# Exogenous Kisspeptin Administration as a Probe of GnRH Neuronal Function in Patients With Idiopathic Hypogonadotropic Hypogonadism

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**Context**: Idiopathic hypogonadotropic hypogonadism (IHH) results from defective synthesis, secretion, or action of GnRH. Kisspeptin is a potent stimulus for GnRH secretion.

**Objective**: We probed the functional capacity of the GnRH neuronal network in patients with IHH.

**Participants**: Eleven subjects with congenital IHH (9 men and 2 women) and one male subject who underwent reversal of IHH were studied. Six of the twelve subjects had an identified genetic cause of their IHH: *KAL1* (n = 1), *FGFR1* (n = 3), *PROKR2* (n = 1), *GNRHR* (n = 1).

**Intervention**: Subjects underwent q10 min blood sampling to measure GnRH-induced LH secretion at baseline and in response to intravenous boluses of kisspeptin (0.24 nmol/kg) and GnRH (75 ng/kg) both pre- and post-six days of treatment with exogenous GnRH (25 ng/kg sc every 2 h).

**Results**: All subjects with abiding IHH failed to demonstrate a GnRH-induced LH response to exogenous kisspeptin. In contrast, the subject who achieved reversal of his hypogonadotropism demonstrated a robust response to kisspeptin.

**Conclusions**: The functional capacity of the GnRH neuronal network in IHH patients is impaired, as evidenced by their inability to respond to the same dose of kisspeptin that effects a robust GnRH-induced LH response in healthy men and luteal-phase women. This impairment is observed across a range of genotypes, suggesting that it reflects a fundamental property of GnRH neuronal networks that have not been properly engaged during pubertal development. In contrast, a patient who had experienced reversal of his hypogonadotropism responded to exogenous kisspeptin. **(*J Clin Endocrinol Metab* 99: E2762–E2771, 2014)**

Idiopathic hypogonadotropic hypogonadism (IHH) is a clinical syndrome characterized by abnormal pubertal progression and low sex steroids in the setting of low/normal gonadotropins. In the vast majority of cases, IHH is due to abnormal GnRH synthesis, secretion or action (1, 2). Thus, the administration of exogenous pulsatile GnRH can stimulate pituitary gonadotropin and gonadal sex steroid secretion, as well as folliculogenesis and spermatogenesis (1, 2). In addition to its use as a fertility treatment, pulsatile GnRH can also be used as an investigative tool to probe the physiology of pituitary gonadotropin release. For example, administration of pulsatile GnRH to IHH patients has revealed novel insights into the effect of GnRH frequency and amplitude on differential gonadotropin release, as well as the relative roles of sex steroids and nonsteroidal hormones on negative feedback at the

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level of the hypothalamus and pituitary (3–9). While exogenous GnRH administration has served as a powerful probe of pituitary physiology, the absence of a comparable probe for hypothalamic GnRH neurons has limited investigation into the biology of the GnRH neuron and the underlying pathophysiology of IHH in humans.

Nonetheless, genetic studies of hypogonadotropic patients, either with anosmia (Kallmann syndrome) or with a normal sense of smell (normosmic idiopathic hypogonadotropic hypogonadism), have revealed much about the underlying pathophysiology that can affect the GnRH neurons as they differentiate, migrate, and secrete GnRH. Mutations in several genes responsible for these processes have been uncovered over the last 20 years (10), including but not limited to *KAL1* (11, 12), which influences GnRH neuronal migration (13), *GNRH1*, which affects GnRH synthesis (14, 15), and *KISS1R*, which impacts GnRH secretion (16, 17). Incomplete expressivity and variable penetrance are characteristic of mutations in several of the genes implicated in congenital hypogonadotropism (18, 19); in a subset of cases, mutations in more than one gene may be required for phenotypic manifestations (20, 21). While most IHH patients have lifelong hypogonadotropism, ie, "abiding" IHH, up to 22% of patients with IHH undergo "reversal," characterized by spontaneous activation of the hypothalamic-pituitary-gonadal axis as evidenced by normalization of sex-steroid levels, increased testicular volume, menstrual cycles, and/or fertility in the absence of any therapy (22, 23). A direct physiologic probe of GnRH function may inform genotype-phenotype correlations, assess propensity for reversal, and uncover new insights into the pathophysiology of IHH.

