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Late onset of obesity in male androgen receptor-deficient (AR KO) mice

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Abstract

An androgen receptor (AR) null mutant mice line was generated by means of a Cre-lox P system. The male ($AR^{L-/Y}$) (KO) mice exhibited typical features of testicular feminization mutant (Tfm) disease in external reproductive organs with growth retardation. The growth curve of the male AR KO mice was similar to that of the wild-type female littermates until the 10th week of age, but thereafter a drastic increase in the growth was observed with development of obesity. Clear increase in the wet weights of white adipose tissues, but not of brown adipose tissue, was found in the 30-week-old male AR KO mice. However, no significant alteration in serum lipid parameters and food intake was observed. Thus, the present results suggest that AR may serve as a negative regulator of adipose development in adult males.

Keywords: Androgen; Androgen receptor; AR KO mice; Tfm disease; Obesity; Adipocyte; Lipid accumulation

Androgens exert a wide variety of actions in target tissues like male reproductive organs, brain for expression of sexual behaviors, and skeletal tissues as anabolic actions [1,2]. Most of such androgenic actions are believed to be mediated by tissue-specific transcriptional controls of a particular set of target genes through nuclear androgen receptor (AR) [3,4]. AR is a member of the nuclear receptor gene superfamily and acts as a ligand-inducible transcription factor [5,6]. Upon ligand binding, AR, like the other nuclear receptors, recruits distinct classes of co-regulators and co-regulator complexes for ligand-dependent transcriptional control [6,7].

Such androgen actions are well documented especially in male animals, however, little is clear about the molecular basis of androgen actions in the target tissues, due to the lack of a null mutant animal line deficient in AR (AR KO). As the mammalian AR gene locates on the X chromosome and is a single copy gene in males, loss of AR function by genetic mutations leads to androgen insensitivity and infertility in males with female-like phenotypes known as testicular feminization syndrome (Tfm) [8,9]. Such infertility in male AR mutant has prevented the generation of AR KO ($AR^{-/Y}$) mice line by a conventional gene disruption method.

To define physiological functions of AR in male and female animals, we applied a Cre-lox P system to establish AR KO mice line [10]. Male AR KO ($AR^{L-/Y}$) mice exhibited typical Tfm abnormalities, which have been well documented in the Tfm rodents and patients [8,9]. Most notably, unlike the reported Tfm mice, neither AR transcript nor AR protein was detectable in AR KO ($AR^{L-/Y}$) mice to date [10], indicating that our AR KO mice have advantage over naturally mutated Tfm animals as an AR null mutant to evaluate the physiological function of AR.

During analyses of the male AR KO mice [10], we became aware that the AR KO mice develop obesity, but its onset appeared only after growth maturation. Although the growth curve of the male AR KO mice was similar to that of the wild-type female littermates until 10 weeks of age, thereafter it caught up in a following couple of weeks and came over that of the wild-type male littermates. Remarkable increases in white adipose tissues were seen, but the levels of serum lipid

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markers and the food intake appeared unaffected by AR inactivation. Such a clear accumulation of lipids in adipocytes was not found in female $AR^{(L-/L-)}$ mice. Thus, these results suggest that AR serves as a negative regulator of adipocyte development in adult males.

Materials and methods

Animal conditions and food intake. AR KO mice were generated by targeting disruption of AR gene by means of a Cre-lox P system, as previously described [10,11]. All mice were given a standard laboratory chow diet (4.4% w/w fat) and water ad libitum. The growth rates of male $AR^{L-/Y}$ and $AR^{+/Y}$ were monitored from the birth. Food intakes of $AR^{L-/Y}$ and $AR^{+/Y}$ mice were monitored for 12 weeks from the 12th week. For hormone treatments, a 60-day time-release pellet (Innovative Research of America) containing either DHT (10 mg/pellet), E2 (0.25 mg/pellet) or placebo (PLA) was implanted subcutaneously in 10-week-old $AR^{L-/Y}$ and $AR^{+/Y}$ mice 2 weeks after gonadectomy under avertin anesthesia. The body fat contents were evaluated by a dual-energy X-ray absorptiometry (DXA, PIXImus2; LUNAR) according to the methods by Sjogren et al. [12].

Histological Analysis. Subcutaneous, infrarenal, intraperitorial, and gonadal fat pads were taken from mice and the wet weights were measured. The tissues were fixed with 4% paraformaldehyde and frozen in tissue-Tek OCT compound. Ten-micrometer cryosections were stained with hematoxylin and eosin, and examined by light microscopy [13].

Serum measurements. Blood was collected by cardiac puncture from anesthetized mice after 20 h fasting. Serum levels of cholesterol, triglycerides, and free fatty acids were determined by an enzymatic colorimetric method (ACS-ACOD; Eiken Chemicals) [13].

