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Androgen (dihydrotestosterone)–mediated regulation of food intake and obesity in female mice

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ABSTRACT

To better understand how elevated androgen levels regulate food intake and obesity in females, we treated ovariectomized female mice with dihydrotestosterone (DHT) (non-aromatazable androgen), measured food intake and body weight, and evaluated physiological changes in liver function, glucose tolerance, and leptin resistance.

Ovariectomized mice were treated with DHT or placebo. Mice were then fed a high fat diet under free-feeding or pair-feeding conditions for 3 months. We found that when DHT-treated ovariectomized mice had free access to food (free-feeding), they had increased food intake and higher body weight compared with control animals. These mice also had a significantly greater accumulation of fat in the liver and exhibited increased fasting glucose, impaired glucose tolerance, and resistance to leptin. However, when these mice were placed on a restricted diet and fed the same caloric amounts as controls (pair-feeding), their body weight increased at the same rate as control animals. This suggests that androgen regulates food intake through altered leptin sensitivity, and this increase of food intake could significantly contribute to an obesity phenotype.

In summary, we demonstrated a role for androgen in the regulation of food intake and weight gain in females using a mouse model. This model will be useful to further elucidate the role of elevated androgen in females.

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1. Introduction

Although women have lower basal levels of androgen compared with men, several studies suggest that an increase in androgen levels can also affect metabolism and food intake in women, resulting in metabolic imbalances and weight gain [1–9]. Elevated androgen (testosterone) levels are associated with polycystic ovary syndrome (PCOS), a condition caused by an imbalance of sex hormones that can lead to menstrual cycle changes, ovarian cysts, difficulty conceiving, and other health changes. Women with PCOS are hyperandrogenic [1], and 50% are obese and have an increase in visceral adipose tissue and insulin resistance [2], as well as altered appetite regulation including impaired ghrelin and cholecystokinin secretion [3]. Elevated androgen (testosterone) levels are also associated with bulimia nervosa in women, an eating disorder characterized by frequent binge-eating episodes. Bulimic women have higher levels of testosterone but lower meal-related satiety peptide secretion than those without the disorder [4]. The use of anti-androgenic oral contraceptives in women with bulimia decreases testosterone and lowers the frequency of binge-eating episodes, suggesting that androgen plays a role in bulimic behavior [4]. Elevated androgen levels are also associated with greater food cravings and increased fat mass in young women [5–8]. All of these observations suggest that androgen may regulate food intake and play a role in obesity in women.

In the brain, the arcuate nucleus (ARC) of the hypothalamus plays a key role in the control of food intake, through opposing orexigenic and anorexigenic neuronal circuits. The anorexigenic neurons express pro-opiomelanocortin (POMC) and cocaine- and amphetamine-regulated transcript (CART). When activated, POMC/CART neurons signal to downstream neuronal pathways that suppress food intake. Androgens have been found to affect POMC gene expression, and androgen receptors regulate the transcription of target genes by interacting with DNA response elements [10].

Abbreviations: PCOS, polycystic ovary syndrome; ARC, arcuate nucleus; POMC, pro-opiomelanocortin; MCH, melanin concentration hormone-1; DHT, dihydrotestosterone; OVX, ovariectomized; IACUC, Institutional Animal Care and Use Committee; ALT, serum alanine aminotransferase; PCR, polymerase chain reaction; *Fas*, fatty acid synthetase; *SCD*, stearoyl-CoA desaturase; *ACC1*, acetyl-CoA carboxylase 1; *ACC2*, acetyl-CoA carboxylase 2; *SREBP1c*, sterol regulatory element-binding protein 1c; *PEPCK*, phosphoenolpyruvate carboxykinase; *G6Pase*, glucose 6-phosphatase; WAT, white adipose tissue.

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Moreover, melanin concentration hormone-1 (MCH) is an appetite-stimulating peptide produced by neurons in the lateral hypothalamus. The effects of MCH are inhibited by leptin [11], which inhibits food intake and stimulates metabolism. MCH was shown to be significantly up-regulated in the hypothalami of ovariectomized (OVX) female mice treated with dihydrotestosterone (DHT) [12]. This suggests that DHT treatment may increase food intake through the central nervous system leading to obesity in females. However, the mechanism by which androgen regulates food intake and obesity in females remains poorly understood. This is mainly because testosterone is converted to estrogen by the aromatase enzyme in females, and it is therefore difficult to determine the precise role of testosterone. Additionally, the sex steroid hormones estrogen and progesterone are also important modulators of food intake and energy balance, and the level and ratios of these hormones may also be important to regulate food intake and energy expenditure.

To better understand how elevated androgen regulates food intake and obesity in females, we developed an important mouse model by treating OVX mice with DHT and examined food intake, body weight, fatty acid biosynthesis, glucose tolerance, and leptin sensitivity.

2. Materials and methods

2.1. Mice

C57Bl/6J mice were obtained from the Jackson Laboratory (Bar Harbor, ME) and housed at the City of Hope Animal Resources Center in ventilated cage racks with free access to water. Mice were maintained on a 12 h light/dark cycle. All institutional guidelines for animal care and use were followed. All animal research procedures used in this study were approved by the Institutional Animal Care and Use Committee (IACUC) at City of Hope. Facilities are credited by AAALAC (Association for Assessment and Accreditation of Laboratory Animal Care), and operated according to NIH guidelines.

