Quantifying the variability in the assessment of reproductive hormone levels

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Objective: To quantify how representative a single measure of reproductive hormone level is of the daily hormonal profile using data from detailed hormonal sampling in the saline placebo-treated arm conducted over several hours.
Design: Retrospective analysis of data from previous interventional research studies evaluating reproductive hormones.
Setting: Clinical Research Facility at a tertiary reproductive endocrinology centre at Imperial College Hospital NHS Foundation Trust.
Patients: Overall, 266 individuals, including healthy men and women (n = 142) and those with reproductive disorders and states (n = 124 [11 with functional hypothalamic amenorrhoea, 6 with polycystic ovary syndrome, 62 women and 32 men with hypoactive sexual desire disorder, and 13 postmenopausal women]), were included in the analysis.
Interventions: Data from 266 individuals who had undergone detailed hormonal sampling in the saline placebo-treated arms of previous research studies was used to quantify the variability in reproductive hormones because of pulsatile secretion, diurnal variation, and feeding using coefficient of variation (CV) and entropy.
Main Outcome Measures: The ability of a single measure of reproductive hormone level to quantify the variability in reproductive hormone levels because of pulsatile secretion, diurnal variation, and nutrient intake.
Results: The initial morning value of reproductive hormone levels was typically higher than the mean value throughout the day (percentage decrease from initial morning measure to daily mean: luteinizing hormone level 18.4%, follicle-stimulating hormone level 9.7%, testosterone level 9.2%, and estradiol level 2.1%). Luteinizing hormone level was the most variable (CV 28%), followed by sex-steroid hormone levels (testosterone level 12% and estradiol level 13%), whereas follicle-stimulating hormone level was the least variable reproductive hormone (CV 8%). In healthy men, testosterone levels fell between 9:00 AM and 5:00 PM by 14.9% (95% confidence interval 4.2, 25.5%), although morning levels correlated with (and could be predicted from) late afternoon levels in the same individual (r^2 = 0.53, $P<.0001$). Testosterone levels were reduced more after a mixed meal (by 34.3%) than during ad libitum feeding (9.5%), after an oral glucose load (6.0%), or an intravenous glucose load (7.4%).
Conclusion: Quantification of the variability of a single measure of reproductive hormone levels informs the reliability of reproductive hormone assessment. (Fertil Steril® 2024;121:334-45. ©2024 by American Society for Reproductive Medicine.)
El resumen está disponible en Español al final del artículo.

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Diagnosis of most reproductive disorders includes assessment of reproductive hormone levels; however, because of practical limitations, a single measure of reproductive hormone levels is often used, often with confirmation on a second occasion in borderline cases (1). Notably, reproductive hormones vary during the day because of diurnal rhythm (2), pulsatile secretion (3), and nutrient intake (4), which has significant implications for the diagnostic accuracy of reproductive hormones. However, there is a scarcity of reports quantifying the variability of reproductive hormones to inform the accuracy of these measures for the diagnosis of reproductive disorders.

Many hormones exhibit both basal tonic secretion and pulsatile release (3, 5). For instance, the periodic release of gonadotropin-releasing hormone (GnRH) causes temporally-coupled luteinizing hormone (LH) release on the background of tonic LH secretion (6). Luteinizing hormone pulses typically have a duration of 60–90 minutes (6), and thus a single LH level will vary depending on the time point during the pulse cycle at which the measure was taken. Additionally, LH pulsatility may be altered in certain conditions, e.g., increased in polycystic ovary syndrome (PCOS) and decreased in functional hypothalamic amenorrhea (FHA) (7). Increased GnRH pulse frequency favors LH-predominant secretion, whereas reduced GnRH pulse frequency favors follicle-stimulating hormone (FSH)-predominant secretion. Therefore, a single LH and FSH level could vary with the number of pulses during the day; however, to date, there is only limited data describing the relationship between baseline reproductive hormone levels and LH pulsatility.

For the diagnosis of male hypogonadism, morning testosterone levels are recommended because testosterone is recognized to have diurnal variation, with levels peaking between 05:30 and 08.00 hours and reaching a nadir from approximately 5:30 PM to 8:00 PM (2). Indeed, up to 30% of men with a low testosterone level on the first measurement taken during the day demonstrate value within the reference range on a repeat measurement in the morning (8). Further, it is recommended to measure testosterone levels in the fasted state (1) because feeding can decrease serum testosterone concentrations for >2 hours (9, 10).

Quantification of the variability in reproductive hormone levels would therefore enable a more precise assessment of how closely they reflect the hormonal profile during the day, with relevance for the diagnosis of reproductive disorders and states. Here, we used data from detailed hormonal sampling conducted over several hours from 13 research studies (11–20) comprising 266 individuals to quantify how representative a single measure of reproductive hormone level is of the daily hormonal profile and to assess the impact of pulsatile secretion, diurnal rhythm, and nutrient intake.

MATERIALS AND METHODS

Study approval

Each study was conducted in accordance with the Declaration of Helsinki, and written consent was obtained from all participants before commencement. Ethical approval was gained from the Research Ethics Committee for each study, with the following references: 04/Q406/151, 17/LO/1504, 16/LO/0391, 13/LO/1807, 12/LO/0507, 05/Q0406/142, 19/LO/116,1 and 15/LO/1481.

Participants

The studies totaled 266 participants, including 164 men and 102 women, for whom baseline characteristics and source research studies are summarized in Supplemental Table 1 (available online) (21). Details on recruitment, study-size calculations, participants, and data collection for the 13 research studies are detailed in the relevant publications and summarized in Supplemental Table 1 (11–20).

The inclusion criteria for healthy men were the absence of hypogonadal symptoms and morning fasting total testosterone level of >11 nmol/L because the odds of having sexual symptoms are increased below this level (22). The inclusion criteria for healthy women were the presence of a regular menstrual cycle length between 28 and 35 days. Women with FHA and women with PCOS were diagnosed according to the Endocrine Society guidelines (23) and modified Rotterdam criteria (24), respectively. Postmenopausal women were aged between 40 and 62 years and had not had a menstrual period for at least 12 months. Men and women with hypoactive sexual desire disorder (HSDD) were diagnosed according to the Diagnostic and Statistical Manual of Mental Disorders-IV (25) and Diagnostic and Statistical Manual of Mental Disorders-V (26) criteria, respectively, and did not have any endocrine abnormalities. Participants with HSDD were otherwise free of current or past psychiatric illness and free of current medications or psychoactive substances (prescribed or illicit) for ≥6 months (15, 18, 19). Each cohort was analyzed in isolation when there were differences in the duration and/or frequency of blood sampling.

Protocols

We analyzed data from the saline-placebo arms of these research studies to assess the hormonal variability in cohorts of healthy men and women and in those with reproductive disorders and states. The duration and blood sampling intervals of the included studies are outlined in Supplemental Table 1 (21). All studies started between 8:00 and 10:00 AM, and regular blood sampling enabled monitoring of serum LH, FSH, estradiol (in women), and total testosterone (in men) levels. All healthy premenopausal women had their

studies performed during the follicular phase between days 1 and 7 of the menstrual cycle (16, 18, 19).

