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**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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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.

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1 **Introduction**

2 The kisspeptins are a group of arginine-phenylalanine amide peptide hormones named

3 according to their amino acid length (1), which are released from specialized hypothalamic

4 neurons and trigger the secretion of gonadotrophin releasing hormone (GnRH) (2). Kisspeptin

5 is critical for reproductive function, since patients with genetic inactivation of kisspeptin or its

6 receptor have pubertal failure and infertility (3). Exogenous kisspeptin potently stimulates the

7 secretion of the pituitary gonadotrophin hormones, luteinising hormone (LH) and follicle

8 stimulating hormone (FSH) in numerous mammalian species including rats (4), mice (5),

9 sheep (6), cows (7), and monkeys (8). In keeping with these animal studies, we and other

10 investigators have demonstrated that kisspeptin stimulates gonadotrophin secretion and LH

11 pulsatility in healthy men, women, and patients with certain forms of infertility (9-16).

12 Furthermore, clinical trials are underway, investigating the potential of the most common

13 circulating isoform of kisspeptin, kisspeptin-54, to trigger oocyte maturation women during *in*

14 *vitro* fertilisation treatment. It is therefore therapeutically important to determine whether

15 kisspeptin-54 also has physiological effects on other endocrine axes in women.

16 In addition to its role stimulating reproductive function, a number of animal studies suggest

17 that kisspeptin may also stimulate the secretion of growth hormone (GH) and prolactin. The

18 kisspeptin receptor is expressed in the pituitary gland, from where GH and prolactin are

19 secreted (1). Kisspeptin-10 stimulates growth hormone (GH) and prolactin secretion from

20 cultured anterior pituitary cells taken from 8 month old male calves (17). Kisspeptin-10 also

21 stimulates GH secretion when incubated with pituitary cells of female baboons (18), and

22 increases prolactin secretion when incubated with pituitary cells of male and female goldfish

23 (19). Furthermore, intravenous bolus injection of kisspeptin-10 acutely increases circulating

24 levels of GH in heifers (7), and intracerebroventricular bolus injection of kisspeptin-10

25 acutely increases circulating prolactin in male and female rats (20). However, a number of

26 other animal studies have failed to identify non-gonadotrophin secretory effects of kisspeptin

27 on the pituitary gland. Circulating levels of GH, prolactin, and thyroid stimulating hormone

28 (TSH) were unaltered following intravenous bolus administration of kisspeptin-10 to adult

1 male rhesus monkeys (21). Intravenous bolus injection of kisspeptin-10 had no effect on
2 circulating GH in pre-pubertal gilts (22), cattle (23) or goats (24). The current animal
3 literature therefore implies a possible role for kisspeptin to stimulate prolactin and GH
4 secretion, but this remains controversial. No previous study has examined the effects of
5 kisspeptin administration on non-reproductive pituitary hormone secretion in humans.
6 Hypersecretion of GH, prolactin and TSH each have serious adverse health consequences for
7 patients (25-28). It is therefore important to establish whether kisspeptin modulates GH,
8 prolactin and TSH secretion in healthy female volunteers. We performed a post-hoc analysis
9 of a prospective single-blinded placebo-controlled clinical study (13), to determine for the
10 first time whether kisspeptin-54 modulates the secretion of GH, prolactin and TSH in healthy
11 female volunteers, at a dose known to stimulate gonadotrophin secretion potently.

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2
3 **1 Methods**

4
5 *2 Subjects*

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7 This is a post-hoc analysis of a single-blinded, placebo-controlled, one-way crossover study
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9 designed to investigate the effects of twice-daily administration of kisspeptin-54 on
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11 reproductive hormone secretion in healthy women (13). Following ethical approval by the
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13 Hammersmith and Queen Charlotte’s and Chelsea Hospitals Research Ethics Committee
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15 (registration number: 05/Q0406/142), written informed consent was obtained from five
16
17 subjects. This study was performed in accordance with the Declaration of Helsinki. Subjects
18
19 had regular menstrual cycles, and were recruited as described previously (age in years:
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21 31.6±2.6, range 24-37; weight in kg: 60.4±2.5, range 50.4-63.8) (13).
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25 *12 Protocol*

