Twice-Daily Subcutaneous Injection of Kisspeptin-54 Does Not Abolish Menstrual Cyclicity in Healthy Female Volunteers

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Background: Kisspeptin is a critical hypothalamic regulator of reproductive function. Chronic kisspeptin administration causes profound tachyphylaxis in male monkeys and in women with functional hypothalamic amenorrhea. The pharmacological effects of chronic kisspeptin exposure in healthy women with normal menstrual cycles have not been studied previously.

Aim: Our aim was to determine the effects of follicular-phase kisspeptin-54 treatment on menstrual cyclicity in healthy women.

Methods: We performed a prospective, single-blinded, 1-way crossover study. Healthy women received twice-daily sc injections of kisspeptin (6.4 nmol/kg) or 0.9% saline during menstrual days 7–14 (n = 5 per treatment arm). Serial assessments of basal reproductive hormones, ultrasound parameters, LH pulsatility, and acute sensitivity to GnRH and kisspeptin-54 injection were performed.

Results: Menstrual cyclicity persisted in all women after follicular-phase kisspeptin-54 treatment. Chronic exposure to kisspeptin-54 did not abolish acute stimulation of LH after injection of kisspeptin-54 or GnRH. In addition, kisspeptin-54 treatment was associated with a shorter mean length of the menstrual cycle (mean length of menstrual cycle was 28.6 ± 1.4 days with saline vs 26.8 ± 3.1 days with kisspeptin, P < .01), earlier onset of highest recorded serum LH (mean menstrual day of highest LH was 15.2 ± 1.3 with saline vs 13.0 ± 1.9 with kisspeptin, P < .05), and earlier onset of the luteal phase (mean menstrual day of progesterone increase was 18.0 ± 2.1 with saline vs 15.8 ± 0.9 with kisspeptin, P < .05).

Conclusion: Our data suggest that 1 week of exogenous kisspeptin-54 does not abolish menstrual cyclicity in healthy women. Further work is needed to determine whether kisspeptin could be used to treat certain anovulatory disorders. (J Clin Endocrinol Metab 98: 4464–4474, 2013)
Subjects and Methods

Subjects

Ethical approval was granted by the Hammersmith and Queen Charlotte’s and Chelsea Hospitals Research Ethics Committee (registration number 05/Q0406/142). Written informed consent was obtained from all subjects. This study was performed in accordance with the Declaration of Helsinki. Five healthy female subjects with regular menstrual cycles were recruited through advertisements placed in local newspapers (age 31.6 ± 2.6, range 24–37 years; weight 60.4 ± 2.5, range 50.4–63.8 kg) as described previously (22, 23).

Protocol

A single-blinded, placebo-controlled, 1-way crossover design study of 10 menstrual cycles was performed (Figure 1). During month 1 of the study protocol, five healthy female subjects self-administered twice-daily sc saline injections between days 7 and 14 of their menstrual cycle (see Subject Characteristics in Supplemental Table 1, published on The Endocrine Society’s Journals Online website at http://jcem.endojournals.org). During month 2 of the study protocol, the same five healthy female subjects self-administered twice-daily sc kisspeptin-54 injections (6.4 nmol/kg; equivalent to 37 μg/kg) (19) between days 7 and 14 of their menstrual cycle; (see Supplemental Methods for kisspeptin-54 peptide synthesis and testing). Day 1 of each month was defined as the first day of menstrual bleeding.

Kisspeptin injections

All subjects were trained in self-administration of sc injections by an investigator at the start of the study protocol. At the beginning of each week when injections were to be performed, a box containing unlabeled vials of freeze-dried saline (month 1) or vials of freeze-dried kisspeptin-54 (month 2), alcohol wipes, saline ampoules for reconstitution of freeze-dried vial contents, 0.5-mL insulin syringes with needles, and needle disposal bins was given to each subject. For injection, vial contents were reconstituted in 0.5 mL of 0.9% saline. A 0.5-mL insulin syringe was then used to inject saline alone (month 1) or 6.4 nmol/kg kisspeptin-54 (month 2) into the lower anterior abdominal region sc. This kisspeptin-54 dose was the same as used in our previous work in healthy women and women with HA (25, 26). Volunteers were instructed to prepare and perform injections in the morning after breakfast (unless attending for a study in which case it was done by the volunteer at time 0 of the study) and in the evening before bed. The volume of the saline or kisspeptin injections was identical. Subjects were instructed to refrigerate vials stored at home. Before commencing study visits, injection sites were inspected, and numbers of returned vials, insulin syringes, and saline or kisspeptin vials were counted to monitor compliance. Plasma kisspeptin immunoreactivity (IR) was also assessed throughout the study protocol to confirm compliance.

