Acute and chronic effects of kisspeptin-54 administration on GH, prolactin and TSH secretion in healthy women

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Title: Acute and chronic effects of kisspeptin-54 administration on GH, prolactin and TSH secretion in healthy women

Short title: Kisspeptin and pituitary hormones

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Key words: Kisspeptin, pituitary, GH, TSH, PRL

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Abstract

Background: The peptide hormone kisspeptin is essential for human reproduction, acting on the hypothalamus to stimulate gonadotrophin releasing hormone (GnRH) secretion. Kisspeptin is currently being evaluated as a novel therapeutic for women with infertility. However some animal studies suggest that kisspeptin may also stimulate growth hormone (GH), prolactin and thyroid stimulating hormone (TSH) secretion, with implications for its safety; no previous study has investigated whether kisspeptin stimulates these pituitary hormones in humans.

Aim: To determine whether kisspeptin-54 modulates GH, prolactin and TSH secretion in healthy women.

Design and participants: Prospective, single-blinded, placebo-controlled, one-way cross-over study. Five healthy women received 7 days of twice-daily subcutaneous bolus vehicle (month 1) or 6.4nmol/kg kisspeptin-54 (month 2).

Measurements: Serum samples were analysed post-hoc for GH, prolactin and TSH.

Results: Mean serum GH, PRL and TSH did not change during the first 4h following kisspeptin-54 injection when compared with vehicle. The mean frequency or amplitude of GH pulses (which influence GH function) did not change acutely following kisspeptin-54 injection when compared with vehicle. No chronic changes in serum GH, PRL or TSH were observed over the 7 day period of twice-daily kisspeptin-54 injections when compared with vehicle.

Conclusion: While we cannot exclude any effect of kisspeptin-54 on GH, prolactin or TSH secretion, we observed no significant changes in these hormones at a dose of kisspeptin-54 administration known to stimulate gonadotrophin secretion in a small study of healthy women. This data has important implications for the potential of kisspeptin to treat patients with infertility.
**Introduction**

The kisspeptins are a group of arginine-phenylalanine amide peptide hormones named according to their amino acid length (1), which are released from specialized hypothalamic neurons and trigger the secretion of gonadotrophin releasing hormone (GnRH) (2). Kisspeptin is critical for reproductive function, since patients with genetic inactivation of kisspeptin or its receptor have pubertal failure and infertility (3). Exogenous kisspeptin potently stimulates the secretion of the pituitary gonadotrophin hormones, luteinising hormone (LH) and follicle stimulating hormone (FSH) in numerous mammalian species including rats (4), mice (5), sheep (6), cows (7), and monkeys (8). In keeping with these animal studies, we and other investigators have demonstrated that kisspeptin stimulates gonadotrophin secretion and LH pulsatility in healthy men, women, and patients with certain forms of infertility (9-16). Furthermore, clinical trials are underway, investigating the potential of the most common circulating isoform of kisspeptin, kisspeptin-25, to trigger oocyte maturation women during *in vitro* fertilisation treatment. It is therefore therapeutically important to determine whether kisspeptin-25 also has physiological effects on other endocrine axes in women.

In addition to its role stimulating reproductive function, a number of animal studies suggest that kisspeptin may also stimulate the secretion of growth hormone (GH) and prolactin. The kisspeptin receptor is expressed in the pituitary gland, from where GH and prolactin are secreted (1). Kisspeptin-10 stimulates growth hormone (GH) and prolactin secretion from cultured anterior pituitary cells taken from 8 month old male calves (17). Kisspeptin-10 also stimulates GH secretion when incubated with pituitary cells of female baboons (18), and increases prolactin secretion when incubated with pituitary cells of male and female goldfish (19). Furthermore, intravenous bolus injection of kisspeptin-10 acutely increases circulating levels of GH in heifers (7), and intracerebroventricular bolus injection of kisspeptin-10 acutely increases circulating prolactin in male and female rats (20). However, a number of other animal studies have failed to identify non-gonadotrophin secretory effects of kisspeptin on the pituitary gland. Circulating levels of GH, prolactin, and thyroid stimulating hormone (TSH) were unaltered following intravenous bolus administration of kisspeptin-10 to adult...
male rhesus monkeys (21). Intravenous bolus injection of kisspeptin-10 had no effect on circulating GH in pre-pubertal gilts (22), cattle (23) or goats (24). The current animal literature therefore implies a possible role for kisspeptin to stimulate prolactin and GH secretion, but this remains controversial. No previous study has examined the effects of kisspeptin administration on non-reproductive pituitary hormone secretion in humans. Hypersecretion of GH, prolactin and TSH each have serious adverse health consequences for patients (25-28). It is therefore important to establish whether kisspeptin modulates GH, prolactin and TSH secretion in healthy female volunteers. We performed a post-hoc analysis of a prospective single-blinded placebo-controlled clinical study (13), to determine for the first time whether kisspeptin-54 modulates the secretion of GH, prolactin and TSH in healthy female volunteers, at a dose known to stimulate gonadotrophin secretion potently.
Methods