Statistical analysis. The data were evaluated by Student's *t* test and a one-way analysis of variance (ANOVA) followed by post hoc comparison using Fisher's protected least significant difference (Fisher's PLSD) test.

Results

Late onset of obesity of male AR KO mice

The AR floxed male ($AR^{L3/Y}$) mice grew up normally with no overt abnormality in behaviors and metabolisms, and appeared completely normal in reproduction. Male AR floxed mice were then crossed with CMV-Cre transgenic mice [11] to generate female heterozygotes ($AR^{+/L-}$) for further production of male AR $KO^{(L-/Y)}$ mice [10].

The male AR ($AR^{L-/Y}$) KO mice exhibited growth retardation and the growth curve was indistinguishable from that of the wild-type female littermates up to the 10th week (Fig. 1A). However, thereafter, the rapid increase in the growth of male AR KO mice was seen, and until the 12th week the body weights of the male AR KO mice exceeded over those of the wild-type male littermates. The late onset of drastic increase in the growth curve in the male AR KO mice was understandable as development of clear obesity (Fig. 1B, upper panel).

Increased adipocytes in male AR KO mice

Reflecting obesity of male AR KO mice, significant increases of wet tissue weights in subcutaneous, infrarenal, and intraperitorial white adipose tissues (WATs) were observed at the 30th week of age (Fig. 1B lower panel, and Fig. 2A) and a clear lipid accumulation was seen in the subcutaneous WAT (Fig. 2B) and infrarenal and intraperitorial WATs (data not shown). In contrast, the sizes of gonadal adipocyte and brown adipocyte tissue (BAT) appeared unchanged by AR inactivation. Such a clear increase was not detected in the WATs of the 8-week-old male AR KO mice, consistent with the total body fat contents (Fig. 2C). To get an insight into the rapid increases in WATs of male AR KO mice after the 8th week of age, we first monitored the food intake for 12 weeks from the 12th to 24th week. Inconsistent with the drastic lipid accumulation in the male AR KO mice, no increase in food intake was seen (Fig. 3A). Likewise, no significant alteration in serum markers related to lipid metabolism was detected in the male AR KO mice at the 8th week (data not shown) and at the 10th week of age (Fig. 3B).

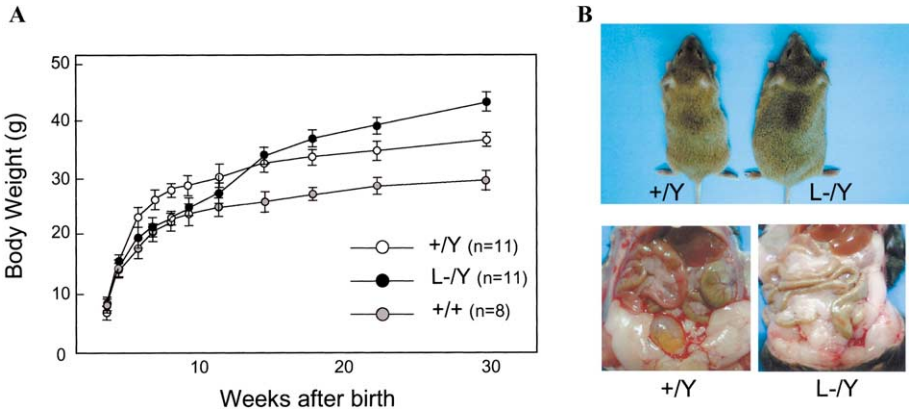

Fig. 1. Late onset of obesity in male AR KO mice. (A) Growth curves of the 30-week-old $AR^{+/Y}$ and $AR^{L-/Y}$ male mice and female $AR^{+/+}$ mice. Circles represent means ± SE. (B) External and intraabdominal appearance of 30-week-old $AR^{+/Y}$ and $AR^{L-/Y}$ mice.

