Diet-induced obesity exacerbates metabolic and behavioral effects of polycystic ovary syndrome in a rodent model

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Ressler IB, Grayson BE, Ulrich-Lai YM, Seeley RJ. Diet-induced obesity exacerbates metabolic and behavioral effects of polycystic ovary syndrome in a rodent model. Am J Physiol Endocrinol Metab 308: E1076–E1084, 2015. First published April 14, 2015; doi:10.1152/ajpendo.00182.2014.—Polycystic ovary syndrome (PCOS) is the most common endocrinopathy affecting women of reproductive age. Although a comorbidity of PCOS is obesity, many are lean. We hypothesized that increased saturated fat consumption and obesity would exacerbate metabolic and stress indices in a rodent model of PCOS. Female rats were implanted with the nonaromatizable androgen dihydrotestosterone (DHT) or placebo pellets prior to puberty. Half of each group was maintained ad libitum on either a high-fat diet (HFD; 40% butter fat calories) or nutrient-matched low-fat diet (LFD). Irrespective of diet, DHT-treated animals gained more body weight, had irregular cycles, and were glucose intolerant compared with controls on both diets. HFD/DHT animals had the highest levels of fat mass and insulin resistance. DHT animals demonstrated increased anxiety-related behavior in the elevated plus maze by decreased distance traveled and time in the open arms. HFD consumption increased immobility during the forced-swim test. DHT treatment suppressed diurnal corticosterone measurements in both diet groups. In parallel, DHT treatment significantly dampened stress responsivity to a mild stressor. Brains of DHT animals showed attenuated c-Fos activation in the ventromedial hypothalamus and arcuate nucleus; irrespective of DHT-treatment, however, all HFD animals had elevated hypothalamic paraventricular nucleus c-Fos activation. Whereas hyperandrogenism drives overall body weight gain, glucose intolerance, anxiety behaviors, and stress responsivity, HFD consumption exacerbates the effect of androgens on adiposity, insulin resistance, and depressive behaviors.

POLYCYSTIC OVARY SYNDROME (PCOS) is the most common endocrine disorder in women of reproductive age with substantial metabolic, reproductive, and psychological impacts. Associated metabolic disturbances include insulin resistance and hyperinsulinemia, impaired glucose tolerance, gestational diabetes, type 2 diabetes, dyslipidemia, and cardiovascular disease (8, 16, 18, 19, 33, 36, 40, 49, 56, 57). Reproductive comorbidities relate largely to oligo- or anovulation; these include infertility (20) and increased risk of endometrial cancer (15). Psychological associations include depression, anxiety, decreased quality of life, and altered response to stress (11, 28, 29, 39, 41). Although these clinical data are clear, we have little in the way of a mechanistic understanding of the underlying etiology. The pathophysiology has been associated with hyperandrogenism and insulin resistance, and the disorder presents itself as several phenotypes.

There is a clear association between PCOS and obesity (BMI \(\geq 30 \text{ kg/m}^2\)) (14, 23). The prevalence of obesity within the PCOS population has been reported to be as high as 61–76% in the US and Australia (11, 24). PCOS women, even those who are of normal BMI, are more likely to have abdominal or visceral adiposity, which contributes to adverse metabolic effects, including insulin resistance (34). Obesity and PCOS increase synergistically (17, 20). Obesity has been hypothesized to play a role in the underlying pathophysiology of the disorder by creating insulin resistance (44). Weight loss has been shown to improve several clinical components, including insulin sensitivity (1, 25), hyperandrogenemia (3, 4), sex hormone-binding globulin levels (12, 32), and ovarian function (30). However, not all women with PCOS are obese. It has been suggested that the pathophysiology of PCOS in lean women differs from those who are obese, relating to hypothalamic pituitary dysfunction and the increased levels of LH rather than insulin (2, 14). However, lean PCOS women demonstrate metabolic differences compared with counterparts of similar BMI without PCOS, including insulin resistance (10, 17) and hyperinsulinemia (42).

A key underlying feature of PCOS irrespective of BMI is hyperandrogenism, particularly testosterone and dihydrotestosterone levels (43). Insulin resistance and compensatory hyperinsulinemia contribute to increased androgen production by the ovaries (7). Hyperinsulinemia also decreases sex hormone-binding globulin synthesis, furthering elevated free testosterone concentrations (7). The shift of gonadotropin secretions to an LH-dominant environment may also contribute to the insulin resistance of this phenotype (45).