Subjects

This is a post-hoc analysis of a single-blinded, placebo-controlled, one-way crossover study designed to investigate the effects of twice-daily administration of kisspeptin-54 on reproductive hormone secretion in healthy women (13). Following ethical approval by the Hammersmith and Queen Charlotte’s and Chelsea Hospitals Research Ethics Committee (registration number: 05/Q0406/142), written informed consent was obtained from five subjects. This study was performed in accordance with the Declaration of Helsinki. Subjects had regular menstrual cycles, and were recruited as described previously (age in years: 31.6±2.6, range 24-37; weight in kg: 60.4±2.5, range 50.4-63.8) (13).

Protocol

During month 1 of the protocol, all subjects self-administered twice-daily subcutaneous injections of saline (vehicle) between days 7 and 14 of their menstrual cycle. During month 2 of the protocol, the same five healthy female subjects self-administered twice-daily subcutaneous injections of kisspeptin-54 (6.4nmol/kg; equivalent to 37mcg/kg) (13), between days 7 and 14 of their menstrual cycle. Day 1 of each month was defined as the first day of menstrual bleeding. Full details of the protocol have been previously published (13). Methods for self-injection of kisspeptin-54 are described previously (10, 11, 13).

Study 1: Acute effects of vehicle or kisspeptin-54 on GH, prolactin and TSH: Subjects underwent blood sampling during the 4 hour period immediately following the first injection of the vehicle (menstrual day 7, month 1) or kisspeptin-54 (menstrual day 7, month 2) treatment period. Vehicle or kisspeptin-54 6.4nmol/kg was administered subcutaneously at 0 minutes, and blood was sampled for serum GH, prolactin, TSH and plasma kisspeptin-IR at 30, 0, 10, 20, 40, 60, 90, 120, 150, 180, 210 and 240 minutes. Results are presented for 4/5 subjects, since insufficient sample volume was available to analyse the results of one subject.
Study 2: Effects of vehicle or kisspeptin-54 on GH pulsatility: Subjects underwent blood sampling during an 8 hour period on the fourth injection day of twice-daily vehicle or kisspeptin-54 treatment (i.e. during menstrual day 11). Blood samples were taken every 10 minutes after subcutaneous injection of vehicle or kisspeptin-54 for 480 minutes and were analysed for serum levels of GH, prolactin and TSH. Results are presented for 5 subjects.

Study 3: Chronic effects of twice-daily vehicle or kisspeptin-54 administration on GH, prolactin and TSH: Serum levels of GH, prolactin and TSH immediately before the first injection of vehicle or kisspeptin-54 (day 7 of the menstrual cycle) were compared with levels immediately before the final injection (day 14 of the menstrual cycle). Hormone levels were calculated as a mean level from two serum samples. Results are presented for 4/5 subjects, since insufficient sample volume was available to analyse the results of one subject.

Analytical Methods

Serum GH was assayed on the Immulite 2000XPi platform (Siemens, Germany), with inter and intra-assay coefficients of variation (CV) of 5% and 6% respectively. Serum PRL and TSH were assayed on the Architect ci8000SR platform, (Abbott Diagnostics, USA). The inter- and intra-assay CV for both the PRL and TSH assays is 5% respectively. Measurement of the GH, PRL and TSH analytes on these automated platforms is based upon solid-phase; two-site chemiluminescent immunometric assays (ICMAs).

