



## Investigation of Gender Difference of Physiological Response in Gonadectomy in Sprague-Dawley (SD) Rats

K. Miyajima<sup>1\*</sup>, Y. Toriniwa<sup>1</sup>, Y. Motohashi<sup>1</sup>, Y. Ishii<sup>1</sup>, S. Kimura<sup>1</sup>, M. Shinohara<sup>2</sup>,  
H. Yamashiro<sup>3</sup>, T. Yamada<sup>4</sup> and T. Ohta<sup>1,4</sup>

<sup>1</sup>Japan Tobacco Inc., Central Pharmaceutical Research Institute, Osaka, Japan.

<sup>2</sup>CLEA Japan Inc., Planning and Development Section, Tokyo, Japan.

<sup>3</sup>Laboratory of Animal Reproduction, Faculty of Agriculture, Niigata University, Niigata, Japan.

<sup>4</sup>Laboratory of Animal Genetics, Graduate School of Science and Technology, Niigata University, Niigata, Japan.

### Authors' contributions

*This work was carried out in collaboration between all authors. Authors KM, TY and TO design the study, wrote the protocol, and wrote the first draft of the manuscript. Authors KM, YT, YM, YI, SK, MS, HY and TO managed the analyses of the study, and performed the statistical analyses. All authors read and approved the final manuscript.*

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### ABSTRACT

**Aim:** Sex hormones, including testosterone and estrogen, result in various pathophysiological changes in the body. To evaluate the pathophysiological changes following gonadectomy in male and female rats, we performed gonadectomy at the same age in male and female Sprague-Dawley (SD) rats.

**Methods:** Male and female Sprague-Dawley rats castrated by bilateral orchidectomy and ovariectomy at 6 weeks of age (six animals of each sex per group). Food intake, body weight, and clinical chemical parameters such as glucose, insulin, triglyceride and total cholesterol levels, were examined every 4 weeks from 8 to 40 weeks of age. Statistical analysis of differences between

\*Corresponding author: Email: [katsuhiko.miyajima@jt.com](mailto:katsuhiko.miyajima@jt.com), [katsu0612@gmail.com](mailto:katsu0612@gmail.com);

control and gonadectomized rats was performed using the F-test, followed by the Student's t-test or Aspin-Welch's t-test.

**Results:** In orchidectomized (ORX) rats, food intakes and body weights were decreased, whereas in ovariectomized (OVX) rats, the body weights were significantly elevated without an obvious change in food intake. In clinical chemical analysis, hypercholesterolemia was observed in both ORX and OVX rats, but the triglyceride level was obviously decreased only in ORX rats during the observational period. In OVX rats, decrease of insulin sensitivity and significant increase of adipose tissue weights were observed. In bone metabolic analysis, bone mineral content in ORX rats and bone mineral density in OVX rats were decreased, respectively.

**Conclusion:** Both orchidectomy and ovariectomy in rats affect glucose/lipid and bone metabolism, and especially, the glucose metabolism was deteriorated in OVX rats. Both male and female sex hormones play a key role in metabolic disease, such as diabetes, hyperlipidemia and osteoporosis.

**Keywords:** Orchidectomy; ovariectomy; sprague-dawley rat.

## 1. INTRODUCTION

Sex hormones, such as testosterone and estrogen, have a variety of physiological characteristics and are considered to affect various metabolic states, including glucose/lipid metabolism and bone metabolism. Therefore, castrated animals, such as orchidectomized (ORX) and ovariectomized (OVX) animals are used to evaluate effects on the pathophysiology, including diabetes mellitus, diabetic complications and osteoporosis. The effects of female hormones on diabetes have been examined in streptozotocin-induced diabetic rats [1], Otsuka-Long-Evans-Tokushima-Fatty (OLETF) rats [2], Wistar diabetic fatty rats [3], eSS rats [4] and Spontaneously Diabetic Torii (SDT) rats [5]. Gender difference and the role of sex-hormone related to the diabetes progression were also described in diabetic animal model including Spontaneously Diabetic Torii fatty (SDT fatty) rats [6,7]. From the research findings in these diabetic models, protective effects of female sex hormones for diabetes were reported. On the other hand, testosterone has been found to reduce glucose tolerance [8], and testosterone supplementation in experimental models exacerbates, whereas gonadectomy attenuates, both hypertension and associated non-diabetic renal disease [9].

