Bitter Taste Receptor Ligand Improves Metabolic and Reproductive Functions in a Murine Model of PCOS

Sheng Wu,1 Ping Xue,1 Neile Grayson,2 Jeffrey S. Bland,2 and Andrew Wolfe1

1Division of Endocrinology, Department of Pediatrics, Johns Hopkins University School of Medicine, Baltimore, Maryland 21287; and 2Kindex Pharmaceutical, Bainbridge Island, Washington 98110

ORCID numbers: 0000-0003-2944-0475 (S. Wu); 0000-0003-1120-1679 (A. Wolfe).

Polycystic ovary syndrome (PCOS) results from functional ovarian hyperandrogenism due to dysregulation of androgen secretion. Cultured theca cells from polycystic ovaries of women with the most common form of PCOS overexpress most androgen producing enzymes, particularly CYP450c17. In this study, a murine model was used of PCOS induced by chronic feeding with a high-fat diet that exhibits the reproductive, hyperandrogenic, and metabolic constellation of PCOS symptoms seen in women. Oral administration of KDT501, a hops-derived bitter taste receptor (Tas2R 108) isohumulone ligand resulted in resolution of PCOS-associated endocrine and metabolic disturbances and restored reproductive function. Pioglitazone, a PPARγ agonist, also improved metabolic and reproductive function, though not to the same degree as KDT501. Specifically, treatment of the murine PCOS model with KDT501 resulted in reduced testosterone and androstenedione levels in the absence of significant changes in LH or FSH, improved glucose tolerance and lipid metabolism, and reduced hepatic lipid infiltration and adiposity. There was significant improvement in estrous cyclicity and an increase in the number of ovarian corpora lutea, indicative of improved reproductive function after exposure to KDT501. Finally, ex vivo exposure of murine ovaries to KDT501 attenuated androgen production and ovarian expression of CYP450c17. Interestingly, the ovaries expressed Tas2R 108, suggesting a potential regulation of ovarian steroidogenesis through this chemosensory receptor family. In summary, a therapeutic strategy for PCOS possibly could include direct influences on ovarian steroidogenesis that are independent of gonadotrophic hormone regulation. (Endocrinology 160: 143–155, 2019)

Female reproductive function is acutely sensitive to the external environment but is also exquisitely responsive to the status of the internal hormonal and metabolic milieu. Modest disruptions in the timing or magnitude of the dynamic hormone levels can negatively affect female reproductive function (1, 2).

The female reproductive hormone axis controls follicular development and ovulation of an ovum from the ovary. Pulsatile GnRH secretion from the hypothalamus regulates the secretion of the pituitary gonadotropins, LH, and FSH from the gonadotroph cells of the anterior pituitary. FSH stimulates follicular development, and the preovulatory surge of LH stimulates the mechanical event of ovulation. The joint action of these two pituitary hormones, each regulating a different ovarian cell type, describes the two-cell, two-gonadotropin hypothesis for estrogen biosynthesis (3). Specifically, LH stimulates the theca cells of the ovary and drives synthesis of androgens, predominantly androstenedione, by stimulating expression of cytochrome P450 family 17 subfamily A member 1 (CYP17A1), the rate-limiting enzyme for androgen synthesis. Androgens produced by the theca cell can diffuse freely to the ovarian granulosa cell and can be converted to estradiol under the control of the aromatase.
enzyme encoded by the Cyp19 gene. Cyp19 expression in the granulosa cell is regulated by FSH. Reproductive dysfunction can occur if pathology occurs at any level of the hypothalamic-pituitary-gonadal axis.

Metabolic status has a profound effect on reproductive function in all species. In mammals, caloric deprivation can rapidly affect neuroendocrine activity and ovarian function, specifically by reducing the activity of the GnRH pulse generator via central sensing of peripheral energy status (1, 2, 4, 5). Reduced GnRH secretion results in attenuated secretion of the pituitary gonadotropins, leading to impaired reproductive cyclicity, reduced ovulatory capacity of the ovaries, and infertility (6–8).