Data analysis

Area under curve (AUC) levels of hormone secretion were calculated using Prism software (GraphPad Inc., Ja Jolla, CA, USA). GH pulses were defined as elevations in serum GH consisting of at least two measurements, one of which was at least 3-fold higher than the assay CV. All data are expressed as mean ± standard error of mean (SEM). Hormone profiles during 4 hour blood sampling studies were analysed using repeated measures 2-way ANOVA with Bonferonni post hoc correction. Pairs of means were analysed using two-tailed t-tests.
all cases, P<0.05 was considered statistically significant.
Results

Study 1: Acute effects of the first injection of vehicle or kisspeptin-54 on circulating kisspeptin immunoreactivity, GH, prolactin and TSH

Blood sampling for plasma kisspeptin-54, serum GH, prolactin and TSH was performed during the first 4 hours immediately after the first subcutaneous bolus injection of vehicle or kisspeptin-54. Plasma levels of kisspeptin-IR were markedly increased following subcutaneous bolus injection of kisspeptin-54 (mean AUC plasma kisspeptin-IR during 4 hours following injection in h.pmol/L: 1684 ± 164, vehicle; 375000 ± 47680, kisspeptin-54, P<0.001 vs. vehicle), and kisspeptin-54 significantly increased serum LH when compared with vehicle (mean AUC serum LH during 4 hours following injection in h.IU/L: -151.0 ± 45.7, vehicle; 1102.0 ± 588.0, kisspeptin-54, P<0.05 vs. vehicle) (13). No significant changes in serum GH, prolactin and TSH were observed following subcutaneous bolus injection of kisspeptin-54, (mean AUC serum level during 4 hours following injection: 57 ± 26 h.mcg/L, GH post-vehicle; 111 ± 57 h.mcg/L, GH post-kisspeptin-54, P=0.51 vs. GH post-vehicle; 41356 ± 6176 h.mIU/L, prolactin post-vehicle; 42868 ± 7175 h.mIU/L, prolactin post-kisspeptin-54, P=0.78 vs. prolactin post-vehicle; 300 ± 68 h.mIU/mL, TSH post-vehicle; 326 ± 78 h.mIU/mL, TSH post-kisspeptin-54, P=0.49 vs. TSH post-vehicle) (Figure 1A-I).

Study 2: Effects of vehicle or kisspeptin-54 on GH pulsatility: We then examined in more detail the acute effects of vehicle or kisspeptin-54 on GH secretion in the same healthy women, by performing frequent blood sampling every 10min for 8 hours immediately following injection of vehicle or kisspeptin-54, on the fourth day a twice-daily injection regime. The mean level of serum GH was not significantly altered by subcutaneous bolus injection of kisspeptin-54 when compared with vehicle (AUC serum level during 8 hours following injection in h.mcg/L: 507 ± 174, GH post-vehicle; 399 ± 61, GH post-kisspeptin-54, P= 0.61 vs. GH post-vehicle) (Figure 2A-C). Furthermore, no significant change in the mean number or amplitude of GH pulses was observed (Figure 2D-G).
Study 3: Chronic effects of twice-daily vehicle or kisspeptin-54 administration on GH, prolactin and TSH:

We finally examined whether a 7 day treatment course (between menstrual days 7 and 14) of twice-daily kisspeptin-54 injections altered serum levels of GH, prolactin and TSH in healthy women when compared with vehicle injections. Levels of serum GH were non-significantly higher on menstrual day 14 when compared with menstrual day 7, but no significant differences were observed between vehicle and kisspeptin-54 treatment groups (mean serum level of GH in mcg/L: 0.39± 0.10, vehicle day 7; 0.27± 0.07, kisspeptin-54 day 7, P= 0.64 vs. vehicle day 7; 1.02 ± 0.24, vehicle day 14; 0.72 ± 0.38, kisspeptin-54 day 14, P= 0.45 vs. vehicle day 14) (Figure 3A-B). Levels of serum prolactin and TSH were similar on menstrual day 14 when compared with menstrual day 7, and no significant differences were observed between vehicle and kisspeptin-54 treatment groups (Figure 3C-F).
Discussion

Kisspeptin is a hypothalamic neuropeptide, which plays a pivotal role in human reproduction. Kisspeptin potently stimulates gonadotrophin secretion in humans, which could be used to treat patients with reproductive disorders. However, kisspeptin has been shown recently to modulate the secretion of the pituitary hormones, GH, TSH and prolactin, during in vitro and in vivo animal studies. It is clinically important to determine whether exogenous kisspeptin also modulates the secretion of non-gonadotrophin pituitary hormones in humans. We report for the first time that subcutaneous bolus injection of kisspeptin-54 does not modulate GH, prolactin or TSH secretion significantly in healthy women, using a dose known to stimulate gonadotrophin secretion potently.

