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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 (non-aromatizable 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 roles of elevated androgen in females.

Keywords

Androgen; Dihydrotestosterone; Ovariectomized mice; Females; Obesity; Food intake

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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 hyper-androgenic [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 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]. Moreover, melanin-concentrating 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 hypothalamus 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 the 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 hour 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 for assessment and accreditation of laboratory animal care, and were in accordance with NIH guidelines.

Sixteen female C57Bl/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 5 α -DHT pellets (Innovative Research of America, Sarasota, 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 subtraction the food weight at the 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 hours 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 μ m 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 hours, euthanized, and blood was collected via cardiac puncture and centrifuged at 4000 rpm for 10 minutes 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'-AGACCGTTATGCCAGACAG-3'; stearyl-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'-CTGGTGCCGGCTGTCTC-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 hours 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 minutes) were measured using a glucometer (Bayer, Germany).

2.6 In vivo leptin sensitivity test

In vivo leptin sensitivity tests were performed in free-feeding mice at 3 months of age. Days 1-3: body weight was measured and all groups of mice were injected with vehicle (PBS) in the morning (8.00am) and in the afternoon (4.30pm). Initial body weight was calculated as the average weight during the initial (3 days) PBS injections. Days 4-6: body weight was

measured and all mice were intraperitoneally injected with recombinant murine leptin (1 mg/kg) (PeproTech, Rocky Hill, NJ) in the morning (8.00am) and in the afternoon (4.30pm). Days 7-8: body weight was measured and all mice were injected with PBS in the morning (8.00am) and in the afternoon (4.30pm).

2.7 Statistical analysis

For multiple comparisons, analysis was followed by comparison of all treatment groups with the control group (Dunnett's test) or by comparison of all pairs of treatment (Tukey's test). Statistical significance was defined as $P < 0.05$. All data are expressed as mean $\pm$ standard error (SEM). Data sets were analyzed for statistical significance using Prism GraphPad 4 software (GraphPad Software, Inc., La Jolla, CA).

3. Results

3.1 Body weight and food intake

The average body weight and food intake of each group of mice (OVX-DHT, OVX-control, and sham) after pair-feeding and free-feeding is shown in Figure 1. After pair-feeding, OVX-control mice weighed more than sham mice, however, there was no body weight difference between OVX-DHT mice and OVX-control mice (Figure 1A). After free-feeding, OVX-control mice weighed more than sham mice, and DHT treatment significantly increased body weight in OVX-DHT mice compared to OVX-controls (Figure 1B). During free-feeding, food intake of a 45% kcal high fat diet was not different between sham and OVX-control mice, however OVX-DHT mice had significantly greater food intake than both OVX-control and sham mice (Figure 1C).

3.2 Liver studies

3.2.1 Liver weight, ALT levels, and liver vacuolation—The average liver weight, ALT levels, and liver vacuolation of each group of mice (OVX-DHT, OVX-control, and sham) after pair-feeding and free-feeding are shown in Figure 2. Liver weight was slightly increased in OVX-control and OVX-DHT mice compared to sham mice during pair-feeding; ALT, a liver injury marker, did not change (Figure 2A and 2B). However, during free-feeding, liver weight and ALT levels in OVX-DHT mice were significantly higher than OVX-control and sham mice. There was no difference in liver weight and ALT levels between OVX-control and sham mice (Figure 2A and 2B).

We observed a significant difference in liver weight between groups only under free-feeding conditions. Therefore, we analyzed liver vacuolation (and expression of fatty acid biosynthesis and gluconeogenesis genes; *Section 3.2.2*) in free-feeding mice. A minimal amount of vacuolation was present in hepatocytes of sham mice (1.52 ± 0.20), and the vacuoles varied in their distribution within the hepatic lobules (Figure 2C). The severity of vacuolation among the livers from the OVX-control group was more variable than among those from the sham group; however, the mean severity was only minimally higher in the OVX-control group (2.00 ± 1.22) than in the sham group. Vacuolation in the OVX-control group was generally located in the mid-zonal region of the hepatic lobule (Figure 2C). The severity of vacuolation in hepatocytes of the OVX-DHT group was significantly worse than

in the other treatment groups (4.62 ± 0.40). Vacuolation in the livers from this group was most intense in the centrilobular and mid-zonal regions and sometimes extended into the portal regions of the hepatic lobules (Figure 2C).

