Western-style diet, with and without chronic androgen treatment, alters the number, structure, and function of small antral follicles in ovaries of young adult monkeys

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Objective: To examine the small antral follicle (SAF) cohort in ovaries of adult rhesus monkeys after consumption of a Western-style diet (WSD), with or without chronically elevated androgen levels since before puberty.
Design: Cholesterol or T (n = 6 per group) implants were placed SC in female rhesus macaques beginning at 1 year of age (prepubertal), with addition of a WSD (high fat/fructose) at 5.5 years (menarche approximately 2.6 years). Ovaries were collected at 7 years of age. One ovary per female was embedded in paraffin for morphologic and immunohistochemical analyses. The SAFs (<2.5 mm) were dissected from the other ovary obtained at or near menses in a subgroup of females (n = 3 per group) and processed for microarray analyses of the SAF transcriptome. Ovaries of adult monkeys consuming a standard macaque diet (low in fats and sugars) were obtained at similar stages of the menstrual cycle and used as controls for all analyses.
Setting: Primate research center.
Animal(s): Adult, female rhesus monkeys (*Macaca mulatta*).
Intervention(s): None.
Main Outcome Measures: Histologic analyses, SAF counts and morphology, protein localization and abundance in SAFs, transcriptome in SAFs (messenger RNAs [mRNAs]).
Result(s): Compared with controls, consumption of a WSD, with and without T treatment, increased the numbers of SAFs per ovary, owing to the presence of more atretic follicles. Numbers of granulosa cells expressing cellular proliferation markers (pRb and pH3) was greater in healthy SAFs, whereas numbers of cells expressing the cell cycle inhibitor (p21) was higher in atretic SAFs. Intense CYP17A1 staining was observed in the theca cells of SAFs from WSD with or without T groups, compared with controls. Microarray analyses of the transcriptome in SAFs isolated from WSD and WSD plus T–treated females and controls consuming a standard diet identified 1,944 genes whose mRNA levels changed twofold or more among the three groups. Further analyses identified several gene pathways altered by WSD and/or WSD plus T associated with steroid, carbohydrate, and lipid metabolism, plus ovarian processes. Alterations in levels of several SAF mRNAs are similar to those observed in follicular cells from women with polycystic ovary syndrome.
Conclusion(s): These data indicate that consumption of a WSD high in fats and sugars in the presence and absence of chronically elevated T alters the structure and function of SAFs within primate ovaries. (Fertil Steril® 2016;105:1023–34. ©2016 by American Society for Reproductive Medicine.)
Key Words: Androgen, metabolism, ovarian function, small antral follicles, Western-style diet

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The disorder polycystic ovary syndrome (PCOS) is a major cause of infertility in women. The main characteristics of women with PCOS include elevated androgen levels, hirsutism, and alterations to the hypothalamic–pituitary–ovarian axis, manifesting as oligo- or anovulation and polycystic ovary morphology (PCOM) (1). The ovarian morphology of many women with a clinical diagnosis of PCOS is multi-follicular; the current guidelines from the Androgen Excess and PCOS Society indicate that $\geq$25 small- to medium-sized (<10 mm) antral follicles (SAFs) in each ovary is sufficient for diagnosis of PCOM by ultrasonography (2). Classically, these SAFs are observed in the periphery of the ovary, leading to the "string of pearls" appearance (3). Growth of these follicles is typically arrested at the small antral stage, and they may persist as cystic structures even after degeneration of granulosa cells lining the interior of the follicle (4).

There is also a high incidence of obesity in women with PCOS, leading to an altered metabolic state, including increased prevalence of insulin resistance and development of metabolic syndrome (5, 6), compared with individuals with a lean PCOS phenotype. Obesity alone imparts a well-documented detrimental impact on a woman's health (7), and research is now focusing on the impact of alterations to the metabolic state on fertility and the contribution of obesity to onset and severity of PCOS phenotype (8).