Sixteen female C57B1/6J mice were ovariectomized (OVX mice) at 7 weeks of age and 8 mice had sham surgery according to an established protocol (control sham mice). The sham operation was exactly the same as the ovariectomy procedure, but the ovaries were not removed from the mice. OVX mice were divided into two groups: OVX-DHT and OVX-control (8 mice per group), and subcutaneously implanted with either 12.5 mg/90 day time-release 5α-DHT pellets (Innovative Research of America, Sarosota, FL) [13,14] (OVX-DHT group) or placebo pellets (OVX-control group). Sham mice were implanted with placebo pellets (Sham group). Pellet implantation was performed at the time of ovariectomy (OVX-DHT, OVX-control) or sham surgery (sham group). All mice were fed a 45 kcal% high fat diet (Research Diet, Inc., New Brunswick, NJ).

2.1.1. Free-feeding studies

Beginning one week after pellet implantation and continuing for 3 months, mice were fed according to a "free-feeding" design with free access to food. Food intake was measured daily and body weight was measured once a week for 3 months.

2.1.2. Pair-feeding studies

Beginning one week after pellet implantation and continuing for 3 months, mice were fed according to a "pair-feeding" design. Based on the food intake in the free-feeding studies, we identified the group that ate the least (OVX-control). For the pair-feeding studies, food intake of the OVX-control group was determined daily by the food weight at the time of measurement from the initially added weight, and all groups were given the same amount of food (by weight); the OVX-DHT and sham groups were fed one day after the OVX-control group to ensure identical caloric intake between groups. Body weight was measured once a week for 3 months.

2.2. Tissue collection and pathological analysis

All mice were fasted for 4 h before euthanasia. Gonadal and inguinal white adipose tissue (WAT) was obtained from all mice and weighed. Livers were collected from all mice and the wet weight was measured. The left lobes of the livers were then fixed with 10% formalin overnight, processed, and embedded in paraffin. 5 μm tissue sections were obtained, stained with hematoxylin and eosin, and examined by light microscopy. (The right lobes of the livers were used for real-time PCR as described in Section 2.4)

Vacuolation of hepatocytes was scored on a 6-point, semi-quantitative, tiered scale where 0 = essentially no vacuolation, and increasing scores of 1 through 5 represented increasing severity of hepatocellular vacuolation. Scoring was performed blindly (without knowledge of treatment group) in 3 different sessions, separated by at least 4 days. The final vacuolation score represented the mean of the 3 session scores. All histopathologic analyses were conducted by a board-certified, veterinary pathologist.

2.3. Liver enzyme measurement

All mice were fasted for 4 h, euthanized, and blood was collected via cardiac puncture and centrifuged at 4000 rpm for 10 min to obtain serum. Serum alanine aminotransferase (ALT) levels were measured by Antech Diagnostics (Irvine, CA).

2.4. Real-time PCR

Total RNA was extracted from the right lobes of liver samples (4 mice per group) using TRIzol reagent (Invitrogen, Carlsbad, CA). cDNA was synthesized from total RNA using reverse transcriptase III (Invitrogen, Carlsbad, CA). Real-time polymerase chain reaction (PCR) was performed to evaluate changes in gene expression. Gene expression was normalized to the expression of the *β-actin* gene. Real-time PCR primers used were: *β-actin*, 5′-CATTGCTGACAGGATGCAGAAGAAG-3′ and 5′-CCTGCTTGCTGATCCACATCTGCT-3′; fatty acid synthetase (*Fas*), 5′-TGGGTTCTAGCCAGCAGAGT-3′ and 5′-AGACCGTTATGCCCAGACAG-3′; stearoyl-CoA desaturase (*SCD*), 5′- AGAGTCAGGAGGGCAGGTTTCC-3′ and 5′-TGATGGTGGTGGTGGTCGTGTA-3′; acetyl-CoA carboxylase 1 (*ACC1*), 5′-CCTCCGTCAGCTCAGATACACTTTC-3′ and 5′-TTTCACTGCTGCAATACCATTGTTG-3′; acetyl-CoA carboxylase 2 (*ACC2*), 5′-GAGCTGCTGTGTAAACACGAGATTG-3′ and 5′-CTGGTGCCGGCTGTCCTC-3′; sterol regulatory element-binding protein 1c (*SREBP1c*), 5′-GCG CTA CCG GTC TTC TAT CA-3′ and 5′-TCC TGC TTG AGC TTC TGG TT-3′; phosphoenolpyruvate carboxykinase (*PEPCK*) 5′-GTG GGC GAT GAC ATT GCC-3′ and 5′-ACT GAG GTG CCA GGA GCA AC-3′; glucose 6-phosphatase (*G6Pase*), 5′-CAT GAG TCA GAC AGG CTG GA-3′ and 5′-CCA TCT CTT TGC CCA GGT AG-3′. Reactions were run in triplicate on the iCycler iQ5 real time PCR detection system (Bio-Rad, Hercules, CA), and results were analyzed with the iQ5 software (Bio-Rad, Hercules, CA).

2.5. Glucose tolerance test

Glucose tolerance tests were performed in free-feeding mice after 2 months of treatment. Mice were fasted with free access to drinking water for 18 h prior to the test. Baseline glucose levels were recorded for each mouse. Mice were then injected with a 1.5 mg glucose/gram body-weight glucose load. The glucose levels pre-injection and post-injection (30, 60, 120, and 180 min) were measured using a glucometer (Bayer, Germany).

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