between the TE group and the control group ($p> 0.05$). Plasma concentrations of total protein, cholesterol, and triglycerides increased, but HDL-C decreased differently in the male TE group compared to the control male group ($^{**}p_{protein} <0.001$; $^{*}p_{cholesterol} = 0.003$; $^{*}p_{triglycerides} = 0.007$; $^{*}p_{HDL-C} = 0.012$). Biochemical parameters in plasma, including glucose, AST and ALT levels, were not statistically different between the male TE group and the control group ($p > 0.05$) (see table 2).

Meanwhile, there was no difference in the concentration of biochemical parameters in plasma, including total protein, glucose, cholesterol, triglycerides,

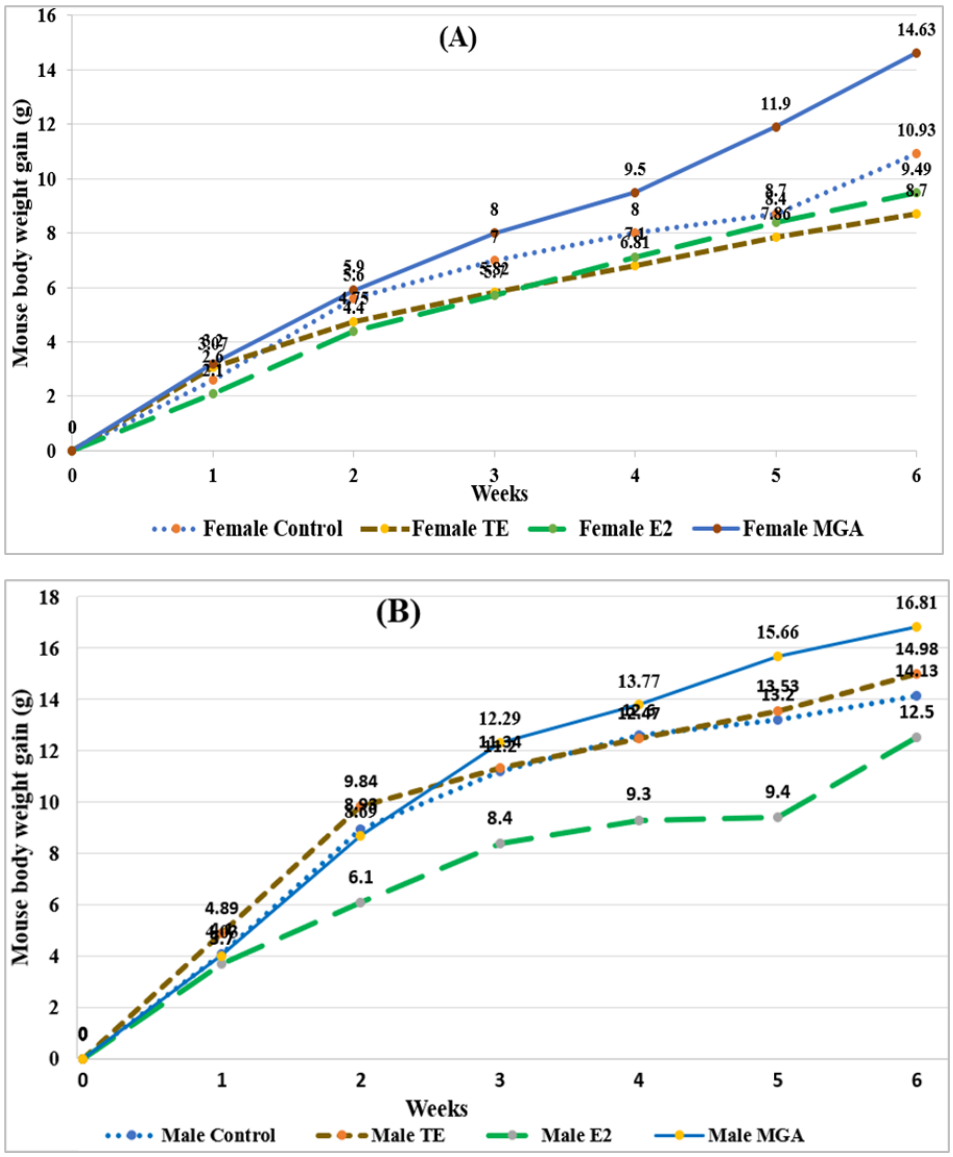

Figure 2. Weekly weight gain rate of female (A) and male (B) mice compared to baseline**.**

HDL-C, AST and ALT between the two groups of male and female mice receiving oral E2 100 µg/kg/day ($p > 0.05$). This was also observed in two groups of male and female mice given MGA 50 µg/kg/day ($p > 0.05$) (see table 2).