Baseline period

During menstrual days 1 to 6 of each month of the study protocol, pituitary responsiveness was assessed by a GnRH test (see Supplemental Methods for protocol), and baseline reproductive hormones and ultrasound markers were measured. The collection, processing, and analysis of blood samples are detailed.
in Supplemental Methods. The baseline period also allowed the acclimatization of subjects to study conditions. No injections were administered during this period.

**Treatment period**

During menstrual days 7 to 14 of each month of the study protocol, subjects self-administered twice-daily, single-blinded sc injections of saline (month 1 of study protocol) or kisspeptin-54 (month 2 of study protocol) as above.

**Posttreatment period**

During menstrual days 15 to 28 of each month of the study protocol, subjects underwent a posttreatment observation period to assess pituitary responsiveness (GnRH test) and measure circulating reproductive hormones and ultrasound markers. No injections were administered during this period.

**Four-hour blood sampling after injection of saline or kisspeptin**

All subjects underwent blood sampling during the 4-hour period immediately after the first injection of the saline (day 7, month 1) or kisspeptin-54 (day 7, month 2) treatment period to confirm that kisspeptin-54 acutely stimulated LH release as previously shown in healthy women (23). Saline or kisspeptin-54 (6.4 nmol/kg) was sc administered at 0 minutes by the subject, and blood was sampled for serum LH, FSH, estradiol, and plasma kisspeptin IR at −30, 0, 10, 20, 40, 60, 90, 120, 150, 180, 210, and 240 minutes.

**Eight-hour blood sampling for assessment of LH pulsatility**

Subjects underwent 3 assessments of LH pulsatility during month 1 and month 2 of the study protocol. Baseline assessment of LH pulsatility was performed on menstrual day 6 (1 day before commencing injections). LH pulsatility was also assessed after injection of saline or kisspeptin-54 on menstrual days 11 and 14. The saline or kisspeptin-54 was reconstituted using one of the vials given to each subject at the beginning of the treatment period for home storage. Blood was sampled every 10 minutes. Studies commenced between 8:00 and 9:00 AM.

**Assessment of pituitary sensitivity before and after injections of saline or kisspeptin**

Subjects each underwent 2 GnRH tests during month 1 and month 2 of the study protocol (see Supplemental Methods for protocol). A baseline GnRH test was performed on menstrual day 1 and was repeated in each subject 24 hours after final saline or kisspeptin injection (menstrual day 15).

**Basal measurement of reproductive hormones**

Basal measurements of serum LH, FSH, estradiol, progesterone, and plasma kisspeptin IR were taken from subjects during days 1, 6, 7, 11, 14, 15, 18, 21, and 28 during month 1 and month 2 of the study protocol. On days 7, 11, and 14, the basal blood sample was taken before the morning injection.

**Ultrasound scans**

Transabdominal ultrasound scans were performed on days 1, 7, 15, 18, 21, and 28 during month 1 and month 2 of the study protocol. Transvaginal scans were not used to minimize discomfort to volunteers during repeated examinations. The ultrasonographer was blinded to treatment for all subjects. During each scan, the following parameters were measured: endometrial thickness (in millimeters), mean ovarian volume (in cubic centimeters), mean follicle number, and maximum diameter of largest follicle in each ovary (in millimeters). Ovulation was defined as a rise in serum progesterone >10 nmol/L together with suggestive radiological features (visualization of a dominant follicle with subsequent appearance of a preovulatory follicle and/or corpus luteum).

