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Determining the Relationship Between Hot Flushes and LH Pulses in Menopausal Women Using Mathematical Modeling

Julia K. Prague,1* Margaritis Voliotis,2,3* Sophie Clarke,1 Alexander N. Comninos,1,4 Ali Abbara,1 Channa N. Jayasena,1 Rachel E. Roberts,1 Lisa Yang,1 Johannes D. Veldhuis,5 Krasimira Tsaneva-Atanasova,2,3,6 Craig A. McArdle,7 and Waljit S. Dhillo1

1Section of Endocrinology & Investigative Medicine, Imperial College, London W12 0NN, United Kingdom; 2College of Engineering, Mathematics, and Physical Sciences, University of Exeter, Exeter EX4 4QF, United Kingdom; 3EPSRC Centre for Predictive Modelling in Healthcare, University of Exeter, Exeter EX4 4QF, United Kingdom; 4Department of Endocrinology, Imperial College Healthcare NHS Trust, London W2 1NY, United Kingdom; 5Mayo Clinic, Rochester, Minnesota 55905; 6Living Systems Institute, University of Exeter, Exeter EX4 4QD, United Kingdom; and 7Bristol Medical School, University of Bristol, Bristol BS1 3NY, United Kingdom

ORCID numbers: 0000-0001-6502-4757 (J. K. Prague); 0000-0001-6488-7198 (M. Voliotis); 0000-0002-6294-7051 (K. Tsaneva-Atanasova); 0000-0003-4836-5351 (C. A. McArdle); 0000-0001-5950-4316 (W. S. Dhillo).

Background: Hypothalamic kisspeptin/neurokinin B/dynorphin (KNDy) neurones regulate LH pulsatility. It is widely accepted that the menopausal hot flush (HF) consistently synchronizes with the LH pulse, implicating the hypothalamic KNDy neurones in generating LH pulsatility and HF. Using a modern immunoassay and mathematical modeling, we investigated if the HF and LH pulse were consistently synchronized in menopausal women.

Methods: Eleven menopausal women (51 to 62 years of age and experiencing ≥7 HF in 24 hours) participated in an 8-hour study. Subjects self-reported HF and underwent peripheral blood sampling every 10 minutes. LH pulsatility was determined using two mathematical models: blinded deconvolution analysis and Bayesian spectrum analysis. The probability that the LH pulse and HF event intervals matched was estimated using the interval distributions observed in our data.

Results: Ninety-six HFs were self-reported, and 82 LH pulses were identified by blinded deconvolution analysis. Using both models, the probability that the two event intervals matched was low in the majority of participants (mean $P = 0.24$; $P = 1$ reflects perfect association).

Interpretation: Our data challenge the widely accepted dogma that HFs consistently synchronize with an LH pulse and therefore have clinically important therapeutic and mechanistic implications. (J Clin Endocrinol Metab 104: 3628–3636, 2019)

Seventy percent of women experience hot flushes (HFs) secondary to the decline in circulating estrogen levels associated with the menopause (1), and 10% describe them as intolerable (2). Symptoms are typically long-lasting (median, 7.4 years) (3) and disrupt all aspects of daily life. Their precise etiology has been of...
LH pulses measured had been associated with a reported flush (32/34), and 26 of the 31 measured skin temperature increases were reported to be in all instances. In the Tataryn cohort, nearly all measurements for HFs (13). The relationship between these patterns of secretion varies between species, age, and context, including disease state (14). This approach can be robustly applied to assessing hormone secretion patterns and more specifically to estimating their pulse intervals along with the associated uncertainty.

