ORIGINAL ARTICLE

Circulating dihydrotestosterone, testosterone, and free testosterone levels and dihydrotestosterone-to-testosterone ratios in healthy women across the menstrual cycle

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Objective: To characterize the circulating androgen levels across the menstrual cycle in healthy women using highly sensitive and accurate methods and report sex differences in the relative levels of dihydrotestosterone (DHT) to testosterone (T) levels.
Design: Prospective cohort study.
Setting: Research clinic, academic teaching hospital.
Patient(s): Twenty-one healthy premenopausal women, aged 19–40 years, with regular menstrual cycles.
Intervention(s): Not applicable.
Main Outcome Measure(s): Serum total T and DHT levels measured using liquid chromatography-tandem mass spectrometry, free T levels measured using a standardized equilibrium dialysis method coupled with measurement of the T levels in the dialysate using liquid chromatography-tandem mass spectrometry, and comparison of the DHT-to-T ratio between healthy women and age-matched healthy men.
Result(s): The serum total and free T levels increased across the follicular phase and peaked at midcycle (total T, 43.6 ± 16.2 ng/dL; free T, 15.6 ± 11.9 pg/mL) and gradually declined in the luteal phase. The DHT level did not significantly change across the menstrual cycle. The DHT-to-T ratios were 1:4 and 1:13 in women and men, respectively.
Conclusion(s): In healthy premenopausal women, the total and free T levels varied significantly across the menstrual cycle, whereas the DHT levels did not change; the peak total and free T levels in the midcycle period were higher than previously reported, underscoring the importance of establishing menstrual phase–specific reference ranges to avoid misdiagnosis of hyperandrogenism. Women have significantly higher DHT levels relative to total T than men; the significance of this sex difference in the DHT-to-T ratio needs further investigation. (Fertil Steril® 2022;■:■–■. ©2022 by American Society for Reproductive Medicine.)
Key Words: Testosterone, dihydrotestosterone, liquid chromatography-tandem mass spectrometry, equilibrium dialysis, menstrual cycle

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Measurements of the circulating testosterone (T) levels in women are important for identifying abnormalities in gonadal and adrenal function in conditions such as polycystic ovary syndrome (PCOS), hirsutism, congenital adrenal hyperplasia, and androgen-secreting tumors. However, accurate measurement of the T levels in women poses significant challenges because women have substantially lower serum T levels than men and these levels progressively decline with age (1, 2). Despite advances in the steroid hormone assay techniques, substantial variability exists among assays, and most commercial radioimmunoassays lack the sensitivity and specificity required to accurately measure the low T levels in women (3). The T levels in women vary substantially during the menstrual cycle (4, 5). The paucity of normative data on the T levels and other androgens across the menstrual cycle has further confounded their interpretation in women. Thus, the development of accurate and sensitive androgen assays is important to define reference ranges and establish thresholds for diagnosing androgen excess and deficiency syndromes in women. An improved understanding of the menstrual phase–specific distribution of the total and free T levels measured using reliable assays will ensure that these cutoffs can be applied appropriately to women in different phases of their menstrual cycle.

Currently, liquid chromatography-tandem mass spectrometry (LC-MS/MS) and equilibrium dialysis are considered the reference methods for the measurement of the total and free T levels, respectively, against which all other methods are compared. Compared with direct immunoassays, these techniques offer the highest sensitivity and specificity to measure the T levels in the low range in women with high accuracy and precision (5, 6). However, these methods require significant expertise, and their results can vary across laboratories owing to methodological and calibrator differences (7). The Centers for Disease Control and Prevention (CDC) has established the Hormone Standardization Program for Testosterone (HoST) to harmonize T assays across laboratories; the hormone levels and reference ranges generated using a HoST-certified assay can be applied to other HoST-certified laboratories (8, 9). Only a few published studies have reported the menstrual phase–specific reference limits in women for total T using the LC-MS/MS technique, and these studies preceded the advent of the HoST (5, 10, 11). Only 1 study used the equilibrium dialysis method to measure the free T levels across the menstrual cycle, but none has measured the free T levels during different phases of the menstrual cycle using equilibrium dialysis coupled with direct measurement of the free T levels in the dialysate using a HoST-certified LC-MS/MS assay (6). Most studies collected samples at only 2 or 3 time points (e.g., follicular, midcycle, and luteal phases); repeated measurements across the entire menstrual cycle would more comprehensively capture the variability in the androgen levels during a menstrual phase.