**Data analysis**

Investigators performing the clinical studies were blinded to results until all subjects had completed the study protocol. J.D.V. used an established, blinded deconvolution method with 93% sensitivity and specificity (27) to identify LH pulses and calculate the secretory mass of LH pulses (integral of LH secretion over time during a secretory burst normalized per liter of distribution volume). Cumulative levels of basal, pulsatile, and total (basal plus pulsatile) LH secretion were also estimated during each study. Data are presented as mean ± SEM. Kisspeptin IR data were log-transformed to normalize data before data analysis. All other analyses included data series, most which had normal distributions assessed using the Kolmogorov-Smirnov test with Dallal-Wilkinson-Lillie analysis. Hormone profiles during 4-hour blood sampling studies were analyzed using repeated-measures 2-way ANOVA with Bonferroni post hoc correction. Pairs of means were analyzed using the unpaired two-tailed t test. Multiple means were compared using 1-way ANOVA with Bonferroni’s multiple-comparison test. In all cases, \( P < .05 \) was considered statistically significant.

**Results**

**Acute effects of saline or kisspeptin-54 injection on plasma kisspeptin at the commencement, midpoint, and end of twice-daily administration in healthy women**

**Acute changes in plasma kisspeptin IR after injection of saline or kisspeptin-54**

Plasma kisspeptin was unchanged at approximately 10 pmol/L after injection of saline on the first (Figure 2A), fourth (Figure 2B), and final (seventh; Figure 2C) days of twice-daily administration. Kisspeptin-54 injection acutely elevated plasma kisspeptin IR on the first day of administration, with peak mean kisspeptin IR levels of 2421 ± 392 pmol/L at 45 minutes after injection (\( P < .001 \) vs saline) (Figure 2A). Similar elevations of kisspeptin IR were observed after injection of kisspeptin on the fourth and last injection days (Figure 2, B and C).

**Plasma kisspeptin IR preinjection of saline or kisspeptin-54**

In subjects receiving saline, plasma kisspeptin IR measured before the morning saline injection remained ap-
proximately 10 pmol/L throughout the study protocol (Figure 2D). In subjects receiving kisspeptin injections, plasma kisspeptin IR was not elevated on menstrual days 1, 6, or 7 because these blood samples were taken before the first kisspeptin-54 injection. Plasma kisspeptin IR was elevated on menstrual days 11 and 14 (during the twice-daily kisspeptin treatment period but just before the morning kisspeptin injection), which suggested compliance with kisspeptin injection the previous evening. As expected, plasma kisspeptin IR was not elevated on the morning of day 15, because it was approximately 24 hours after the final kisspeptin-54 injection. Furthermore, plasma kisspeptin IR remained <10 pmol/L on days 18 to 28 of the study protocol (Figure 1D).

Acute effects of saline or kisspeptin-54 injection on serum reproductive hormones at the commencement, midpoint, and end of twice-daily administration in healthy women

Commencement of treatment period (menstrual day 7)

Saline injection did not change serum LH levels when compared with baseline (Figure 3A, baseline LH 5.20 ± 0.64 IU/L). Kisspeptin-54 injection acutely increased serum LH levels in subjects when compared with saline (Figure 3A, P < .05 at 180–210 minutes, baseline LH 5.68 ± 0.98 IU/L). The mean maximal increase in LH from baseline after kisspeptin injection was observed at 180 minutes and was 8.6 ± 3.4 IU/L above baseline.

Midpoint of treatment period (menstrual day 11)

Saline injection did not change serum LH or FSH compared with baseline (Figure 3B, baseline LH 5.74 ± 1.45 IU/L). Kisspeptin-54 injection acutely increased serum LH levels in subjects when compared with saline (Figure 3B, P < .01 at 210 minutes, and P < .05 at 220 to 240 minutes, baseline LH 9.41 ± 4.89 IU/L). The mean maximal increase in LH from baseline after kisspeptin injection was observed at 210 minutes and was 8.3 ± 2.4 IU/L above baseline.