Because the etiology of PCOS remains elusive, rodent models have produced pharmacological hyperandrogenism, i.e., exposure to dihydrotestosterone prior to puberty (54), to probe various aspects of the disease or more recently with the aromatase inhibitor letrozole (37). A recent study comparing multiple murine models of PCOS found that long-term exposure to DHT created the best mouse model for the study of PCOS, as it most closely replicated the endocrine, metabolic, and ovarian manifestations of human PCOS (9).

It is evident that both obesity and hyperandrogenism are key components in the PCOS condition; however, the direct contributions of each and their interplay remain unclear. Given the divergent populations of lean and obese women affected by
PCOS, we sought here to further understand the underlying mechanisms. The goal of the present study was to determine what effects obesity and hyperandrogenism, both independently and synergistically, have on the metabolic, behavioral, and reproductive consequences in a rat model of PCOS. First, we induced hyperandrogenemia using chronic exposure to elevated levels of dihydrotestosterone (DHT), as it has previously shown metabolic and reproductive effects (e.g., irregular cycles and insulin resistance) that resemble women with PCOS (21, 37). The animals were placed on either an obeseogenic high-fat diet (HFD) to represent a model of obese PCOS or a nutrient-matched low-fat diet (LFD) to model lean PCOS. We assessed the effects of hyperandrogenism and diet on metabolic parameters (body weight, body composition, glucose, and insulin tolerance) and on hormones and behavior (anxiety, depression, and stress response). We used behavioral measures of stress and anxiety [elevated plus maze (EPM), forced-swim test (FST), and novel environment stressors] to parse the contribution of hyperandrogenism and saturated fat consumption to the behavioral phenotype. c-Fos expression was measured to examine possible brain regions involved in mediating observed differences. We hypothesized that obesity would exacerbate metabolic, stress, and reproductive indices in a rat model of PCOS.

METHODS

Animals. All procedures for animal use were approved by the University of Cincinnati Institutional Animal Care and Use Committee. Fifty young (20- to 24-day-old) female Long-Evans rats (35–49 g; Harlan Laboratories, Indianapolis, IN) were individually housed and maintained in a room on a 12:12-h light-dark cycle at 25°C and 50–60% humidity. Following acclimatization to the facilities, rats were divided into two body weight- and fat mass-matched groups (DHT and placebo). Pellet implantation occurred 4 days after arrival. Animals were given ad libitum access to water and either a palatable HFD (no. D03082706, 4.54 kcal/g, 40% fat, 45% carbohydrate, and 15% protein; Research Diets, New Brunswick, NJ) or LFD (no. D03082705, 3.81 kcal/g, 9% fat, 76% carbohydrate, and 15% protein; Research Diets). Animals were divided into four body weight- and fat mass-matched groups: 1) placebo pellet and receiving LFD (CON-LFD; n = 13, modeling lean non-PCOS), 2) placebo pellet and receiving HFD (CON-HFD; n = 12, modeling obese non-PCOS), 3) DHT pellet and receiving LFD (DHT-LFD; n = 12, modeling lean PCOS), and 4) DHT pellet and receiving HFD (DHT-HFD; n = 13, modeling obese PCOS). Animals were euthanized after 12 wk.

Pellet implantation. From postnatal days 24 to 28, anesthetized (isoflurane) female pups were implanted subcutaneously with a 90-day continuous release pellet containing 7.5 mg of 5α-DHT or placebo (Innovative Research of America, Sarasota, FL). This model and dose were chosen based on their ability to induce both ovarian and metabolic characteristics of PCOS (37) and to mimic the hyperandrogenic state in women with PCOS. The DHT pellets were intended to last the duration of the study (47).

Body weight, composition, and food intake. Food intake (food hopper weights) and body weights were measured weekly for the entirety of the study. Echo magnetic resonance imaging whole body composition analysis (EchoMedical Systems, Houston, TX) (47) was performed on all rats at 1, 6, and 11 wk to determine fat and lean body composition.