In recent years, it has become increasingly clear that states of positive energy balance can also contribute to reproductive dysfunction in women. Obesity, and the associated comorbidities of insulin resistance and type 2 diabetes, are associated with menstrual irregularities, including amenorrhea/oligomenorrhea, lower rates of success in in vitro fertilization, and increased risk of miscarriage (9–12). Animal models of diet-induced obesity (DIO) have shown a clear link between obesity and female infertility (13–16). Our group showed that female mice eating a 60% HFD for 3 months develop acyclicity and infertility associated with increased LH secretion from the pituitary (14, 17, 18), and insulin stimulated androgen synthesis from the theca cells of the ovary (13). The metabolic and reproductive features of this mouse model recapitulate the type B form of polycystic ovary syndrome (PCOS) associated with hyperandrogenemia and infertility but not polycystic ovaries (19). These studies demonstrated that, at least in part, the hyperandrogenemia and infertility that developed in females was a consequence of the compensatory hyperinsulinemia of whole-body insulin resistance.

Extracts from hops (Humulus lupulus) have been widely used as flavoring agents in brewing. Mixtures of hop extracts exhibited antidiabetic effects (20, 21) and could prevent obesity and reduce systemic inflammation in mice fed a HFD (22, 23). We developed KDT501, a stereochemically pure, substituted 1,3-cyclopentadione (24), chemically derived from hop extracts and assigned the correct stereochemistry. KDT501 retains the beneficial metabolic properties of hops, including reducing systemic inflammation and postmeal triglyceride levels in male and female human subjects (25). In this study, we evaluated the effects of KDT501 in a rodent model of PCOS induced by chronic female obesity. We hypothesized that KDT501 would improve the metabolic profile of obese female mice and would result in improved reproductive function. Although we did observe an improvement in metabolic features of obese females treated with KDT501, our data suggest that the effects are not all secondary to improved metabolic status, but may, in fact, be the result of a direct effect of KDT501 at the level of the ovary.

**Materials and Methods**

**DIO in mice**

At 7 weeks of age, female mice on a mixed-strain background (129X1/SvJ, C57BL/6J, CD1) were fed and maintained on a HFD for 11 weeks before and throughout the course of study. In parallel, a group of normal chow–fed mice were maintained. In the HFD, 60% of kilocalories were from fat with an energy density of 5.24 kcal/g (D12492: 60% fat, 20% carbohydrates, 20% protein; Research Diets, New Brunswick, NJ). Wood-chip bedding was maintained in these cages so that mice would not supplement their diet by eating the standard corn cob bedding. Mice were weighed every week. In the regular chow diet (Teklad Global, Envigo, Madison, WI; 24% calories from protein, 18% calories from fat and 58% calories from carbohydrates, and an energy density of 3.1 kcal/g). All procedures were approved by the Johns Hopkins Animal Care and Use Committee and performed under standard light and dark cycles. The experimental design is illustrated in Fig. 1.

**Oral gavage to HFD-fed mice**

At 80 days after introduction to HFD, the test compounds were administered orally to the mice once a day. Mice were randomly allocated on the basis of body weight to three groups (n = 10 to 14 mice in each group). KDT501 (KDT; 100 mg/kg), pioglitazone (PIO; 30 mg/kg), and vehicle [VEH; 0.5% methylcellulose and 0.2% Tween 80 (w/v)] were administered by oral gavage (in 150-μL volume). In parallel, a group of chow-fed mice was administered VEH daily (Chow-VEH).

**Glucose and insulin tolerance tests**

One month after drug treatment, mice were tested for glucose tolerance by intraperitoneal-glucose tolerance test (IP-GTT). The mice were fasted overnight for 16 hours, blood was collected through the tail vein and baseline blood glucose levels were determined, the test compound was administered, and blood was collected again after 15, 30, 60, and 120 minutes. The area under the curve (AUC) was calculated to determine glucose tolerance. Insulin tolerance tests were performed similarly with the addition of insulin (0.75 U/kg) to the test compound. Glucose and insulin levels were determined at baseline and at 15, 30, 60, and 120 minutes after the injection of the test compound. The AUC for glucose and insulin levels was calculated to determine insulin sensitivity. The results were analyzed by one-way analysis of variance (ANOVA) with Tukey’s multiple comparisons test. The significance level was set at p < 0.05. The results are presented as mean ± SEM.