Discovered as a key gatekeeper of the timing of sexual maturation across mammalian species (16, 17), the hypothalamic neuropeptide kisspeptin is potentially one such probe. Kisspeptin is a potent stimulus for GnRH secretion (24), and evidence suggests it may be secreted in a pulsatile manner (25, 26). Considerable data regarding the neuroendocrine response to exogenous kisspeptin administration in healthy men and women has been assembled. In men, regardless of the formulation or method of administration, kisspeptin has been shown by our group and others to induce a robust GnRH-induced LH response (27–30, 59). In contrast, in reproductive age women, kisspeptin's effects are more variable and depend on the phase of the menstrual cycle (59). Kisspeptin administration in the luteal phase results in an LH response comparable to that observed in healthy men; however, administration in the follicular phase results in an almost negligible rise in LH (59). While this variability in response to kisspeptin administration has not been fully explained, enough data has been assembled to serve as a normative backdrop against which data from pathophysiologic populations can be compared. Thus, just as the discovery of GnRH allowed investigators to chart the biology of the pituitary gonadotrope, exogenous kisspeptin administration was utilized in this study as an in vivo probe of the GnRH neuronal network in patients with IHH. We hypothesized that patients with genetic mutations leading to an absence of GnRH neurons (ie, *KAL1*), would not demonstrate any GnRH-induced LH response to exogenous kisspeptin. In contrast, we hypothesized that patients with mutations leading to a reduced complement of GnRH neurons or reduced secretory capacity would exhibit an attenuated response to this hypothalamic hormone.

## Materials and Methods

### Subjects and eligibility criteria

This study was approved by the Institutional Review Board of Massachusetts General Hospital (MGH)/Partners Healthcare, and all subjects gave written informed consent. All subjects had been diagnosed with congenital IHH as evidenced by absent or incomplete pubertal development by age 18 years and low sex steroids in the setting of low or inappropriately normal gonadotropins. DNA extracted from subjects' blood had previously been screened for rare sequence variants in *CHD7* (MIM 608892), *FGF8* (MIM 600483), *FGFR1* (MIM 136350), *GNRH1* (MIM 152760), *GNRHR* (MIM 138850), *HS6ST1* (MIM 604846), *KAL1* (MIM 300836), *KISS1* (MIM 603286), *KISS1R* (MIM 604161), *NSMF*, previously called *NELF* (MIM 60813), *PROK2* (MIM 607002), *PROKR2* (MIM 607123), *TAC3* (MIM 162330), and *TACR3* (MIM 162332) by PCR amplification of exons followed by Sanger sequencing, as described previously (21). Rare sequence variants (RSVs) were defined as having a minor allele frequency of less than 1% in the 1000 Genomes Project and the National Heart, Lung, and Blood Institute Grand Opportunity Exome Sequencing Project (32). RSVs were reported if they were predicted to be damaging by at least 3 out of 4 in silico prediction programs: PolyPhen-2 (33), SIFT (34), Mutation Taster (35), Panther (36). To determine whether multiple mutations were located on one allele or separate alleles, PCR products were cloned using the TOPO TA Cloning Kit for Sequencing (Life Technologies), followed by amplification and sequencing of individual clones.

### Study design

The main study protocol, schematized in Figure 1, consisted of two admissions to the Harvard Catalyst Clinical Research Center (CRC) at Massachusetts General Hospital. Prior to the first CRC admission, the subjects' sex-steroid therapy was withheld to allow them to return to their hypogonadal state; this "washout" period lasted 2 weeks for transdermal testosterone, 6 weeks for injected testosterone enanthate or cypionate, and 8 weeks for oral contraceptive (OC) pills.