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27 During month 1 of the protocol, all subjects self-administered twice-daily subcutaneous
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29 injections of saline (vehicle) between days 7 and 14 of their menstrual cycle. During month 2
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31 of the protocol, the same five healthy female subjects self-administered twice-daily
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33 subcutaneous injections of kisspeptin-54 (6.4nmol/kg; equivalent to 37mcg/kg) (13), between
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35 days 7 and 14 of their menstrual cycle. Day 1 of each month was defined as the first day of
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37 menstrual bleeding. Full details of the protocol have been previously published (13). Methods
38
39 for self-injection of kisspeptin-54 are described previously (10, 11, 13).
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44 *21 Study 1: Acute effects of vehicle or kisspeptin-54 on GH, prolactin and TSH:* Subjects
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46 underwent blood sampling during the 4 hour period immediately following the first injection
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48 of the vehicle (menstrual day 7, month 1) or kisspeptin-54 (menstrual day 7, month 2)
49
50 treatment period. Vehicle or kisspeptin-54 6.4nmol/kg was administered subcutaneously at 0
51
52 minutes, and blood was sampled for serum GH, prolactin, TSH and plasma kisspeptin-IR at -
53
54 30, 0, 10, 20, 40, 60, 90, 120, 150, 180, 210 and 240 minutes. Results are presented for 4/5
55
56 subjects, since insufficient sample volume was available to analyse the results of one subject.
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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 $\pm$ 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. In

1 all cases, $P < 0.05$ was considered statistically significant.

For Peer Review

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).

1 *Study 3: Chronic effects of twice-daily vehicle or kisspeptin-54 administration on GH,*
2 *prolactin and TSH:*

3 We finally examined whether a 7 day treatment course (between menstrual days 7 and 14) of
4 twice-daily kisspeptin-54 injections altered serum levels of GH, prolactin and TSH in healthy
5 women when compared with vehicle injections. Levels of serum GH were non-significantly
6 higher on menstrual day 14 when compared with menstrual day 7, but no significant
7 differences were observed between vehicle and kisspeptin-54 treatment groups (mean serum
8 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.
9 vehicle day 7; 1.02 ± 0.24 , vehicle day 14; 0.72 ± 0.38 , kisspeptin-54 day 14, $P = 0.45$ vs.
10 vehicle day 14) (Figure 3A-B). Levels of serum prolactin and TSH were similar on menstrual
11 day 14 when compared with menstrual day 7, and no significant differences were observed
12 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

1 volunteers (13), we did not observe any acute or chronic changes in GH, prolactin or TSH
2 secretion in the same subjects.
3 It is interesting to consider why subcutaneous administration of kisspeptin-54 was not
4 observed to significantly change the secretion of GH, prolactin and TSH significantly in
5 healthy female participants. Firstly, it is important to recognise that this study was performed
6 in a small number of subjects, which was neither designed nor statistically powered to
7 exclude an effect of kisspeptin-54 on GH, prolactin or TSH secretion. For instance, the mean
8 number of GH pulses during kisspeptin-54 administration was nearly two-fold, but non-
9 significantly, higher when compared with saline. This study therefore cannot exclude that
10 kisspeptin-54 modulates secretion of any of these hormones; rather, it suggests that no
11 obvious change in GH, prolactin or TSH secretion is observed at the dose of kisspeptin-54
12 known to potently stimulate reproductive hormones in healthy women. Although the tested
13 dose of kisspeptin-54 potently stimulated gonadotrophin secretion (13), we cannot exclude
14 that a higher dose of kisspeptin-54 would have stimulated GH, prolactin or TSH secretion.
15 Furthermore, although plasma kisspeptin immunoreactivity was markedly increased
16 following subcutaneous injection of kisspeptin-54, we cannot exclude that an intravenous
17 route of administration of kisspeptin-54 would have been more effective in stimulating GH,
18 prolactin and TSH secretion. It is possible that sex steroids potentiate the stimulatory action
19 of kisspeptin on GH and prolactin secretion in non-human mammals; plasma concentrations
20 of GH were increased following peripheral administration of kisspeptin-10 to ovariectomised
21 female cows, but only following oestradiol and/or progesterone supplementation (30).
22 Furthermore, Szawka et al showed that intracerebroventricular administration of kisspeptin-
23 10 only stimulated prolactin secretion in ovariectomised rats if they were pre-treated with
24 estradiol (20). In light of these observations, it is interesting to note that we examined the
25 effects of exogenous kisspeptin-54 on GH and prolactin secretion in healthy female
26 volunteers during day 7 and day 11 of the menstrual cycle, when serum oestradiol levels were
27 approximately 300pmol/L and 800pmol/L, respectively. It is therefore unlikely that
28 kisspeptin-54 failed to stimulate significant GH or prolactin secretion in female subjects due