The OVX rat model is most commonly used in research on postmenopausal osteoporosis. After ovariectomy, bone resorption exceeds bone formation, causing bone loss [10,11]. It is reported that male sex hormones, such as androgen, dihydrotestosterone and testosterone, stimulate mineralization and increase mineral content in bone and bone volume [12-15]. In ORX dogs, the bone volume was significantly decreased [16].

To evaluate the pathophysiological changes following gonadectomy in male and female rats, we performed gonadectomy at the same age in male and female Sprague-Dawley (SD) rats.

## 2. MATERIALS AND METHODS

### 2.1 Animals

SD rats were purchased from CLEA Japan, Inc. (Tokyo, Japan). Rats were housed in suspended bracket cages and given a standard laboratory diet (CRF-1, Oriental yeast co., Ltd. Tokyo, Japan) and water *ad libitum* in a room with controlled temperature, humidity and lightning. Male and female rats were castrated by bilateral orchidectomy and ovariectomy, respectively, under sodium pentobarbitone anesthesia (50 mg/kg i.p.) at 6 weeks of age, and sham-operated rats were prepared as control rats (six animals of each sex per group).

### 2.2 Biophysiological Parameters

Food intake, body weight and clinical chemical parameters, such as glucose, insulin, triglyceride (TG) and total cholesterol (TC) levels were examined every 4 weeks from 8 to 40 weeks of age. Blood samples were collected from the tail vein of non-fasted rats. Serum glucose, TG and TC levels were measured using commercial kits (Roche Diagnostics, Basel, Switzerland) and automatic analyzer (Hitachi, Tokyo, Japan). Serum insulin level was measured with a rat-insulin enzyme-linked immunosorbent assay (ELISA) kit (Morinaga Institute of Biological Science, Yokohama, Japan).

Oral glucose tolerance test (OGTT) was performed at 16 weeks of age. Glucose solution (2 g/kg) was administered to 4 hours fasted rats.

Blood samples were collected before, and 30, 60 and 120 min after glucose loading. Serum glucose and insulin levels were measured by using commercial kits and ELISA kit, as described above.

### 2.3 Adipose Tissue Weight

Visceral and subcutaneous fat weights in each rat were determined at 16 weeks of age by computed tomography (CT) analysis. The fat weights were measured by a laboratory X-ray CT device (LaTheta, ALOKA Co., LTD., Osaka, Japan). Rats were anesthetized with an intraperitoneal injection of 50 mg/kg pentobarbital (Tokyo chemical industry, Tokyo, Japan), and about 20 CT photographs per rat were taken at 5 mm intervals between diaphragm and lumbar vertebrae. Total fat weight was calculated from visceral and subcutaneous fat weights.

### 2.4 Bone Mineral Densitometry

Bone mineral density (BMD) and bone mineral content (BMC) of the right tibia were evaluated at 40 weeks of age. BMD and BMC were measured by quantitative computed tomography (QCT) using a LaTheta LCT-100A micro-CT scanner (Aloka, Tokyo, Japan) with a pixel size of 250 × 250 μm and a slice thickness of 1 mm. The rats were anesthetized with pentobarbital (50 mg/kg) during the measurement.

### 2.5 Statistical Analysis

Results were expressed as the mean ± standard deviation. Statistical analysis of differences between mean values was performed using the F-test, followed by the Student's t-test or Aspin-Welch's t-test. Differences from the Sham-SD rats were defined as significant at  $p < 0.05$ .

