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Effects of Testosterone on White Adipose and Liver Tissues in Male Castrated C57BL/6J Mice

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Obesity is defined as increased mass of adipose tissue, conferring a higher risk of cardiovascular and metabolic disorders such as diabetes, hyperlipidemia, and coronary heart disease. To get a better understanding of the role of a male sex hormone testosterone on obesity, we thus measured the effects of testosterone on white adipose tissue (WAT) mass, adipocyte histology and hepatic lipid accumulation in male castrated (CAST) C57BL/6J mice. Compared to male CAST control mice, testosterone-treated mice had the decreased WAT mass and the increased the number of adipocytes. Especially, histological data showed that the adipocyte size was reduced in a dose-dependent manner and was most effective at dose 150 μg per mouse for testosterone. In addition, the administration of testosterone resulted in the inhibition of hepatic lipid accumulation compared with control mice. Our results suggest that testosterone regulates adipocytes development and hepatic lipid metabolism, resulting in the prevention of obesity in male CAST mice.

Key Words: Testosterone, White adipose tissue, CAST, Male mice

INTRODUCTION

Adipose tissue is composed of adipocytes, which store energy in the form of triglycerides and release it as free fatty acids (for reviews, see Spiegelman et al., 1996; Fajas et al., 1998). Together with muscle, adipose tissue is the major regulator of energy balance of the body. Excessive accumulation of adipose tissue leads to obesity, whereas its absence is associated with lipodystrophic syndromes.

There are reports that development of adipocyte is related to sex steroid hormone. Regional fat distribution differs between men and women and is also modified during various physiopathological situations (pregnancy, postmenopause, and transsexualism), suggesting a potential role for sex steroid hormones in determining the site specificities of fat deposition (Rebuffe-Scrive et al., 1985; Bjorntrop 1991; Elbers et al., 1997). There is a tendency to increase adipose tissue mass with a decrease in sex steroid hormones, as occurs with ageing or gonadectomy (Bjorntorp, 1996) whereas testosterone replacement has been shown to reduce fat tissue mass and to increase muscle size and strength in hypogonadal and eugonadal men (Bhasin et al., 1997; Snyder et al., 2000; Alexandersen and Christiansen, 2004). In 3T3-L1 and 3T3-F442A preadipocyte cell lines and pig preadipocytes, high concentrations of dehydroepiandrosterone (DHEA) and other androgen-related steroids were shown to block the adipose conversion process, as followed by measurement of glycrol-3-phosphate dehydrogenase (GPDH) activity, a late marker of differentiation (Gordon et al., 1986; Mclntosh et al., 1998; Lea-Currie et al., 1999).

It seems now clear that fat distribution and accumulation are under the control of male sex steroid hormones. But histological analysis of the changes in adipose tissue and liver tissues by testosterone in CAST mice is still poorly understood.

Therefore, the objective of the present study was to determine whether testosterone induces morphological changes of WAT and liver. Thus, we examined WAT mass, adipocyte and liver histology in male CAST mice. Here we report that testosterone affects WAT weight, adipocyte size and number, as well as hepatic lipid accumulation, suggesting that testosterone may regulate obesity of male CAST

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C57BL/6J mice.

MATERIALS AND METHODS

1. Animal treatments

For all experiments, eight-week-old male C57BL/6J mice were housed and bred at the Korea Research Institute of Bioscience and Biotechnology under pathogen-free conditions with a standard 12-h light/dark cycle. Prior to the administration of testosterone, mice were fed standard rodent chow and water *ad libitum*. Mice were CAST and each randomly divided into 4 groups. Fore groups received once daily intraperitoneal injections of testosterone (Sigma) at indicated doses for 8 days. Testosterone dissolved with corn oil and chow diet-fed control mice were administrated corn oil. All the animals were sacrificed by cervical dislocation, tissues were harvested, weighed, snap frozen in liquid nitrogen and stored at -80℃ until use.