When comparing the female E2 group with the female control group, plasma AST activity increased in the E2 group with statistical significance compared to the control group ($^{*}p_{AST} = 0.006$), while biochemical parameters in plasma including total protein, glucose, cholesterol, triglycerides, HDL-C, ALT were not statistically different between the two groups ($p > 0.05$). When comparing the male E2 group with the male control group, the increase in AST activity and HDL-C concentration in the male E2 group was statistically different from that in the male control group ($^{*}p_{AST} = 0.036$; $^{*}p_{HDL\text{-}C} = 0.026$), while plasma biochemical parameters including total protein, cholesterol, triglycerides, glucose, AST and ALT were not statistically different between the two groups ($p > 0.05$) (see table 2).

When comparing the female MGA group with the control group, the increased plasma glucose concentration in the female MGA group was statistically different from the female control group ($^{**}p_{glucose} < 0.001$), while biochemical parameters in plasma including total protein, cholesterol, triglycerides, HDL-C, AST, and ALT were not statistically different between the two groups ($p > 0.05$). When comparing the MGA male group with the control male group, the increased glucose and cholesterol concentrations in the MGA male group were statistically different from the control male group ($^{*}p_{AST} = 0.036$; $^{*}p_{HDL\text{-}C} = 0.026$), while biochemical parameters in plasma including total protein,

Table 2. Plasma concentrations of biochemical parameters in mice after 5 weeks of receiving oral steroid hormones.

Plasma concentration	Groupe	Control (Mean ± SEM)	TE (Mean ± SEM)	E2 (Mean ± SEM)	MGA (Mean ± SEM)
Total protein (g/dL)	F	6.20 ± 0.26	7.00 ± 0.38	6.13 ± 0.19	6.04 ± 0.29
	M	6.09 ± 0.10	**7.37± 0.22**	6.39 ± 0.19	6.29 ± 0.20
Glucose (mg/dL)	F	238.90 ± 6.51	262.51 ± 11.10	222.29 ± 12.07	**331.10±5.87****
	M	234.37 ± 16.51	246.57 ±23.71	230.32 ± 6.06	**332.98±10.07****
Cholesterol (mg/dL)	F	92.46 ± 2.19	**140.55± 9.11***	112.78 ± 10.31	102.99 ± 6.29
	M	97.66 ± 3.24	**113.57± 2.69*,i**	95.60 ± 1.67	**111.80 ± 2.74***
Triglycerides (mg/dL)	F	77.92 ± 5.27	**103.22± 6.91***	73.36 ± 2.65	78.17 ± 4.92
	M	75.34 ± 6.22	**139.14± 11.64*,i**	73.69 ± 3.29	79.00 ± 0.66
HDL-C (mg/dL)	F	53.47 ± 4.85	52.52 ± 5.62	57.50 ± 6.21	54.81 ± 4.73
	M	58.09 ± 3.84	**47.17± 1.66***	**68.70 ± 1.12***	59.72 ± 1.75
LDL-C (mg/dL)	F	42.97 ± 14.57	60.38 ± 12.63	55.62 ± 12.80	46.99 ± 8.05
	M	40.31 ± 2.96	43.25 ± 2.61	27.18 ± 2.74	37.41 ± 4.32
AST (U/L)	F	74.74 ± 6.04	**122.65±10.41***	**145.26 ± 5.53***	70.44 ± 8.80
	M	82.04 ± 11.26	106.23 ±13.79	**126.43 ± 7.46***	77.94 ± 12.47
ALT (U/L)	F	62.75 ± 6.05	104.72 ± 24.34	71.14 ± 3.28	68.77 ± 5.60
	M	57.81 ± 6.19	**48.70 ± 8.87i**	61.89 ± 3.40	64.33 ± 7.61
Creatinine (mg/dL)	F	0.58 ± 0.04	0.51 ± 0.05	0.75 ± 0.01	0.54 ± 0.07
	M	0.62 ± 0.02	0.55 ± 0.06	0.57 ± 0.01	0.59 ± 0.02