**Figure 1.** Timeline of the in vivo study. Female mice, 7 wk old, were placed on a HFD regimen. They were cycled at the beginning of the dietary regimen to assess normal estrous cyclicity. Only cycling mice were further evaluated. Mice were maintained on the HFD throughout the study. Chow-fed controls were analyzed at the same ages as their HFD counterparts. Mice were then evaluated for estrous cyclicity, LH, FSH, insulin, leptin, and IL-6 levels, glucose metabolism, and insulin sensitivity, and then euthanized and tissues collected for further analysis. GTT, glucose tolerance test; ITT, insulin tolerance test.
level was measured using an OneTouch Ultra glucometer. A dose of 20% dextrose was injected IP (2 g/kg body weight). Blood glucose samples were measured at 15, 30, 60, 90, and 120 minutes. One week after IP-GTT, the mice were tested for whole-body insulin sensitivity by insulin tolerance test. Mice were fasted for 7 hours, blood was collected through the tail vein, and baseline blood glucose level was measured as described. Insulin, 0.3 U/kg, was injected IP. Blood glucose levels of each mouse were measured at 15, 30, 60, 90, and 120 minutes. Area under the curve (AUC) was calculated for both the IP-GTT and the ITT as previously described (26, 27).

**Quantitative RT-PCR**

The relative mRNA levels of Cyp17a1 in ovarian explants were examined by quantitative RT-PCR using the CFX Connect Detection System (Bio-Rad). Total RNA was prepared using TRIzol reagent (Invitrogen), and cDNA was synthesized using the iScript cDNA Synthesis Kit (Bio-Rad). Relative cDNA levels were determined using SYBR Green Master Mix Solution (Bio-Rad). Cyp17a1, Cyp19, and 18S mRNA levels were quantified with primers listed in Table 1. PCR conditions were optimized to generate >95% PCR efficiency. Cycle threshold (Ct) was obtained for each sample. A corrected Ct was calculated by

**Hormonal assays**

To measure serum levels of LH, FSH, insulin, and leptin, serum samples were collected from diestrus mice via mandibular bleeding. Samples were obtained between 9:00 AM and 10:00 AM for 15 consecutive days beginning 10 days after the HFD and 10 days after drug treatment. Vaginal cells were collected via saline lavage and then fixed with 100% ethanol and stained with the DIFF Quick Stain Kit (IMEB, San Marcos, CA). Stages were assessed on the basis of vaginal cytology (28): Predominant cornified epithelium indicated the estrus stage, predominant nucleated cells indicated the proestrus stage, both cornified and leukocytes indicated the metestrus stage, and predominant leukocytes indicated the diestrus stage. The number of completed cycles in 15 days was calculated for each mouse and averaged across groups.

**Estrous cycle analysis**

Vaginal cytology was assessed daily between 9:00 AM and 10:00 AM for 15 consecutive days beginning 10 days after starting the HFD and 10 days after drug treatment. Vaginal cells were collected via saline lavage and then fixed with 100% ethanol and stained with the DIFF Quick Stain Kit (IMEB, San Marcos, CA). Stages were assessed on the basis of vaginal cytology (28): Predominant cornified epithelium indicated the estrus stage, predominant nucleated cells indicated the proestrus stage, both cornified and leukocytes indicated the metestrus stage, and predominant leukocytes indicated the diestrus stage. The number of completed cycles in 15 days was calculated for each mouse and averaged across groups.