Dihydrotestosterone (DHT) is a potent 5α-reduced metabolite of T in humans that is typically produced from both T and androstenedione by the action of a class of steroid 5α-reductase enzymes (12). Several women with hirsutism with or without PCOS have normal T levels yet demonstrate increased 5α-reductase activity in ovarian tissue as well as increased serum and urinary 5α-reduced metabolites of T (13). An alternative "backdoor pathway" that involves the 5α reduction of 17α-hydroxyprogesterone and its conversion to 5α-DHT via a biochemical pathway that bypasses T as an intermediate has been proposed as the source of increased DHT production in women with disorders of androgen excess, such as congenital adrenal hyperplasia and PCOS (14–16). Clinically, the DHT-to-T ratio has been suggested by some experts to be potentially useful in the evaluation of female androgen excess disorders although others have questioned its usefulness (17). Dihydrotestosterone is recognized as an important androgen in the skin; the formation of DHT and its metabolite, 3 alpha, 17 beta-androstanediol glucuronide (3-alpha diol G), is increased in vitro in the skin of women with idiopathic hirsutism (18). Thus, the circulating 3-alpha diol G levels have been proposed as a biomarker of increased peripheral androgen action in women with hirsutism. The serum DHT levels are not routinely measured largely because of the inability to measure the DHT levels in women accurately because of the limited sensitivity of the available radioimmunoassays and even some first-generation LC-MS/MS assays (19). In recent years, the LC-MS/MS methods with improved sensitivity and specificity have been developed to measure the DHT levels in the low range in women and minimize interference from other sex steroids (20–22). However, there is a paucity of data on the distribution of the DHT levels during different phases of the menstrual cycle.

The present study aimed to examine the changes in the T and DHT levels across the menstrual cycle in carefully characterized healthy young women using LC-MS/MS assays certified by the HoST with a high level of precision and accuracy in the low range of these hormones prevalent in women. We also measured the free T levels using a standardized equilibrium dialysis method to separate bound and unbound fractions of T and then directly measure the T levels in the dialysate using a sensitive LC-MS/MS assay. We also examined the sex differences in relative levels of total T to DHT levels using these validated techniques.

MATERIALS AND METHODS

Study Participants

We recruited 21 healthy women, aged 19–40 years, with regular menstrual cycles for at least 3 months. Women were excluded if they had a history of irregular menses, symptoms of hyperandrogenism (i.e., hirsutism, androgenic alopecia, or severe acne), and use of medications known to alter sex hormone levels (i.e., oral contraceptives, glucocorticoids, androgens, and opioids). Those with a body mass index (BMI) of $\geq$30 kg/m^2, aspartate aminotransferase (AST) or alanine transaminase (ALT) level $>$1.5 times the upper limit of normal, diabetes mellitus (taking diabetes medication, fasting glucose level of $>$126 mg/dL, A1c level of $>$6.4%), or serum creatinine level of $>$1.5 mg/dL were excluded. Subjects with hypertension, hypothyroidism, hyperlipidemia, or depression on stable doses of medications for at least 3 months were included.

These 21 healthy women who met all eligibility criteria were then asked to come every 3 days for 2 consecutive menstrual cycles for blood sample collection of fasting blood

samples before 10 AM; the first sample was drawn during the early follicular phase (days 1–5). Serum was separated, aliquoted, and stored at −80°C until assayed. The participants were asked to maintain menstrual diaries, and the luteinizing hormone (LH) levels were measured every 3 days to track the timing of ovulation (i.e., LH peak). Consensus on the occurrence and timing of the LH peak for each subject was established after independent adjudication by 2 physician investigators.

Of the 21 women enrolled, 4 were excluded from the analysis for the following reasons: 1 subject did not have an identifiable midcycle LH peak and different phases of the menstrual cycle could not be ascertained; 1 subject had persistently elevated LH levels of >40 IU/L for the entire menstrual cycle; 1 subject had very low LH levels throughout the menstrual cycle without an identifiable midcycle LH peak; and 1 subject had persistently elevated LH levels of >20 IU/L and high total T levels (range, 50–98 ng/dL) throughout the menstrual cycle. The remaining 17 participants with complete data for at least 1 entire menstrual cycle with an identifiable LH peak were included in the analysis.