During the first CRC admission, blood sampling was performed every 10 min for 12–14 h. The first 6 h assessed the subjects' baseline LH secretion in the absence of any intervention. Subjects then received an intravenous (IV) bolus of kiss-

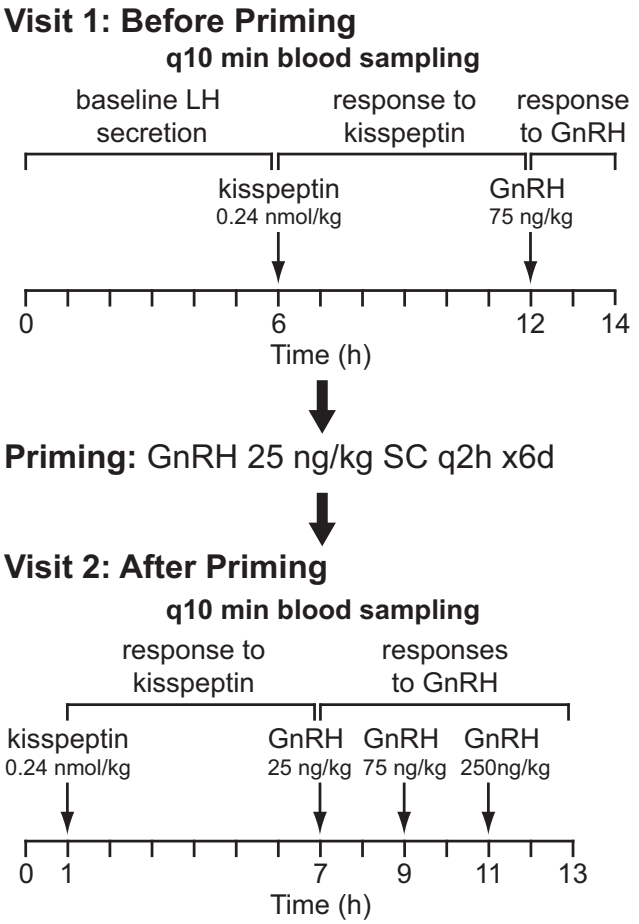


**Figure 1.** Schematic of the study protocol.

peptin-10 0.24 nmol/kg (0.313 μg/kg), followed by 4–6 h of blood sampling. This dose of kisspeptin was chosen because prior work by our group demonstrated that this dose consistently elicits GnRH-induced LH pulses of physiologic amplitude in healthy men and healthy luteal phase women (29, 31). A bolus of GnRH 75 ng/kg was then given, followed by an additional 2 h of blood sampling. Kisspeptin and GnRH were synthesized by Polypeptides, Inc., and the 10-amino-acid isoform of kisspeptin (corresponding to amino acids 112–121 of the preprohormone) was used in these studies.

Although the vast majority of patients with hypogonadotropic hypogonadism have hypothalamic and not pituitary defects in hormone secretion, many patients will fail to respond to an initial bolus of exogenous GnRH. Therefore, even if kisspeptin induced a GnRH secretory event, the subsequent GnRH stimulation of a "sleepy" pituitary gland might result in absence of an LH response. To avoid the possibility of such false negative interpretations, we capitalized on the long-standing knowledge that exposure to intermittent, exogenous GnRH administration "primes" pituitary gonadotrophs (37). Thus, either immediately or within a few days after discharge from their first CRC admission, 8 subjects underwent pituitary "priming" with GnRH 25 ng/kg administered subcutaneously every 2 h by a Crono F portable infusion pump (Canè S.p.A) for 6 days. The last dose of GnRH was given 48 h prior to a second, "postpriming" admission to allow circulating sex steroids to return to baseline concentrations. During this second admission, subjects underwent q10 min blood sampling × 1 h, kisspeptin-10 0.24 nmol/kg IV administration followed by 6 h of blood sampling, and finally three boluses of GnRH (25, 75, 250 ng/kg in ascending order) IV followed by 2 h of blood sampling after each bolus.

Four subjects returned for additional studies in which they received multiple boluses of kisspeptin at doses ranging from 0.24 to 2.4 nmol/kg. Subject 10 received five boluses and subject 7 received two boluses of the same dose of kisspeptin used in the single-bolus protocol above (0.24 nmol/kg). Subjects 9 and 12 received escalating doses of kisspeptin at 0.24, 0.72, and 2.4 nmol/kg.