Estrous cycle. The stage of the estrous cycle was determined for 5 consecutive days at 4, 11, and 12 wk. Cells were obtained by vaginal lavage and stained with the DipQuick staining kit (Jorgensen Laboratories, Loveland, CO) for the determination of the estrous cycle phase (5). Animals were rated as anovulatory when they remained in the diestrus phase indefinitely. Animals were rated as irregular when an abnormal ordering of the phases occurred (Fig. 1E). The stage of the cycle was merged for weeks 4 and 11 (pellets were active), and the percentage of time spent in each portion of the cycle was assessed for this time frame (Fig. 1F).

Glucose tolerance tests. Glucose tolerance test (GTT) was performed at 7 wk. Rats were fasted for 8 h. After a baseline blood sample was taken (0 min), 50% d-glucose (Phoenix Pharmaceutical, St. Joseph, MO) was intragastrically gavaged. Blood glucose was measured at baseline (0 min) and 15, 30, 45, 60, and 120 min after glucose administration on duplicate samples using Accu-chek glucometers and test strips (Roche, Indianapolis, IN). All blood samples were obtained from the tip of the tail vein of freely moving rats. Each rat received a dose of glucose equal to 1.5 g/kg average body weight of the animals. Plasma insulin was measured at 0 and 15 min.

Insulin tolerance test. Insulin tolerance test (ITT) was performed at 11 wk. Eight-hour-fasted rats were injected ip with insulin (0.5 U/kg). Blood glucose was measured at baseline (0 min) and 15, 30, 45, and 60 min after injections with Accu-chek glucometers and test strips. All blood samples were obtained from the tip of the tail vein of freely moving rats.

Elevated plus maze. An EPM test was performed during week 5 to assess anxiety-related behavior. Animals were placed in a holding room ~4 h before the onset of the dark phase to acclimate. The challenge was performed at 6-min intervals commencing 15 min after the onset of the dark phase. The apparatus comprised of a polystyrene maze with two open (40 × 10) and two enclosed (40 × 10 × 20) arms. The arms radiated from a 10-cm central square. The entire apparatus was elevated 60 cm off the floor. For testing, each animal was placed on the center square of the maze facing the same open arm. Behavior was recorded from an overhead ceiling camera for 5 min. Video files were captured and saved for later scoring. The maze was cleaned after each rat was tested. A number of behavioral measures were analyzed, which included standard measures of exploration and anxiety-like behavior as well as additional measures of fear and arousal. Parameters were measured using the Topscan program CleverSys (Reston, VA), using an automated rating system. Several parameters were scored, including arm time (open and closed), arm entries (open and closed), and general locomotor activity.

Novel environment challenge. Novel environment challenge was performed during week 6 to test hypothalamic-pituitary-adrenal (HPA) activation by stress. At 2 h after the onset of the light cycle, each animal was brought from the housing room into the procedure room. Prestress tail blood samples were quickly collected by tail clip, with the sampling completed within 3 min of first disturbing the rat to ensure assessment of prestress plasma ACTH and corticosterone (53). Rats were then placed immediately into the bottom of a large, unfamiliar Plexiglas cylinder (~25 cm in diameter and lined with excretory paper) for 5 min, after which the animal was returned to the home cage. Additional tail blood samples were quickly collected at 15, 30, 60, and 120 min from the unrestrained rat and returned to the home cage.

Forced-swim test. A modified FST was conducted during week 10 to assess depressive-like behavior. At 3 h after the start of the light cycle (13), rats were placed into a Plexiglas cylinder (45 cm high and 20 cm in diameter) filled with 31 cm of water (23–25°C) for 10 min. Each session was videotaped, and behavior was later scored by two independent observers blind to the treatment conditions. In brief, behavior was scored every 5 s, and the total count of each behavior during the 10-min testing session was summed for each animal and averaged within each treatment group. The behaviors scored are defined as follows: 1) mobility, i.e., either climbing/rapid movement of limbs in and out of the water with the body parallel to the apparatus or swimming/moving limbs in an active manner and making circular movements around the apparatus; and 2) immobility, i.e., rat making minimal movements to keep its nose above water or floating in the...
water without struggling. Because the data collected represented a continuous parametric variable with a normal distribution, we used a Pearson’s correlation coefficient as an assessment of inter-rater reliability based on individual raw scores from each rater. That test yielded a Pearson’s correlation of 0.934, indicating high reliability between the two raters. The average of their scores was used for the graphs and further analysis.