*, **: significant difference between the groups receiving oral TE, E2 and MGA with control groups ($p < 0.05$ and < 0.001, respectively, t-test).
^{i}: significant difference between male (M) and female mice (F) in the control group, groups of receiving oral TE, E2 and MGA ($p < 0.05$, t-test).

triglycerides, HDL-C, AST, and ALT were not statistically different between the two groups ($p > 0.05$). Plasma creatinine and LDL-C concentrations of mouse groups receiving TE, E2 and MGA were not significantly different between groups of male and female mice, and with the control group ($p > 0.05$) (see table 2).

3.4. Evaluation of changes in biochemical parameters after 1 week of stopping oral steroid hormones

For the group of mice discontinued oral TE for 1 week (stopped-TE group), the plasma protein and HDL-cholesterol concentrations of mice in the female-stopped-TE group were statistically different from those in the male stopped-TE group ($^{i}p_{protein}=0.003$; $^{i}p_{HDL\text{-}C}=0.026$). Plasma biochemical parameters including glucose, cholesterol, triglycerides, LDL-C, AST, ALT, and creatinine were not statistically different between the two stopped-TE groups ($p> 0.05$). When compared with the female control group, protein activity, cholesterol and blood triglyceride levels continued to increase significantly in the female stopped-TE group ($^{**}p_{protein} <0.001$; $^{*}p_{cholesterol} = 0.046$; $^{*}p_{triglycerides} = 0.004$), while AST levels decreased no differently. When compared with the control male group, plasma triglyceride concentration increased in the male stopped-TE group with statistical significance ($^{*}p_{triglycerides} = 0.049$), while total protein, cholesterol, and blood triglyceride concentrations decreased and the HDL-C increase was not different ($p > 0.05$) (see table 3).

For the groups of mice discontinued oral E2 (stopped-E2 group) and MGA (stopped-MGA group) for 1 week, HDL-C concentrations were found to differ between male and female mice in both of the stopped-E2 and stopped-MGA groups. The other parameters did not differ between the two groups of male and female mice in both of the stopped-E2 and stopped-MGA groups ($p > 0.05$) (see table 3).

Plasma AST activity continued to increase significantly in the female stopped-E2 group ($^{*}p_{AST} = 0.006 < 0.05$), while biochemical parameters in plasma including total protein, glucose, cholesterol, triglycerides, HDL-C, and ALT were not significantly different compared with the female control group ($p > 0.05$). Plasma AST activity continued to increase ($p < 0.05$) in the male stopped-E2 group, while HDL-C concentration increased insignificantly compared with the control male group ($p > 0.05$) (see table 3).

Plasma glucose concentration continued to increase in the female stopped-MGA group with a statistically significant difference (*pglucose < 0.001), while biochemical parameters in plasma including total protein, cholesterol, triglycerides, HDL-C, AST, and ALT were not significantly different from the control female group ($p > 0.05$). Plasma glucose concentration continued to increase significantly ($p < 0.05$), but cholesterol increased insignificantly ($p> 0.05$) in the male stopped-MGA group compared to the control male group ($p > 0.05$) (see table 3).

Plasma creatinine and LDL-C concentrations of groups of mice discontinued oral TE, E2 and MGA for

Table 3. Plasma concentrations of biochemical parameters in mice after 1 week of stopping oral steroid hormones.