3.2.2 Fatty acid biosynthesis and gluconeogenesis pathways—Expression of fatty acid biosynthesis and gluconeogenesis genes (*Fas*, *SCD*, *ACCI*, *ACC2*, *SREBPc1*, *PEPCK*, and *G6Pase*) in each group of mice (OVX-DHT, OVX-control, and sham) after free-feeding is shown in Figure 3. Expression of all of the enzymes for fatty acid biosynthesis (*Fas*, *SCD*, *ACCI* and *ACC2*) was significantly increased in the livers of OVX-DHT mice compared to OVX-control mice by real time PCR. There was no difference in the liver expression of these enzymes between OVX-control and sham mice. For gluconeogenesis enzymes, the level of *SREBPc1* was increased in OVX-control mice compared to sham mice; and an additional increase was seen in the livers of OVX-DHT mice. Expression of *PEPCK* and *G6Pase* was increased in the livers of OVX-control mice compared to sham mice; however expression of these genes was not increased in OVX-DHT mice compared to sham mice.

3.3 White adipose tissue weight

The weight of gonadal and inguinal white adipose tissue (WAT) in each group of mice (OVX-DHT, OVX-control, and sham) after pair-feeding and free-feeding is shown in Figure 4. The WAT was increased in OVX-control mice compared to sham mice in both pair-feeding and free-feeding, and DHT treatment did not change gonadal WAT weight in OVX-DHT mice (Figure 4A). The inguinal WAT was increased in OVX-control mice compared to sham mice in both pair-feeding and free-feeding. DHT treatment did not change inguinal WAT weight in free-feeding, but inguinal WAT weight decreased in OVX-DHT mice during pair-feeding (Figure 4B).

3.4 Physiological studies

We observed a significant increase in body weight in OVX-DHT mice only under free-feeding conditions. Therefore, we evaluated physiological changes by glucose tolerance test and *in vivo* leptin sensitivity test in free-feeding mice.

3.4.1 Glucose tolerance—Serum glucose concentrations after glucose injection in each group of mice (OVX-DHT, OVX-control, and sham) after free-feeding are shown in Figure 5. In OVX-DHT mice, baseline glucose concentrations were significantly higher than in OVX-control mice. Compared to the sham group, OVX-control mice showed higher serum glucose concentrations 30 and 60 minutes post glucose injection, however there was no significant difference between the serum glucose concentrations in these groups. OVX-DHT mice had significantly higher glucose levels after injection compared to OVX-control mice, and there were significant differences between these groups in the ability to clear glucose over time.

3.4.2 Leptin sensitivity—Body weight gain after leptin treatment in each group of mice (OVX-DHT, OVX-control, and sham) after free-feeding is shown in Figure 6. After injection of leptin, the body weight of sham mice significantly decreased (by day 7)

compared to baseline (day 1). OVX-control mice showed a smaller decrease in body weight after leptin treatment (compared to sham mice). However, leptin treatment did not change body weight in OVX-DHT mice. There were significant differences in body weight between the sham/OVX-control groups and the OVX-DHT group at day 7 (Figure 6).

4. Discussion

In females, estrogen controls energy balance, food intake, and body fat distribution, and low estrogen can cause obesity in rodent models as well as humans [15]. The aromatase knockout (ArKO) mouse presents with age-progressive obesity and hepatic steatosis, and by one year of age both male and female ArKO mice develop hypercholesterolemia [16]. This suggests that estrogen can play a similar role for both males and females in the progression of obesity. For androgen, in males, several studies have demonstrated an inverse relationship between indicators of obesity and testosterone levels over all age groups [17]. However clinical findings suggest that an increase in androgen levels can increase food intake in women, resulting in metabolic imbalances and weight gain [1-9]. These findings suggest that the roles of androgen in females and males are different.