**AM and PM nonstress blood collections.** During week 10, tail blood was collected within 1 h after light onset (AM) and 2 days later at 45 min before dark onset (PM). The animals were not disturbed for 18 h prior to each bleed, and blood samples were collected quickly and quietly by trained personnel to minimize stress, as described previously (53).

**Insulin, lipids, and plasma hormone assays.** Insulin was measured from plasma taken at the zero time point from the oral GTT. Insulin was measured using ELISA (Crystal Chem, Downers Grove, IL). Cholesterol and triglycerides were measured from plasma taken after an 8-h fast. Blood was cold centrifuged, and plasma was stored at −80°C until assessment. Cholesterol and triglycerides were measured via colorimetric assays using Infinity Reagents (Thermo Fisher Scientific, Waltham, MA). Corticosterone was measured by 125I radioimmunoassay (RIA) kits from ICN Biochemicals (Cleveland, OH).

For each assay performed, control samples with known concentrations of hormone (usually low, normal, and high; provided by the manufacturer) were included to assess performance and reliability. The corticosterone assay has an intra-assay coefficient of variation (CV) of 8.6%, an interassay CV of 13.6%, and a minimum sensitivity of 12.5 ng/ml. The cytokines were measured using a multiplex assay (no. RADPK-81K; Millipore).

**Novel environment stressor.** During week 12, animals were exposed to a final novel stressor and then euthanized 90 min afterward to determine the brain regions activated by the stressor. At 3 h after the onset of the light cycle, each animal was placed into a small, clean mouse cage with the lid in place for 15 min to provide a novel stressor. Ninety minutes after the onset of the stress, rats were given lethal injections of pentobarbital sodium (1 mg/g body wt), and a vaginal lavage was done, followed by cardiac puncture and removal of one ovary. The animal was then transcardially perfused with normal saline followed by 4% paraformaldehyde in PBS (pH 7.4). Brains were collected for c-Fos immunohistochemistry, postfixed at 4°C for 24 h in 4.0% paraformaldehyde, and then transferred to 30% sucrose-PBS. At the time of euthanization, tissues were extracted and weighed as indices of chronic HPA axis tone (right and left adrenals, spleen and thymus).

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Fig. 1. Metabolic parameters postimplant. **A:** body weights of placebo and dihydrotestosterone (DHT)-treated rats on low- (LFD) or high-fat diet (HFD); n = 12–13. Main effect of group and time, repeated-measures 2-way ANOVA. Effect of diet: #P < 0.05, ##P < 0.01. Effect of drug: ***P < 0.001. **B:** cumulative food intake during study. Main effect of group and time and significant interaction of group × time by repeated-measures 2-way ANOVA. Each time point was evaluated by 1-way ANOVA with Bonferroni post hoc test. *Time = 4–10 wk.* Control (CON)-LHD vs. DHT-HFD; *time = 5–10 wk.* CON-HFD vs. DHT-HFD. **C:** fat mass as measured by NMR, 2-way ANOVA. Main effect of diet (a vs. b) and drug (c vs. d). D: lean mass as measured by NMR, 2-way ANOVA. Main effect of diet (a vs. b). Differing letters denote statistical differences between treatment groups; n = 12–13/group. Data are presented as means ± SE.

**E:** % animals categorized as either normally cycling, irregularly cycling, or acyclic from week 4. **F:** merged data from weeks 4 and 11 and % animals found on each day of the cycle over the 10-day time frame.
**c-Fos immunohistochemistry.** Brains were sectioned on a freezing/sliding microtome at 30 μm and collected into cryoprotectant [30% ethylene glycol, 30% sucrose in 0.1 M NaPO4 (~20°C)]. Free-floating sections were rinsed in 0.1 M PBS. Endogenous peroxidases were inactivated using 3% H2O2 in methanol (10 min). Sections were preincubated in blocking buffer consisting of 0.1 M PBS with 4% normal goat serum (NGS) in 0.4% Triton X-100 for 1 h. Primary c-Fos antibody (no. sc-52, 1:2,500; Santa Cruz Biotechnology, Santa Cruz, CA) was diluted in 4% NGS and 0.4% Triton X-100 in 0.1 M PBS and incubated overnight. Following several 0.1 M PBS washes, tissues were incubated in biotinylated goat anti-rabbit IgG (1:300, no. BA1000; Vector Laboratories, Burlingame, CA) in blocking buffer consisting of 0.1 M PBS with 4% NGS in 0.4% Triton X-100 for 1 h. After several 0.1 M PBS washes, tissues were incubated in ABC (1:300, no. PK6100, Vectastain; Vector Laboratories) for 1 h. Antigens were visualized with 3,3-diaminobenzidine enhanced with nickel chloride.