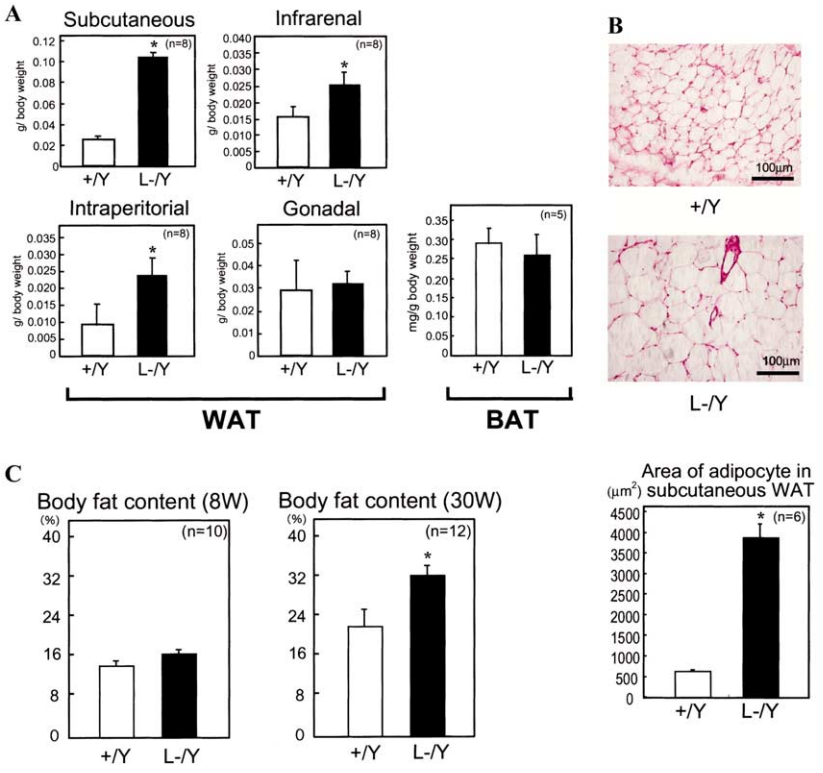

Fig. 2. Increased wet weights in white adipose tissues in male AR KO mice. (A) Wet weights of WATs and BAT from 30-week-old $AR^{+/Y}$ (empty bars) and $AR^{L-/Y}$ (filled bars) mice. Bars represent means ± SE (* $P < 0.005$). (B) Subcutaneous WATs from 30-week-old $AR^{+/Y}$ and $AR^{L-/Y}$ were fixed and sectioned and stained with hematoxylin and eosin (scale bars: 50 μm). Area of adipocyte in subcutaneous WAT from 30-week-old $AR^{+/Y}$ (empty bars) and $AR^{L-/Y}$ (filled bars) is displayed. (C) Fat proportion of 8- and 30-week-old $AR^{+/Y}$ (empty bars) and $AR^{L-/Y}$ (filled bars). Bars represent means ± SE (* $P < 0.005$).

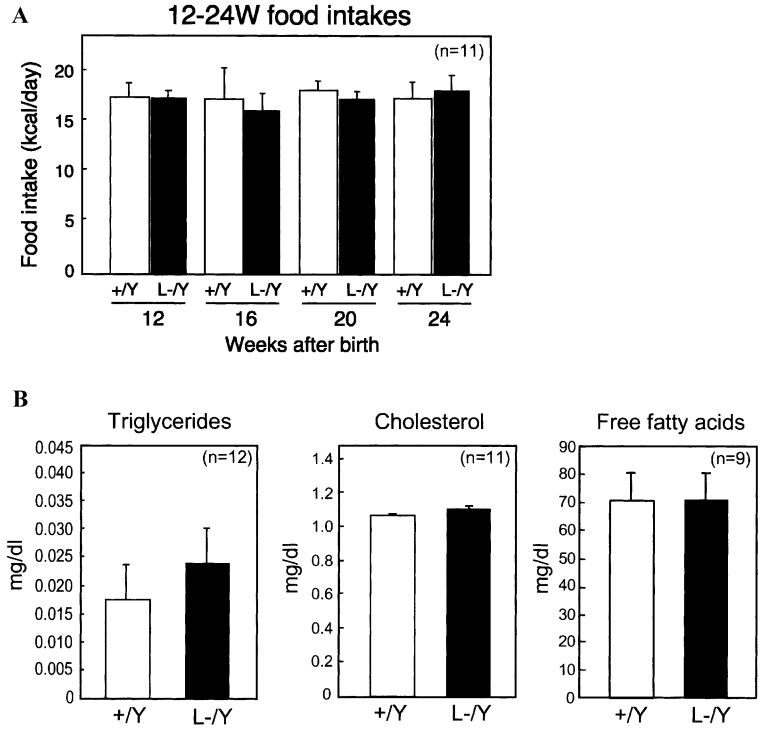

Fig. 3. No alteration in serum lipid markers and food intake in male AR KO mice. (A) Serum contents of triglycerides, total cholesterol, and free fatty acids in 30-week-old $AR^{+/Y}$ (empty bars) and $AR^{L-/Y}$ (filled bars) mice. Bars represent means ± SE. (B) Daily food intakes of 12–24-week-old $AR^{+/Y}$ (empty bars) and $AR^{L-/Y}$ (filled bars) mice. Bars represent means ± SE.