## 3. RESULTS

### 3.1 Biophysiological Parameters

Changes of biological parameters, such as food intake, body weight and serum parameters are shown in Figs. 1 and 2. In ORX rats, food intakes tended to be lower after 12 weeks of age, and the body weights also tended to be lower after 12 weeks (Figs. 1A and 1B). In OVX rats, food intakes tended to be higher from 12 to 20 weeks of age, but obvious changes in food intake were not observed after 24 weeks of age (Fig. 2A). Body weights in OVX rats were significantly

higher during the observational period, except for the starting point at 8 weeks of age (Fig. 2B). Neither ORX nor OVX rats showed a significant difference in basal glucose levels (Figs. 1C and 2C). Basal insulin levels in OVX rats were significantly higher at 12, 20, and 24 weeks of age (Fig. 2D). TG levels in ORX rats were lower during the experimental period (Fig. 1E). TC levels were significantly higher in both ORX and OVX rats (Figs. 1F and 2F). There were no apparent changes in TG levels in OVX rats.

OGTT was performed at 16 weeks of age (Fig. 3). No change was observed in ORX rats, but glucose intolerance was observed in OVX rats. The insulin levels at 30 min after glucose loading was elevated in OVX rats, but the glucose level after glucose loading did not decrease, and the glucose level at 120 min after glucose loading was obviously elevated (Figs. 3C and 3D). There were no apparent changes in the insulin levels in ORX rats.

### 3.2 Adipose Tissue Weight

In OVX rats at 16 weeks of age, both visceral and subcutaneous fat tissue weights were significantly elevated, and the total fat tissue weight was about 3 times higher as compared with that in sham SD rats (Table 1). In ORX rats at 16 weeks of age, subcutaneous fat tissue weight was higher, but the lean body mass was lower.

### 3.3 Bone Mineral Densitometry

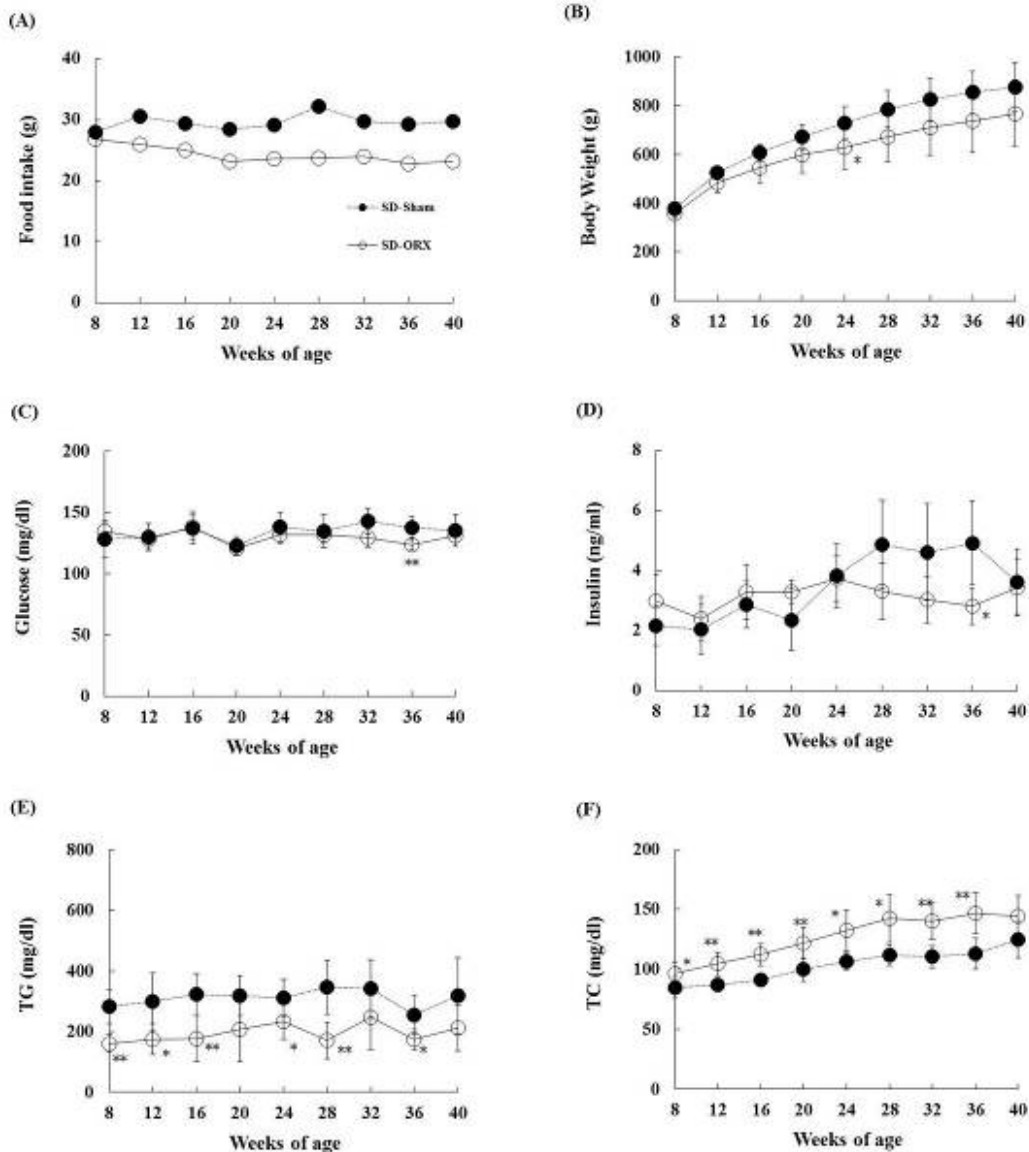
Both orchidectomy and ovariectomy affected bone metabolism, showing lower BMC in ORX rats and lower BMD in OVX rats (Fig. 4).