We used available baseline hormone data from healthy age-matched men from the 5α-Reductase Trial that is published. Men who had androgen deficiency or who were receiving glucocorticoids, growth hormone, androgens, or 5α-reductase inhibitors were excluded (23). Fifty-nine healthy men aged 19–40 years with normal T levels (300–1,200 ng/dL) were included in the analyses.

The studies were approved by the Institutional Review Board of the Massachusetts General Brigham Healthcare System. The participants provided written informed consent.

Hormone Measurements

The serum total T level was measured using a highly sensitive and specific LC-MS/MS assay, which has been certified by the CDC's HoST (23–26); details are published. Briefly, T in human serum was extracted by solid-phase extraction, separated by high-performance liquid chromatography, and measured by mass spectrometry in electrospray ionization source. Deuterated T (16,16, 17-D3 T), added to each sample, was used as an internal standard. The lower limit of quantitation (LLOQ) was 1 ng/dL, and the linear range was 1–1,000 ng/dL. The cross-reactivity of dehydroepiandrosterone, dehydroepiandrosterone sulfate, DHT, androstenedione, and estradiol in the T assay is negligible at 10 times the circulating levels of these hormones. The interassay coefficients of variation (CVs) were 7.9%, 7.7%, 4.4%, and 3.3% at 48.6, 241, 532, and 1,016 ng/dL, respectively. The interassay CVs were <5% for the National Institute of Standards and Technology female standard 971 and <2% for National Institute of Standards and Technology male standard 971. As part of the CDC's HoST, quality control samples provided by the CDC were run every 3 months. The mean bias was −0.8% (95% confidence interval, −2.1% to +0.5%) in the values reported by the laboratory relative to the values assigned by the CDC in samples with levels ranging from 7.7 to 941.0 ng/dL: −0.5% (−2.7% to +1.7%) in the female samples with the level range of 7.7–60.8 ng/dL and −0.9% (−2.6% to +0.3%) in the male samples with the level range of 243–941 ng/L. The imprecision (CV%) values were 2.9% in the female samples (level range, 7.7–60.8 ng/dL) and 1.9% in the male samples (level range, 243–941 ng/dL).

The serum sex hormone binding globulin (SHBG) was measured using a 2-site immunofluorometric assay (Beckman Instruments, Pasadena, CA). In the low, medium, and high pools, interassay CVs were 8.3%, 7.9%, and 10.9%, and intra-assay CVs were 7.3%, 7.1%, and 8.7%, respectively. The analytic sensitivity was 0.5 nmol/L. The LH level was measured using immunofluorometric assay with a sensitivity of 0.1 U/L (27, 28).

The free T level was measured by equilibrium dialysis (23, 25). Subsequently, 200 μL of serum was dialyzed for 24 hours at 37°C against a buffer mimicking the composition of protein-free plasma using a dialysis membrane with a cutoff of 10,000 daltons. The T level was measured directly in the dialysate using an LC-MS/MS assay and expressed as absolute level (pg/mL) as well as a percent of the total T level. In the dialysate of the equilibrium dialysis procedure, the LLOQ was 1 pg/mL. The intra-assay CV at this level (1 pg/mL) was 6.0%, and the interassay CV was 9.9%.

The serum DHT level was measured using an LC-MS/MS assay (20). Serum samples were extracted using solid-phase extraction, and the eluate was separated by high-performance liquid chromatography and analyzed by mass spectrometry using an electrospray ionization source. Deuterated DHT added to each sample served as an internal standard for assay calibration. The LLOQ was 1 ng/dL. The interassay CVs in the quality control samples with levels of 5.2, 22.0, and 44.1 ng/dL were 6.1%, 6.5%, and 8.6%, respectively.

Statistical Analyses

The distributional properties of hormone values were graphically inspected. The total and free T, DHT, and SHBG levels across different phases of the menstrual cycle were tabulated, and the mean (standard deviation), median, and range were determined for the follicular, midcycle peak and luteal phases. The menstrual cycle phases were determined by the LH peak levels for each woman. The mean follicular and luteal phase hormone levels were calculated as means before and after the midcycle peak. Differences in the hormone levels across the menstrual cycle were assessed using repeated-measures analysis of covariance. In addition, we performed sensitivity analyses using the nonparametric Wilcoxon signed-rank test and natural log-transformed hormone levels and with models adjusted for age and BMI.