There are few animal models currently available to accurately study the effects of androgen on food intake and obesity in females because in most, the conversion of testosterone to estrogen can confound the mechanism by which elevated androgen regulates food intake and weight gain. Our model overcomes this problem because DHT cannot be converted to estrogen, and it is therefore a useful way to assess the specific role of androgen on food intake and obesity. In this study we showed that when OVX mice treated with androgen (OVX-DHT) have free access to food (free-feeding), they have increased food intake and higher body weight compared with control mice (OVX-control). These mice also have a significantly greater accumulation of fat in the liver and exhibit increased fasting glucose, impaired glucose tolerance, and resistance to leptin. However, when OVX-DHT mice are placed on a restricted diet and fed the same caloric amounts as controls (pair-feeding), their body weight increases at the same rate as control animals (OVX-control). Moreover, our preliminary results showed that sham mice treated with DHT under a free-feeding diet were heavier than OVX mice, and slightly lighter than OVX-DHT mice (data not shown). It was also reported that intact female rat treated with DHT as PCOS model increased body weight [13]. These results suggest that obesity phenotype can be defined by estrogen/androgen ratio.

In previous studies, ovariectomy was shown to increase body weight through increased food intake [18] and reduced energy expenditure [19]. In our study, OVX-control mice did not have increased food intake compared with sham mice, yet OVX-control mice were significantly heavier than sham mice when mice were fed under pair-feeding conditions. This corresponds with previous studies [19] and suggests that OVX mouse weight gain was not caused by increased food intake. In contrast, we found that DHT treatment led to an increase in body weight through increased food intake (free-feeding); but DHT treatment did not change body weight when mice were fed under pair-feeding conditions. We also found that the fatty acid biosynthesis pathway was increased in OVX-DHT mice however the gluconeogenesis pathway was not increased by DHT treatment. In contrast, gluconeogenesis

was increased in OVX-control mice compared to sham mice; and this increase was inhibited by DHT treatment. Although high androgen and low estrogen can both induce body weight gain, these results suggest that androgen induces obesity via a different mechanism that is independent of low estrogen. Estrogen repletion of OVX mice can decrease energy expenditure, leading to obesity [19]. In contrast, DHT treatment can influence appetite behavior (hyperphagia) through altering leptin sensitivity resulting in obesity.

Mclinnnes et al. reported that DHT-treated mice exhibit increased body weight and visceral fat mass associated with triglyceride accumulation, and no significant differences were observed for food intake in their model [20]. In their study, a regular chow diet was used. Women with PCOS exhibit a dietary pattern marked by consumption of a greater amount of specific foods with a high glycemic index [21]. The food used in our study (45% kcal high fat diet) was chosen to ensure we would observe a clear obesity phenotype if present, and mice might have preference for a high fat diet over chow diet under DHT treatment. Women with hyperandrogenemia had greater high fat food cravings independent of age, BMI and PCOS status [22]. Therefore, it would be expected that hormonal changes affect food craving in females. Also this can be one of the reasons Mclinnnes et al. did not see increase of food intake in their model but we did.

Our results showed that OVX mice, under free-feeding condition, treated with DHT showed greater fat accumulation in the liver compared to OVX-control mice, as well as increased fasting glucose and impaired glucose tolerance. In addition, OVX-DHT mice did not show an obesity phenotype under pair-feeding conditions, suggesting that increased food intake greatly contributed to the obese phenotype (body weight gain and liver steatosis) by DHT treatment, leading to insulin resistance.

The mechanism by which DHT increases food intake can be partially explained by the leptin resistance observed in our model. It has been reported that OVX mice do not respond to injected leptin, whereas estradiol-supplemented mice show a significant reduction in body weight [23]. Our results show moderate leptin resistance in OVX-control mice compared to sham mice. These findings suggest that estrogen plays an important role in regulating leptin sensitivity. Manneras et al. reported that DHT-treated intact female rats exhibited the metabolic characteristics of PCOS. In this model, DHT-treated female rats showed increased leptin levels [13]. In our study, mice treated with DHT did not decrease their body weight after leptin treatment, suggesting that DHT alters leptin sensitivity. DHT treatment also induced obesity in both ours and the model by Manneras et al.