## 4. DISCUSSION

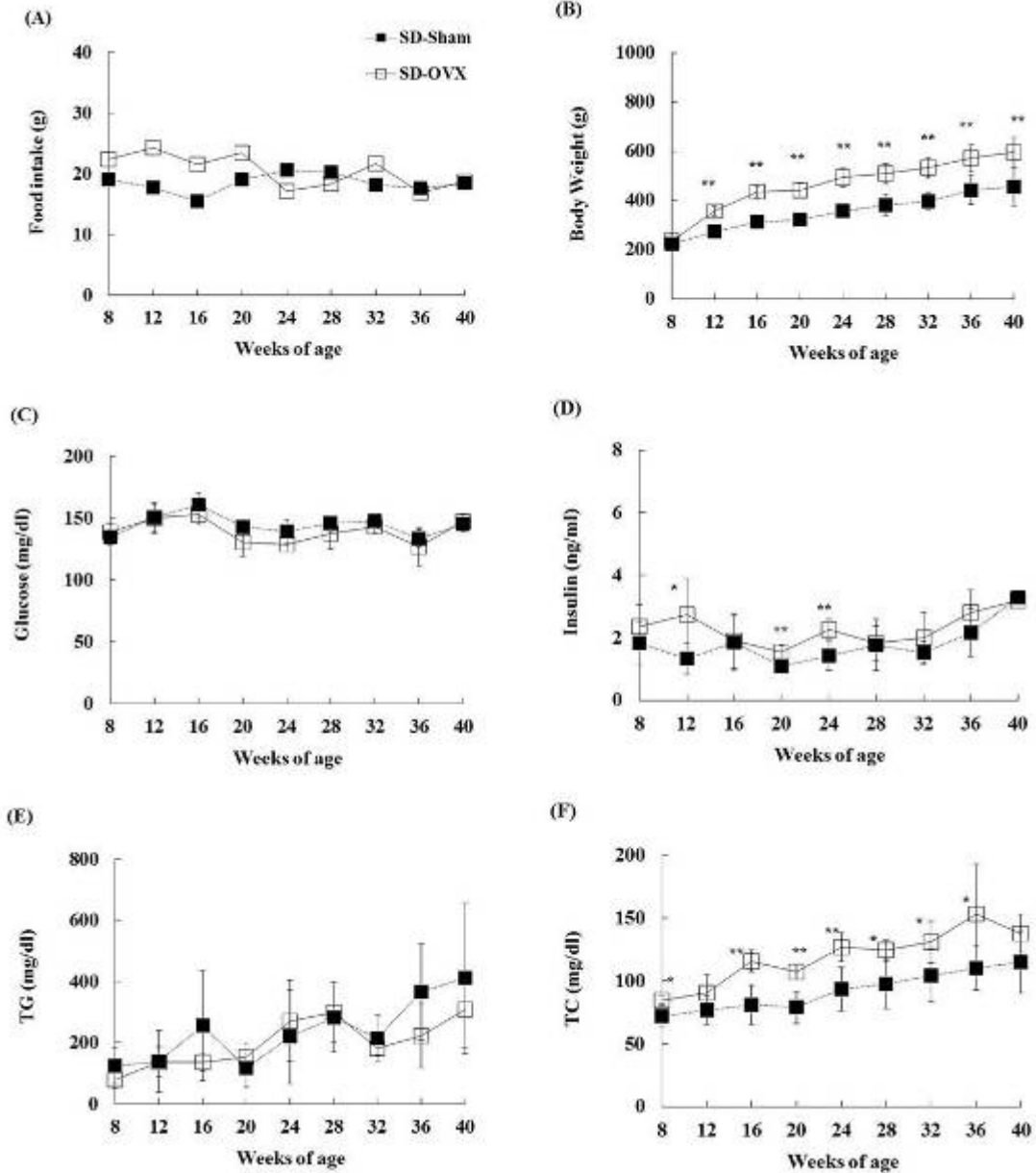
Decreases of food intake and body weight were observed in ORX rats, and a significant increase of body weight was observed in OVX rats. In ORX rats, lower of food intake resulted in lower body weights, whereas the higher body weights in OVX rats were not accompanied by significantly higher food intake. The higher body weights in OVX rats may be related to the elevation of fat tissue weights. Similar differences in food intake and body weight on ORX and OVX rats were observed in diabetic models, such as OLETF rats and SDT rats [2,5]. Basal insulin levels in OVX rats were occasionally higher, and lower insulin sensitivity may be caused by