The nutrient-intake intervention regimens were conducted as part of Izzi-Engbeaya et al. (13) 2018Click or tap here to enter text., and ad libitum feeding was conducted as part of the remaining studies in healthy men (11, 12, 15). For the cohort receiving nutrient interventions, participants fasted from 10:00 PM on the preceding night (14). At 11:00 AM, participants either had a mixed meal (MM), an oral glucose load (OGL) of 75 g oral glucose, or an intravenous glucose load (IVGL) (0.3 g/kg of 20% dextrose intravenously infused over 120 seconds). The MM consisted of either Waitrose "spaghetti Bolognese" (125 kcal/100 g) or "mushroom risotto" (124 kcal/100 g) and participants were asked to eat until comfortably full. For all other studies, participants were offered a ready-made meal of their choice and could eat freely during the day (ad libitum feeding).

Hormonal assays

Serum LH, FSH, estradiol, and testosterone levels were measured using chemiluminescent immunoassays (Abbott Diagnostics, Maidenhead, UK) and analyzed in the same Clinical Chemistry Laboratory of Imperial College Healthcare NHS Trust between March 2012 and February 2022. During this time period, there was a phased transition from Abbott Architect to the Abbott Alinity platform between 2018 and 2019. Validation against the previous Abbott Architect analyzers was completed in compliance with ISO 15189. Because the results generated using the Abbott Alinity analyzers are directly comparable to the previous Abbott Architect analyzers, there was no change in the local reference ranges for serum gonadotropins or sex-steroid analytes. Reference ranges were as follows: LH (follicular phase) 2–10 IU/L; FSH (follicular phase) 1.5–8.0 IU/L; estradiol (early follicular phase) 100–300 pmol/L; and testosterone 10–30 nmol/L. The highest interassay coefficients of variation for either platform were $< 8.9\%$ for LH; $<4.6\%$ for FSH; $<7.7\%$ for estradiol; and $\leq 8.1\%$ for testosterone. The limits of detection for the assays were LH 0.04 IU/L, FSH 0.02 IU/L, estradiol 92 pmol/L, and testosterone 0.05 nmol/L.

Statistical analysis

Statistical analysis was performed using GraphPad PRISM Version 9.0. Descriptive statistics were obtained for each participant and then averaged across each cohort, including the coefficient of variation (CV), which is a measure of the relative dispersion of the measurements around the mean, and entropy, which is a measure of disorder within a dataset where a higher number represents greater variability. Entropy was calculated using the entropy package (version 1.3) for R software (version 4.1.2). For each participant, hormone levels were normalized between 0 and 1, using the maximum and minimum levels across all cohorts, and discretized in 100 bins. Hence, the entropy of the data is bounded between 0 and log (100) = 6.51 nats. Estimation was performed using the bias-corrected maximum likelihood method (provided by the entropy package v1.3). The 95% confidence interval (CI) for the mean, percentage change from the baseline value, and entropy were calculated with bootstrapping, a resampling method that uses repeated sampling with replacement to give robust estimates of the sampling distributions for a statistic of interest (e.g., mean, variance, or CIs) without assuming a particular distribution for the data. Bootstrapping was performed using the boot function in R software (version 4.1.2). In all instances, 10,000 bootstrap replicates were generated from the dataset using resampling with replacement, and the statistic of interest (e.g., mean and variance) was calculated for each replicate, resulting in a bootstrap sampling distribution for that statistic. The (100 − a)% bootstrap CI was obtained from the bootstrap distribution as the (a/2%, [100 − a/2]%) percentile interval. This enables these estimates to be more representative of the true variability in the population. Entropy and bootstrapping could only be calculated in studies with a duration of at least 270 minutes because of the minimum number of sampling points required for these calculations.

The HormoneBayes framework was used to analyze features of LH pulsatility (number of pulses, secretion rate, and mean pulse mass) (3). Hormone relationships and the determination of the quantity and frequency of hormonal samples were analyzed using simple linear regression. Diurnal variation was assessed using the Wilcoxon signed-rank test, comparing the 9 AM and 5 PM measures. Feeding intervention data were nonparametrically distributed and therefore summarized using the median and interquartile range (IQR). The Kruskal-Wallis test and Dunn multiple comparison test were used to compare more than two groups of nonparametric data. A P value of $<.05$ was regarded as signifying statistically significant differences.

RESULTS

The summary statistics for each cohort are presented for LH in Table 1, FSH in Table 2, and testosterone and estradiol in Supplemental Table 2 (21). The initial morning values of reproductive hormones were typically higher than the mean value throughout the day (percentage decrease: LH 18.4%, FSH 9.7%, testosterone 9.2%, and estradiol 2.1%) (Supplemental Tables 3 to 5).

Determining overall variability in the daily reproductive hormone profile

The variability of reproductive hormone levels was quantified using CV and entropy. Across the entire cohort (both men and women), FSH level was the least variable reproductive hormone (CV 8%) (Table 2), followed by sex-steroid hormone (testosterone 12% and estradiol 13%) levels (Supplemental Table 2) (21), whereas LH level was the most variable (CV 28%) (Table 1). Testosterone levels were highly variable and pulsatile in some individuals, with levels varying by as much as 15 nmol/L within 30 minutes (Supplemental Fig. 1, available online) (21). Women with FHA had the highest entropy for LH (1.31–1.32 nats) (Table 1) and FSH (0.80 nats) (Table 2). Healthy men had consistently high entropy for sex-steroid hormones between 0.79 and 0.87 nats (Supplemental Table 2) (21). Estradiol had the highest entropy

TABLE 1

Statistics for luteinizing hormone in each cohort.

