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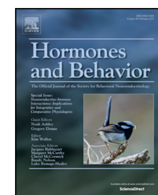

The effects of chronic testosterone administration on body weight, food intake, and adipose tissue are changed by estrogen treatment in female rats

Takeshi Iwasa *, Toshiya Matsuzaki, Kiyohito Yano, Rie Yanagihara, Altankhuu Tungalagsuvd, Munkhsaikhan Munkhzaya, Yiliyasi Mayila, Akira Kuwahara, Minoru Irahara

Department of Obstetrics and Gynecology, Institute of Biomedical Sciences, Tokushima University Graduate School, 3-18-15 Kuramoto-Cho, Tokushima 770-8503, Japan

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ABSTRACT

In females, estrogens play pivotal roles in preventing excess body weight (BW) gain. On the other hand, the roles of androgens in female BW, appetite, and energy metabolism have not been fully examined. We hypothesized that androgens' effects on food intake (FI) and BW regulation change according to the estrogens' levels. To evaluate this hypothesis, the effects of chronic testosterone administration in ovariectomized (OVX) female rats with or without estradiol supplementation were examined in this study. Chronic testosterone administration decreased BW, FI, white adipose tissue (WAT) weight, and adipocyte size in OVX rats, whereas it increased BW, WAT weight, and adipocyte size in OVX with estradiol-administered rats. In addition, chronic testosterone administration increased hypothalamic CYP19a1 mRNA levels in OVX rats, whereas it did not alter CYP19a1 mRNA levels in OVX with estradiol-administered rats, indicating that conversion of testosterone to estrogens in the hypothalamus may be activated in testosterone-administered OVX rats. Furthermore, chronic testosterone administration decreased hypothalamic TNF-α mRNA levels in OVX rats, whereas it increased hypothalamic IL-1β mRNA levels in OVX with estradiol-administered rats. On the other hand, IL-1β and TNF-α mRNA levels in visceral and subcutaneous WAT and liver were not changed by chronic testosterone administration in both groups. These data indicate that the effects of chronic testosterone administration on BW, FI, WAT weight, and adipocyte size were changed by estradiol treatment in female rats. Testosterone has facilitative effects on BW gain, FI, and adiposity under the estradiol-supplemented condition, whereas it has inhibitory effects in the non-supplemented condition. Differences in the responses of hypothalamic factors, such as aromatase and inflammatory cytokines, to testosterone might underlie these opposite effects.

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1. Introduction

Energy balance and reproductive function are closely linked in most species. The sex hormones, estrogens and androgens, are particularly related in the regulation of food intake (FI), energy metabolism, and body weight (BW) in mammals and humans (Hirschberg, 2012). Ovariectomy increases FI and BW in female animals, and these effects are prevented by estradiol replacement, indicating that estrogens play pivotal roles in preventing excess BW gain in females (Asarian and Geary, 2013; Blaustein and Wade, 1976; Hirschberg, 2012). These estrogens' effects on BW are primarily mediated by estrogen receptor-α (ER-α) in the hypothalamic area and brain stem. Estradiol injection into the hypothalamic paraventricular nucleus decreases BW and FI in ovariectomized rats (Palmer and Gray, 1986; Butera and Beikirch, 1989). Mutations of ER-α genes induce obesity in humans and mice, and deletion of ER-α blocks the effects of estradiol on BW (Heine et al., 2000; Okura et al., 2003; Gao et al., 2007).

On the other hand, the roles of androgens in female FI, energy metabolism, and BW regulation have not been fully examined, although some studies have shown that androgens increase FI and fat weight in females (Hirschberg et al., 2004; Naessen et al., 2007; Lim et al., 2009; Hirschberg, 2012) and increase the risk of visceral obesity in women and experimental animals (Evans et al., 1988; Dieudonne et al., 1998; Ibanez et al., 2003; Gambineri et al., 2004; Nohara et al., 2014). Androgen-administered animals have been used as a model of polycystic ovary syndrome (PCOS), which is one of the common causes of anovulation and metabolic abnormalities in women of reproductive age, because these animals show disruption of ovulation, as well as increased BW and fat mass (Walters et al., 2012). However, the physiological mechanisms by which androgens disturb FI and BW regulation in females at this age have not been fully evaluated. Interestingly, it has been reported that the effects of androgens on energy metabolism and BW in males are different from those in females of reproductive age.

