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The effects of chronic testosterone administration on body weight, food intake, and fat weight were age-dependent

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ABSTRACT

Previously, we showed that chronic testosterone administration increased body weight (BW) and food intake (FI), but did not alter fat weight, in young female rats. To examine our hypothesis that the effects of androgens on BW, FI and body composition might be age-dependent, the effects of chronic testosterone administration were evaluated in rats of different ages; i.e., young and middle-aged rats. Although chronic testosterone administration increased BW gain, FI, and feed efficiency in both young and middle-aged rats, it increased visceral fat weight in middle-aged rats, but not in young rats. Therefore, it is possible that testosterone promotes the conversion of energy to adipose tissue and exacerbates fat accumulation in older individuals. In addition, although the administration of testosterone increased the serum leptin level, it did not alter hypothalamic neuropeptide Y mRNA expression in middle-aged rats. On the contrary, the administration of testosterone did not affect the serum leptin levels of young rats. Thus, testosterone might induce hypothalamic leptin resistance, which could lead to fat accumulation in older individuals. Testosterone might disrupt the mechanisms that protect against adiposity and hyperphagia and represent a risk factor for excessive body weight and obesity, especially in older females.

1. Introduction

Energy balance and reproduction are closely linked in most species. The sex hormones, estrogen and androgens, are involved in the regulation of food intake (FI), body weight (BW), body composition, and energy metabolism in mammals and humans [1]. In females, estrogen plays pivotal roles in preventing excess BW gain and adiposity by suppressing FI and increasing energy metabolism. For example, ovariectomy increases FI and BW in female mammals, and these effects can be prevented by estradiol (E2) replacement [2]. Some of estrogen's effects are mediated by estrogen receptor-α (ER-α) in the hypothalamus and brainstem, and the injection of E2 into the hypothalamic paraventricular nucleus decreased BW and FI in ovariectomized (OVX) rats [3,4]. However, as most of these studies involved young animals it remains unclear whether older animals continue to be responsive to the nutritional and metabolic effects of estrogen. Recently, Santollo et al. have shown that young and middle-aged female rats exhibit similar sensitivity to the anorexigenic effects of E2, suggesting that the response to estrogen is maintained in older individuals [5].

On the other hand, the roles of androgens in female FI and BW regulation have not been fully examined, although some studies have shown that androgens increase FI and BW in females [1,6–8] and raise the risk of visceral obesity in women and experimental animals [9–13]. In our previous study, we evaluated the effects of chronic testosterone administration on BW, FI, and body composition in young female rats [14]. As a result, we found that BW gain and FI were increased by chronic testosterone administration, but the weights of visceral and subcutaneous fat were not changed. Interestingly, BW gain, FI, and fat weight were not altered by chronic testosterone administration in OVX rats in the latter study. Therefore, these findings indicate that, similar to the actions of estrogen, the effects of androgens on BW, FI, and body composition might differ in an age-dependent manner.

To further investigate this hypothesis, we evaluated the effects of the chronic administration of testosterone in different age groups; i.e., young and middle-aged rats, in the present study. The effects of chronic testosterone administration on BW, FI, and visceral and subcutaneous fat accumulation were assessed. In addition, the levels of neuropeptide Y (NPY) and prepro-orexin (pp-orexin), which are peripheral and hypothalamic orexigenic factors, respectively, and leptin, proopiomelanocortin (POMC), and the leptin receptor (OBRb), which are anorexigenic factors, were also investigated because it has been reported that sex steroids affect the serum and hypothalamic levels and actions of these factors [15–18]. The hypothalamic mRNA expression level of the androgen receptor (AR) was also measured because the AR plays

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pivotal roles in the regulation of BW and FI [1].

2. Experimental

2.1. Animals

Eighteen young (10 weeks of age) and 16 middle-aged (12 months of age) Sprague-Dawley female rats (Charles River, Kanagawa, Japan) were housed in a room under controlled lighting (12 h light, 12 h darkness; lights turned on at 0800 and turned off at 2000) and temperature (24 °C) conditions with free access to food and water. All animal experiments were conducted in accordance with the ethical standards of the institutional animal care and use committee of the University of Tokushima. Rats were randomly assigned to either the testosterone-administered (T) or the no testosterone (control) group (n = 8 or 9 per group). In the testosterone group, a silastic tube filled with crystalline testosterone was implanted into each rat (inner diameter: 3 mm, outer diameter: 5 mm, length of the filled part: 30 mm) (As One Co., Ltd., Tokyo, Japan) (De Vries et al., 1994). In the control group, an empty tube was implanted into each rat. BW and cumulative FI were assessed every week, and the rats were sacrificed by decapitation at 4 weeks (wk) after the implantation procedure. The implantation and decapitation were carried out under sevoflurane anesthesia. The brain, blood, visceral fat (parametrial, perirenal, and mesenteric deposits), and subcutaneous fat (inguinal deposits) were collected. The weights of visceral and subcutaneous fat were assessed immediately after removal, and samples (around 300–400 mm^3) of visceral (parametrial) fat were dissected. The serum was separated by centrifugation and stored at −20 °C, and the tissue samples were stored at −80 °C.