Cohort	*n*	Baseline value (IU/L)	Min baseline value	Max baseline value	Arithmetic mean ± SD	Geometric mean	Median (IQR)	SEM	95% CI	Bootstrapped 95% CI	CV (%)	Group entropy mean (nats) (95% CI)
Healthy men	68	3.42	2.14	4.36	3.03 ± 0.77	2.94	2.91 (2.42–3.64)	0.26	2.44–3.63	I.D.P	27	I.D.P
Healthy men	31	3.48	2.11	4.21	3.01 ± 0.66	2.95	2.95 (2.47–3.49)	0.18	2.61–3.41	2.64–3.44	22	1.14 (1.08–1.19)
Healthy men	7	3.49	2.11	5.39	3.39 ± 0.92	3.27	3.13 (2.64–3.39)	0.16	3.06–3.71	2.69–4.15	27	1.29 (1.25–1.33)
Healthy men	26	3.50	1.75	5.12	2.97 ± 0.84	2.85	2.79 (2.31–3.46)	0.12	2.73–3.19	2.64–3.34	30	1.30 (1.25–1.34)
Men with HSDD	32	3.12	2.02	3.74	2.76 ± 0.62	2.70	2.65 (2.25–3.32)	0.21	2.30–3.24	I.D.P	23	I.D.P
All men	164	3.40	2.03	4.56	3.03 ± 0.76	2.94	2.89 (2.42–3.58)	0.19	2.63–3.44	2.66–3.64	25.62 (CI: 24.33–27.13)	1.24 (1.20–1.28)
Healthy women	10	4.48	1.86	5.39	3.28 ± 1.00	3.09	3.15 (2.40–3.98)	0.20	2.86–3.69	2.59–4.04	36	1.25 (1.08–1.44)
Women with FHA	6	3.08	0.82	4.28	1.91 ± 0.97	1.68	1.69 (1.12–2.50)	0.19	1.50–2.34	1.16–2.80	57	1.32 (1.11–1.48)
Women with FHA	5	1.43	0.52	1.74	0.94 ± 0.29	0.90	0.89 (0.73–1.11)	0.04	0.86–1.04	0.67–1.27	34	1.31 (1.05–1.50)
Women with PCOS	6	7.18	3.50	8.30	5.35 ± 1.37	5.22	5.17 (4.40–6.58)	0.27	4.92–6.03	4.33–6.70	34	1.27 (1.09–1.47)
Women with HSDD	30	4.04	2.59	4.83	3.68 ± 0.71	3.60	3.70 (3.08–4.24)	0.21	3.19–4.18	3.12–3.99	22	1.05 (0.94–1.16)
Women with HSDD	32	4.48	3.05	4.83	3.87 ± 0.65	3.82	3.72 (3.36–4.48)	0.21	3.38–4.38	I.D.P	19	I.D.P
Postmenopausal women	13	29.06	20.05	31.77	26.46 ± 2.66	26.23	26.69 (24.77–28.46)	0.37	25.62–27.15	24.76–27.98	10	0.72 (0.67–0.78)
All women	102	7.68	4.63	8.73	6.50 ± 1.09	6.36	6.43 (5.69–7.34)	0.21	6.05–6.97	6.11–7.80	30.10 (CI: 26.37–34.63)	1.16 (1.09–1.23)
All men and women	266	5.90	3.54	7.00	5.05 ± 0.96	4.94	4.95 (4.33–5.77)	0.20	4.62–5.50	4.96–6.41	27.90 (CI: 26.01–29.97)	1.20 (1.15–1.24)

Note: Values were calculated for each individual over the sampling period, and the mean is presented. Additionally, bootstrapped values for the CIs and group entropy mean with 95% confidence intervals are presented. These average values are presented according to gender (all men and all women) and for the entire cohort (all men and women). CI = confidence interval; CV = coefficient of variation; FHA = functional hypothalamic amenorrhea; HSDD = hypoactive sexual desire disorder; IDP = insufficient data points; IQR = interquartile range; PCOS = polycystic ovary syndrome; SD = standard deviation; SEM = standard error of the mean.

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TABLE 2

Statistics for follicle-stimulating hormone in each cohort.

Cohort	*n*	Baseline value (IU/L)	Min baseline value	Max baseline value	Arithmetic mean ± SD	Geometric mean	Median (IQR)	SEM	95% CI	Bootstrapped 95% CI	CV (%)	Group Entropy mean (nats) (95% CI)
Healthy men	68	2.86	2.27	2.99	2.95 ± 0.52	2.88	2.60 (2.42–2.79)	0.18	2.54–3.35	I.D.P	10	I.D.P
Healthy men	31	2.46	2.17	2.57	2.34 ± 0.13	2.33	2.35 (2.25–2.45)	0.04	2.26–2.41	2.09–2.63	5	0.22 (0.14–0.31)
Healthy men	7	2.43	2.00	2.54	2.26 ± 0.13	2.26	2.24 (2.17–2.37)	0.02	2.21–2.31	2.16–2.36	6	0.35 (0.20–0.51)
Healthy men	26	2.70	1.93	2.83	2.39 ± 0.18	2.38	2.41 (2.29–2.52)	0.03	2.35–2.45	2.30–2.48	7	0.51 (0.41–0.60)
Men with HSDD	32	3.87	3.17	3.94	3.52 ± 0.26	3.51	3.47 (3.30–3.77)	0.09	3.31–3.69	I.D.P	7	I.D.P
All men	164	2.86	2.31	2.97	2.69 ± 0.24	2.67	2.61 (2.49–2.78)	0.07	2.53–2.84	2.18–2.49	7.17 (CI: 6.67–7.82)	0.36 (0.29–0.42)
Healthy women	10	4.48	3.39	5.18	4.28 ± 0.41	4.25	4.30 (4.02–4.52)	0.08	4.10–4.44	3.96–4.53	11	0.64 (0.54–0.78)
Women with FHA	6	5.02	3.68	5.65	4.43 ± 0.50	4.38	4.37 (4.05–4.75)	0.10	4.22–4.65	4.04–4.88	11	0.80 (0.68–0.92)
Women with FHA	5	3.70	2.38	3.78	3.08 ± 0.32	3.06	3.10 (2.86–3.30)	0.05	3.00–3.20	2.79–3.36	10	0.80 (0.67–0.92)
Women with PCOS	6	4.14	3.18	4.63	3.77 ± 0.32	3.75	3.72 (3.57–3.95)	0.06	3.63–3.90	3.51–4.08	9	0.52 (0.24–0.77)
Women with HSDD	30	4.96	3.87	5.22	4.57 ± 0.43	4.55	4.60 (4.24–4.92)	0.13	4.30–4.87	4.32–4.91	9	0.42 (0.29–0.56)
Women with HSDD	32	4.67	4.04	4.93	4.43 ± 0.33	4.41	4.34 (4.15–4.74)	0.11	4.17–4.68	I.D.P	8	I.D.P
Postmenopausal women	13	69.16	54.08	72.46	61.54 ± 3.70	61.46	61.38 (59.15–6.77)	0.52	60.54–62.62	59.09–64.51	6	0.38 (0.25–0.51)
All women	102	13.73	10.66	14.55	12.30 ± 0.86	12.27	12.26 (11.72–12.85)	0.15	11.99–12.62	12.95–14.38	9.20 (CI: 8.27–10.31)	0.59 (0.52–0.67)
All men and women	266	9.20	7.18	9.73	8.30 ± 0.60	8.27	8.24 (7.87–8.65)	0.12	8.05–8.55	9.36–10.42	8.22 (CI: 7.70–8.80)	0.48 (0.42–0.53)

Note: Values were calculated for each individual over the sampling period, and the mean is presented. Additionally, bootstrapped values for the CIs and group entropy mean with 95% confidence intervals are presented. These average values are presented according to gender (all men and all women) and for the entire cohort (all men and women). CI = confidence interval; CV = coefficient of variation; FHA = functional hypothalamic amenorrhea; HSDD = hypoactive sexual desire disorder; IDP = insufficient data points; IQR = interquartile range; PCOS = polycystic ovary syndrome; SD = standard deviation; SEM = standard error of the mean.