End of treatment period (menstrual day 14)

Saline injection did not change serum LH or FSH compared with baseline (Figure 3C, baseline LH 17.79 ± 9.69 IU/L). The mean maximal increase in LH from baseline after kisspeptin injection was observed at 220 minutes and was 12.7 ± 8.1 IU/L above baseline (baseline LH 6.01 ± 1.60 IU/L). Kisspeptin-54 injection showed a trend toward stimulating serum LH, but this did not reach statistical significance compared with saline. During days 7, 11, and 14 of the treatment period, total LH secretion was
increased significantly after kisspeptin-54 when compared with saline ($P < .01$ using 2-way ANOVA) (Figure 3D). We also compared the magnitude of serum LH increase 1 hour after kisspeptin-54 injection with the peak increase in serum LH after kisspeptin-54 injection (Supplemental Figure 1). On menstrual day 7, the peak LH response was 3-fold higher when compared with the LH response 1 hour after kisspeptin-54 injection (increase in serum LH was 9.2 ± 4.5 IU/L at 1 hour; peak 3.1 ± 1.8 IU/L, $P = .063$ vs 1 hour). On menstrual days 11 and 14, there was virtually no change in mean serum LH 1 hour after kisspeptin-54 injection (<1 IU/L), whereas peak increases in serum LH of 19.7 ± 9.5 IU/L ($P = .059$ vs 1 hour) and 23.0 ± 12.9 IU/L ($P = .067$ vs 1 hour) were later observed, respectively.

**Effects of twice-daily saline or kisspeptin injections on length of the menstrual cycle and biochemical markers of reproductive activity in healthy women**

**Length of menstrual cycle**

Subjects had the same menstrual cycle length before commencing the study when compared with menstrual cycle length during saline administration (mean menstrual cycle length was 28.6 ± 1.1 days before study commencement and 28.6 ± 1.4 days with saline; $P = 1.00$). However all subjects had a shorter menstrual cycle (by approximately 2 days) during kisspeptin-54 treatment when compared with saline (mean length of menstrual cycle was 28.6 ± 1.4 days with saline vs 26.8 ± 3.1 days with kisspeptin, $P < .01$) (Figure 4A).

**Timing of peak serum LH**

During kisspeptin-54 treatment, observed peak levels of serum LH and estradiol, but not FSH, were earlier during the menstrual cycle when compared with the saline group (Figure 4, B–D). Furthermore the menstrual day of highest recorded serum LH was approximately 2 days earlier during kisspeptin-54 treatment when compared with saline treatment (mean menstrual day of highest recorded serum LH was 15.2 ± 1.3 with saline vs 13.0 ± 1.9 with kisspeptin, $P < .05$) (Figure 4E).

**Timing of luteal phase of menstrual cycle**

We examined the onset of the luteal phase, which is characterized by release of a mature oocyte from the ovary and secretion of progesterone by the residual corpus luteum. During kisspeptin-54 treatment, levels of serum progesterone became elevated (>10 nmol/L) earlier during the menstrual cycle when compared with the saline group (Figure 4F). The menstrual day of onset of the luteal phase (defined as beginning when serum progesterone was
Effects of twice-daily saline or kisspeptin on LH pulsatility in healthy women

LH pulsatility was determined immediately before commencing saline or kisspeptin-54 treatment (menstrual day 6 of study protocol) and during saline or kisspeptin-54 treatment (menstrual days 11 and 14 of study protocol). Mean secretory mass was slightly lower before kisspeptin-54 treatment when compared with saline treatment (mean secretory mass was 5.9 ± 0.5 IU/L with saline vs 4.1 ± 0.4 IU/L with kisspeptin, P < .01) (Figure 6A). Despite this, mean secretory mass was increased significantly during menstrual day 11 (fourth day of treatment) during kisspeptin-54 treatment when compared with the saline treatment (mean secretory mass was 3.4 ± 0.4 IU/L with saline vs 14.5 ± 3.6 IU/L with kisspeptin, P < .01). On the last day of treatment (menstrual day 14), no significant differences in secretory mass were observed between saline or kisspeptin-54 treatment. No significant differences in pulse frequency were observed between saline and kisspeptin-54 treatment (Figure 6B). Pulsatile LH secretion was increased significantly during menstrual day 11 (fourth day of treatment) in the kisspeptin-54 group when compared with the saline group (mean secretory mass was 17.9 ± 3.6 IU/L with saline vs 78.2 ± 22.8 IU/L with kisspeptin, P < .05) (Figure 6C). However, basal and total LH secretion were not significantly different between groups (Figure 6, D and E).