ovariectomy. TG levels in ORX rats decreased during the experimental period, and the changes are considered to be related with the lower food intake. In ORX OLETF rats, TG levels were significantly lower, but TC levels did not show a significant difference [2]. The reason is unknown, but it is considered that effects on cholesterol metabolism in orchidectomy may be different between normal and diabetic rats. In OVX OLETF rats, both TG and TC levels were significantly higher [2].

In OGTT, the insulin level at 30 min after glucose loading was elevated in OVX rats, but the glucose level at 120 min after glucose loading did not show the decrease. In OVX rats, the insulin sensitivity is considered to be decreased. Also, in castrated female diabetic rat, the glucose tolerance was impaired [2]. In castrated male diabetic rats, the glucose tolerance was improved, but a similar change was not observed in normal ORX rats in this study.



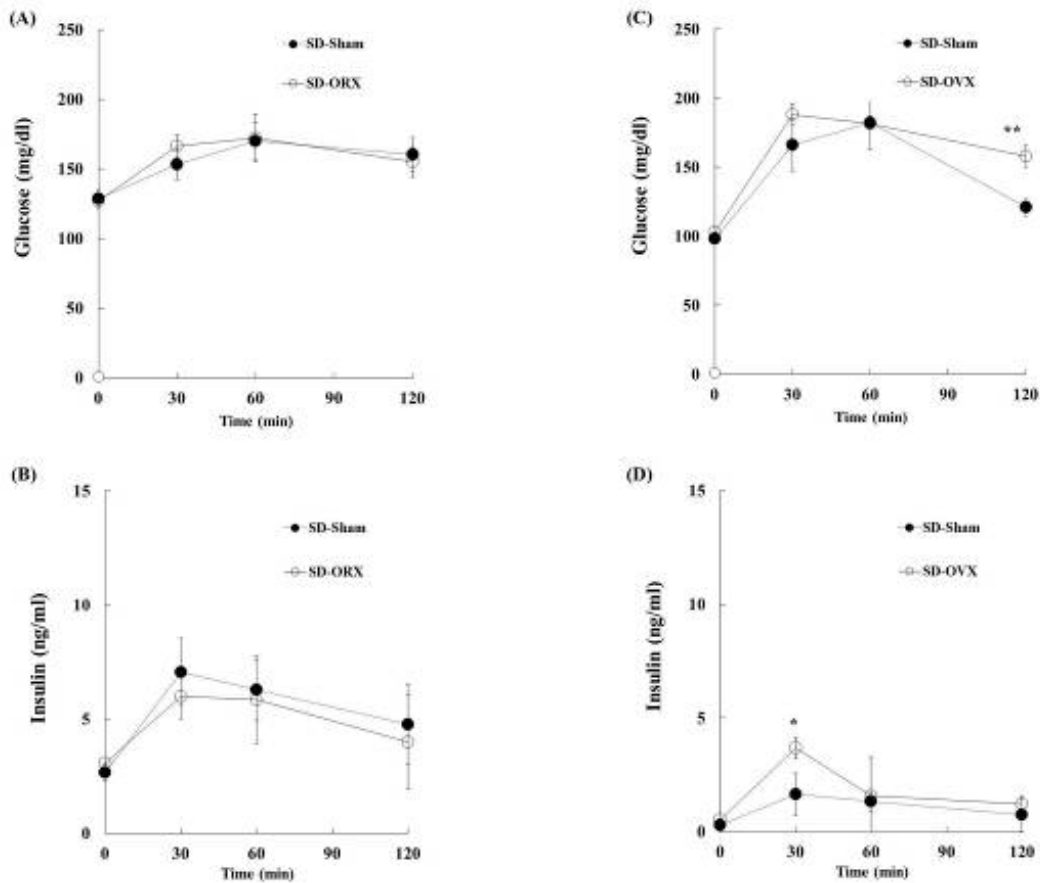
**Fig. 1. Changes in food intake (A), body weight (B), and serum glucose (C), insulin (D), triglyceride (TG) (E), and total cholesterol (TC) (F) levels in Sham rats and ORX rats. Data represent means  $\pm$  standard deviation (n=6). \*p<0.05, \*\* p<0.01; significantly different from the Sham rat (t-test)**



**Fig. 2. Changes in food intake (A), body weight (B), and serum glucose (C), insulin (D), triglyceride (TG) (E), and total cholesterol (TC) (F) levels in Sham rats and OVX rats. Data represent means  $\pm$  standard deviation (n=6). \*p<0.05, \*\* p<0.01; significantly different from the Sham rat (t-test)**

In both ORX and OVX rats, the fat tissue weights increased. Sex hormones are considered to play a role in the structure and function of adipose tissue [17,18]. OVX mice gained body fat, whereas exposure to estrogen decreased fat mass [19]. It is considered that the endogenous estrogens decrease body fat. In estrogen-deficiency with ovariectomy, induces an increase of fat tissue weight. Moreover, an inhibitory effect

of testosterone on adipocyte differentiation has been reported [20]. In testosterone-deficiency with orchidectomy, also induces an increase of fat tissue weight, but, in this study, the visceral fat tissue weight did not change. The exact functions of sex hormones in adipocyte differentiation remain poorly understood and are often controversial.



**Fig. 3.** Changes of serum glucose and insulin levels after glucose loading in ORX rats (A), (B) and OVX rats (C), (D) at 16 weeks of age. Blood samples were taken via the tail vein before, and 30, 60, and 120 min after glucose loading. Data represent means  $\pm$  standard deviation (n=6). \*p<0.05, \*\* p<0.01; significantly different from the Sham rat (t-test)