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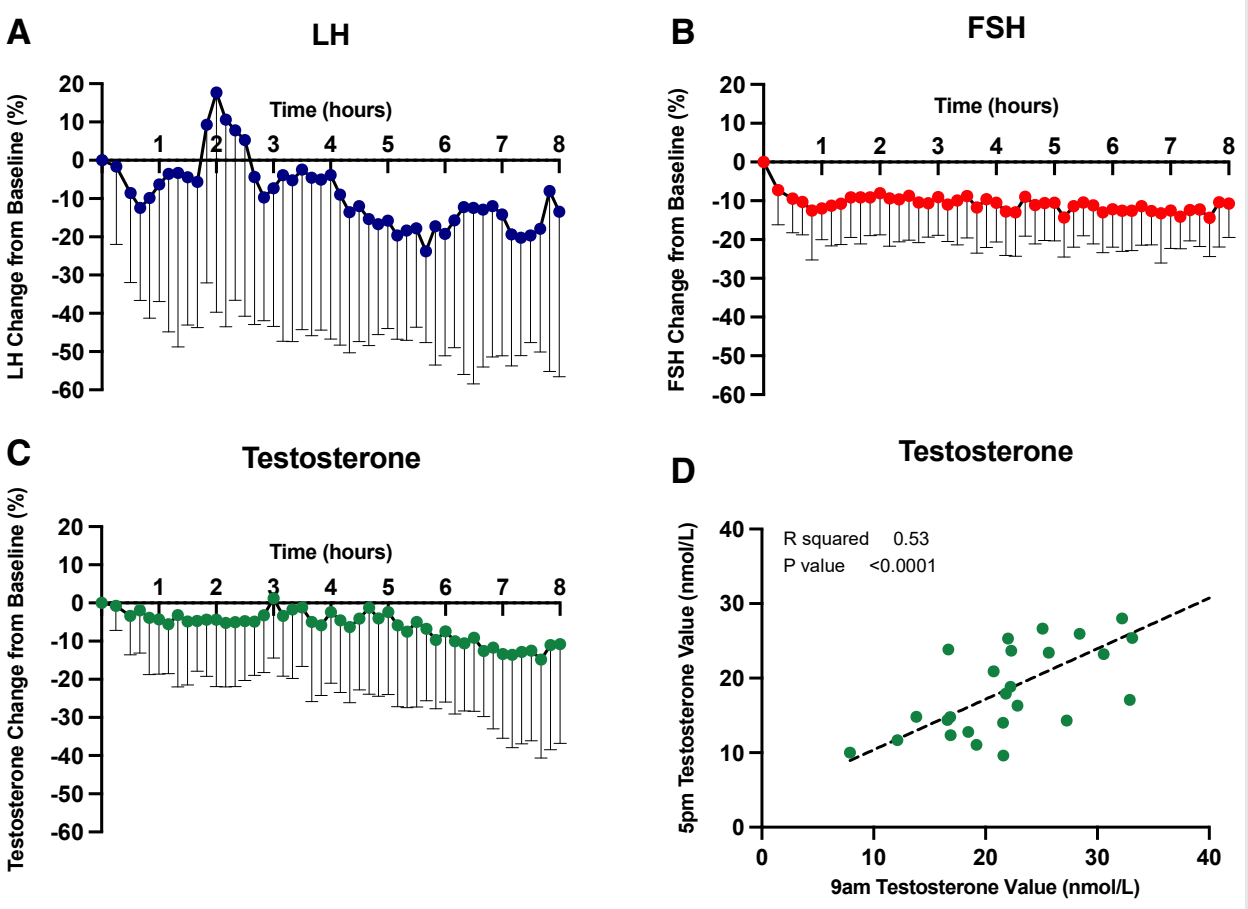
FIGURE 1



Diurnal variation in testosterone and luteinizing hormone (LH) levels in healthy men. Reproductive hormone levels were monitored for 8 hours for a cohort of healthy men (n = 26), from which the percentage change from the first value at each sampling point from the baseline was calculated. The mean percentage change of LH (**A**), FSH (**B**), and testosterone (**C**) levels from baseline levels. (**D**) The relationship between the baseline testosterone level at 9 AM and the testosterone level at the end of the monitoring period at 5 PM. Data are presented as mean ± SD.

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(0.79–0.92 nats) in healthy women and women with FHA, and the lowest entropy (0.07 nats) in postmenopausal women, where estradiol levels were low and undetectable (Supplemental Table 2) (21). Similarly, postmenopausal women also had the lowest entropy for LH (0.72 nats) (Table 1) and FSH (0.38 nats) (Table 2).

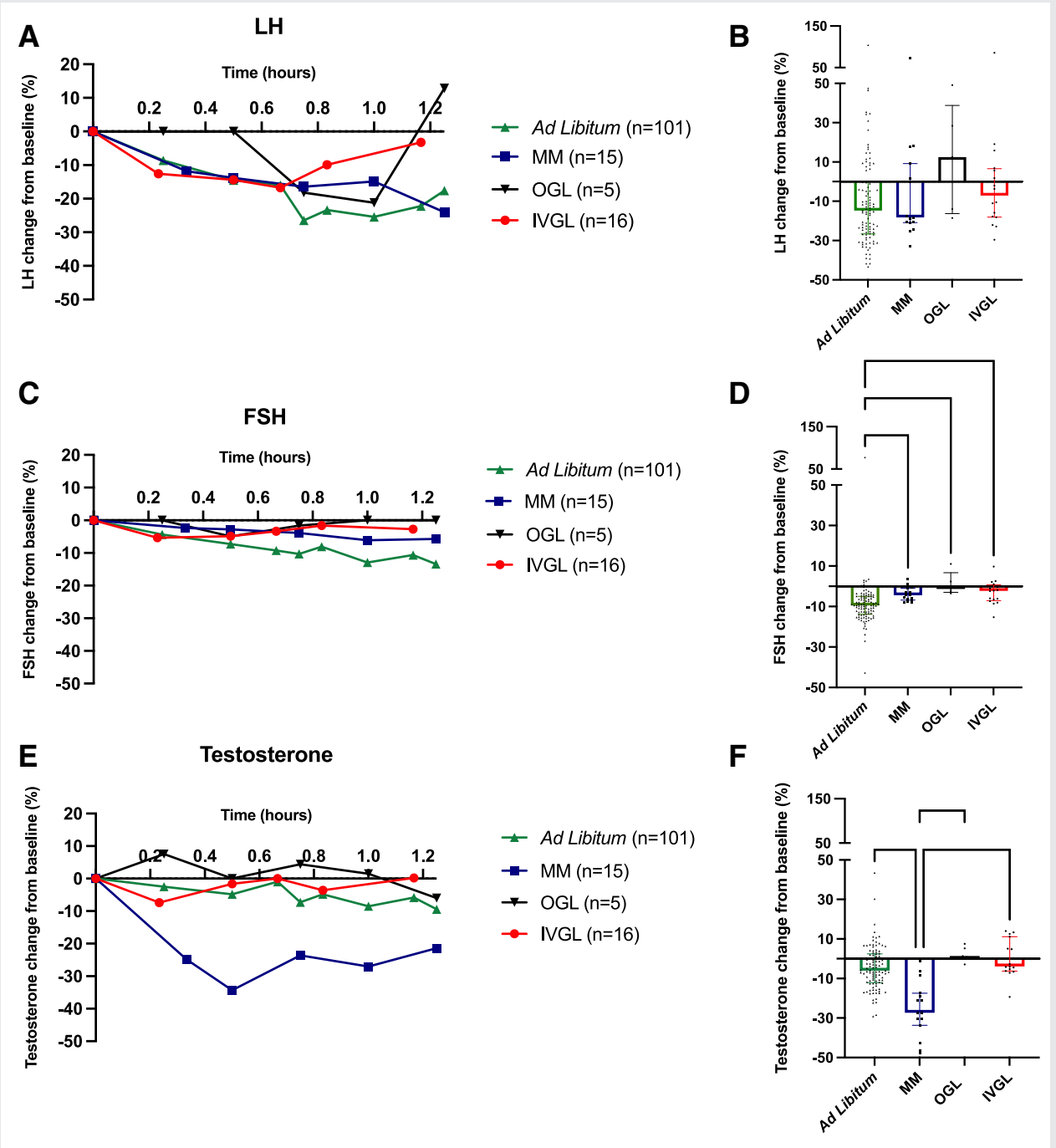
Number of samples needed for accurate assessment