2.2. Hormone assay

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. Serum estradiol (E2) levels were measured by a commercial laboratory (SRL, Tokyo, Japan.) using a chemiluminescence immunoassay. Detection limit of E2 kit is 10 pg/mL and coefficient of variations is smaller than 7%.

2.3. Quantitative real-time polymerase chain reaction

Whole hypothalamic explants were dissected from the frozen brains, as described previously [14]. 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 the 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 StepOnePlus™ real-time PCR system (PE Applied Biosystems, Foster City, CA, USA) and FAST SYBR® green. The hypothalamic mRNA levels of OBRb, the AR, NPY, POMC, and pp-orexin and the leptin mRNA level in visceral fat were measured. The mRNA expression level of each factor was normalized to that of GAPDH or the 18S rRNA level. Dissociation curve analysis was also performed for each gene at the end of the PCR. Each amplicon generated a single peak. The relevant primer sequences, product sizes, and annealing temperatures are shown in Table 1. The PCR conditions were as follows: initial denaturation and enzyme activation were carried out at 95 °C for 20 s, followed by 45 cycles of denaturation at 95 °C for 3 s, and annealing and extension for 30 s.

2.4. Statistical analysis

All results are presented as mean ± SEM values. Two-way repeated or factorial ANOVA was used to compare the effects of testosterone between young and middle-aged rats. Tukey-Kramer *post-hoc* analysis was used to determine individual group differences following the detection of significant main or ANOVA effects ($P < 0.05$).

3. Results

At the beginning of the study, the mean BW of the young groups were significantly lower than those of the middle-aged groups (young-control: 222.7 ± 3.1 g, young-T: 221.3 ± 3.3 g, middle-control: 423.7 ± 12.2 g, middle-T: 426.3 ± 8.8 g). At 4 wk after the implantation procedure, the serum E2 levels of the examined groups did not differ (young-control: 32.4 ± 3.2 pg/mL, young-T: 32.9 ± 3.6 g, middle-control: 42.4 ± 6.2 g, middle-T: 28.3 ± 6.8 g).

Repeated-measures ANOVA revealed a significant interactive effect of treatment × time on the change in BW ($F = 69.3$, $P < 0.01$) (Fig. 1A). Both T ($F = 58.0$, $P < 0.01$) and age ($F = 4.70$, $P < 0.01$) had significant effects on the BW change seen at 4 wk after the implantation procedure (Fig. 1B). *Post-hoc* analysis showed that the BW changes observed at 4 wk after the implantation procedure in the young-T and middle-T groups were significantly greater than those seen in the young-control and middle-control groups, respectively (Fig. 1B). It also showed that the BW changes observed at 4 wk after the implantation procedure in the young-control and young-T groups were significantly greater than those seen in the middle-control and middle-T groups, respectively. Repeated-measures ANOVA revealed a significant interactive effect of treatment × time on cumulative FI ($F = 163.3$, $P < 0.01$) (Fig. 1C). Both T ($F = 31.0$, $P < 0.01$) and age ($F = 28.0$, $P < 0.01$) had significant effects on cumulative FI at 4 wk after the implantation procedure (Fig. 1D). *Post-hoc* analysis showed that cumulative FI at 4 wk after the implantation procedure was significantly greater in the young-T and middle-T groups than in the young-control and middle-control groups, respectively (Fig. 1D). It also showed that the young-control and young-T groups exhibited significantly greater cumulative FI at 4 wk after the implantation procedure than the middle-control and middle-T groups, respectively.

Both T ($F = 171.7$, $P < 0.01$) and age ($F = 7.16$, $P < 0.01$) had significant effects on feed efficiency (FE). *Post-hoc* analysis showed that FE was significantly higher in the young-T and middle-T groups than in the young-control and middle-control groups, respectively (Fig. 2A). It also demonstrated that FE was significantly higher in the young-control and young-T groups than in the middle-control and middle-T groups, respectively. Both T (visceral fat: $F = 5.40$, $P = 0.03$; total fat: $F = 6.13$, $P = 0.02$) and age (visceral fat: $F = 5.82$, $P < 0.01$; total fat: $F = 7.95$, $P < 0.01$) had significant effects on relative visceral fat weight and total fat weight. *Post-hoc* analysis showed that the relative visceral fat weight and total fat weight of the middle-T group were significantly heavier than those of the middle-control group (Fig. 2B and D). It also demonstrated that the relative visceral fat weight and total fat weight of the young-control and young-T groups were significantly lighter than those of the middle-control and middle-T groups, respectively.

Both T ($F = 6.13$, $P < 0.01$) and age ($F = 7.95$, $P < 0.01$) had significant effects on relative lean body mass. *Post-hoc* analysis showed that the relative lean body mass of the middle-T group was significantly lighter than that of the middle-control group (Fig. 2E). It also demonstrated that the relative lean body mass values of the young-control and young-T groups were significantly heavier than those of the middle-control and middle-T groups, respectively.

Both T ($F = 11.8$, $P < 0.01$) and age ($F = 7.64$, $P < 0.01$) had significant effects on the serum leptin level. *Post-hoc* analysis showed that the serum leptin levels of the middle-T group were significantly higher than those of the middle-control group (Fig. 3A). It also