<table>
<thead>
<tr>
<th>Table 1. Primer Sequences</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gene</td>
</tr>
<tr>
<td>------</td>
</tr>
<tr>
<td>Cyp17</td>
</tr>
<tr>
<td>Cyp19</td>
</tr>
<tr>
<td>TstR108</td>
</tr>
<tr>
<td>18S</td>
</tr>
</tbody>
</table>

**Figure 2.** (A) Female mice were fed an HFD (60% calories from fat) or chow (18% calories from fat) for 80 d. HFD-fed mice were treated with VEH (HFD-VEH; black triangles), KDT (HFD-KDT; 100 mg/kg; red circles), or pioglitazone (HFD-PIO; 30 mg/kg; green squares). The Chow-VEH group is indicated by inverted blue triangles. The weight of the mice (in grams) in each group was assessed for 8 wk. Chow-VEH mice weighed significantly less than all HFD groups. (B) Data are presented as change in weight (weight gain) from week 0 over the 8-wk period. (A, B) Data are from repeated measures one-way ANOVA; n = 5 to 14 mice. *P < 0.05 for HFD-PIO vs HFD-VEH and HFD-KDT mice; **P < 0.05 for HFD-VEH vs Chow-VEH and HFD-KDT.
subtracting the 18S Ct from the relevant gene Ct for each sample. Relative differences from the VEH-treated sample were calculated by using the following formula: fold change = 2^(control Ct minus sample Ct). The presence of Tas2r108 mRNA was detected using PCR with primers shown in Table 1. RNA was treated with DNase before amplification. As a negative control, RNA not treated with reverse transcription was used. As a positive control, genomic DNA was used. The Tas2r108 gene is intronless.

**Ovarian morphology**

Ovaries were collected and placed immediately in 10% PBS-buffered formalin. Ovaries were sectioned to a thickness of 5 μm, every 10th section was collected for a total of five sections collected, and sections were stained with hematoxylin and eosin (H&E) by the Johns Hopkins Molecular and Comparative Pathobiology Phenotyping Core. Five sections from each animal were examined and the highest number of corpora lutea (CL) counted on an individual slide were averaged for each group.

**Ex vivo ovarian culture**

An ovarian ex vivo preparation was used as previously described (13). Briefly, 10 wild-type mice (129X1/SvJ, stock no. 000691; Jackson Laboratory) were euthanized and isolated ovaries were incubated in a 24-well tissue-culture plate with tissue-culture well inserts (Millicell-CM, 0.4-mm pores; EMD Millipore) with McCoy’s 5A medium. Medium was replaced after 3 hours of incubation and one ovary was incubated with KDT (25 μM) and the other ovary from the same mouse was incubated with VEH for 24 hours as a control. Medium was collected after 24 hours and assayed for androstenedione; ovaries were collected and frozen at −80°C until RNA isolation was performed.

**Liver histology**

Liver tissues were collected and placed immediately in 10% formalin. The 5-μm sections were stained with H&E by the Johns Hopkins Molecular and Comparative Pathobiology Phenotyping Core. Every 10th section was analyzed, for a total of five sections per animal. Quantification of the area of fat in liver tissue was measured with ImageJ software (https://imagej.nih.gov/ij/) and compared across groups.

**Statistical analysis**

All data were analyzed using GraphPad, version 5 (Prism Software, La Jolla, CA) and are expressed as mean ± SEM. Significance was determined via unpaired two-tailed Student t test, one-way ANOVA, or one-way ANOVA with repeated measures with the appropriate post hoc tests. *P* ≤ 0.05 was considered statistically significant.

**Results**

**KDT attenuated weight gain in HFD-fed mice**

Mice fed an HFD were significantly heavier than chow-fed controls at the start of drug treatment [HFD (n = 11), 35.41 ± 1.012 g; chow (n = 5), 21.64 ± 0.3140 g;...
independent of treatment. Mice fed an HFD and treated with VEH (HFD-VEH) gained approximately 4 g of weight during the 8-week drug treatment period (Fig. 2). Chow-VEH mice of the same age did not gain weight over the 8-week study (Fig. 2). There was a significant difference in weight gain between HFD-fed mice treated with PIO (HFD-PIO) or HFD-VEH. HFD-fed, KDT-treated (HFD-KDT) mice exhibited no weight gain over the 8 weeks, and their change in weight was significantly attenuated when compared with PIO- or VEH-treated mice (Fig. 2B).