To determine the minimum number of samples required to reflect mean levels during the day more closely, the coefficient of determination (r^2) was calculated for the mean of the first two or three time points at 30–60 minute intervals (e.g., T = 0, T = 30, T = 60, and T = 120 minutes) and compared with the average levels of each hormone throughout the entire sampling period (Supplemental Table 6) (21).

Male cohort. The inclusion of additional LH level measures (T = 0 and T = 60 minutes) increased the r^2 from 0.38 (a single baseline measure) to $r^2 = 0.55$ (two measures) and to $r^2 = 0.70$ (three measures at T = 0, T = 60, and T = 120 minutes). For the FSH level, a single baseline measure was already highly representative of the mean levels throughout the day ($r^2 = 0.95$). For the testosterone level, a single baseline measure had an $r^2 = 0.81$, which was increased to $r^2 = 0.91$ with two measures (T = 0 and T = 120 minutes), but there was no meaningful improvement from incorporating a third measure ($r^2 = 0.92$).

Female cohort. A single baseline LH level had an $r^2 = 0.80$, which was increased to $r^2 = 0.91$ with two measures (T = 0 and T = 120 minutes), but there was no meaningful advantage from incorporating a third measure ($r^2 = 0.92$). For the FSH level, a single baseline measure had an $r^2 = 0.82$, which was increased to $r^2 = 0.94$ with two measures (T = 0 and T = 120 minutes), but there was no

FIGURE 2



The effect of four nutrient-intake interventions (ad libitum, mixed meal (MM), oral glucose load (OGL), and intravenous glucose load (IVGL)) on luteinizing hormone (LH), follicle-stimulating hormone (FSH), and testosterone levels in healthy men. (**A**) Median percentage change of LH levels from baseline levels (initial morning value); (**C**) Median percentage change of FSH from baseline levels; and (**E**) Median percentage change of testosterone from baseline levels. Groups were analyzed using a two-way ANOVA test. The Kruskal-Wallis test with a post hoc Dunn test was used for multiple comparisons of median percentage of LH (**B**), FSH (**D**), and testosterone (**F**) levels change from baseline levels after different nutrient regimes. ***P*<.01, ****P*<.001, *****P*<.0001.

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meaningful improvement from incorporating a third measure ($r^2 = 0.95$). Similarly, a single baseline level of estradiol was already highly representative of the mean levels during the day ($r^2 = 0.99$).

Diurnal variation in reproductive hormones

To assess diurnal variation, the percentage change of LH, FSH, and testosterone levels (from 9:00 AM to 5:00 PM) was analyzed in healthy men. Testosterone levels fall during the day, with other studies conducted over a 24-hour interval indicating that the nadir for testosterone levels is detected between 07:00 PM and 9:00 PM in young men. Thus, testosterone levels had yet to reach their nadir by the end of the sampling period, and thus we used the latest value available at 5:00 PM within the limits of the duration of the sampling conducted. Baseline 9:00 AM values of LH ($P=.011$), FSH ($P<.0001$), and testosterone ($P=.0043$) were significantly higher than their respective 5:00 PM measures (Fig. 1A to C). The largest percentage decrease in testosterone levels compared with the baseline value was 14.9% (7.7 hours after the 9:00 AM baseline value) (Fig. 1C). Notably, baseline testosterone levels were significantly correlated with the testosterone levels at 5:00 PM ($r^2 = .53$, $P<.0001$) (Fig. 1D). The pattern of decline in both LH and testosterone levels likely indicates evidence of diurnal variation (Fig. 1A and C), although not in FSH levels, which fell within the first hour after sampling and then remained relatively steady (Fig. 1B).

A small but significant change in LH levels between the baseline value and the 5:00 PM value was also observed in healthy women ($P=.037$) (Supplemental Fig. 2A) (21). However, there was no significant change in either FSH or estradiol levels (Supplemental Fig. 2B and C) (21).

Effect of nutrient interventions on LH, FSH, and testosterone levels

The effects of four nutrient-intake interventions—ad libitum feeding, MM, OGL, and IVGL—on LH, FSH, and testosterone levels over a 1.25-hour monitoring period were examined in healthy men.

The median nadir of LH level was −26.4% at 0.75 hours after ad libitum feeding, −24.0% at 1.25 hours after an MM, −21.2% at 1 hour after an OGL, and −16.7% at 0.67 hours after an IVGL (Fig. 2A). There was no significant difference in the change in LH levels between these interventions (Fig. 2B).

The median nadir of FSH level was −13.4% at 1.25 hours after ad libitum feeding, −6.1% at 1 hour after a MM, −4.9% at 0.5 hours after an OGL, and −5.4% at 0.23 hours after an IVGL (Fig. 2C). Percentage FSH level changes after ad libitum feeding was greater after a MM ($P=.0052$), OGL ($P=.0051$), or IVGL ($P=.0018$) (Fig. 2D).

Healthy men had the greatest fall in testosterone levels after a MM (median nadir −34.3% at 0.5 hours after ingestion) compared with −9.5% during ad libitum feeding at 1.25 hours, −6.0% after OGL at 1.25 hours, and −7.4% at 0.23 hours after IVGL (Fig. 2E and F).

Relationship between baseline reproductive hormone levels and LH pulsatility

The relationship between baseline reproductive hormone levels and the following three measures of LH pulsatility was analyzed: the number of LH pulses over 8 hours; secretion rate of LH; and LH pulse mass.

In premenopausal women, there were no relationships between baseline LH level (Supplemental Fig. 3A) (21) or FSH-LH level (Supplemental Fig. 3G) (21) and the number of LH pulses, nor FSH, or any of the three measures of pulsatility (Supplemental Fig. 3D to F) (21). There was a negative association between baseline FSH-LH levels, LH secretion rate, and mean LH pulse mass ($P<.0001$; Supplemental Fig. 3H and I) (21). Positive associations between baseline LH levels, LH secretion rate, and mean LH pulse mass were observed ($P<.0001$; Supplemental Fig. 3B and C) (21).

In postmenopausal women, apart from a significant association between baseline LH and LH secretion rate ($P=.0001$; Supplemental Fig. 4B (21)), there were no associations between baseline LH, FSH, and FSH-LH levels or other measures of LH pulsatility (Supplemental Fig. 4A and C to I (21)).