* Corresponding author.
E-mail address: iwasa.takeshi@tokushima-u.ac.jp (T. Iwasa).

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Deficiency of testosterone, one of the most important androgens, increases the amount of visceral adipose tissue and insulin resistance in males, and it increases the risk of diabetes and metabolic syndrome (Navarro et al., 2015). These data suggest that testosterone plays pivotal roles in preventing excess BW gain and obesity in males.

In a previous study, we evaluated the effects of chronic testosterone administration on BW, FI, and body composition in female rats of different reproductive ages and gonadal status, i.e., pre-pubertal age, ovarian-intact reproductive age, and ovariectomized reproductive age (Iwasa et al., 2016). We found that BW gain and FI were increased by chronic testosterone administration in pre-pubertal and ovarian-intact reproductive age rats, but not in ovariectomized rats. In this study, all ovariectomized rats showed undetectable levels of serum estradiol, whereas 25% of pre-pubertal rats showed detectable levels of serum estradiol (unpublished data). In addition, because the ovaries were not removed in pre-pubertal rats, other kinds of estrogen may be produced and secreted into the plasma. Thus, it is speculated that pre-pubertal rats may have higher estrogen levels than ovariectomized rats. Therefore, we hypothesized that testosterone's effects on FI and BW regulation may be changed by the estrogen level.

To further investigate this hypothesis, we evaluated the effects of chronic administration of testosterone in ovariectomized female rats with or without estradiol supplementation in this study. Because estradiol could be stably supplemented, the interaction between testosterone and estradiol may be more precisely evaluated in this model. The effects on BW, FI, body composition, histological findings of fat and liver were evaluated. In addition, the peripheral and hypothalamic orexigenic factor, neuropeptide Y (NPY), and anorexigenic factors, leptin, proopiomelanocortin (POMC), and leptin receptor (OBRb), were evaluated, because it has been reported that sex steroids affect serum and hypothalamic levels and actions of these factors (Kimura et al., 2002; Anukulkitch et al., 2007; Santollo and Eckel, 2008; Li et al., 2016). Hypothalamic estrogen and androgen receptors and aromatase gene expressions were also measured, because these factors also play pivotal roles in the regulation of BW and FI (Jones et al., 2000; Wu et al., 2009; Hirschberg, 2012). Furthermore, gene expressions of peripheral and hypothalamic pro-inflammatory cytokines were also measured, because these factors were involved in the disruption of the regulation of BW and FI in the obesity models, including the model induced by ovariectomy (Carvalheira et al., 2003; De Souza et al., 2005; Rogers et al., 2009; Thaler et al., 2013).

2. Materials and methods

2.1. Animals

Eight-week-old Wistar female adult rats (200–230 g) were purchased from Charles River Laboratories Japan, Inc. (Kanagawa, Japan) and housed in a room under controlled light (12 h light, 12 h darkness; lights turned on at 0800 and turned off at 2000) and temperature (24 °C) with free access to food and water. In total, 32 rats were used in this study. All animal experiments were conducted in accordance with the ethical standards of the institutional Animal Care and Use Committee of the University of Tokushima. Ovariectomy was carried out under sodium pentobarbital (60–80 mg/kg, intraperitoneal, i.p.), and tube implantations and decapitation were carried out under sevoflurane anesthesia. At 9 weeks of age, rats were ovariectomized bilaterally and individually housed after surgery. Water containing ibuprofen (0.1 mg/mL) was provided during the 3 days after surgery to reduce the rats' postoperative pain.