Glucose and insulin tolerance in HFD-fed mice
To begin to assess the effects of drug treatment on glucose metabolism in DIO mice, IP-GTT tests were performed (Fig. 3A). It is well established that glucose tolerance is impaired in DIO female mice compared with chow-fed female mice (17, 30) and this was observed when comparing glucose tolerance between HFD-VEH mice and Chow-VEH mice (Fig. 3A). Treatment with KDT or PIO resulted in improved glucose tolerance relative to HFD-VEH (Fig. 3A). When AUC data were calculated for each mouse, there was a significant improvement in glucose tolerance in HFD- KDT mice.
relative to HFD-VEH controls, but did not improve glucose tolerance to levels seen for Chow-VEH (Fig. 3). Although glucose tolerance improved, the change in glucose tolerance was not significant after treatment with PIO (Fig. 3B).

Whole-body insulin sensitivity was assessed by an insulin tolerance test with glucose levels measured after an injection of insulin (Fig. 3C). Here, we recorded whole-body insulin resistance when comparing HFD-VEH mice with Chow-VEH mice (Fig. 3C and 3D). The AUC was calculated and findings are displayed in Fig. 3D. HFD-PIO and HFD-KDT mice exhibited significantly improved insulin sensitivity relative to HFD-VEH mice (Fig. 3C and 3D).

**KDT and PIO attenuated fatty liver in HFD-fed mice**

Chronic feeding of an HFD increases fat accumulation in the liver in female mice (31). H&E staining of livers clearly revealed lipid accumulation in HFD-VEH mice (Fig. 4A) relative to Chow-VEH mice (Fig. 4B). HFD-KDT mice had significantly less lipid accumulation than HFD-VEH mice (Fig. 4C and 4E). There was also a significantly lower amount of lipid deposition in HFD-PIO mice than in HFD-VEH mice, although there was a more variable response in the HFD-PIO group (Fig. 4D and 4E).

**Hormone and cytokine profile was not affected by KDT**

Fasting serum insulin and leptin levels were significantly elevated in HFD-VEH mice compared with Chow-VEH mice. Leptin and insulin levels were higher in HFD-KDT and HFD-PIO mice when compared with Chow-VEH controls, although statistical significance was only demonstrated for leptin (Fig. 5A). Levels of insulin and leptin were not significantly different among the HFD-PIO, HFD-KDT, or HFD-VEH groups (Fig. 5A and 5B). Multiple proinflammatory cytokines and chemokines are increased in obese, diabetic mice and may mediate some of the deleterious effects on metabolism and reproduction. Short-term (7-day) KDT treatment of chow-fed mice did not change serum levels of 19 different cytokines measured by Luminex multiple-ligand assay (Fig. 6).

**KDT did not change pituitary gonadotropin levels**

Morning levels of LH (Fig. 7A) and FSH (Fig. 7B) were not significantly altered in HFD-PIO or HFD-KDT mice when compared with HFD-VEH. Because the ratio of LH to FSH can influence ovarian steroidogenesis, we calculated this value; no significant difference was observed among groups (Fig. 7C). Increased levels of circulating androgens are a hallmark feature of PCOS and are observed in the chronic HFD-fed model (2.6-fold higher testosterone in HFD-VEH than Chow-VEH; $P = 0.07$; Fig. 7D). Testosterone levels in HFD-KDT and HFD-PIO mice were reduced relative to HFD-VEH (33% and 12.9%, respectively).

**KDT and PIO increased the number of CL in HFD-fed mice**

Sectioned and stained ovaries (Fig. 7E–7H) were evaluated for numbers of CL. CL develop from follicles that have ovulated and therefore represent the recent ovulatory history of the mouse. Few CL were observed in HFD-VEH mice (Fig. 7E) when compared with Chow-VEH mice (Fig. 7H), as expected, based on our previous studies (13, 14). There was a statistically significant near doubling of the number of CL present in ovaries from HFD-KDT mice (Fig. 7F) and HFD-PIO mice (Fig. 7G) than in HFD-VEH mice (Fig. 7E).