**RESULTS**

**DHT treatment causes increased body weight, food intake, and lean and fat mass.** DHT-treated animals had significantly greater body weight starting 10 days after pellet placement (P < 0.001; Fig. 1A). This was accompanied by greater food intake by DHT-treated animals starting 3 days postimplant (P < 0.01; Fig. 1B). Those animals eating a HFD also consumed more starting 3 days postimplant (P < 0.001). With respect to fat mass, there was a main effect of both diet (P < 0.001) and hormone treatment (P < 0.001), as well as a significant interaction between the two (Fig. 1C), with HFD animals having greater fat mass. DHT-treated animals had greater lean mass than placebo-treated animals (Fig. 1D).

**DHT treatment causes abnormal cyclicity irrespective of diet.** DHT-treated animals lavaged during week 4 exhibited complete loss of estrous cyclicity and remained consistently in the diestrus phase. Seventy-five percent of placebo-treated animals displayed normal cyclicity (linearly moving through the phases, metestrus, diestrus, proestrus, and estrus), whereas 25% displayed out-of-order vaginal cytology (Fig. 1E). We did not continuously cycle animals because of the various other testing the animals underwent. We did cycle the animals a second time at the end of the study, during week 11. We merged the week 4 and week 11 data in terms of the day of the estrus cycle to show the percentage of days they were found in each phase. Because the DHT-treated animals were found in the continuous diestrus phase, the placebo-treated animals spent considerably more time in each of the other phases than the DHT-treated animals. (P < 0.001) for all stages (Fig. 1F).

**DHT with HFD causes impaired glucose tolerance and insulin resistance.** An oral GTT, performed at week 7, showed impaired glucose tolerance in DHT-treated animals, which was worse in DHT-HFD animals (P < 0.05; Fig. 2A). The glucose area under the curve (AUC) demonstrated elevations based on drug treatment (P < 0.05) for DHT-treated animals (Fig. 2B). Fasting plasma insulin showed no statistical differences (Table 1). An ITT performed in week 11 demonstrated insulin resistance in DHT-HFD animals (P < 0.05; Fig. 2C). Fasting glucose levels (t = 0; Fig 2C) were significantly elevated for DHT-HFD animals by this time (P < 0.05).

**DHT treatment causes increased anxiety, and HFD causes increased depression-like behaviors.** Animals performed in the EPM for a 5-min interval following the onset of the dark phase during week 5. DHT-treated animals showed greater anxiety-
Table 1. Circulating hormones measured in the plasma

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<th>2-Way ANOVA Statistics</th>
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<td>HFD</td>
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<td>Triglycerides, mg/dl</td>
<td>131.3 ± 28.3</td>
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<td>134.6 ± 16.5</td>
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<td>Cholesterol, ng/ml</td>
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<td>64.2 ± 2.9</td>
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<td>TNF, pg/ml</td>
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<td>IL-6, pg/dl</td>
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<td>IL-1β, pg/dl</td>
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<td>141.8 ± 31.1</td>
<td>77.3 ± 25.5</td>
<td>159.4 ± 34.8</td>
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Data are presented as means ± SE; n = 12–13/group. CON, control; DHT, dihydrotestosterone; LFD, low-fat diet; HFD, high-fat diet; NS, not significant.

like behavior, as evidenced by spending significantly less time in the open arms [P (drug) < 0.05; Fig. 3A] and traveling less distance in the open arms [P (drug) < 0.01; Fig. 3B]. No differences emerged in entrances to the open arms or in the latency to enter the open arms (data not shown). A modified FST was performed in week 10 to assess depressive-like behavior. HFD animals demonstrated greater depressive-like behavior with increased immobility [P (diet) < 0.05; Fig. 3C] compared with LFD animals.