Analyses comparing the hormone levels among healthy women and healthy men were conducted using analysis of covariance models. To assess robustness of our findings, we performed sensitivity analyses using the Wilcoxon signed-rank test, natural log-transformed hormone data, and models with age and BMI adjustments.

Statistical analyses were conducted using SAS 9.3 (SAS Institute, Inc., Cary, NC) and R software version 3.2.5 (R Foundation).

TABLE 1

Baseline characteristics of healthy cycling women (n = 17)

Patient Characteristics	Mean ± SD	Median	Range
Age (y)	26.6 ± 4.91	27.0	19.0–40.0
Cycle length (d)	26.3 ± 2.26	27.0	23–30
Weight (kg)	62.7 ± 6.29	62.2	52.4–70.8
BMI (kg/m^2)	23.1 ± 2.69	22.9	19.0–28.1
Hormone levels[a]			
Total T (ng/dL)	29.7 ± 12.1	27.3	10.7–58.2
Free T (pg/mL)	8.54 ± 2.03	8.43	5.41–13.2
DHT (ng/dL)	8.81 ± 5.78	6.71	2.71–23.5
SHBG (nmol/L)	59.8 ± 14.3	59.4	41.6–80.7
DHT-to-T ratio	4.37 ± 2.55	3.44	1.29–8.43

BMI = body mass index; DHT = dihydrotestosterone; SD = standard deviation; SHBG = sex hormone binding globulin; T = testosterone.
[a] Hormone levels measured during the early follicular phase (cycle days 1–5). The range is shown as minimum–maximum.

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RESULTS

Baseline Characteristics

The baseline characteristics of the 17 participants with data for at least 1 complete menstrual cycle are shown in Table 1. The mean ± standard deviation age and BMI of women were 26.6 ± 4.9 years and 23.1 ± 12.1 kg/m^2, respectively. Table 1 shows the mean baseline androgen levels from the early follicular phase (days 1–5) of the menstrual cycle.

Androgen Levels in Women Across the Menstrual Cycle

Comparison of the mean androgen levels overall across the different phases of the menstrual cycle is shown in Table 2. As expected in normally cycling healthy women, the LH levels increased gradually during the follicular phase of the menstrual cycle and peaked at midcycle (LH surge) indicating timing of ovulation (Fig. 1). The mean total and free T levels significantly changed over the menstrual cycle. The mean total T levels increased gradually from 15.6 ± 11.9 ng/dL in the early follicular phase to a midcycle peak level of 43.6 ± 16.2 ng/dL (P=.01; Fig. 1 and Table 2). The mean midcycle peak free T levels were significantly higher than those in the early follicular phase (15.6 ± 11.9 pg/mL vs. 9.00 ± 3.12 pg/mL; P=.05; Fig. 1 and Table 2). The serum total T levels were higher during the early luteal phase than in the follicular phase but declined gradually during the luteal phase; therefore, the mean T levels did not significantly differ between the luteal and follicular phases.

The mean DHT levels were not significantly different across the menstrual cycle (9.3 ± 3.7 ng/dL vs. 10.8 ± 5.0 ng/dL; P=.23; Fig. 1 and Table 2). The total T to DHT ratio did not change significantly across the menstrual cycle (P=.27; Table 2).

Although there was significant variation in the SHBG levels across the menstrual cycle overall, the mean values were not significantly different between the follicular and luteal phases.

The numerical distribution of the androgen levels by menstrual cycle day (averaged in 3-day intervals) relative to the LH peak (day 0) are shown in Supplemental Table 1 (available online).

Sensitivity analyses using the nonparametric Wilcoxon signed-rank test and comparison of outcomes after natural log-transformation provided similar results (Supplemental Table 2). Given low statistical power because of our small sample size, the significance of our results was attenuated after adjusting for age and BMI and when adjusted analyses were performed on natural log-transformed data (Supplemental Table 3).