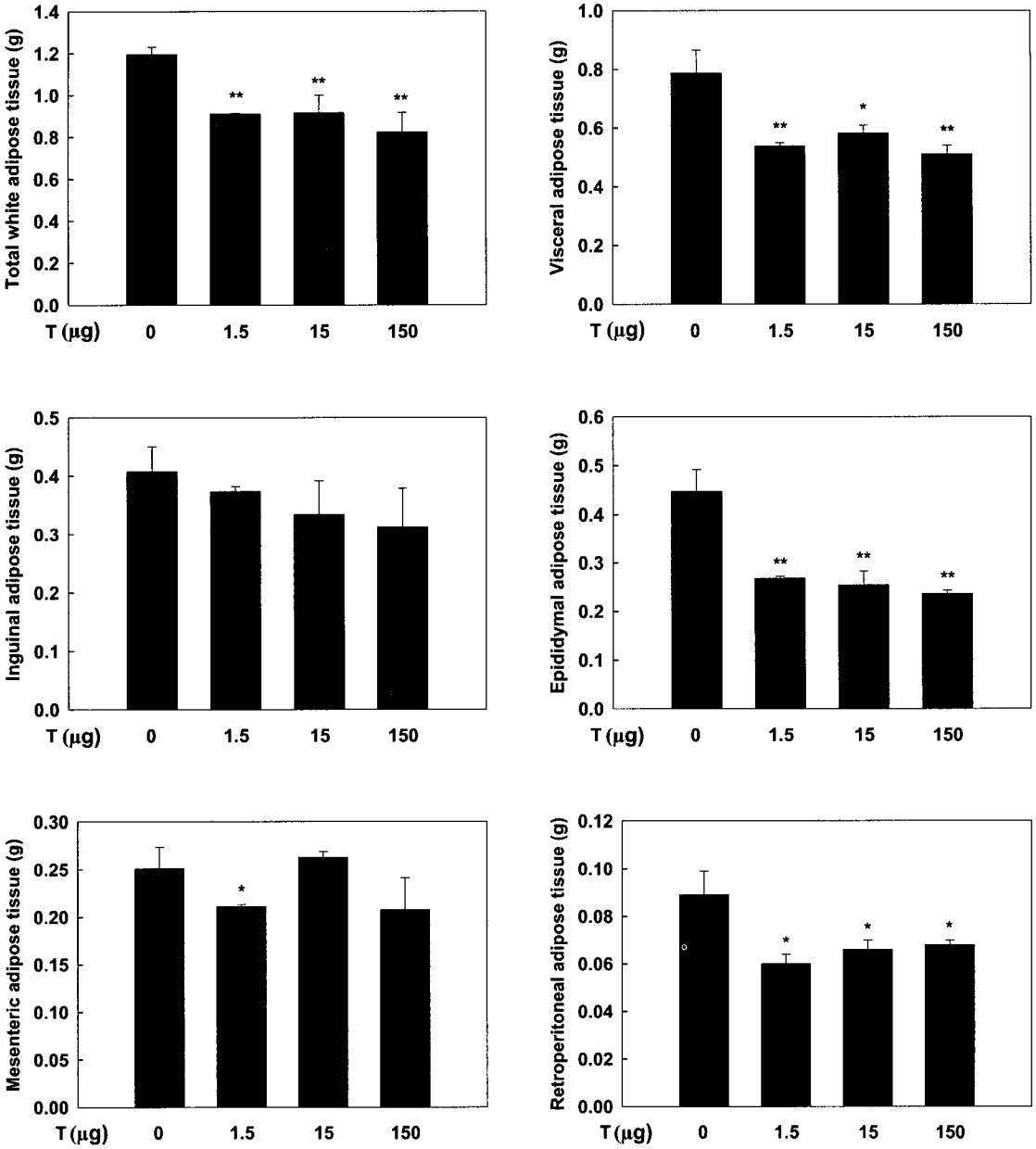

Fig. 1. Effects of testosterone on adipose tissue mass in male CAST mice. Mice (n=3/group) received intraperitoneal injections of testosterone at the indicated doses for 8 days. Chow diet-fed control mice were administrated corn oil. All values are expressed as mean ± SD. *, Significantly different *versus* control group ($P<0.05$). **, Significantly different *versus* control group ($P<0.005$).

2. Histologic analysis and morphometry

Adipose and liver tissues were fixed in 10% phosphate-buffered formalin for 1 day and processed in a routine manner for paraffin section. Sections (5 μm) were stained with hematoxylin and eosin for microscopic examination. For the quantitation of number and size of adipocytes, the sectional areas of adipose tissues in the hematoxylin and eosin-stained preparations were analyzed with image analysis system (Image pro-plus, MD, USA).