DHT causes blunted corticosterone response to stress. Basal morning and evening plasma corticosterone levels were lower in DHT-treated animals [P (drug) < 0.01], with HFD animals having elevated morning levels compared with LFD animals [P (diet) < 0.05; Fig. 3D]. During week 6, a novel environment stress test demonstrated a blunted corticosterone response in DHT-treated animals at 15 [P (drug) < 0.0001], 30 [P (drug) < 0.0001], and 60 min [P (drug) < 0.01] (Fig. 3E). The AUC was lower in both DHT groups compared with controls [P (drug) < 0.0001; Fig. 3F].

Stress induces differences in c-Fos immunoreactivity. Animals were exposed to one final novel environment test prior to being euthanized, and brains were processed for immediate early gene c-Fos. c-Fos immunohistochemistry revealed differences in c-Fos activation between groups. c-Fos-positive cells were counted in the PON, SCN (not shown), PVN of the hypothalamus (Fig. 4A), VMH (Fig. 4B), and ARC of the hypothalamus (Fig. 4C). A significant difference was found in the PVN, where greater c-Fos expression occurred in HFD-fed

Fig. 3. Behavioral measures of anxiety and depression and the stress axis. A: %time spent in open arms of the elevated plus maze; 2-way ANOVA. Main effect of drug (a vs. b). B: distance travelled in open arms of the elevated plus maze; 2-way ANOVA. Main effect of drug (a vs. b). C: immobility counts during forced-swim test; 2-way ANOVA. Main effect of drug (a vs. b); D: plasma corticosterone levels within 2 h of lights on (AM) and 1 h prior to dark onset (PM); 2-way ANOVA. Main effect of diet (a vs. b); main effect of drug in the AM measurements (c vs. d); main effect of drug in the PM measurements (e vs. f). E: plasma corticosterone levels during acute novel environment test; 2-way ANOVA, repeated measures with 1-way ANOVA with Bonferroni post hoc. **P < 0.01 and ***P < 0.001, control-LFD vs. DHT-LFD and DHT-HFD; time = 15 and 30 min. F: Area under the curve for measurements in E. Main effect of drug by 2-way ANOVA (a vs. b). Differing letters denote statistical differences between treatment groups; n = 12–13/group. Data are presented as means ± SE.
animals \( P \) (diet) < 0.01; Fig. 4A]. Compared with placebo-treated controlled animals, DHT-treated animals showed reduced c-Fos expression in the VMH \( P \) (drug) < 0.01; Fig. 4B and ARC \( P \) (drug) < 0.05; Fig. 4C.

**Organ weights are affected by DHT treatment.** At the time of euthanization, despite DHT-treated animals having greater body weights than controls (Fig. 1A), they had smaller adrenal glands \( P \) (drug) < 0.0001; Fig. 5A and thymus glands \( P \) (drug) < 0.0001; Fig. 5B both by raw weight (not shown) and as a percent of body weight (as shown). Spleens were smallest in DHT-HFD animals, with an effect of both drug \( P \) < 0.0001 and diet \( P \) < 0.001 (Fig. 5C). DHT also affected reproductive organ size, with DHT-treated animals having both smaller uteri \( P \) < 0.0001 (Fig. 5D) and ovaries \( P \) < 0.0001 when normalized to body weight (Fig. 5E). Interestingly, diet also had an effect on the ovaries, with HFD causing decreased weight compared with LFD \( P \) < 0.05.

**DHT and HFD affect lipids and inflammation.** Serum fasting triglyceride and cholesterol levels were highest in DHT-HFD animals \( P \) < 0.01; Table 1. Serum IL-1β levels were greater in HFD-fed animals than in LFD-fed animals \( P \) (diet) < 0.01; Table 1. No differences were observed in serum TNFα or IL-6 levels.

**DISCUSSION**

The etiology of PCOS remains unknown, with several human phenotypes characterizing the disorder. Given that PCOS has a known association with hyperandrogenism but is present in both lean and obese women, we sought to distinguish what metabolic and behavioral manifestations of the condition are related to diet exposure and the resulting differences in body weight. We hypothesized that increased saturated fat consumption and obesity would exacerbate the overlying disease-related manifestations of hyperandrogenism alone. We found that DHT-treated animals gained more lean body weight and were glucose intolerant independently of diet. DHT-HFD animals had the highest levels of fat mass, insulin resistance, and dyslipidemia.