Assessment of pituitary sensitivity before and after injections of saline or kisspeptin

We examined the sensitivity of all healthy female subjects to iv GnRH, both 6 days before (menstrual day 1 of study protocol) and 24 hours after saline or kisspeptin treatment (menstrual day 15 of study protocol). On menstrual day 1, no significant difference in pituitary sensitivity was observed after GnRH administration between subjects before commencing saline of kisspeptin treatment (mean maximal LH increase during first 2 hours after GnRH injection was 13.1 ± 1.1 IU/L before saline and 13.4 ± 1.1 IU/L before kisspeptin-54; P value was not significant) (Supplemental Figure 2A). Furthermore, 24
hours after cessation of twice-daily saline or kisspeptin-54 injections, no significant difference in pituitary sensitivity was observed after GnRH administration between saline and kisspeptin-54 treatment (mean maximal LH increase during first 2 hours after GnRH injection was 39.9/11006 8.9 IU/L after saline and 41.0/11006 16.4 IU/L after kisspeptin-54; P value was not significant) (Supplemental Figure 2B).

The expected physiological increase in pituitary sensitivity to GnRH during menstrual day 15 vs menstrual day 1 (28) was observed in healthy female subjects, whether receiving saline or kisspeptin treatment (Supplemental Figure 2C).

**Discussion**

Genetic studies demonstrate that kisspeptin peptides are necessary for pubertal maturation in humans (8–10). We and other investigators have recently demonstrated that exogenous kisspeptin acutely stimulates gonadotropin secretion in women (23–26). However, the pharmacological effects of chronic kisspeptin exposure in healthy women with regular menstrual cycles have not been studied previously. Chronic kisspeptin administration causes profound tachyphylaxis in male monkeys and in women in functional HA (16, 25, 26). We present novel data suggesting that menstrual cyclicity persists in healthy women after twice-daily kisspeptin-54 treatment during the follicular phase of the menstrual cycle.

In the current study, kisspeptin treatment was associated with an advanced timing of the serum progesterone increase and onset of menstrual bleeding by approximately 2 days when compared with saline. Animal studies demonstrate that kisspeptin stimulates gonadotropin secretion in a GnRH-dependent manner because its action is abolished by GnRH antagonist (14). Exogenous GnRH is sufficient to trigger ovulation (29). More studies with daily follicle morphology assessment and daily serum LH measurement would be required to confirm our findings. However, our study data raise the possibility that kisspeptin-54 administration may advance the onset of ovulation in healthy women by stimulating endogenous hypothalamic GnRH secretion. In addition to stimulating GnRH, kisspeptin itself is implicated in generation of the LH surge needed for ovulation. In rodents, a subpopulation of hypothalamic anteroventral periventricular nucleus kisspeptin neurons are implicated in generating the LH surge needed for ovulation (30) and are positively rather than negatively regulated by estradiol (31). Central administration of a monoclonal antibody to kisspeptin is sufficient to block ovulation in rats (32). Furthermore, administration of kisspeptin-10 has been shown to stimulate ovulation in the musk shrew (33), rat (34), and sheep (17). Humans have no anatomical equivalent of the anteroventral periventricular nucleus. Nevertheless, it is possible that exogenous kisspeptin-54 may have advanced the onset of the luteal phase in our female subjects by increasing kisspeptin signaling in a subpopulation of hypothalamic kisspeptin neurons that stimulate the LH surge. Several lines of evidence suggest that in humans, unlike in lower species, a change in pituitary responsiveness to GnRH rather than an actual GnRH surge is responsible for the LH surge (35, 36). It is therefore possible that kisspeptin-54 advances ovulation in women by increasing the prevailing levels of estradiol, which are needed to trigger the LH surge at a pituitary level.