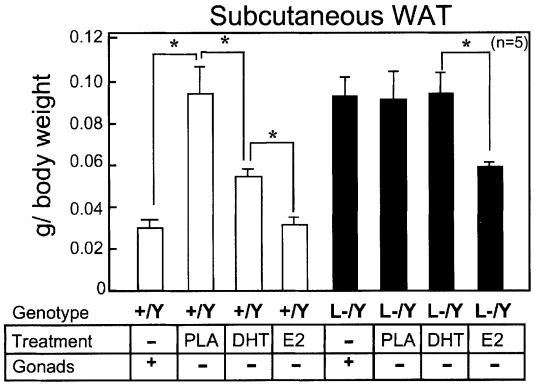

Fig. 4. Suppressive actions of sex steroid hormones in male adipose development. Wet weights of subcutaneous WAT from intact and hormone (DHT, E2, or placebo) treated $AR^{+/Y}$ (empty bars) and $AR^{L-/Y}$ (filled bars) mice. Bars represent means ± SE (*$P < 0.005$).

Treatment of estrogen attenuated the late onset of lipid accumulation of the male AR KO mice

As the estrogen signaling mediated through estrogen receptor α (ERα) is shown to suppress the development of WATs by the observations of male mice deficient in either ERα or aromatase [14,15], we wondered if such estrogenic action is intact or not in the male AR KO mice. As expected, a treatment with dihydrotestosterone (DHT) for 12 weeks to the 10-week-old male AR KO mice had no suppressive action, but 17β-estradiol (E2) was potent enough to attenuate the increase of wet weights in subcutaneous WAT (Fig. 4). In contrast, both of DHT and E2 were effective in preventing the subcutaneous WAT development in the wild-type male littermates. Thus, these findings suggest that the androgen-AR system has a negatively regulatory role in adipocyte development in male adult animals.

Discussion

We have succeeded in generating a null mutant mouse line by means of a Cre-lox P system [10], and the male AR KO ($AR^{L-/Y}$) mice exhibited the typical features of Tfm syndrome [8,9] including female-like outlook of external reproduction organs, degenerated testes, no ovary, and blunted vagina [10]. Male AR KO mice showed growth retardation, but the growth curve was similar to that of the wild-type female littermates up to the 8th week. However, thereafter obesity developed in the male AR KO mice with remarkable increases in WATs. Moreover, a DHT treatment with wild-type male mice attenuated adipogenesis, in agreement with previous studies in vitro [16–18]. Thus, these results suggest that the androgen-AR signaling system is a negative factor for adipocyte development in the adult male animals.

There are a number of factors involved in adipogenesis [19]. Among them, an estrogen-ER system has been recently shown to play a negative role in adipocyte development like the androgen-AR signaling presented here. Interestingly, the suppressive actions of these sex hormones were seen in WATs, but not in BAT [14,15]. However, there was a clear sex difference between two sex hormone actions in adipogenesis. Impaired estrogen signaling induced either by ERα or aromatase inactivation in mice resulted in increases in WATs in both sexes [14,15]; indeed an estrogen treatment was suppressive for adipocyte development in the male AR KO mice. However, obesity did not develop in the female AR KO ($AR^{L-/L-}$) mice at 30 weeks old with no significant difference in body weights when compared to the wild-type female mice (data not shown). Moreover, the onset of the obesity in male AR KO mice was only after growth maturation, while increases in WATs were detected in the ERα KO mice at the 8th week of age [14]. It is also notable that no wet weight increase in gonadal WAT was detected in the male AR KO mice even at 30 weeks of age, unlike male ERα KO mice. Thus, it is likely that the suppressive action of androgen in adipocyte differentiation is male-specific and late-onset.

Irrespective of clear increases in WATs in the adult AR KO male mice, food intake appeared unaffected with no alterations in serum lipid parameters. But glucose tolerance in the adult male AR KO may be impaired with insulin resistance. These observations point out a possibility that energy expenditure may decrease by AR inactivation, leading to the accumulation of lipid in WATs. As the ERα KO mice are the case [14], it is of great interest to evaluate the energy expenditure in the male AR KO mice. Alternative possibility is that the adipocyte development in the adults may be impaired by AR inactivation through an alteration in the expression of critical factors in adipogenesis.

The molecular basis of male-specific androgen actions in preventing adipogenesis in adult males is unknown at the present time. However, it is possible that the expressions of adipogenic factors are under the control of androgen-AR signaling. These transcriptional controls are expected to include positive and negative regulations, since like the other members of the nuclear receptor gene superfamily, AR may serve as a dual transcriptional regulatory factor upon the target gene promoters [3–7].

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