In this study, we determined whether the LH peak was associated with HFs in menopausal women using a modern LH immunoassay and two independent and established mathematical models. Blinded deconvolution analysis was used to identify LH peaks and the likelihood that they coincide with self-reported HFs, and Bayesian spectrum analysis was used to determine the probability distribution for LH pulse intervals and the probability of a match between the LH peak and self-reported HF intervals.
Patients and Methods

Protocol

Eleven participants attended our temperature-controlled clinical research facility for a single 8-hour study visit. Once a participant was introduced to the unit, a cannula was inserted in a peripheral vein under aseptic conditions (time - 30 minutes), through which all blood samples were taken every 10 minutes from time 0 until time 480 minutes. All participants were ambulatory and could eat and drink freely during the study visit. Precise timings of self-reported HF episodes were recorded in real time. All blood samples were left to clot for at least 30 minutes prior to centrifugation at 503rcf for 10 minutes, after which the serum supernatant was extracted and immediately frozen at -20°C for analysis using an automated chemiluminescent immunoassay method (Abbott Diagnostics, Maidenhead, UK) in batches after study completion. Reference ranges were as follows: LH, 4 to 14 IU/L; respective intra-assay and interassay coefficients of variation, 4.1% and 2.7%; analytical sensitivity, 0.5 IU/L.

Statistical analysis

LH pulsatility was determined from the raw data of LH measurements over the 8-hour study period using two independent mathematical models. Using a blinded deconvolution (empirical) method with 93% sensitivity and 93% specificity, we calculated onset times and pattern regularity of the LH pulses (orderliness) as per Veldhuis et al. (14). Using Bayesian spectrum analysis (BSA), we calculated the probability density function for interpulse interval for each participant conditional on the observed data. The BSA method was then applied to the accompanying self-reported HF event data to obtain the posterior distribution for the interval between episodes. To achieve this, HF event data were transformed into a binary (0-1) time series, where 1 indicates an HF episode and 0 indicates no episode. BSA was performed in R using the BaSaR library (Bayesian Spectrum Analysis in R) (16).

For each participant, the two distributions of LH pulse and HF intervals were used to investigate the association between HFs and LH pulses. A perfect association between LH pulses and HF events requires that the corresponding intervals are equal. Therefore, it is possible to use the two distributions to calculate the probability that the intervals match (i.e., are “approximately” equal to each other) and use this probability to denote the degree of correlation between the occurrence of LH pulses and HF events. This approach is illustrated for simulated (i.e., artificially created or synthetic) data in the online repository (17), where the probability was calculated using the following formula:

\[
P_{\text{match}} = \int_{-\infty}^{\infty} P_{LH}(t|D) \left( \int_{t - \varepsilon/2}^{t + \varepsilon/2} P_{HF}(t'|D)dt' \right) dt
\]

where \(P_{LH}(t|D)\) and \(P_{HF}(t'|D)\) are the posterior distributions for \(T_{LH}\) (LH pulse intervals) and \(T_{HF}\) (HF intervals); \(T_{\text{min}} = 20\) minutes and \(T_{\text{max}} = 240\) minutes specify the range for \(T_{LH}\) and \(T_{HF}\); and the parameter \(\varepsilon\) determines the acceptable discrepancy between the intervals (i.e., intervals match if \(|T_{LH} - T_{HF}| \leq \varepsilon/2\)). We estimated the probability for \(e = 10\) minutes, which is the LH sampling interval in our clinical study, and for \(e = 20\) minutes, which is twice the LH sampling interval. This allowed us to test the robustness of our method against variations of this parameter.

To further supplement our analysis, we estimated the probability that the LH pulse and HF event intervals match (i.e., are equal) using the empirical distributions of intervals observed in our data. To do so, for each participant we calculated (i) the intervals between self-reported HF events and (ii) the intervals between the onset of LH pulses that were identified by the blinded deconvolution method (14). Then, we calculated the probability that the LH pulse and HF intervals match as the fraction of pairs from the two interval groups (LH pulse and HF) that are matched within \(e\) minutes, where, similar to the Bayesian analysis, \(e\) was set to 10 minutes and 20 minutes. Ten minutes was selected because this was the frequency of blood sampling, and 20 minutes was selected because this was double the length of the frequency of blood sampling.

Study approval

Ethical approval was obtained from the West London Regional Ethics Committees (15/LO/1481). All participants provided written informed consent prior to inclusion. The study was performed in accordance with Good Clinical Practice guidelines. Eligible participants were healthy women aged 40 to 62 years, experiencing at least seven HFs per 24-hour period (some of which were bothersome or severe) who had not had a menstrual period for at least 12 months and who had not been taking any medication shown to improve menopausal flushes in the preceding 8 weeks.