Excessive secretion of GH, TSH and prolactin are associated with a number of adverse health consequences. Increased circulating levels of GH are associated with hypertension, myocardial hypertrophy (25) and diabetes mellitus (26). Hyperprolactinaemia leads to galactorrhoea and infertility (27). Furthermore, TSH hyper-secretion has sympathomimetic actions leading to tachyarrhythmias, tremor, insomnia, and osteoporosis (28). A number of studies performed in animals have identified that kisspeptin stimulates GH and prolactin. Gutierrez-Pascual et al. demonstrated that kisspeptin-10 stimulates secretion of GH from rat pituitary cells in vitro in a dose-dependent manner (29). Kadokawa et al demonstrated that kisspeptin-10 increased prolactin release from bovine anterior pituitary cells by 23% (17). Yang et al. observed that incubation of goldfish pituitary cells with kisspeptin-10 stimulated secretion of both GH and prolactin (19). In vivo studies by Kadokawa et al. showed that circulating concentrations of GH acutely increased over 4-fold following IV administration of 1mg kisspeptin-10 in pre-pubertal female cows. (7). Furthermore, Szawka et al. observed that intracerebroventricular bolus injection of 3nmol kisspeptin-10 acutely increased circulating prolactin levels over 2-fold in male and female rats (20). There is a paucity of data examining the effects of kisspeptin on TSH secretion, but Luque et al. observed no stimulation of TSH following incubation of female baboon pituitary cells with kisspeptin-10 (18). Although we have previously reported that kisspeptin-54 acutely stimulated LH in these healthy female
volunteers (13), we did not observe any acute or chronic changes in GH, prolactin or TSH secretion in the same subjects.

It is interesting to consider why subcutaneous administration of kisspeptin-54 was not observed to significantly change the secretion of GH, prolactin and TSH significantly in healthy female participants. Firstly, it is important to recognise that this study was performed in a small number of subjects, which was neither designed nor statistically powered to exclude an effect of kisspeptin-54 on GH, prolactin or TSH secretion. For instance, the mean number of GH pulses during kisspeptin-54 administration was nearly two-fold, but non-significantly, higher when compared with saline. This study therefore cannot exclude that kisspeptin-54 modulates secretion of any of these hormones; rather, it suggests that no obvious change in GH, prolactin or TSH secretion is observed at the dose of kisspeptin-54 known to potently stimulate reproductive hormones in healthy women. Although the tested dose of kisspeptin-54 potently stimulated gonadotrophin secretion (13), we cannot exclude that a higher dose of kisspeptin-54 would have stimulated GH, prolactin or TSH secretion. Furthermore, although plasma kisspeptin immunoreactivity was markedly increased following subcutaneous injection of kisspeptin-54, we cannot exclude that an intravenous route of administration of kisspeptin-54 would have been more effective in stimulating GH, prolactin and TSH secretion. It is possible that sex steroids potentiate the stimulatory action of kisspeptin on GH and prolactin secretion in non-human mammals; plasma concentrations of GH were increased following peripheral administration of kisspeptin-10 to ovariectomised female cows, but only following oestradiol and/or progesterone supplementation (30). Furthermore, Szawka et al showed that intracerebroventricular administration of kisspeptin-10 only stimulated prolactin secretion in ovariectomised rats if they were pre-treated with estradiol (20). In light of these observations, it is interesting to note that we examined the effects of exogenous kisspeptin-54 on GH and prolactin secretion in healthy female volunteers during day 7 and day 11 of the menstrual cycle, when serum oestradiol levels were approximately 300pmol/L and 800pmol/L, respectively. It is therefore unlikely that kisspeptin-54 failed to stimulate significant GH or prolactin secretion in female subjects due
to low circulating levels of oestradiol. Age may also play a role in modulating the effects of kisspeptin on GH secretion; kisspeptin has been shown to be more effective at stimulating GH in anterior pituitary cells from juvenile cows when compared with adult cows. It would therefore be interesting to study whether kisspeptin-54 stimulated GH secretion in children; observing such an effect might imply that kisspeptin has distinct, developmental effects on non-gonadotrophin pituitary function, which are subsequently lost during adulthood.