DHT-to-T Ratio in Healthy Women Compared with Age-Matched Healthy Men

As expected, the total and free T and DHT levels were substantially higher in men than in women (P<.001; Table 3). Notably, the total T to DHT ratio was significantly higher in men than in women in all phases of the menstrual cycle (13.6 ± 5.7 vs. 4.4 ± 2.6; P<.001), indicating the substantially higher levels of DHT relative to total T in women than in men. Women had significantly higher SHBG levels than men.

These results remained unchanged after adjusting for age and BMI, when performed on natural log-transformed levels (Supplemental Table 4) and when cohorts were compared using the Wilcoxon signed-rank test (data not shown).

DISCUSSION

Using state-of-the-art assays that exhibit a high level of precision and accuracy in the low range, we conducted comprehensive profiling of the total and free T and DHT levels in healthy women across the entire menstrual cycle. We found that the serum total and free T levels increased progressively across the follicular phase and peaked to its highest levels at midcycle. During the luteal phase, the total and free T levels gradually declined to levels similar to the follicular phase. These changes in the total and free T levels across the menstrual cycle are consistent with previously published data (5, 6, 29) although the absolute mean T levels in our study are higher than values reported in some prior studies.

TABLE 2

Hormone levels for phases of the menstrual cycle (n = 17.)

Hormone Levels	Follicular Phase	LH Peak	Luteal Phase	*P* Value (Follicular vs. Peak)	*P* Value (Follicular vs. Luteal)	*P* Value (Overall)
Total T (ng/dL)						
Mean ± SD	34.6 ± 13.0	43.6 ± 16.2	35.7 ± 10.0	.014	.529	.005
Median	33.5	40.0	34.1			
Range	15.8–57.5	23.9–82.8	20.1–52.3			
Free T (pg/mL)						
Mean ± SD	9.99 ± 3.12	15.6 ± 11.9	9.55 ± 3.43	.050	.560	.023
Median	8.83	11.8	9.05			
Range	5.27–17.3	6.75–53.4	5.64–17.0			
DHT (ng/dL)						
Mean ± SD	9.26 ± 3.71	9.33 ± 3.67	10.8 ± 4.98	.926	.130	.233
Median	7.82	8.72	9.35			
Range	4.32–16.5	2.83–16.2	2.99–20.9			
SHBG (nmol/L)						
Mean ± SD	63.9 ± 24.0	56.4 ± 23.5	65.9 ± 26.2	.061	.577	.013
Median	57.3	49.1	56.1			
Range	40.7–122	35.7–116	41.3–135			
DHT-to-T ratio						
Mean ± SD	4.68 ± 1.76	5.46 ± 3.05	4.36 ± 2.02	.346	.521	.273
Median	3.98	4.18	4.21			
Range	2.33–7.93	2.43–12.1	1.52–9.66			

Comparisons between the menstrual cycle phases were performed using repeated-measures analysis of covariance. Range is shown as minimum–maximum. DHT = dihydrotestosterone; SD = standard deviation; SHBG = sex hormone binding globulin; T = testosterone