Results

The cohort of 11 participants had a mean age of 56 years (range, 51 to 62 years) and a mean body mass index of 26.0 kg/m² (range, 19.7 to 36.7 kg/m²). Eight of the participants were white, and three were Black Caribbean. The mean time since onset of HFs was 103 months (range, 20 to 192 months), and the mean time since last menstrual period was 104 months (range, 36 to 192 months). Three subjects were current smokers, and mean LH at screening was 28 IU/L (SD, 7.2). All possible covariates were included in exploratory analyses of LH pulse number, amplitude, and orderliness as per our a priori statistical plan but were removed from the final analysis models when not shown to be significant.

The total number of HFs reported was 96, and the total number of LH pulses identified by deconvolution analysis was 82. The mean number of HFs per participant was nine (SD, 4.38; range, 2 to 19), and the mean number of LH pulses per participant was eight (SD, 1.97; range, 4 to 10) (Table 1). For both methods (BSA and blinded deconvolution analysis), the probability of a match for the intervals between the LH pulses and HFs varied greatly between participants (lowest \(P = 0.062\) to highest \(P = 0.816\), where \(P = 1\) reflects a perfect match) (Table 1). For the majority of participants (8/11), the probability of a match was <0.5 (irrespective of the method used and of whether intervals were matched within 10 or 20 minutes), suggesting a weak association.
between the occurrence of the HF and the LH pulse in the majority of menopausal women (Table 1).

The individual participant histograms for HF and LH pulse interval enable an estimate of certainty to be deduced from the shape of the distribution (i.e., narrow distribution suggests increased certainty; wide distribution with multiple peaks suggests low certainty), which reflects the extent of the variation in the certainty of the frequency estimate for both intervals between participants. This is illustrated for simulated (i.e., artificially created or synthetic) data in the online repository (17). Figure 1 incorporates all raw data for all 11 menopausal women in our study.

Good concordance was demonstrated between the BSA and empirical method in calculating the probability that the intervals between LH pulses and HF episodes matched (Fig. 2), although the match probabilities were typically a little higher when intervals where matched within 20 minutes rather than within 10 minutes.

Figure 1. Individual raw data for all participants and the corresponding histograms for LH pulses and HF event intervals as determined by BSA. For each participant: (Left panel) Black line: LH (mIU/L) measured every 10 min from a peripheral indwelling venous cannula; red line: self-report of an HF. (Right panel) Posterior distribution of LH pulse interval (black) and HF event interval (red) as determined by BSA. The probability of the two intervals (LH pulse and HF) matching is calculated, where \( P = 1 \) reflects a perfect association.

Discussion

Using two independent and established mathematical models, we have demonstrated that, in the majority of menopausal women in this study, there was no clear association between LH pulse and HF interval (Table 1). This is in contrast to the repeatedly referenced and accepted long-held conclusion that the LH pulse synchronizes with the onset of the menopausal HF. In only one menopausal woman out of 11 did we determine a high probability (P > 0.8) that the intervals between LH pulse and HF episodes matched. Furthermore, in contrast to Casper et al. (9), we did not find that the women who had the most frequent LH pulses had the highest frequency of HF episodes. Because the methodological detail regarding how LH pulses were defined is not included in the paper by Casper et al. (9), further interpretation or inference as to why these two analyses have conflicting results is problematic. Furthermore, using improved methodology (modern LH assay and robust mathematical analysis of LH pulsatility), we did not confirm the close temporal association between LH pulse and HF episode reported.
in the paper by Tataryn et al. (10), where LH pulses were defined as increases of LH of 20% over nadir.