**Estrous cyclicity improved in HFD-KDT and HFD-PIO mice**

We have previously reported that HFD-induced obesity impairs estrous cyclicity in female mice (13, 14). Mice were assessed for estrous cycle stage for 15 consecutive days beginning 10 days after drug or VEH treatment. Figure 8A plots the percentage of mice that completed one, two, or three complete estrous cycles during the 15 days. Of HFD-VEH mice, 37% did not have even one complete estrous cycle. Treatment with PIO resulted in 20% of HFD-fed mice having no cycles, and only 7.1% of HFD-KDT mice had no complete estrous cycles. No HFD-VEH female mice completed three complete estrous cycles, whereas 20% of KDT-treated mice ($n = 2$ of 10) and 21% of PIO-treated mice ($n = 3$ of...
14) completed three complete estrous cycles. All Chow-VEH mice completed at least two cycles, and 40% completed three cycles (Fig. 8A).

In Fig. 8B, the number of cycles each mouse completed during the 15 days of analysis was plotted. HFD-VEH mice had significantly fewer cycles than Chow-VEH mice. The HFD-KDT mice had significantly more complete estrous cycles than did HFD-VEH mice. HFD-PIO mice also had more complete cycles than HDF-VEH mice, but the difference did not achieve statistical significance.

**KDT directly regulated androgen synthesis from isolated ovaries**

To directly assess the effects of KDT on ovarian function, an *ex vivo* isolated ovary preparation was used. Androstenedione secretion from ovaries treated with KDT was significantly reduced compared with ovaries treated with VEH (34% reduction; Fig. 9A). The CYP17 enzyme is rate limiting for androgen synthesis and reduced androstenedione secretion could be due to reduced Cyp17 expression. Relative mRNA levels of Cyp17a1 were significantly lower in the 25-uM KDT-treated ovaries (49% reduction; Fig. 9B). No significant difference in Cyp19 (Fig. 9C) or ribosomal 18S (data not shown) mRNA levels was observed. Recently, a role for KDT signaling via the TAS2R108 bitter taste receptor in gut epithelium has been proposed as a mechanism for stimulated GLP-1 secretion and improved metabolic health in DIO male mice (32). Using RT-PCR, we have detected Tas2r108 mRNA in mouse ovaries (Fig. 9D).

**Figure 6.** Serum cytokine levels in study mice. Luminex assay was used to measure serum levels of (A) GCSF; (B) TNFa; (C) eotaxin; (D) GM-CSF; (E) interferon-γ; (F) IL-1α; (G) IL-1β; (H) IL-2; (I) IL-4; (J) IL-3; (K) IL-6; (L) IL-10; (M) IL-12P70; (N) IL-13; (O) IL-17; (P) KC; (Q) MCP-1; (R) MIP-1α; (S) MIP-1β; and (T) TNFa in female mice treated with KDT (100 mpk) or VEH for 7 d (n = 10). IFNG, interferon-γ; KC, keratinocyte chemoattractant.
Discussion

PCOS is a complex, heterogeneous disorder affecting 5% to 21% of premenopausal women. PCOS is characterized by hyperandrogenism and anovulation or oligoovulation and is frequently associated with hirsutism, obesity, insulin resistance, and type 2 diabetes (33, 34). First-line therapy includes treatment with oral contraceptives to control hyperandrogenemia (35) or clomiphene or letrozole used for fertility treatment (36, 37). Lifestyle modifications and weight loss are effective in improving fertility and may be a result of improved insulin sensitivity (38).

The insulin resistance and hyperinsulinemia often associated with PCOS have been proposed to play a role in contributing to the endocrinopathies associated with the disease (13, 14, 39, 40); thus, insulin-sensitizing drug treatments have been tried as therapeutic options. Results obtained using metformin and the thiazolidinediones (TZDs) have been inconclusive and are not standard of care for fertility treatment of women with PCOS (41).