All of the previous studies investigating the effects of kisspeptin on GH, PRL and TSH studied the decapeptide isoform, kisspeptin-10, whereas we studied the effects of the 54 amino acid isoform, kisspeptin-54. Kisspeptin-10 is actually the fragment of kisspeptin-54 needed for binding to the kisspeptin receptor, and for biological activity (1). We therefore cannot exclude that kisspeptin-10 would stimulate GH, PRL and TSH secretion more potently when compared with kisspeptin-54 in healthy women. However, we have previously demonstrated in healthy subjects that kisspeptin-54 has a longer plasma half-life (approx. 27 min) when compared with kisspeptin-10 (approx. 4 min) (9, 12). Furthermore, levels of plasma kisspeptin immunoreactivity are over 15-fold higher following an injection of kisspeptin-54 when compared with an equimolar injection of kisspeptin-10 (12). We would therefore not expect kisspeptin-54 to be less potent in its biological actions when compared with kisspeptin-10.

As expected, GH was secreted in discrete pulses in our healthy female subjects (31). It was therefore important to confirm that kisspeptin-54 did not modulate the secretory pattern of GH secretion over an 8 hour period, rather than merely assessing area under curve GH secretion. During these 8 hour blood sampling studies (study 2), subjects had already received three previous days of twice-daily kisspeptin-54 injections regime; we therefore cannot exclude that tachyphylaxis to the effects of kisspeptin-54 on pituitary hormone (GH, Prolactin, TSH) secretion may have occurred, as has been demonstrated previously in women with gonadotrophin secretion (10). However, none of the subjects had changes in GH, Prolactin or TSH in response to kisspeptin-54 on the first day of treatment (study 1), which suggests that tachyphylaxis did not contribute to our observations.
We observed that levels of serum GH levels were non-significantly higher during menstrual day 14 when compared with menstrual day 7, in both vehicle and kisspeptin-54 treatment groups. Higher levels of serum GH have been observed previously in women during the periovulatory phase when compared with early follicular phase of the menstrual cycle (32). Furthermore, oestrogen administration increases GH secretion in oestrogen-deficient women with Turner’s syndrome (33). During the current study, serum GH may have therefore been elevated on menstrual day 14 due to increased levels of circulating oestrogen. The mechanism by which oestrogen influences GH secretion is currently unclear (34). However, a study by Ovesen et al. suggests that the rate of pulsatile GH secretion and GH pulse frequency are both increased whereas basal GH secretion is unchanged during the periovulatory phase of menstrual cycle when compared with the early follicular phase in healthy young women (35). It is therefore possible that oestrogen may promote GH pulse generation within the hypothalamus. However, it is also possible that oestradiol acts indirectly on GH by influencing the production of IGF-1 in the liver (34).

In summary, this study is the first to examine the acute and chronic effects of exogenous kisspeptin-54 on GH, prolactin and TSH secretion in healthy female volunteers. While we cannot exclude any effect of kisspeptin-54 on GH, prolactin or TSH secretion, we observed no significant changes in these hormones at a dose of kisspeptin-54 administration known to stimulate gonadotrophin secretion in a small study of healthy women. This data has important implications for the safety of kisspeptin-54 as a potential therapeutic for patients with reproductive disorders.
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Figure Legends:

**Figure 1:** Acute effects of the first injection of vehicle or kisspeptin-54 on GH, prolactin and TSH (study 1). 5 healthy women were given a subcutaneous bolus injection of vehicle (saline) on day 7 of their menstrual cycle (follicular phase) in month 1 and kisspeptin-54 (6.4nmol/kg) in month 2 of the study. Frequent blood sampling was conducted for 4 hours after the injection for pituitary hormones and kisspeptin levels. **Results are presented for only 4 subjects,** since insufficient sample volume was available to analyse the results of one subject. The hormone level results of all participants were collated for GH (A), PRL (D) and TSH (G). Grouped mean results for each time point of the 4 hour study following the subcutaneous injection of vehicle in month 1 and kisspeptin-54 in month 2 are shown for GH (B), PRL (E) and TSH (H). Individual results are shown for GH (C), PRL (F), and TSH (I). There was no significant difference in hormone levels between the vehicle and kisspeptin groups. Data presented as mean±SEM.