The mechanism(s) leading to development of a PCOS phenotype are currently unclear and, because of the heterogeneous nature of the disorder, are likely multifaceted. However, exposure of female monkeys and sheep to exogenously elevated androgens in utero can cause a PCOS-like phenotype that manifests in the offspring during adulthood (9, 10). We recently developed a nonhuman primate model to test the hypothesis that a three- to fourfold elevation in circulating T levels, beginning before/during puberty, leads to development of symptoms associated with PCOS in young adults. This model recapitulates the scenario when endogenous T levels begin to rise in adolescent girls at risk for PCOS (1). Female macaques received silastic implants containing cholesterol or T beginning at 1 year of age (11, 12). Chronic exposure to elevated T levels altered hypothalamic–pituitary function by 4 years of age (increased LH pulse frequency), similar to reported alterations observed in adolescents with PCOS, but there was no major effect on ovarian morphology/function in these peripubertal monkeys (11).

Given the strong association between PCOS and metabolism, it was postulated that addition of a Western-style diet (high fat and fructose; WSD) and subsequent changes in metabolism would induce or exacerbate PCOS-like symptoms. Thus, a WSD was fed to these control and T-treated females beginning at 5.5 years of age until the end of the study, 1.5 years later (12). The WSD increased body weight and percent body fat in all females (12, 13). Reduced sensitivity to insulin was observed in females receiving both WSD and T, but not with WSD alone (12). All females displayed reduced levels of E_2 and shorter follicular phases compared with before onset of WSD, but evidence of ovulatory activity was detected in these females according to patterns of E_2 and P secretion (12). Ovarian ultrasonography performed at menstruation observed increased numbers of SAFs in all females when compared with before onset of WSD. Larger numbers of small, non-ovulatory antral follicles ("subordinate antral follicles"; approximately seven) were also detected at late follicular phase in WSD-exposed females compared with those (three to four subordinates) of adult rhesus monkeys consuming a standard diet (14). The size of the largest (putatively dominant) antral follicle was also markedly less in WSD with or without T (WSD±T) animals than in controls (12).

Because of the observed alterations in the ovarian phenotype induced by WSD±T of these macaque females (12), the ovaries were collected from these females to [1] histologically analyze the impact of WSD with and without chronically elevated T on the SAF cohort in the ovary, and [2] characterize alterations to the SAF transcriptome. These data were compared with that obtained from control monkeys in our colony (i.e., adult macaques with regular menstrual cycles receiving a standard low-fat/low-sugar diet, and without chronic elevation of androgens).

MATERIALS AND METHODS

Animals

All procedures were reviewed and approved by the Oregon Health & Science University/Oregon National Primate Research Center (ONPRC) Institutional Animal Care and Use Committee. A cohort of female rhesus monkeys (*Macaca mulatta*; n = 6 per treatment group), detailed in McGee et al. (11, 12), received silastic implants containing either cholesterol or T; the latter maintained a three- to fourfold elevation of circulating T from 1 year of age until the end of the study (prepubertal to adult). From 1 until 5.5 years of age, females consumed a standard diet (15% calories from fat, 27% from protein, 59% from carbohydrates; no. 5000; Purina Mills), supplemented with fresh fruits and vegetables. Then, at 5.5 years of age, these females were transitioned to a WSD (33% calories from fat, 17% from protein, 51% from carbohydrates; 5A1F, Purina Mills) supplemented with high-fructose treats (12, 13), which they consumed until the end of the study (approximately 7 years old).

At the end of the study, WSD ± chronic T exposure females were randomly divided into two subgroups for ovarian collection: either at menses (early follicular phase, days 1–3 of the menstrual cycle; onset of frank menses = day 1, n = 3 per group) or at mid-luteal phase (n = 3 per group). There were no differences in serum E_2 levels between WSD and WSD+T females collected at menses, but WSD+T females had reduced mid-luteal phase serum P levels compared with WSD alone (13). Ovaries were collected from anesthetized females as previously described (15).

One ovary per female was selected for fixation: either randomly from females at/near onset of menses, or the non-corpus luteum–bearing ovary from the luteal phase of the menstrual cycle, and processed as described below (histologic evaluation). Ovaries at these stages of the menstrual cycle were also obtained from adult macaques with regular menstrual cycles (7.3 ± 0.5 years of age; control), either as whole tissue (n = 3) or prestained hematoxylin and eosin