To explore the complex tissue and hormone interactions underlying the development of infertility in PCOS, our group has developed a mouse model of DIO associated with female infertility that shares many features with type B manifestation of PCOS, including anovulation, acyclicity, high LH level, hyperandrogenemia, and infertility (13, 14, 17). A similar observation has been made in the development of a rat model of PCOS (46). Using this model in combination with genetically modified mouse models, we demonstrated that obesity-induced hyperinsulinemia contributes to neuroendocrine dysfunction and ovarian dysfunction associated with infertility (13, 14, 18).

The TZDs are a class of drug that target PPARγ and are used to treat diseases featuring insulin resistance, including type 2 diabetes. PPARγ is a member of the nuclear hormone receptor family and plays an important role in adipocyte differentiation, lipid metabolism, insulin sensitivity, and glucose homeostasis (47, 48). Improved glucose tolerance and insulin sensitivity were
observed in PIO-treated mice in our study (Fig. 3). However, TZDs, such as PIO or troglitizone, also induce weight gain in humans and rodent models not only by promoting adipogenesis and fluid retention but also by increasing food intake (49–51). These limitations have prompted investigators to seek other therapeutic options.
Previous work had suggested that the stereochemically pure, substituted 1,3-cyclopentadione, KDT could improve glucose tolerance and reduce body fat in male HFD-fed mice (32, 52); however, the impact on metabolic function in female mice had not been explored. We sought to determine if KDT could contribute to improved metabolic status in HFD-fed female mice and concurrently ameliorate reproductive dysfunction. Although HFD-VEH mice in this study gained weight (Fig. 2), it was not to the magnitude we had previously observed (13, 14, 17), suggesting that the oral gavage procedure used to deliver the drug affected feeding behavior. This is supported by the lack of weight gain over the course of 8 weeks observed in Chow-VEH mice (Fig. 2). This could be due to the volume of the drug filling the stomach (which is not likely, because the volume was only 150 μL) or possibly to chronic low-level stress-axis activation due to the gavage process, which reduces weight gain in HFD-fed rodent models (53). This may also explain the lack of weight gain in female mice (Fig. 2), as has been described for males (25). Whether this is a sex-specific difference in the effects of stress or a more general sex difference in drug effects is not clear. However, treatment of female mice with KDT resulted in a significant attenuation of weight gain in those fed an HFD relative to HFD-VEH controls. As demonstrated by Kok et al. (32), this is not due to reduced food intake. The effects of KDT on weight in females closely resembles the effects observed in males (32, 52), suggesting sex difference in the pharmacology of PIO could explain the lack of weight gain in females.

Further evidence for sex-specific differences in the pharmacological impact of PIO is the dramatic difference in hepatic steatosis in HFD-fed male mice vs HFD-fed female mice. In males, there is a significant increase in lipid content in the liver in response to PIO vs VEH treatment (53, 54). However, in our current study, PIO significantly reduced lipid accumulation in the liver relative to VEH treatment in female mice (Fig. 4). As with the effects on weight, KDT had similar hepatic lipid-reducing effects in males (32) and, in our study, females (Fig. 4). Although HFD-fed females are partly protected from developing insulin resistance by estrogen (31, 55), they still exhibit impaired insulin sensitivity when compared with chow-fed females, as shown in our study and in others (17, 31) (Fig. 3).

Both PIO and KDT improved glucose tolerance (Fig. 3A and 3B) and whole-body insulin sensitivity in HFD-fed mice in the current study, as was reported previously for males (32, 52). Improved insulin sensitivity in HFD mice may contribute to the observation that HFD-KDT and HFD-PIO mice in the current study had lower fasting glucose levels than did HFD-VEH mice (Fig. 3C) and may be the reason that fasting insulin levels did not differ (Fig. 5B) between the HFD-fed groups.