However, the presence of HFs in other clinical conditions that affect the secretion of LH from the pituitary and/or GnRH neurones suggests that, in some circumstances, the synchronicity between LH secretion and HFs suggested by Casper et al. (9) and Tataryn et al. (10) is not seen. For example, women with hypopituitarism still experience HFs if administered exogenous estrogen that is subsequently withdrawn despite having no ability to secrete LH (12). Similarly, premenopausal women who are administered GnRH agonists to suppress secretion of LH and sex steroids for a condition such as endometriosis also experience HFs on treatment (18). However, whereas LH pulses are due to the pulsatile secretion of GnRH (19), GnRH secretion itself cannot be the sole causative factor of HFs because exogenous estrogen withdrawal in female patients with a genetic cause of hypogonadotrophic hypogonadism secondary to failure of the hypothalamic GnRH neurones to develop and migrate appropriately (Kallman syndrome) causes HFs (20).
Furthermore, HFs do not occur in hypothalamic amenorrhea where estrogen and GnRH levels are both low in response to a physiological stress such as undernutrition (20).

The recent discovery that highly conserved hypothalamic kisspeptin/neurokinin B/dynorphin (KNDy) neurones that colocalize kisspeptin, NKB, and dynorphin act upstream of the GnRH neurones to regulate the secretion of LH from the anterior pituitary has changed the understanding of the neuroendocrine control of reproduction (21, 22). Within this context, our data indicate two possible scenarios. The first scenario is that the pulsatile kisspeptin output from KNDy neurones that drives pulsatile GnRH and LH secretion also drives the HFs, in which case we would have to

![Figure 1. (Continued)](image)

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Outcomes and probabilities that the intervals between LH pulses and HF episodes match within 10 and 20 min as determined by BSA and an empirical method (blinded deconvolution analysis) as per Veldhuis et al. (14). A probability match of 1 reflects a perfect association between timing of LH pulses and menopausal HFs.
Correlation coefficient $r = 0.92$ ($r = 1$ reflects a perfect linear association), suggesting good concordance between the two mathematical models.

![Figure 2. Summary plot of the probability that the intervals between LH pulses and HF episodes are matched within 10 min as determined by BSA (x-axis) and an empirical method (blinded deconvolution analysis; x-axis) for all 11 participants (each marked by a black circle). Correlation coefficient $r = 0.92$ ($r = 1$ reflects a perfect linear association), suggesting good concordance between the two mathematical models.](image)

invoke an additional regulator of the HFs to explain the uncoupling. The second and more likely scenario is that the HFs are driven by a separate, independent output from the KNDy neurones so that the HFs are not necessarily synchronized with kisspeptin, GnRH, or LH pulses. NKB is from the KNDy neurones so that the HFs are not necessarily that the HFs are driven by a separate, independent output

In summary, using a modern LH immunoassay and two independent, established mathematical models, we have demonstrated that LH pulses and HFs were not consistently synchronized in the majority of menopausal women in this study. It is well established that these events can be uncoupled in conditions such as hypothalamic amenorrhea or estradiol withdrawal from women with hypopituitarism, but our data clearly suggest that such uncoupling is also highly prevalent in menopausal women. The clear implication is that other factors contribute to their etiology, raising the future possibility of more specific therapeutic approaches, such as NKB antagonists, for menopausal HFs.

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**Clinical Trial Information:** Clinicaltrials.gov no. NCT02668185 (registered 29 January 2016).

**Author Contributions:** J.K.P., A.N.C., A.A., C.N.J., and W.S.D. designed the study. J.K.P., S.C., R.E.R., and L.Y. completed the study visits and laboratory processing. J.D.V. completed the blinded deconvolution analysis. M.V. completed the Bayesian spectrum analysis under the supervision of K.T.-A. and C.A.M. J.K.P. and M.V. wrote the first draft of the manuscript. All authors contributed to subsequent versions.

**Correspondence and Reprint Requests:** Waljit S. Dhillo, PhD, Imperial College School of Medicine, 6th Floor Commonwealth Building, Hammersmith Campus 150 Du Cane Rd., London, London W12 0NN, United Kingdom. E-mail: w.dhillo@imperial.ac.uk.

**Disclosure Summary:** The authors have nothing to disclose.
References and Notes