Chronic kisspeptin administration has also been implicated as a potential novel therapy for inhibiting reproductive hormone secretion in contraception or the treatment of hormone-sensitive cancer; our data suggest that kisspeptin-54 may have limited therapeutic potential in this regard, at least in women at the dose of kisspeptin tested.
However, our observation that kisspeptin may advance the menstrual cycle raises the possibility that women with certain forms of infertility could be treated with kisspeptin. A recent study suggests that kisspeptin is sufficient to restore ovulation in a mouse model of anovulatory hyperprolactinemia (37). More studies are required to determine whether kisspeptin-54 treatment could be used to stimulate ovulation in women with anovulatory reproductive disorders other than HA.

Traditional ovulatory drugs such as clomiphene citrate have low pregnancy rates (38, 39). Although efficacious, in vitro fertilization confers a risk of ovarian hyperstimulation (40). Kisspeptin acts by stimulating endogenous, rather than pharmacological, levels of reproductive hormone secretion. Kisspeptin might therefore offer a fertility treatment with lower risk of ovarian hyperstimulation syndrome when compared with in vitro fertilization.

Comparison of our data with other clinical studies of kisspeptin administration is complicated by the investigation of its 2 peptide forms (kisspeptin-54 and -10) and sexual dimorphism of the effects of kisspeptin-10; iv bolus administration consistently stimulates LH men (20–22), but women are much less sensitive to the effects of kisspeptin-10 during the follicular phase of the menstrual cycle when compared with the preovulatory phase (22, 24). Although kisspeptin-54 stimulates LH in both sexes, its effects have never been compared directly between men and women (19, 23). Consistent with our findings in healthy women, George et al (21) recently observed that a 22.5-hour iv infusion of the shorter form of kisspeptin, kisspeptin-10, stimulates LH secretion without apparent tachyphylaxis in healthy men. However, in contrast to the current findings, twice-daily kisspeptin-54 treatment has been shown to rapidly cause tachyphylaxis in women with HA. Women with HA are acutely 4-fold more sensitive to the effects of kisspeptin-54 when compared with healthy women in the follicular phase of their menstrual cycle. A higher dose of kisspeptin-54 may therefore be necessary to achieve tachyphylaxis in healthy women with HA. Women with HA are acutely 4-fold more sensitive to the effects of kisspeptin-54 when compared with healthy women in the follicular phase of their menstrual cycle. A higher dose of kisspeptin-54 may therefore be necessary to achieve tachyphylaxis in healthy men. However, in contrast to the current findings, twice-daily kisspeptin-54 treatment has been shown to rapidly cause tachyphylaxis in healthy men (21, 23). Nevertheless, our data suggest that menstrual cyclicity persists in healthy women using the currently tested regimen of follicular-phase kisspeptin-54 treatment. Preliminary data have emerged suggesting that chronic administration of a kisspeptin analog causes tachyphylaxis in healthy men (41). It would be interesting to determine whether tachyphylaxis to kisspeptin-54 is also sexually dimorphic or dependent on the dose and precise form of kisspeptin receptor agonist administered.

It is interesting to note that although iv bolus injection of the shorter kisspeptin fragment, kisspeptin-10, acutely stimulates LH secretion within an hour of administration, we observed that sc injection of kisspeptin-54 acutely
stimulated peak LH secretion 3 to 4 hours after administration despite a rapid elevation of kisspeptin IR within minutes of administration. On the first injection day, the increase in serum LH 1 hour after kisspeptin-54 injection was 3-fold lower when compared with the peak increase in serum LH after kisspeptin-54 injection. Furthermore, on the fourth and final injection days, there was negligible stimulation of LH secretion 1 hour after kisspeptin-54 injection; however, serum LH later increased to peak levels 3 to 4 hours after kisspeptin injection. By comparison, Chan et al (20) observed that kisspeptin-10 stimulated peak LH secretion within an hour of commencing administration in healthy men. It is possible that these data suggest differences between the biological actions of kisspeptin-10 and -54. For instance, one might speculate that kisspeptin-10 stimulates a readily releasable pool of GnRH stored within nerve terminals of the median eminence (42, 43), whereas kisspeptin-54 might act more proximally in the GnRH neuron to stimulate de novo GnRH synthesis. Alternatively, it is possible kisspeptin-54 might require breakdown into a smaller kisspeptin fragment before becoming biologically active. However, liquid chromatography has only identified kisspeptin-54 in the human circulation acutely after kisspeptin-54 administration (19).