Plasma concentration	Groupe	Control (Mean ± SEM)	Stopped-TE (Mean ± SEM)	Stopped-E2 (Mean ± SEM)	Stopped-MGA (Mean ± SEM)
Total protein (g/dL)	F	6.23 ± 0.26	**10.57± 0.46****	6.20 ± 0.34	6.01 ± 0.18
	M	6.13 ± 0.17	**6.56 ± 0.12**^{i}	6.36 ± 0.12	6.34 ± 0.13
Glucose (mg/dL)	F	232.87 ± 7.57	267.12 ± 15.33	226.30 ± 6.92	**327.03 ± 9.65***
	M	238.26 ± 10.89	241.12 ± 2.20	237.77 ± 8.54	**332.72 ± 11.30****
Cholesterol (mg/dL)	F	99.44 ± 3.80	**114.54± 6.19***	112.67 ± 3.23	104.18 ± 3.76
	M	104.66 ± 3.39	116.92 ± 6.35	111.34 ± 6.06	116.30 ± 5.69
Triglycerides (mg/dL)	F	76.85 ± 5.91	**124.52± 8.54***	74.70 ± 2.43	77.66 ± 1.51
	M	72.90 ± 3.98	**105.05± 12.36***	69.34 ± 2.58	76.96 ± 2.45
HDL-C (mg/dL)	F	52.83 ± 2.22	49.44 ± 2.63	59.40 ± 5.64	53.60 ± 2.65
	M	62.54 ± 1.98	63.03 ± 5.12 ^{i}	66.90 ± 2.73	**65.58 ± 2.81** ^{i}
LDL-C (mg/dL)	F	41.92 ± 5.63	37.83± 4.39	44.59 ± 4.42	45.46 ± 6.08
	M	36.71 ± 2.13	38.54 ± 1.86	35.78 ± 4.32	37.41 ± 4.32
AST (U/L)	F	72.99 ± 3.82	90.69 ±15.64	**145.76 ± 8.71***	70.44 ± 9.44
	M	77.91 ± 8.09	96.90 ±8.69	**117.01 ± 5.69***	72.58 ± 4.84
ALT (U/L)	F	69.06 ± 4.73	83.07 ± 14.37	76.65 ± 6.72	71.16 ± 5.17
	M	61.74 ± 5.23	83.15 ± 11.14	68.20 ± 8.00	70.89 ± 6.27
Creatinine (mg/dL)	F	0.61 ± 0.04	0.61 ± 0.02	0.67 ± 0.04	0.58 ± 0.07
	M	0.70 ± 0.05	0.53 ± 0.05	0.65 ± 0.04	0.62 ± 0.03

*, **: significant difference between the groups of mice stopped receiving TE, E2, and MGA with control groups ($p < 0.05$ and $p < 0.001$, respectively, t-test).

^{i}: significant difference between male (M) and female mice (F) in the control group and the groups of mice stopped receiving TE, E2, and MGA ($p < 0.05$, t-test).

1 week were not significantly different between groups of male and female mice, and with the control group ($p > 0.05$) (see table 3).

4. DISCUSSION

Steroid compounds used for anabolic purposes in animals include estradiol, progesterone, and testosterone. Numerous previous toxicity studies of TE, E2, and MGA have been conducted on various animal species to predict their toxicity to humans [8-11]. This study evaluates the impact of growth hormone on changes in biochemical parameters in Swiss albino mice, a breed widely used in pharmacology, toxicology, and nutrition research [8]. After the first 5 weeks of testing, oral TE 100 µg/kg/day, E2 100 µg/kg/day and MGA 50 µg/kg/day administered to mice did not cause severe toxicity symptoms and affected to mouse daily activity. Although no fat accumulation was found in the liver, the perirenal fat accumulation in mice in the test group was a sign of poor metabolism. The thickness of the perirenal fat layer is associated with metabolic risk factors in patients with chronic kidney disease [16]. Since cell membrane progesterone receptors are thought to be expressed in the kidneys, the sex hormone may have potential effects on the kidneys [17].

Growth hormones play a crucial role in regulating appetite, eating behavior, and energy metabolism. E2 inhibits food intake, while TE stimulates appetite. This study allowed mice to eat and drink freely (*ad libitum*) to assess the effects of these hormones on weight gain [18,19]. Animal sex and maturity affect growth rate and body composition. The results of this study showed that TE influenced body mass in mice, E2 decreased the body mass of testing mice of both sexes for 5 weeks, while the weight of mice exposed to MGA increased very rapidly compared to the control group. TE, produced in the testes of male cattle, contributes to this increased feed efficiency and lower carcass fat content. TE binds to receptors in muscle and stimulates the increased incorporation of amino acids into proteins, thus increasing muscle mass without increasing adipose tissue. Naturally occurring endogenous steroids are inactive when taken orally [19]. E2 is thought to reduce food and water intake in mice through genetic mechanisms typically involving estrogen receptors in the cell nucleus [20]. Estradiol may increase energy expenditure by promoting thermogenesis of brown adipose tissue via the hypothalamic-sympathetic nervous system. This mechanism leads to greater weight loss compared to groups with lower or no estrogen exposure [21]. MGA is a synthetic progestogen that is about 30 times more potent than progesterone, and is therefore used to improve body weight and feed conversion efficiency in female beef cattle [22]. MGA is hypothesized to stimulate various groups of muscle-derived cells to switch to the adipogenesis pathway in both bovine and