DISCUSSION

A single measure of reproductive hormone levels can be impacted by several factors, including pulsatile secretion, diurnal rhythm, and nutrient intake, but there are only limited reports quantifying the impact of these factors. Existing studies have examined the day-to-day variation of reproductive hormone levels (2, 27), but there is limited data on the variability (incorporating both biologic and assay variabilities) in levels over a single day. Here, we analyzed data from research studies that employed detailed sampling of reproductive hormone levels over several hours in both healthy cohorts and those with reproductive disorders and states to enable quantification of this variability.

We found that a single measure of LH was the most variable, predominantly because of its pulsatile secretion. Although FSH level is also subject to control by pulsatile GnRH secretion, it has a longer half-life than LH (1 hour vs. 20 minutes) and thus is less susceptible to marked fluctuations with each GnRH pulse. Consequently, a single measure of FSH level was more representative of mean levels during the day.

Baseline (initial morning level) of LH, FSH, and testosterone were usually the highest measures over the day. In men, a single measure of FSH level, two measures of testosterone levels, and three measures of LH levels more accurately approximated mean levels during the day. In women, a single measure of estradiol level and two measures of LH and FSH levels were sufficient to approximate mean levels during the day accurately. The feasibility and cost of implementing additional sampling into clinical practice remain to be determined, but these data could be of value to inform the accurate evaluation of patients with borderline reproductive hormone levels. This observation is of clinical relevance for clinical guidelines on the assessment of male hypogonadism, with current recommendations (1) indicating that two early morning, fasting testosterone level measurements on separate days are required in the presence of symptoms. Conversely, a

single testosterone level measure within the healthy reference range is often deemed sufficient to exclude hypogonadism. However, testosterone levels can be highly variable in some individuals, changing by as much as 15 nmol/L within 30 minutes. For practical reasons, large cohort studies used to generate reference ranges were based on single morning measures of testosterone level (22). Up to 30% of men in the European Male Aging Study with testosterone levels within the reference range had symptoms consistent with hypogonadism (22), which is often interpreted to suggest that symptoms of hypogonadism are nonspecific. However, a single early morning testosterone level is often the highest level encountered during the day, and some men could conceivably have low testosterone levels during the remainder of the day which could be associated with hypogonadal symptoms. Measurements of testosterone levels are recognized to have lower accuracy, particularly at lower levels of testosterone, as found in women or men affected by hypogonadism (28, 29). Testosterone assays can be also challenged by factors, including assay interference and a lack of calibrating standards (30). With advancing technologies for continuous sampling, the possibility of characterizing hypogonadal symptomatology against multiple measures of testosterone levels throughout the day may become possible to more precisely characterize the relationship between sex-steroid levels and symptoms (31).

Diurnal variation

Diurnal variation occurs in multiple hormone levels, including testosterone levels. Ahokoski et al. (27) reported a decrease in estradiol-17β and testosterone in healthy men over the day but no definitive diurnal variation in LH or FSH levels. Interestingly, some men do not show any diurnal variation in testosterone levels (32) and others can even have higher levels in the afternoon than in the morning (2, 33). Notably, we found no evidence of a significant fall in estradiol in women during the day, whereas Panico et al. (34) showed that estradiol can fall by 23.8 pg/mL over the day (34). Existing reports in healthy men and women concluded that there is only minimal diurnal variation in LH and FSH levels (2, 35, 36). In the present study, we found evidence of a diurnal fall in both testosterone and LH levels in healthy men, with baseline (9:00 AM) testosterone levels being correlated with that at 5:00 PM in each man. The initial 12.5% drop in FSH levels occurred within the first hour, followed by relatively steady levels, suggesting that this fall could be more consistent with a stress response e.g., because of cannulation rather than a diurnal effect.

Nutrient intake

In published data, OGL or MM induced an 18% and 26% fall in testosterone levels, respectively (9). Another study reported an even lower nadir testosterone level after an OGL of 24.7% in a mixed cohort of men, including those with type 2 diabetes mellitus (10). Therefore, overdiagnosis of hypogonadism could occur when testosterone levels are assessed in the fed state. Previous studies have drawn conflicting conclusions on whether other reproductive hormones such as LH levels are also affected by feeding, with some reporting decreased LH pulsatility (4, 9) and others reporting no effect (37).

In the present study, an MM resulted in the largest negative impact on testosterone levels, with a median fall of 34.3% at 0.5 hours after ingestion. This was greater than those observed after ad libitum feeding (−9.5%), OGL (−6.0%), or IVGL (−7.4%), indicating that other components of nutrient intake aside from glucose, such as fat (38, 39) or protein (40), contribute to the fall in testosterone levels. These results corroborate those of a randomized control trial whereby ingestion of protein caused a greater decline in serum testosterone levels than glucose levels (4). A further report also found that MM decreased serum testosterone levels by 26%, whereas OGL decreased by only 18% (9). One proposed theory is that ingestion of amino acids such as leucine causes upregulation of androgen receptors, increasing muscle tissue uptake of testosterone, and consequently decreasing serum testosterone levels (4).

It is feasible that oral ingestion can stimulate gut hormone secretion, with protein and fat components recognized to increase postprandial rises of gastrointestinal incretin hormones such as gastric inhibitory peptides more than glucose load (41). However, recent studies were unable to demonstrate that glucagon-like peptide 1 (14), peptide YY (42), or glucagon (43) had acute effects on reproductive hormone secretion in healthy men. Another proposed mechanism is due to a rise in insulin inhibiting the hypothalamic-pituitary-gonadal axis and decreasing testosterone production (44), although this is inconsistent with the lesser impact of an IVGL than an MM. The constancy of LH and FSH levels in the fed and fasted states could indicate that the effect of nutrient intake could be mediated via testicular sensitivity to gonadotropins rather than central suppression of pituitary LH secretion, although the absence of a compensatory increase in LH levels in response to decreased testosterone levels could suggest a central component.

Pulsatility

LH pulsatility is a sensitive marker of gonadal status, being altered in several endocrine disorders (45). A significant relationship between baseline LH levels and LH secretion rate was present in our female cohorts. Postmenopausal women displayed a positive relationship between FSH level predominance and LH secretion rate, consistent with increased GnRH pulsatility secondary to a lack of estradiol-induced negative feedback (46); however, the inverse was true in premenopausal women.

Strengths of this study include a comprehensive assessment of variability in hormonal profiles (not usually conducted in clinical practice) of a relatively large sample of both healthy individuals and those with reproductive disorders and states. The hormonal assays were conducted in the same laboratory with frequent quality controls, minimizing variability because of the use of different assays between studies. The use of entropy and bootstrapping of 95% confidence intervals increased the robustness of the assessment. Limitations include the retrospective study design, different

sampling intervals, and durations between some cohorts. Because of the small number of individuals with specific reproductive conditions, such as PCOS, additional data are needed to fully capture whether there are differences in the variability of reproductive hormone levels in women with different PCOS phenotypes. Therefore, future studies with larger subgroup sizes could be more conclusive. The sampling after nutrient-intake interventions was of short duration; therefore, the full effect of nutrient-intake may not have been captured. All feeding studies were conducted only in healthy men; however, the ad libitum group did not have any constraints around feeding time or amount and were not the same individuals who had dedicated feeding interventions, which could result in some residual unidentified confounding.