3. Statistics

Unless otherwise noted, all values are expressed as mean ± standard deviation (SD). All data were analyzed by ANOVA for statistically significant differences between each group.

RESULTS

To determine the effects of pharmacological doses of testosterone on adipose tissue mass, male CAST C57BL/6J mice were treated once daily with testosterone for 8 days in a dose-dependent experiment. Compared with chow-fed control mice, mice treated with testosterone for 8 days significantly reduced total WAT mass at all doses (P<0.05 and P<0.005) (Fig. 1). The inhibitory effects of testosterone on WAT mass showed the site specificities. Compared with control mice, testosterone treatment significantly decreased visceral adipose tissue mass at all doses (P<0.05 and P< 0.005), but not inguinal adipose tissue mass. Among visceral adipose tissues, this effect was most effective in epididymal adipose tissue (47.2%).

We determined whether testosterone regulates adipocyte size and number in epididymal adipose tissue of male CAST mice (Figs. 2 and 3). Compared with chow-fed control mice, mice treated with testosterone for 8 days significantly reduced adipocyte size and increased adipocyte number in a dose-dependent manner (P<0.01 and P<0.001), and a maximal effect was achieved at dose of 150 μg per mouse for testosterone, a 48.3% reduction of adipocyte size and a

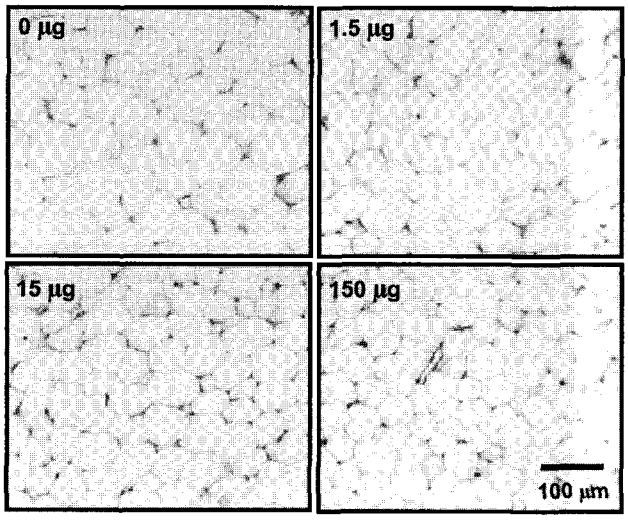

Fig. 2. Effects of testosterone on morphological changes of adipose tissue in male CAST mice. Mice (n=3/group) received intraperitoneal injections of testosterone at the indicated doses for 8 days. Chow diet-fed control mice were administrated corn oil. The epididymal adipose tissues stained with hematoxylin and eosin (original magnification ×200).