Leptin is effective in reducing food intake and body fat in leptin-deficient and normal mice, however mice with induced obesity are relatively resistance to treatment [11]. Similarly, women with PCOS have elevated circulating leptin levels, especially the obese women [24]. There are two mechanisms that may explain the observed leptin resistance; decreased leptin transport to the brain or impaired leptin signal transduction in target neurons [25]. Interestingly, it was reported that leptin receptor Try985Leu homozygous mutant mice showed a lean phenotype only in females but not in males [26], suggesting that gonadal hormones determine central sensitivity of leptin. However, the limitation of our study is that the study design does not distinguish between leptin resistance caused by obesity or direct

DHT-regulated leptin sensitivity. Based on previous studies [25, 26], the latter hypothesis is supported. However, more evidence will be needed to evaluate if DHT itself causes leptin resistance, and leads to obesity in females.

Conclusions

In our study we developed a useful mouse model with which to study the precise role of elevated androgen in female food intake and obesity. Using this novel approach, we demonstrated a role for androgen in the regulation of food intake and weight gain in females. This model will be useful to further elucidate the role of elevated androgen in females by evaluating whether increased androgen influences the choice of diet, and whether androgen directly alters leptin sensitivity.

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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
<i>Fas</i>	Fatty acid synthetase
<i>SCD</i>	Stearoyl-CoA desaturase
<i>ACC1</i>	Acetyl-CoA carboxylase 1
<i>ACC2</i>	Acetyl-CoA carboxylase 2
<i>SREBP1c</i>	Sterol regulatory element-binding protein 1c
<i>PEPCK</i>	Phosphoenolpyruvate carboxykinase
<i>G6Pase</i>	Glucose 6-phosphatase
WAT	White adipose tissue

Highlights

Ovariectomized (OVX) female mice were treated with androgen.

Androgen regulated food intake through altered leptin sensitivity.

Increase of food intake by androgen caused an obesity phenotype.

This model is useful to further elucidate the role of elevated androgen in females.

Figure 1. Average body weight and food intake of each group of mice (OVX-DHT, OVX-control, and sham) after pair-feeding and free-feeding (A) Average body weight in pair-fed mice. (B) Average body weight free-fed mice. (C) Average food intake in free-fed mice. Values are expressed as mean and standard error for 8 mice. * $P < 0.05$. OVX: ovariectomized; DHT: dihydrotestosterone.

Figure 2. Average liver weight, ALT levels, and liver vacuolation of each group of mice (OVX-DHT, OVX-control, and sham) after pair-feeding and free-feeding
 (A) Average liver weight. (B) Average ALT serum levels. (C) Liver vacuolation in after free-feeding. * $P < 0.05$. Representative hematoxylin and eosin staining of livers from each group are shown. Bar 100 μm in representative picture. OVX: ovariectomized; DHT: dihydrotestosterone.

Figure 3. Expression of fatty acid biosynthesis and gluconeogenesis enzymes in each group of mice (OVX-DHT, OVX-control, and sham) after free-feeding
 Gene expression was normalized to β -actin. * $P < 0.05$. Values are expressed as mean and standard error for 3 mice. OVX: ovariectomized; DHT: dihydrotestosterone.

Figure 4. Average weight of gonadal and inguinal white adipose tissue (WAT) in each group of mice (OVX-DHT, OVX-control, and sham) after pair-feeding and free-feeding (A) Average gonadal and (B) inguinal WAT weight. Values are expressed as mean and standard error for 8 mice. * $P < 0.05$. OVX: ovariectomized; DHT: dihydrotestosterone.

Figure 5. Serum glucose concentrations after glucose injection in each group of mice (OVX-DHT, OVX-control, and sham) after free-feeding

Values are expressed as mean and standard error for 8 mice. * $P < 0.05$. OVX: ovariectomized; DHT: dihydrotestosterone.

Figure 6. Body weight gain after leptin treatment in each group of mice (OVX-DHT, OVX-control, and sham) after free-feeding

Initial body weight was calculated as average weight during the initial (3 days) PBS injections before leptin injection. Values are expressed as mean and standard error for 8 mice.* $P < 0.05$.