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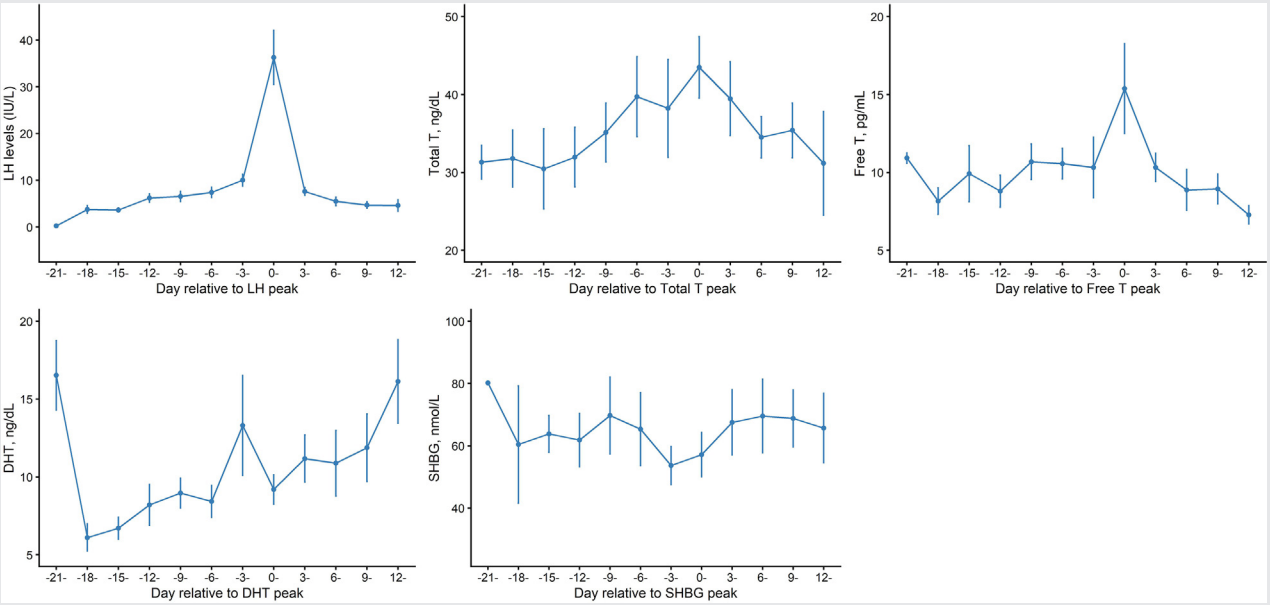
In a study of healthy women using LC-MS/MS where samples were collected at 3 time points (early follicular, midcycle, and midluteal phases), Rothman et al. (5) reported a mean total T level during the midcycle peak of 22.7 ng/dL vs. a mean midcycle peak total T level of 43.6 ng/dL in our study. The differences in the absolute total T levels could be because of differences in the assay calibrators or the more frequent blood sampling every 3 days across the entire menstrual cycle in our study, which may have enabled a more comprehensive assessment of the mean levels across the

FIGURE 1



Luteinizing hormone (LH), total and free testosterone (T), dihydrotestosterone (DHT), and sex hormone binding globulin (SHBG) levels across the menstrual cycle. Menstrual cycle phases assessed by LH peak. For the follicular and luteal phases, data were averaged in 3-day time intervals. The dots represent the mean values at each 3-day time interval, and the bars indicate standard errors..

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TABLE 3

Comparison of the hormone levels in healthy women and age-matched healthy men

Hormone Levels	Healthy Women[a] (N = 17)	Healthy Men (N = 59)	*P* (Men vs. Women)
Total T (ng/dL)			
Mean ± SD	29.7 ± 12.1	683 ± 212	<.001
Median	27.3	668	
Range	10.7–58.2	205–1,231	
Free T (pg/mL)			
Mean ± SD	8.54 ± 2.03	134 ± 47	<.001
Median	8.43	129	
Range	5.41–13.2	64–285	
DHT (ng/dL)			
Mean ± SD	8.81 ± 5.78	55.2 ± 20.0	<.001
Median	6.71	51.3	
Range	2.71–23.5	4.70–129	
SHBG (nmol/L)			
Mean ± SD	59.8 ± 14.3	40.3 ± 15.2	<.001
Median	59.4	40.1	
Range	41.6–80.7	1.70–88.3	
DHT-to-T ratio			
Mean ± SD	4.37 ± 2.55	13.5 ± 5.72	<.001
Median	3.44	11.6	
Range	1.29–8.43	5.88–43.5	

DHT = dihydrotestosterone; SD = standard deviation; SHBG = sex hormone binding globulin; T = testosterone.
[a] Values measured during the early follicular phase. The range is shown as minimum–maximum.

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different phases. As the LC-MS/MS assay for the measurement of the total T levels used in our analyses is certified by the CDC's HoST, which is based on an accuracy standard, these ranges can be used across other laboratories and assays that are also certified by the HoST. The intensive sampling across 2 menstrual cycles enabled a more detailed characterization of the changes in these hormones than has been performed in most previous studies.