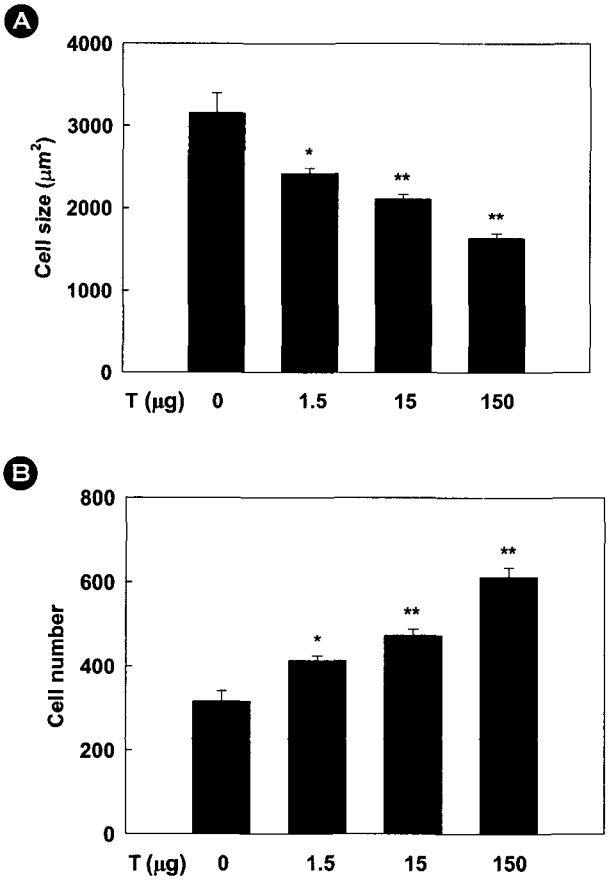

Fig. 3. Effects of testosterone on size and number of epididymal adipocytes in male CAST mice. Size of adipocytes and their numbers in a fixed area (1,000,000 μm²) were quantified by an image analysis system. Mice (n=3/group) received intraperitoneal injections of testosterone at the indicated doses for 8 days. Chow diet-fed control mice were administrated corn oil. Size **(A)** and number **(B)** of adipocytes were measured and all values are expressed as mean ± SD. *, Significantly different *versus* control group (P<0.01). **, Significantly different *versus* control group (P<0.001).

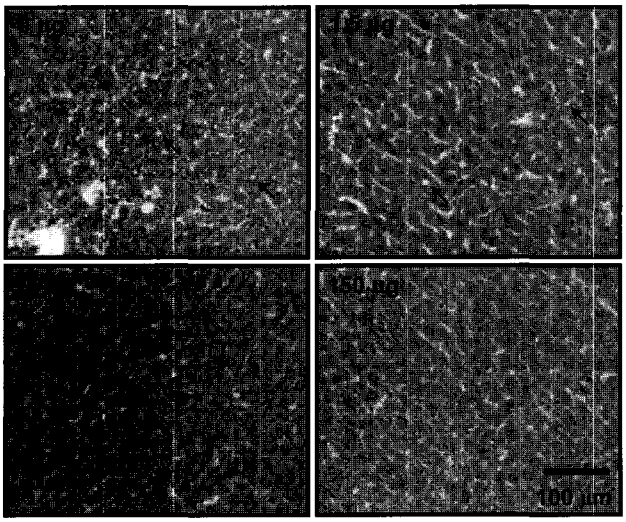

Fig. 4. Effects of testosterone on morphological changes of hepatic lipid accumulation in male CAST mice. Mice (n=3/group) received intraperitoneal injections of testosterone at the indicated doses for 8 days. Chow diet-fed control mice were administrated corn oil. The livers were stained with hematoxylin and eosin (original magnification ×200). Arrows indicate the fatty changes in hepatocytes.

92.8% induction of adipocyte number.

We determined whether testosterone regulates hepatic lipid accumulation (Fig. 4). The hepatic accumulation of lipids was inhibited in testosterone-treated mice compared with chow-fed control mice. Testosterone prevented almost completely hepatic lipid accumulation at dose of 150 μg per mouse for testosterone.

DISCUSSION

Obesity, which is an important risk factor for cardiovascular diseases, is characterized by excessive adipose tissue deposition in abdominal and visceral regions (Bjorntrop, 1991). The clinical evidence strongly suggests a major role for sex steroid hormones in the regulation of adipose tissue distribution (Bjorntorp, 1996; Tchernof et al., 2000). This study was therefore undertaken to determine whether testosterone modulates adipose tissue development and hepatic lipid accumulation in male CAST C57BL/6J mice. Our results demonstrate that testosterone-treated male CAST mice decreased white adipose tissue mass, size of adipocyte, and hepatic lipid accumulation compared with control mice, and these effects of testosterone were dose-dependent, suggesting that testosterone treatment prevents white adipocyte development and stimulates hepatic lipid catabolism.

Our present results are supported by other previous reports. In ageing men, low testosterone has been associated with an increase in central obesity (Jorgensen et al., 1996; Vermeulen et al., 1999; Tsai et al., 2000). Testosterone treatment in men has been shown to decrease central obesity and total fat content (Marin et al., 1992; Boyanov et al., 2003). This prevention of central obesity by testosterone may be site specific, as testosterone inhibits triglyceride assimilation in intra-abdominal fat depots, but not in subcutaneous fat depots (Marin et al., 1996). Moreover, androgen receptor concentration is higher in visceral fat than subcutaneous fat in both males and females (Mayes and Watson, 2004). This difference in concentrations of AR in adipose tissue may offer a possibility that androgen is effective in prevention of central fat accumulation. In addition to antiadipogenic effects of testosterone *in vivo*, testosterone promotes the commitment of pluripotent precursor cells into the myogenic lineage and inhibits their differentiation into the adipogenic lineage (Singh et al., 2003). Testosterone and dihydrotestosterone (DHT) treatment of epididymal preadipocytes *in vitro* inhibits the activity of GPDH, an adipose-specific enzyme (Dieudonne et al., 2000). These results suggest that testosterone prevents adipocyte development via direct or indirect mechanism in adipocytes.