Whereas the hyperandrogenism in both the LFD and HFD groups created several physiological effects, including increased body weight, lean mass, and fat mass and increased anxiety-like behavior, HFD did exacerbate some effects of DHT. DHT plus HFD worked synergistically to create the highest body weight, greatest fat mass, greatest food consumption, worst glucose tolerance, insulin resistance, and dyslipidemia. This is consistent with human data, which has shown
that obesity exaggerates the degree of hyperandrogenism in women with PCOS (26, 31). Additionally, obesity exaggerates insulin resistance in PCOS (48, 50), consistent with our findings. However, hyperandrogenism alone results in greater glucose intolerance, as demonstrated by oral GTT. Together these data suggest that hyperandrogenism induced by DHT treatment may be responsible for a component of the metabolic symptoms of PCOS even without obesity and may be why lean PCOS women still exhibit significant insulin resistance, but undoubtedly, obesity exaggerates the metabolic symptoms induced by the hyperandrogenemia.

With regard to stress behaviors, DHT animals demonstrated increased anxiety-related behavior as manifested by reduced time in the open arms in the elevated plus maze and had dampened stress responsivity to a mild stressor. Following a different mild stressor, brains of DHT animals showed attenuated stress-induced c-Fos activation in the VMH and ARC; irrespective of DHT-treatment, however, all HFD animals had elevated PVN c-Fos activation. DHT additionally led to elevated levels of inflammatory markers, with other inflammatory marker elevation due to HFD. Overall, these suggest an overall dampened stress responsivity with hyperandrogenism.

Women with PCOS have increased rates of anxiety and depression (28, 29, 39) as well as an altered HPA response to a stressor (6, 22). However, obesity is a risk factor for depression and anxiety (35, 58). Therefore, we sought to clarify whether these PCOS-related manifestations are related specifically to the hyperandrogenism or the obesity. This PCOS rat model allowed us to investigate this potential relationship without the confounding psychosocial effects of the condition that cannot be separated from women with PCOS and are often suggested as the culprit of these associations. We used the elevated plus maze to test anxiety-related behaviors and found significantly greater anxiety-related behavior in the DHT-treated animals compared with controls. This suggests a link between hyperandrogenism and anxiety, which is consistent with some human studies (39, 55) but not with others (27). To examine depressive-like behavior, we used a modified FST and found that those on HFD had greater depressive-like behavior with increased immobility. We also found that hyperandrogenism had a significant impact on the HPA axis. DHT-treated animals demonstrated lower basal (AM and PM) corticosterone levels. Furthermore, the DHT-treated animals had smaller adrenal glands, corresponding with decreased basal HPA tone, which has been reported previously (38).

During the novel environment test, DHT-treated animals had a dampened corticosterone response. This is consistent with the reduced anatomic localization in the brain of c-Fos (both VMH and the ARC) to the acute stressor. However, within the PVN (a major site of integration of metabolic and stress stimuli), HFD diet enhances the activation of these neurons. Furthermore, the DHT-treated animals had smaller adrenal glands, corresponding with decreased basal HPA tone, which has been reported previously (38). Overall, these data suggest that in
PCOS, hyperandrogenism plays a greater role in the anxiety symptoms. In this animal model, the DHT treatment dampened the HPA axis response, and there was no apparent interaction between diet and treatment. Taken together, the data suggest that a significant component of the psychological symptoms of PCOS is directly attributable to hormone imbalance (hyperandrogenia).

CONCLUSIONS

Taken together in our rodent model of DHT-induced PCOS, hyperandrogenism drives overall body weight gain, glucose intolerance, anxiety behaviors, and stress responsivity. Palatable HFD consumption, however, exacerbates adiposity, insulin resistance, and depressive behaviors. Therefore, in treatment and characterization of human PCOS, stratifying by metabolic profiling and appropriate dietary intervention may be beneficial in managing the constellation of reproductive, metabolic, and psychological aspects of PCOS.

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DISCLOSURES

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AUTHOR CONTRIBUTIONS


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