1 to low circulating levels of oestradiol. Age may also play a role in modulating the effects of
2 kisspeptin on GH secretion; kisspeptin has been shown to be more effective at stimulating GH
3 in anterior pituitary cells from juvenile cows when compared with adult cows. It would
4 therefore be interesting to study whether kisspeptin-54 stimulated GH secretion in children;
5 observing such an affect might imply that kisspeptin has distinct, developmental effects on
6 non-gonadotrophin pituitary function, which are subsequently lost during adulthood.

7 All of the previous studies investigating the effects of kisspeptin on GH, PRL and TSH
8 studied the decapeptide isoform, kisspeptin-10, whereas we studied the effects of the 54
9 amino acid isoform, kisspeptin-54. Kisspeptin-10 is actually the fragment of kisspeptin-54
10 needed for binding to the kisspeptin receptor, and for biological activity (1). We therefore
11 cannot exclude that kisspeptin-10 would stimulate GH, PRL and TSH secretion more potently
12 when compared with kisspeptin-54 in healthy women. However, we have previously
13 demonstrated in healthy subjects that kisspeptin-54 has a longer plasma half-life (approx.
14 27min) when compared with kisspeptin-10 (approx. 4min) (9, 12). Furthermore, levels of
15 plasma kisspeptin immunoreactivity are over 15-fold higher following an injection of
16 kisspeptin-54 when compared with an equimolar injection of kisspeptin-10 (12). We would
17 therefore not expect kisspeptin-54 to be less potent in its biological actions when compared
18 with kisspeptin-10.

19 As expected, GH was secreted in discrete pulses in our healthy female subjects (31). It was
20 therefore important to confirm that kisspeptin-54 did not modulate the secretory pattern of
21 GH secretion over an 8 hour period, rather than merely assessing area under curve GH
22 secretion. During these 8 hour blood sampling studies (study 2), subjects had already received
23 three previous days of twice-daily kisspeptin-54 injections regime; we therefore cannot
24 exclude that tachyphylaxis to the effects of kisspeptin-54 on pituitary hormone (GH,
25 Prolactin, TSH) secretion may have occurred, as has been demonstrated previously in women
26 with gonadotrophin secretion (10). However, none of the subjects had changes in GH,
27 Prolactin or TSH in response to kisspeptin-54 on the first day of treatment (study 1), which
28 suggests that tachyphylaxis did not contribute to our observations.

1 We observed that levels of serum GH levels were non-significantly higher during menstrual
2 day 14 when compared with menstrual day 7, in both vehicle and kisspeptin-54 treatment
3 groups. Higher levels of serum GH have been observed previously in women during the
4 periovulatory phase when compared with early follicular phase of the menstrual cycle (32).
5 Furthermore, oestrogen administration increases GH secretion in oestrogen-deficient women
6 with Turner's syndrome (33). During the current study, serum GH may have therefore been
7 elevated on menstrual day 14 due to increased levels of circulating oestrogen. The mechanism
8 by which oestrogen influences GH secretion is currently unclear (34). However, a study by
9 Ovesen et al. suggests that the rate of pulsatile GH secretion and GH pulse frequency are both
10 increased whereas basal GH secretion is unchanged during the periovulatory phase of
11 menstrual cycle when compared with the early follicular phase in healthy young women (35).
12 It is therefore possible that oestrogen may promote GH pulse generation within the
13 hypothalamus. However, it is also possible that oestradiol acts indirectly on GH by
14 influencing the production of IGF-1 in the liver (34).
15 In summary, this study is the first to examine the acute and chronic effects of exogenous
16 kisspeptin-54 on GH, prolactin and TSH secretion in healthy female volunteers. While we
17 cannot exclude any effect of kisspeptin-54 on GH, prolactin or TSH secretion, we observed
18 no significant changes in these hormones at a dose of kisspeptin-54 administration known to
19 stimulate gonadotrophin secretion in a small study of healthy women. This data has important
20 implications for the safety of kisspeptin-54 as a potential therapeutic for patients with
21 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. 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

Figure 2