CONCLUSIONS

Collectively, we have quantified the variability in reproductive hormone levels over the day in both healthy men and women, as well as those with reproductive disorders. These data could inform the timing, frequency, and reliability of hormonal assessments when evaluating patients in different reproductive states. Further, these data can inform guideline writers regarding the reliability of a single measure of reproductive hormones in the assessment of hypogonadism.

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REFERENCES

1. Bhasin S, Brito JP, Cunningham GR, Hayes FJ, Hodis HN, Matsumoto AM, et al. Testosterone therapy in men with hypogonadism: an Endocrine Society Clinical Practice Guideline. J Clin Endocrinol Metab 2018;103:1715–44.
2. Brambilla DJ, Matsumoto AM, Araujo AB, McKinlay JB. The effect of diurnal variation on clinical measurement of serum testosterone and other sex hormone levels in men. J Clin Endocrinol Metab 2009;94:907–13.
3. Voliotis M, Abbara A, Prague JK, Veldhuis JD, Dhilo WS, Tsaneva-Atanasova K. LBMON233 Hormonebayes: a novel bayesian toolbox for analysis of pulsatile hormone dynamics. J Endocr Soc 2022;6(Suppl 1):A648–9.
4. Schwartz A, Hunschede S, Lacombe RJS, Chatterjee D, Sánchez-Hernández D, Kubant R, et al. Acute decrease in plasma testosterone and appetite after either glucose or protein beverages in adolescent males. Clin Endocrinol (Oxf) 2019;91:295–303.
5. Voliotis M, Hanassab S, Abbara A, Heinis T, Dhillo WS, Tsaneva-Atanasova K. Quantitative approaches in clinical reproductive endocrinology. Curr Opin Endocr Metab Res 2022;27:100421.
6. Rossmanith WG. Ultradian and circadian patterns in luteinizing hormone secretion during reproductive life in women. Hum Reprod 1993;8(Suppl 2):77–83.
7. Phylactou M, Clarke SA, Patel B, Baggaley C, Jayasena CN, Kelsey TW, et al. Clinical and biochemical discriminants between functional hypothalamic amenorrhoea (FHA) and polycystic ovary syndrome (PCOS). Clin Endocrinol (Oxf) 2021;95:239–52.
8. Bhasin S, Basaria S. Diagnosis and treatment of hypogonadism in men. Best Pract Res Clin Endocrinol Metab 2011;25:251–70.
9. Gagliano-Jucá T, Li Z, Pencina KM, Beleva YM, Carlson OD, Egan JM, et al. Oral glucose load and mixed meal feeding lowers testosterone levels in healthy eugonadal men. Endocrine 2019;63:149–56.
10. Caronia LM, Dwyer AA, Hayden D, Amati F, Pitteloud N, Hayes FJ. Abrupt decrease in serum testosterone levels after an oral glucose load in men: implications for screening for hypogonadism. Clin Endocrinol (Oxf) 2013;78:291–6.
11. Comninos AN, Wall MB, Demetriou L, Shah AJ, Clarke SA, Narayanaswamy S, et al. Kisspeptin modulates sexual and emotional brain processing in humans. Journal of Clinical Investigation 2017;127:709–19.
12. Yang L, Demetriou L, Wall MB, Mills EGA, Zargaran D, Sykes M, et al. Kisspeptin enhances brain responses to olfactory and visual cues of attraction in men. JCI Insight 2020;5:e133633.
13. Izzi-Engbeaya C, Comninos AN, Clarke SA, Jomard A, Yang L, Jones S, et al. The effects of kisspeptin on β-cell function, serum metabolites and appetite in humans. Diabetes Obes Metab 2018;20:2800–10.
14. Izzi-Engbeaya C, Jones S, Crustna Y, Machenahalli PC, Papadopoulou D, Modi M, et al. Effects of glucagon-like peptide-1 on the reproductive axis in healthy men. J Clin Endocrinol Metab 2020;105:1119–25.
15. Mills EG, Ertl N, Wall MB, Thurston L, Yang L, Suladze S, et al. Effects of kisspeptin on sexual brain processing and penile tumescence in men with hypoactive sexual desire disorder: a randomized clinical trial. JAMA Netw Open 2023;6:e2254313.
16. Abbara A, Eng PC, Phylactou M, Clarke SA, Richardson R, Sykes CM, et al. Kisspeptin receptor agonist has therapeutic potential for female reproductive disorders. J Clin Invest 2020;130:6739–53.
17. Jayasena CN, Abbara A, Veldhuis JD, Comninos AN, Ratnasabapathy R, De Silva A, et al. Increasing LH Pulsatility in women with hypothalamic amenorrhoea using intravenous infusion of kisspeptin-54. J Clin Endocrinol Metab 2014;99:E953–61.
18. Thurston L, Hunjan T, Mills EG, Wall MB, Ertl N, Phylactou M, et al. Melanocortin 4 receptor agonism enhances sexual brain processing in women with hypoactive sexual desire disorder. J Clin Invest 2022;132:e133633.
19. Thurston L, Hunjan T, Ertl N, Wall MB, Mills EG, Suladze S, et al. Effects of kisspeptin administration in women with hypoactive sexual desire disorder: a randomized clinical trial. JAMA Netw Open 2022;5:e2236131.
20. Prague JK, Roberts RE, Comninos AN, Clarke S, Jayasena CN, Nash Z, et al. Neurokinin 3 receptor antagonism as a novel treatment for menopausal hot flushes: a phase 2, randomised, double-blind, placebo-controlled trial. Lancet 2017;389:1809–20.
21. Abbara A, Adams S, Phylactou M, Izzi-Engbeaya C, Mills EG, Thurston L, et al. Quantifying the variability in the assessment of reproductive hormone levels_Supplemental figures and tables. Available at. https://doi.org/10.6084/m9.figshare.24226303.v1.
22. Wu FCW, Tajar A, Beynon JM, Pye SR, Silman AJ, Finn JD, et al. Identification of late-onset hypogonadism in middle-aged and elderly men. N Engl J Med 2010;363:123–35.
23. Gordon CM, Ackerman KE, Berga SL, Kaplan JR, Mastorakos G, Misra M, et al. Functional hypothalamic amenorrhea: an endocrine society clinical practice guideline. J Clin Endocrinol Metab 2017;102:1413–39.
24. Rotterdam ESHRE/ASRM-Sponsored PCOS Consensus Workshop Group. Revised 2003 consensus on diagnostic criteria and long-term health risks related to polycystic ovary syndrome. Fertil Steril 2004;81:19–25.
25. American Psychiatric Association. Diagnostic and statistical manual of mental disorders: DSM-IV-TR. American Psychiatric Publication; 2000.