## Laboratory assays and pulse analysis

LH was measured at each time point, on 2-h study pools, and on an all-study quality control (QC) pool, and FSH and either testosterone or estradiol were measured on 2-h study pools. Serum LH, FSH, estradiol and testosterone concentrations were determined by direct immunoassay using the automated Abbott ARCHITECT system (Abbott Laboratories, Inc.).

LH levels are expressed in units of the second International Pituitary Standard WHO [80/552]. The minimal detectable dose (lowest dose distinguishable from zero; 95% confidence interval (CI) of a blank detection) for the LH assay is 0.07 mIU/mL. The limit of quantitation (functional sensitivity defined as lowest concentration with a CV <20%), which was used for reporting all values, for the LH assay is 0.1 mIU/mL. Assay reproducibility was monitored using commercial controls that ranged in LH concentrations from 4 to 50 mIU/mL; the CV for all these controls was <5%. Study specific variance was determined by repeated LH testing of aliquots of a pool made from the timed draws for each study. The normal reference range for adult men is 2.0–12.0 mIU/mL and the normal reference range for normally cycling women in the luteal phase is 0.6–19.0 mIU/mL.

FSH levels are expressed in units of the first International Pituitary Standard WHO [92/510]. The minimal detectable dose (lowest dose distinguishable from zero; 95% CI of a blank detection) for the FSH assay was 0.05 mIU/mL. The limit of quantitation for the FSH assay was 0.1 mIU/mL. In addition, QC sera containing 5–75 mIU/mL were tested daily and CVs were <5%. The normal reference range for adult men is 1.0–8.7 mIU/mL and the normal reference range for normally cycling women in the luteal phase is 2–13 mIU/mL.

Estradiol was measured by second generation immunoassay traceable to mass spectrometry-based assays (38). Functional sensitivity of the estradiol assay was 15 pg/mL; minimal detectable difference was 5 pg/mL. CV was <8% at low control of 45 pg/mL. Testosterone was measured by a second generation immunoassay traceable to mass spectrometry-based assays (39). Functional sensitivity of the testosterone assay was 4.33 ng/dL; the minimal detectable difference was 2.67 ng/dL. CV was <10% for testosterone levels >15 ng/dL.

A validated modification of the method of Santen and Bardin, incorporating the study specific variance, was used to identify LH pulses (40, 41). LH amplitude was calculated as the maximal LH achieved minus the LH at baseline. For the subject who underwent reversal of his IHH and who therefore produced endogenous LH pulses, the binomial probability was used to calculate the likelihood that kisspeptin administration coincided with endogenous pulses by chance. *P* values less than 0.05 were considered significant. Unless otherwise noted, values are reported as mean ± standard deviation.

## Results

### Subject characteristics

Ten men and two women with IHH participated in this study, and their clinical characteristics are described in Table 1. Seven men and one woman had Kallmann syndrome; the remaining three men and one woman had normosmic IHH. One man with normosmic IHH had undergone reversal of his condition, first noted at age 21 as evidenced by normalization of serum testosterone (275 ng/dL) and increased testicular volume (42). Six subjects carried rare sequence variants in IHH genes that were predicted to be deleterious: *FGFR1* (N = 3, p.[F747L; D768H] [normosmic IHH]; p.G687R [KS]; p.L630P [KS]), GNRHR (N = 1 p.[R262Q];[L286P] [normosmic IHH]), *KAL1* (N = 1, p.L601YfsX18 [KS]), *KISS1* (N = 1, p.C53R [KS]), and *PROKR2* (N = 1, p.L173R [KS]) (Table 1). The remaining six subjects had no identified rare sequence variants predicted to be deleterious.