The qualitative changes in the free T levels reported in this study are not dissimilar from those reported previously by others. However, the absolute free T levels have varied substantially among studies largely because of the differences in the methods for determining the free T levels. Some studies estimated the free T levels in these studies using linear binding equations whose underlying assumptions of binding affinity and dynamics have been shown to be inaccurate (30, 31). Although the equilibrium dialysis method is widely believed to be the reference method, the free T levels determined using this method also vary among laboratories depending on the dialysis conditions, buffer composition, dialysis duration, and T assay. Because the commercial laboratories do not report the procedural details of the equilibrium dialysis, sampling method, or characteristics of the participants in which their reference ranges are derived, it is difficult to evaluate their methods or ascertain the sources of differences in their reported reference ranges. The commercial T assays also do not take into account the phase of the menstrual cycle in which the blood was collected. To overcome these challenges, we report here the details of the standardized equilibrium dialysis method used to separate bound and unbound T and using an LC-MS/MS method that has been certified by the CDC's HoST (32). Other laboratories using these standardized conditions for equilibrium dialysis and a CDC-certified assay for T should be able to use the reported free T values in the context of the menstrual phase in which the blood sample was drawn. Our data underscore the importance of establishing cycle-related physiologic reference ranges as well as the need to re-evaluate the reference values provided by commercial laboratories to avoid the potential for misdiagnosis of androgen disorders.

The role of DHT in women is incompletely understood. Dihydrotestosterone is a potent androgen in humans. Routine measurement of the serum DHT levels is not currently recommended because of the inability to measure DHT levels in women accurately and precisely because of the limited sensitivity of the available radioimmunoassays , lack of normative ranges in healthy cycling women, and absence of consensus about its role in hyperandrogenic disorders, such as hirsutism and PCOS (19). Several reports have suggested that DHT plays an important role in mediating androgen effects in the hair follicle and the sebaceous glands (33). Circulating 3-alpha diol G, a metabolite of DHT, has been proposed as a biomarker of increased peripheral androgen action and reported to be increased in women with hirsutism (18). Very few studies have attempted to examine the DHT levels across the menstrual cycle using LC-MS/MS methods. In our study, consistent with an earlier report, the DHT levels did not significantly change across the menstrual cycle with the mean early follicular phase levels of 9 ng/dL (5). Our findings are consistent with 1 prior study using LC-MS/MS (5). Compared with T, it is important to acknowledge that the circulating levels of DHT are generally low because of the efficient mechanisms that metabolize DHT in target tissues to its inactive glucuronide metabolites as well as its higher affinity for SHBG (33). Thus, future studies should include these distal metabolites of DHT (i.e., 3-alpha diol G) because they may be better markers of peripheral androgen production than DHT alone.

It has not been widely appreciated that women have significantly higher DHT levels relative to total T than men. We found that the DHT level was approximately one fourth of the total T level in women in contrast to one thirteenth of the total T level in men. The clinical significance of the substantially higher DHT levels relative to total T in women is not known. In addition, it is not known whether the high DHT levels relative to T in women are the result of higher expression levels or activity of 1 or more isoforms of the steroid 5α-reductase enzyme in women than in men or because of the increased DHT production by the "backdoor pathway" for DHT production (17). Several women with hirsutism and/or PCOS have normal T levels yet demonstrate increased levels of DHT and its urinary metabolites, suggesting the possibility of DHT production through an alternative "backdoor pathway" in women with androgen excess disorders (13, 14, 17). Some reports have linked a higher DHT-to-T ratio to adverse metabolic phenotypes in women with PCOS, highlighting the value of this biomarker in predicting health outcomes (17). The relation of DHT levels and DHT-to-T ratio with androgenic symptoms and metabolic outcomes needs further study. Further studies are also needed to determine if there are sex differences in the relative contribution of androgens derived from conventional vs. alternative backdoor pathways and whether androgens in women with hirsutism or PCOS are exclusively derived from conventional pathway or whether they reflect increased activity of the alternate pathway for DHT synthesis.

Our study has notable strengths and some limitations. We carefully screened for healthy menstruating women and excluded women with hyperandrogenism. In contrast to previous studies where hormone measurements were limited to 2–3 times points, blood samples were taken every 3 days across 2 menstrual cycles, allowing us to capture a wider distribution of androgen levels within the different phases of the menstrual cycle. We measured the total T and DHT levels using LC-MS/MS, widely considered the reference method with the highest sensitivity and specificity. The free T level was measured using a standardized equilibrium dialysis method coupled with direct measurement of the T levels in the dialysate using LC-MS/MS. Our study also highlights the need for menstrual phase–specific reference ranges using these validated methods to differentiate between physiologic and pathologic androgen states in women.

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