26. American Psychiatric Association, DSM-5 Task Force. Diagnostic and statistical manual of mental disorders: DSM-5™. 5th ed. American Psychiatric Publishing, Inc. Washington; 2013. https://psycnet.apa.org/record/2013-14907-000.
27. Ahokoski O, Virtanen A, Huupponen R, Scheinin H, Salminen E, Kairisto V, et al. Biological day-to-day variation and daytime changes of testosterone, follitropin, lutropin and oestradiol-17β in healthy men. Clin Chem Lab Med 1998;36:485–91.
28. Wang C, Catlin DH, Demers LM, Starcevic B, Swerdloff RS. Measurement of total serum testosterone in adult men: comparison of current laboratory methods *versus* liquid chromatography-tandem mass spectrometry. J Clin Endocrinol Metab 2004;89:534–43.
29. Miller KK, Rosner W, Lee H, Hier J, Sesmilo G, Schoenfeld D, et al. Measurement of free testosterone in normal women and women with androgen deficiency: comparison of methods. J Clin Endocrinol Metab 2004;89:525–33.
30. Rosner W, Auchus RJ, Azziz R, Sluss PM, Raff H. Position statement: utility, limitations, and pitfalls in measuring testosterone: an Endocrine Society position statement. J Clin Endocrinol Metab 2007;92:405–13.
31. Zainol Abidin A, Rahim R, Md Arshad MK, Fatin Nabilah MF, Voon CH, Tang TH, et al. Current and potential developments of cortisol aptasensing towards point-of-care diagnostics (POTC). Sensors 2017;17:1180.
32. Shlykova N, Davidson E, Krakowsky Y, Bolanos J, Traish A, Morgentaler A. Absent diurnal variation in serum testosterone in young men with testosterone deficiency. J Urol 2020;203:817–23.
33. Vitzthum VJ, Worthman CM, Beall CM, Thornburg J, Vargas E, Villena M, et al. Seasonal and circadian variation in salivary testosterone in rural Bolivian men. Am J Hum Biol 2009;21:762–8.
34. Panico S, Pisani P, Muti P, Recchione C, Cavalleri A, Totis A, et al. Diurnal variation of testosterone and estradiol: a source of bias in comparative studies on breast cancer. J Endocrinol Invest 1990;13:423–6.
35. Hastings MH. Neuroendocrine rhythms. Pharmacol Ther 1991;50:35–71.
36. van Kerkhof LWM, Van Dycke KCG, Jansen EHJM, Beekhof PK, van Oostrom CTM, Ruskovska T, et al. Diurnal variation of hormonal and lipid biomarkers in a molecular epidemiology-like setting. PLOS ONE 2015;10:e0135652.
37. Lehtihet M, Arver S, Bartuseviciene I, Pousette Å. S-testosterone decrease after a mixed meal in healthy men independent of SHBG and gonadotrophin levels. Andrologia 2012;44:405–10.
38. Meikle AW, Stringham JD, Woodward MG, McMurry MP. Effects of a fat-containing meal on sex hormones in men. Metabolism 1990;39:943–6.
39. Volek JS, Love DM, Avery NG, Sharman MJ, Kraemer WJ. Effects of a high-fat diet on postabsorptive and postprandial testosterone responses to a fat-rich meal. Metabolism 2001;50:1351–5.
40. Habito RC, Ball MJ. Postprandial changes in sex hormones after meals of different composition. Metabolism 2001;50:505–11.
41. Vollmer K, Holst JJ, Baller B, Ellrichmann M, Nauck MA, Schmidt WE, et al. Predictors of incretin concentrations in subjects with normal, impaired, and diabetic glucose tolerance. Diabetes 2008;57:678–87.
42. Izzi-Engbeaya C, Jones S, Crustna Y, Machenahalli PC, Papadopoulou D, Modi M, et al. Effects of peptide YY on the Hypothalamic-pituitary-gonadal axis in healthy men. J Clin Endocrinol Metab 2020;105:833–8.
43. Izzi-Engbeaya C, Jones S, Crustna Y, Machenahalli PC, Papadopoulou D, Modi M, et al. Acute effects of glucagon on reproductive hormone secretion in healthy men. J Clin Endocrinol Metab 2020;105:1899–905.
44. Grossmann M. Low testosterone in men with type 2 diabetes: significance and treatment. J Clin Endocrinol Metab 2011;96:2341–53.
45. Liang S, Kinghorn AB, Voliotis M, Prague JK, Veldhuis JD, Tsaneva-Atanasova K, et al. Measuring luteinising hormone pulsatility with a robotic aptamer-enabled electrochemical reader. Nat Commun 2019;10:852.
46. Hall JE. Endocrinology of the menopause. Endocrinol Metab Clin North Am 2015;44:485–96.

Cuantificando la variabilidad en la valoración de niveles de hormonas reproductivas

Objetivo: Cuantificar cómo de representativa del perfil hormonal diario es una única medición del nivel de hormonas reproductivas, empleando datos de un muestreo hormonal detallado en una rama tratada con placebo salino realizado durante varias horas.

Diseño: Análisis retrospectivo de datos de estudios intervencionistas previos sobre evaluación de hormonas reproductivas.

Marco: Instalación de investicación clínica en un centro terciario de endocrinología reproductiva en Imperial College Hospital NHS Foundation Trust.

Pacientes: Se incluyó a 266 individuos en el análisis, incluyendo a hombres y mujeres sanos (n = 142) y a aquellos con enfermedades reproductivas (n = 124 [11 con amenorrea hipotalámica funcional, 6 con síndrome de ovario poliquístico, 62 mujeres y 32 hombres con desorden de deseo sexual hipoactivo y 13 mujeres postmenopáusicas]).

Intervenciones: Se utilizaron los datos de 266 individuos que habían sido sujetos a un muestreo hormonal detallado en las ramas tratadas con placebo salino de estudios previos para cuantificar la variabilidad en hormonas reproductivas debida a su secreción pulsátil, a la variación diurna y a la alimentación empleando el coeficiente de variación (CV) y la entropía.

Medidas principales de resultados: La capacidad de una única medición del nivel de una hormona reproductiva para cuantificar la variabilidad en niveles de hormonas reproductivas debida a la secreción pulsátil, la variación diurna y la ingesta de nutrientes.

Resultados: El valor matutino inicial de los niveles de hormonas reproductivas fue habitualmente mayor que la media del valor durante el día (porcentaje de descenso desde la medición matutina inicial a la media diaria: hormona luteinizante 18,4%, hormona folículo-estimulante 9,7%, testosterona 9,2% y estradiol 2,1%). El nivel de hormona luteinizante fue el más variable (CV 28%), seguido de las hormonas sexuales esteroideas (testosterona 12% y estradiol 13%), mientras que la hormona folículo-estimulante fue la menos variable (CV 8%). En varones sanos, los niveles de testosterona bajaron un 14,9% (intervalo de confianza del 95% 4,2 a 25,5%) entre las 9:00 y las 17:00, aunque los niveles matutinos se correlacionaron con, y podrían ser predecidos a partir de, los niveles vespertinos del mismo individuo ($r^2 = 0{,}53$; $p < 0{,}0001$). Los niveles de testosterona descendieron más tras una comida mixta (34,3%) que durante alimentación *ad libitum* (9,5%), tras una carga de glucosa oral (6,0%) o una carga de glucosa intravenosa (7,4%).

Conclusión: La cuantificación de la variabilidad de una única medición de los niveles de hormonas reproductivas informa de la fiabilidad de la evaluación de las hormonas reproductivas.