One of the salient features of the female, chronic HFD-fed model of infertility was the high LH secretion induced by insulin action at the level of the GnRH neuron and the
pituitary (14, 18). That there was no KDT-induced change observed in LH and FSH levels in our study (Fig. 7) suggests that improved ovulatory capacity could have resulted from direct ovarian response to improved metabolic function resulting from KDT or PIO treatment. Similarly, improved estrous cyclicity (Fig. 8) in HFD-KDT mice may have be due to improved endocrine function of the ovary. We demonstrated the effect was not a result of changes in serum insulin or leptin in response to drug treatment (Fig. 5). In addition, KDT had no effect on serum levels of a wide array of cytokines (Fig. 6), suggesting a direct drug effect on the ovary. Although improved ovulatory function could be secondary to attenuated metabolic dysfunction, we hypothesized there might be functional regulation at the level of the ovary. The ovary is a likely target for KDT, because we saw no differences in neuroendocrine function in response to treatment (Fig. 7A–7C). Although an increase in circulating testosterone levels was measured in HFD-VEH vs Chow-VEH mice (Fig. 7D), as we have previously reported (14), only a modest reduction in testosterone levels was found when HFD mice were treated with KDT or PIO (Fig. 7D). These changes could result from systemic changes in insulin sensitivity or could be due to action directly at the level of the ovary. In mice, the ovary is the sole source of circulating testosterone in females. Unlike most other mammals, the adrenal gland does not produce androgens in the mouse (56). To directly assess the impact of KDT, we performed a study using ovaries cultured ex vivo. Paired ovaries were removed from normal chow-fed mice and one ovary was cultured in KDT and the other treated with VEH. KDT significantly reduced androstenedione secretion from the KDT treated ovary when compared with the VEH-treated ovary (Fig. 9A). This was

Figure 9. KDT inhibits androstenedione secretion from isolated ovaries. (A) Whole ovaries were removed from lean wild-type mice and cultured in vitro for 24 h; one ovary was incubated in KDT (KDT; 25 μM; black bar) and the other was treated with VEH (VEH; open bar). Levels of androstenedione were measured in the media. A total of 10 mice were used; thus, 10 individual ovaries in each group. (B) Relative levels of Cyp17 mRNA in ovaries treated with VEH (open bar) and KDT (black bar). Values are relative to control and are normalized with 18s mRNA levels (n = 10). (C) Tas2r108 expression is observed in the mouse ovary. (D) A 577-bp amplicon from PCR amplification of representative ovary cDNA. Mouse genomic DNA served as a positive control for this intronless gene.
associated with a significant decrease in mRNA levels of Cyp17 (Fig. 9B), the rate-limiting enzyme in androgen synthesis in the theca cells. These effects are not nonspecific, because mRNA levels of the Cyp19 (aromatase) gene were not altered by KDT treatment (Fig. 9C). Reduced androgen synthesis from the ovary may contribute to improved cyclicity and ovulatory function in DIO female mice, as we have previously shown (13).

Recently, a role for KDT signaling via the TAS2R108 bitter taste receptor in gut epithelium has been proposed as a mechanism for stimulated GLP-1 secretion and improved metabolic health in HFD-fed male mice (32). We detected Tas2r108 mRNA in mouse ovaries (Fig. 9D), raising the intriguing possibility that this is a potential mechanism for direct KDT regulation of androgen synthesis.

In summary, we have documented a marked improvement in metabolic function in a DIO mouse model of PCOS after treatment with KDT. Although PIO treatment also improved glucose tolerance and insulin sensitivity, it was not as efficacious as KDT and was far less effective than KDT in ameliorating lipid accumulation in the liver. PIO and KDT improved reproductive cyclicity and ovulatory function in HFD-fed mice. The effects of KDT could be mediated directly at the level of the ovary via the TAS2R108 receptor. The results of this study suggest KDT could represent a therapeutic option for women struggling with the metabolic and reproductive dysfunction associated with PCOS.

Acknowledgments

We thank Dr. Paul Schimmel for his thoughtful evaluation of this manuscript, Dr. Ya Ping Ma for technical assistance, and Dr. Jennifer Pluznick for sharing her expertise in the field of taste receptors.

Financial Support: This study was funded by Kindex Pharmaceuticals (Seattle, Washington).

Correspondence: Sheng Wu, PhD, Johns Hopkins University School of Medicine, 600 North Wolfe Street, CMSC 406, Baltimore, Maryland 21287. E-mail: Swu24@jhmi.edu.

Disclosure Summary: The authors have nothing to disclose.

References