**Figure 2:** The effects of vehicle or kisspeptin-54 on GH pulsatility. 5 healthy women were given twice daily subcutaneous injections of vehicle (saline) in month 1 and kisspeptin-54 (6.4nmol/kg) in month 2 from day 7 of their menstrual cycle (follicular phase) for 4 days followed by frequent blood sampling for 8 hours. **A:** Grouped mean results for each time point of the 8 hour study following 4 days of twice daily subcutaneous injections of vehicle in month 1 and kisspeptin-54 in month 2. **(B)** Collated and **(C)** Individual GH results for all participants following vehicle injections in month 1 and kisspeptin injections in month 2. **(D)** Collated and **(E)** Individual number of GH pulses in the 8 hour period following a subcutaneous injection of vehicle and kisspeptin-54 collated for the five participants. Three participants had two GH pulses following vehicle and kisspeptin-54 injection (data points nudged for clarity) **(F)** Collated and **(G)** Individual amplitude of the GH pulses collated for the five participants. Data presented as mean±SEM.
Figure 3: Effects of chronic subcutaneous administration of vehicle or kisspeptin. 5 healthy women received twice daily subcutaneous injections of vehicle (saline) from day 7 of their menstrual cycle (follicular phase) for 7 days in month 1 of the study. This was followed in month 2 by twice daily subcutaneous injections of kisspeptin-54 (6.4nmol/kg) from day 7 to day 14 of their menstrual cycle. A blood sample was taken prior to the first subcutaneous injection of vehicle (month 1) and kisspeptin-54 (month 2) on day 7 of their menstrual cycle and again on day 14. This allowed direct comparison between baseline and after 7 days of twice daily injections of vehicle or kisspeptin. Results are presented for only 4 subjects, since insufficient sample volume was available to analyse the results of one subject. The results were collated for all participants and shown for GH (A), PRL (C) and TSH (E). Individual results for all participants are shown for GH (B), PRL (D), and TSH (F). There was no statistical difference comparing the vehicle and kisspeptin groups or between the day 7 and 14 results. Data presented as mean±SEM.
Figure 1

A. Mean AUC serum GH levels (h.mcg/L)

B. Mean serum GH levels (mcg/L)

C. AUC serum GH levels (h.mcg/L)

D. Mean AUC serum PRL levels (h.mIU/L)

E. Mean serum PRL levels (mIU/L)

F. AUC serum PRL levels (h.mIU/L)

G. Mean AUC serum TSH levels (h.mlU/L)

H. Mean serum TSH levels (mIU/L)

I. AUC serum TSH levels (h.mIU/L)
Figure 2

A. Mean serum GH levels (mcg/L) over time (mins)

B. Mean AUC serum GH levels (h.mcg/L) for Vehicle and Kisspeptin-54

C. Number of GH pulses for Vehicle and Kisspeptin-54

D. Mean amplitude of GH pulses (mcg/L) for Vehicle and Kisspeptin-54
Figure 3

A

Mean serum GH (mcg/L)

Vehicle Day 7
Vehicle Day 14
Kisspeptin-54 Day 7
Kisspeptin-54 Day 14

B

Serum GH levels (mcg/L)

Vehicle
Kisspeptin-54

C

Mean serum PRL (mIU/L)

Vehicle Day 7
Vehicle Day 14
Kisspeptin-54 Day 7
Kisspeptin-54 Day 14

D

Serum PRL levels (mIU/L)

Vehicle
Kisspeptin-54

E

Mean serum TSH (mIU/L)

Vehicle Day 7
Vehicle Day 14
Kisspeptin-54 Day 7
Kisspeptin-54 Day 14

F

Serum TSH levels (mIU/L)

Vehicle
Kisspeptin-54