It is interesting to appraise the evidence that exogenous kisspeptin stimulates basal and pulsatile GnRH secretion. Although kisspeptin stimulates rat pituitary gonadotropin secretion in vitro (44), animal studies using GnRH antagonists support the view that exogenous kisspeptin stimulates basal LH secretion through a GnRH-dependent mechanism (45, 46), which is possibly mediated through GnRH nerve terminals at the median eminence (42, 43). However, it is less clear whether exogenous kisspeptin stimulates endogenous GnRH pulsatility. Sustained exposure to exogenous kisspeptin-10 or -54 stimulates pulsatile LH secretion for several hours in healthy men (21) and patients with inactivating mutations of the neurokinin B signaling pathway (47). However, because these studies merely measure LH pulsatility, it is not possible to exclude that effects reflect increasing circulating estrogen and pituitary responsiveness to unchanged, endogenous GnRH pulsatility. Chan and colleagues (20) recently demonstrated that iv bolus kisspeptin-10 increased the time to the next endogenous LH pulse in healthy men; this may represent the only current data suggesting that exogenous kisspeptin can directly modulate endogenous GnRH pulsatility in humans (by increasing the latency period to the next LH/GnRH pulse).

It is important to recognize that the current study is based on observations within a small number of subjects so may have insufficient statistical power to reveal subtle effects of kisspeptin-54 administration on menstrual cyclicity or LH pulsatility. Furthermore, the gonadotropin response to kisspeptin treatment is known to alter with the phase of the menstrual cycle in healthy women (23, 24). A larger study is needed to confirm our findings and determine what factors influence the variability in the response of subjects to kisspeptin treatment. It is possible that chronic kisspeptin-54 treatment might have effects that last beyond the treatment period. For this reason, we designed a 1-way crossover protocol in which subjects self-administered saline during the first month followed by kisspeptin-54 during the second month. It therefore remains possible that an order effect might have contributed to our results. However, it is noteworthy that LH pulse secretory mass was marginally lower at the start of the second month when compared with LH pulse secretory mass at the start of the first month (when greater stress levels would be expected). Furthermore, subjects had the same menstrual cycle length before commencing the study when compared with menstrual cycle length during saline administration. We also recognize that although estradiol measurements were compared with placebo-controlled values, the automated platform assay used to analyze serum estradiol can cross-react with other steroids.

Mean peak levels of LH during the menstrual cycle were lower during kisspeptin treatment when compared with saline treatment. Furthermore, it is important to recognize that kisspeptin-54 did not acutely stimulate significant LH secretion during the final day of twice-daily kisspeptin-54 treatment. We therefore cannot exclude that kisspeptin-54 treatment caused partial tachyphylaxis in healthy female volunteers. Alternatively, the short baseline sampling period involving just 2 blood samples may have reduced our power to detect stimulation of LH after kisspeptin-54 injection. LH levels were highly variable during menstrual day 14, which may have been caused by some but not all subjects experiencing an LH surge and by the potential effect of kisspeptin-54 to advance the onset of the LH surge in subjects.

It is noteworthy that all subjects had peak progesterone levels ≥21 mmol/L after kisspeptin-54 treatment, which is highly suggestive of ovulation. Furthermore, a corpus luteum was observed in all 5 subjects during the month of kisspeptin-54 treatment (Supplemental Table 2). Our data therefore suggest that all 5 subjects had ovulatory cycles during kisspeptin treatment. More studies are required to determine whether menstrual cycles during kisspeptin therapy have subtle physiological differences when compared with natural menstrual cycles and to determine whether anovulation is observed at a different frequency during kisspeptin treatment when compared with placebo treatment.
In summary, our data have important pharmacological implications; menstrual cyclicity persists in healthy women during a treatment regime of kisspeptin-54 previously demonstrated to cause tachyphylaxis in women with HA. Furthermore, kisspeptin-54 treatment is associated with an advanced timing of the luteal phase of the menstrual cycle when compared with placebo. More studies are required to determine whether kisspeptin-54 therapy could have potential to treat patients with anovulatory reproductive disorders.

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