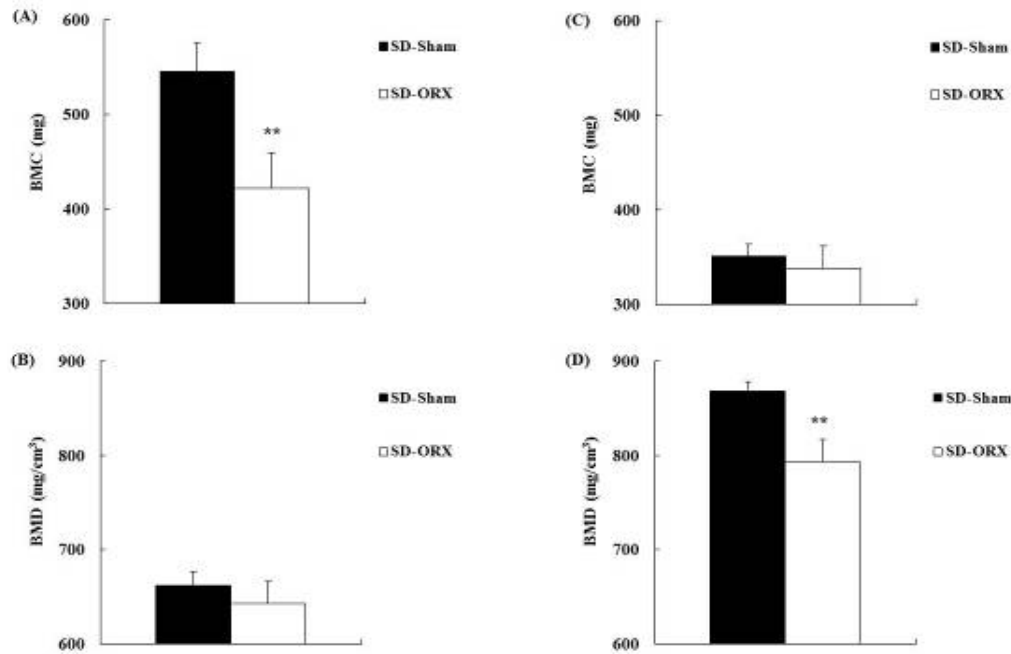
**Table 1.** Changes of fat tissue weights at 16 weeks of age in Gonadectomized rats

	Sham	Gonadectomy
<b>Male SD rat</b>		
Visceral fat (g)	39.5 $\pm$ 7.5	38.7 $\pm$ 9.6
Subcutaneous fat (g)	16.0 $\pm$ 5.9	29.7 $\pm$ 7.2**
Total fat (g)	55.4 $\pm$ 13.0	68.5 $\pm$ 16.5
Lean body mass (g)	237.7 $\pm$ 13.5	201.9 $\pm$ 9.2**
<b>Female SD rat</b>		
Visceral fat (g)	19.9 $\pm$ 5.7	49.5 $\pm$ 13.2**
Subcutaneous fat (g)	6.1 $\pm$ 3.0	25.4 $\pm$ 9.3**
Total fat (g)	26.1 $\pm$ 8.2	74.9 $\pm$ 20.1**
Lean body mass (g)	135.5 $\pm$ 5.5	165.0 $\pm$ 15.8**

Data represents means  $\pm$  standard deviation (n=6). \*\*p<0.01; significantly different from the Sham-SD rats (t-test).

Both orchidectomy and ovariectomy affected bone metabolism, showing lower BMC in ORX rats and lower BMD in OVX rats. It is reported that osteoporosis occurs in postmenopausal women due to the reduction or loss of sex

hormone [21-23]. Also, it is considered that the loss or reduced levels of sex hormones, such as estrogen and testosterone, cause osteoporosis in men. Sex hormones are considered to be closely related with the bone metabolism.



**Fig. 4. Bone mineral density (BMD) and bone mineral content (BMC) of the right tibia in ORX rats (A), (B), and OVX rats (C), (D) at 40 weeks of age. Data represent means  $\pm$  standard deviation (n=6). \*\* p<0.01; significantly different from the Sham rat (t-test)**

## 5. CONCLUSION

Both orchidectomy and ovariectomy in rats affect glucose/lipid and bone metabolism, and especially, the glucose metabolism was deteriorated in OVX rats. Both male and female sex hormones play a key role in metabolic disease, such as diabetes, hyperlipidemia and osteoporosis.

## CONSENT

It is not applicable.

## ETHICAL APPROVAL

All animal protocols used under strict compliance with our own Laboratory Guidelines for Animal Experimentation which is based on the ethical standards laid down in the 1964 Declaration of Helsinki. Gonadectomy is considered useful method for evaluating influence of sex-steroid hormone and is conducted generally in animal experiments.

## FINANCIAL SUPPORT

We have no disclosure and financial support.

## RESEARCH LIMITATIONS

Since animals were subjected to gonadectomy and different from the actual patient's circumstances with sexual dysfunction, our research had several limitations.

## COMPETING INTERESTS

The authors have no conflict of interest to disclose with respect to this research.

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