2.2. Effects of chronic testosterone administration in ovariectomized (OVX) rats not administered estradiol

Four weeks after ovariectomy (13 weeks of age), rats were randomly divided into either the testosterone-administered (Testosterone) or the without testosterone-administered (Control) group ($n = 8$ per group). In the Testosterone group, rats were implanted with a silastic tube filled with crystalline testosterone (inner diameter 3 mm, outer diameter 5 mm, length of the filling part 30 mm) (As One Co., Ltd., Tokyo, Japan) (De Vries et al., 1994). In the Control group, rats were implanted with an empty tube. At 16 days after implantation, the rats were sacrificed by decapitation after measurement of BW and cumulative FI. The fixed amount of normal diet (type MF; Oriental Yeast Co. Ltd., Tokyo, Japan) (359 kcal/100 g) was placed in the food space of the wire mesh top, and the remaining food weight was measured 16 days after implantation. Wood chip bedding was changed 8 days after implantation, and broken food in the bedding was collected at this time point and 16 days after implantation, and their weights were added to the remaining food weight. Brain, blood, visceral fat (parametrial, perirenal, and mesenteric depots, subcutaneous fat (inguinal depot), liver, and uterus were collected. Weights of visceral fat, subcutaneous fat, and uterus were measured immediately after removal, and tissue samples (around 300–400-mm3) of visceral (parametrial) and subcutaneous fat were dissected. Similarly, the same sizes of tissue samples were dissected from the liver. Serum was separated by centrifugation and stored at −20 °C, and tissue samples were stored at −80 °C. Other tissue samples of visceral and subcutaneous fat and liver were fixed in 4% paraformaldehyde.

2.3. Effects of chronic testosterone administration in ovariectomized with estradiol-administered (OVX + E) rats

Four weeks after ovariectomy (13 weeks of age), all rats were implanted with a silastic tube filled with crystalline estradiol (length of the filling part, 3 mm) (Le et al., 2014). At the same time, rats were divided into either the Testosterone or the Control group, as described above ($n = 8$ per group). At 16 days after implantation, the rats were sacrificed by decapitation after measurement of BW and cumulative FI. Tissue weight was measured, and samples were collected and stored or fixed, as described above.

2.4. Hormone assay

Serum estradiol and testosterone levels were measured by a commercial laboratory (SRL, Tokyo, Japan) using an electrochemiluminescence immunoassay (ECLIA; Roche Diagnostics GmbH, Mannheim, Germany). Serum leptin levels were measured using radioimmunoassay kits (multi-species leptin RIA kit, Linco Research Inc., MO, USA). The sensitivity of the assay was 1.0 ng/mL, and its inter- and intra-assay coefficients of variation were 3.2% and 7.8%, respectively.

2.5. Quantitative real-time polymerase chain reaction

Whole hypothalamic explants were dissected from the frozen brains, as described previously (Iwasa et al., 2016). Briefly, the brain sections were dissected out via an anterior coronal cut at the posterior border of the mammillary bodies, parasagittal cuts along the hypothalamic fissures, and a dorsal cut 2.5 mm from the ventral surface. Total RNA was isolated from hypothalamic explants and visceral fat using a TRIzol® reagent kit (Invitrogen Co., Carlsbad, CA, USA) and an RNeasy® mini kit (Qiagen Gmbh, Hilden, Germany). Then, cDNA was synthesized with oligo (deoxythymidine) primers at 50 °C using the SuperScript III first-strand synthesis system for the real-time polymerase chain reaction (PCR; Invitrogen Co.). The PCR analysis was performed using the StepOnePlusTM real-time PCR system (PE Applied Biosystems, Foster City, CA, USA) and FAST SYBR® green. The ER-α androgen receptor (AR), CYP19a1, NPY, POMC, OBRb, interleukin-1β (IL-1β), and tumor necrosis factor-α (TNF-α) mRNA levels in the hypothalamus, leptin, IL-1β, and TNF-α mRNA levels in visceral and subcutaneous WAT, and IL-1β and TNF-α mRNA levels in the liver were measured. The mRNA

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