mouse cell culture models [23]. Furthermore, MGA inhibits the release of luteinizing hormone and α-estradiol, thus suppressing ovulation and the estrous cycle. Conversely, when exogenous testosterone and estrogen are introduced into the body, the brain and glands detect the "excess" and naturally stop producing them.

Total cholesterol and triglyceride concentrations in the experimental mice receiving TE for 5 weeks were significantly higher than in the control group. The increase of plasma total cholesterol, LDL-C, and triglycerides was observed at week 9 in mice injected with TE [24]. Other clinical studies have observed an elevation in total cholesterol and triglycerides in patients using TE for a long time [25]. This effect can be explained by TE inhibiting lipid uptake and lipoprotein lipase activity in fat cells [26]. Our study found that E2 reduced plasma LDL-C concentrations in male mice compared with controls. This result is consistent with the previous study [27]. TE has an effect on increasing protein synthesis and reducing protein degradation, while E2 increased protein synthesis by altering skeletal muscle protein synthesis [19,28]. The mean blood glucose values in both male and female rats increased non-statistically compared with the control group after 5 weeks of oral TE 100 µg/kg/day. This increase in blood glucose may be explained by the long-term use of low doses of TE that can reduce the action of insulin, which is responsible for lowering blood glucose levels [29]. For the effects of E2 on mice in this study, no significant decrease in blood glucose level ($p > 0.05$) in both sexes was observed. However, the blood sugar levels in male and female testing mice with MGA were higher than that of the control. Since MGA has the primary biological effect of progestin, leading to abnormal glucose metabolism [30]. AST activity in the female TE group increased significantly compared to the control. The results of the effects of testosterone on AST and ALT levels were similar to a previous study on mice using synthetic testosterone with an equivalent dose for 6 weeks [31]. Estrogen was proved to increase serum AST and ALT [32]. The increase in ALT levels was also observed in mice exposed to high doses of MGA [33].

There are regulations limiting the maximum residue of MGA in food in Vietnam, but not yet for testosterone and estrogen. However, exposure to low concentrations of exogenous steroid hormones can also lead to the accumulation of excess hormones, leading to a risk of metabolic diseases. Growth hormone stimulants at low doses improve weight gain and feed efficiency in animals, with effects varying significantly depending on the breed, type of hormone, and threshold concentration of growth hormone in the blood [34]. The limitations of this project are only to evaluate the recovery of biochemical parameters in Swiss Albino mice. Further studies could evaluate the effects of steroid hormones at lower doses on other animal species. They could also assess the recovery of biochemical parameters over a longer period after discontinuation of growth hormones.

5. CONCLUSION

The findings of this study may contribute to the warning that long-term exposure to oral steroid hormones at low concentrations can cause changes in blood biochemical parameters. Prolonged testosterone exposure at low doses might affect total protein and lipid metabolisms considerably more than glucose concentration in white mice, where female mice especially experienced much more serious hyperproteinemia than the male mice. Oral E2 increases AST activity, which can affect liver function, while MGA can affect glucose and lipid metabolism.

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Author contribution

Thuan Thi Minh Nguyen: Conceptualization, Methodology, Formal analysis, Project administrator, Supervision, Writing-reviewing and editing.
Nhi Tu Duong: Methodology, Investigation, Data collection, Formal analysis, Writing-original draft preparation.
Nhi Diem Nguyen: Investigation, Data collection, Formal analysis, Writing-reviewing and editing.
Anh Thi Lan Nguyen: Methodology, Investigation.

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Conflict of interest

The authors declare that they have no conflict of interest.

Ethics approval

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