Regulation of some key proteins in adipose tissues by testosterone may also be a mechanism for the treatment and prevention of obesity. Two key proteins that are involved in lipid deposition are lipoprotein lipase (LPL) and leptin. LPL is the key enzyme for the hydrolysis of circulating triglycerides into free fatty acids, which in turn are stored in the adipose tissues (Goldberg and Merkel, 2001). Postheparin LPL activity was decreased by testosterone administration in both male and female rats (Staprans et al., 1990). Testosterone replacement therapy is known to decrease LPL activity in visceral adipocytes, but not in subcutaneous adipocytes (Rebuffe-Scrive et al., 1991). These results suggest that testosterone could be used to possibly prevent central obesity.

Leptin is another adipose protein that could be a target for regulation by sex steroid hormones to control central obesity. Leptin plays a key role in the regulation of food intake, energy expenditure and body weight homeostasis, suggesting that the amount of leptin is related to the amount and distribution of body fat (Friedman and Halaas, 1998). In males, several studies have reported an inverse relation-

ship between serum testosterone and leptin concentrations. Administration of testosterone to men appears to decrease the levels of leptin (Luukkaa et al., 1998; Erfurth and Ahren, 2000). Serum leptin levels were three times higher in hypogonadal men than in normal men and testosterone administration normalized the leptin levels in the hypogonadal men (Jockenhovel et al., 2000). In addition, in male rats of similar body weights, orchidectomy caused a rise in plasma leptin levels, which was abolished with testosterone administration (Pinilla et al., 1999; Watanobe and Suda, 1999).

These genes are activated by peroxisome proliferator-activated receptor γ (PPARγ) (Hollenberg et al., 1997; Desvergne et al., 1999). PPARγ is the most adipose-specific of the PPARs. It is expressed at the highest level in adipose tissue and adipocyte cell lines (Braissant, 1996; Schoonjans et al., 1996). Agonist-induced activation of PPARγ causes adipocyte differentiation and regulates expression of genes for maintaining the fat cell phenotype (Forman et al., 1995; Lehmann et al., 1995; Hollenberg et al., 1997). The fat cells do not develop in the absence of PPARγ in mosaic knock-out mice (Rosen et al., 1999). In heterozygous PPARγ-deficient mice than wild-type mice, body weight gain and an increase in white adipose tissue mass under a high-fat diet was significantly less (Kubota et al., 1999). Histological analyses revealed that the size of adipocytes from heterozygous PPARγ-dificient mice was significantly smaller than that of adipoctyes from wild-type mice under a high-fat diet.

However, the recent reports show that the expression of PPARγ is influenced by gonadal sex steroids. Testosterone and DHT inhibited the differentiation of 3T3-L1 preadipocytes into mature adipocytes, and down-regulated the mRNA and protein expression of key adipogenic transcription factors, C/EBP-δ, C/EBP-α and PPAR-γ2, in a time- and concentration-dependent manner in 3T3-L1 cells as well as mesenchymal C3H 10T1/2 cells (Singh et al., 2003; Singh et al., 2006). In addition, DHEA down-regulated the expression of PPARγ in cultured adipocytes (Kajita et al., 2003). These results suggest that testosterone may be involved in transactivation of PPARγ in adipocyte, resulting in the inhibition of adipoctye differentiation. However, the mechanism for the regulation of adipocyte differentiation by testosterone is not clear.

In conclusion, the results of this study using male CAST C57BL/6J mice indicate that testosterone exert inhibitory effects on adipocyte differentiation of WAT in a dose-dependent manner, providing morphological evidence that testosterone modulates adipocyte differentiation. Moreover, testosterone inhibited hepatic lipid accumulation, showing the involvement of testosterone in lipid catabolism of male mice. Further studies will be necessary to determine the mechanism by which adipocyte differentiation is regulated by testosterone.

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