### "Pre-priming": initial responses to kisspeptin and GnRH

Subjects were admitted to the MGH Clinical Research Center for frequent blood sampling at 10 min intervals to chart endogenous LH secretion and responses to kisspeptin and GnRH (Supplemental Figure 1). Of the 11 subjects with abiding hypogonadotropism, whether normosmic or with KS, none exhibited any LH pulses at baseline. Seven of 11 subjects had LH levels near or at the limit of detection (mean ± SD LH 0.1 ± 0.1 mIU/mL). In contrast, four of 11 subjects had detectable but nonpulsatile LH secretion (LH 1.1 ± 0.1 mIU/mL). Of note, these same 4 subjects demonstrated robust responses to exogenous GnRH 75 ng/kg (LH pulse amplitude 5.9 ± 0.9 mIU/mL) suggesting that their pituitaries were in a greater state of "readiness," likely due to endogenous stimulation from an intact but enfeebled GnRH secretory program (Table 1, subjects 7–10).

After receiving a kisspeptin bolus of 0.24 nmol/kg, no subject with abiding hypogonadotropism responded with an LH pulse. The average change in LH after kisspeptin was 0.1 ± 0.1 mIU/mL. This is in marked contrast to our experience in healthy men, in which administration of the same dose of kisspeptin-10 resulted in an LH pulse with an average amplitude of 5.0 ± 3.0 mIU/mL (29). The lack of response to kisspeptin administration in subjects with

**Table 1.** Subject Characteristics

| # | Sex | Age (y) | BMI (kg/m$^2$) | Diagnosis | Age at Presentation (y)[a] | Rare Sequence Variant[b] | TV (mL) | Change in LH Amplitude in Response to GnRH (mIU/mL)[c] | Prior Hormone Treatment | Other |
|---|---|---|---|---|---|---|---|---|---|---|
| 1 | M | 43 | 38.6 | KS | 16 | *KAL1* p.L601YfsX18 | 9, 8 | 1.3 | GnRH<br>T | |
| 2 | M | 59 | 29.0 | KS | 18 | None identified | 2,2 | 0.1 | GnRH<br>hCG<br>FSH<br>T | Rheumatoid arthritis |
| 3 | M | 20 | 35.1 | KS | 14 | None identified | 2, 3 | 2.6 | hCG<br>T | |
| 4 | M | 38 | 45.7 | KS | 17 | *PROKR2* p.L173R | 4, 5 | 0.5 | GnRH<br>FSH<br>T | |
| 5 | M | 53 | 28.6 | KS | 25 | None identified | 2, 2 | 0.5 | GnRH<br>T | |
| 6 | M | 32 | 31.9 | nIHH | 12 | *FGFR1* p.[F747L; D768H] | 6, 6 | 1.4 | T | |
| 7 | M | 42 | 33.5 | nIHH | 16 | None identified | 10, 12 | 6.8 | hCG<br>FSH<br>T | |
| 8 | M | 47 | 28.5 | nIHH | 21 | None identified | 15, 10 | 5.9 | GnRH<br>hCG<br>T | |
| 9 | M | 22 | 22.7 | KS | 17 | *FGFR1* p.G687R<br>*KISS1* p.C53R | 4, 5 | 4.6 | T | |
| 10 | F | 54 | 31.0 | KS | 18 | *FGFR1* p.L630P | NA | 6.3 | None | Hypothyroidism |
| 11 | F | 22 | 30.2 | nIHH | 14 | *GNRHR* p.[R262Q]; [L286P] | NA | 0.3 | Estrogen/ progesterone OCP | |
| 12[d] | M | 23 | 40.5 | nIHH | 19 | None identified | 15, 20 | 7.3 | T | Reversal |

[a] Age at presentation for delayed puberty. Subjects under the age of 18 were re-evaluated after the age of 18 to confirm the diagnosis of IHH, which was further re-confirmed for subjects 1–11 through participation in this study. Subjects 3 and 6 had been followed from birth due to cryporchidism and therefore presented at an earlier age. [b] Variants are listed if they were predicted to be deleterious by at least three out of four prediction programs. [c] Change in LH amplitude in response to GnRH is peak LH - baseline LH in response to 75 ng/kg bolus of intravenous GnRH prior to pituitary priming. [d] This subject attained serum testosterone in normal adult range in the absence of exogenous treatment. BMI, body mass index; KS, Kallmann syndrome; nIHH, normosmic idiopathic hypogonadotropic hypogonadism; NA, not applicable; OCP, oral contraceptive pills; T, testosterone; TV, testicular volume.

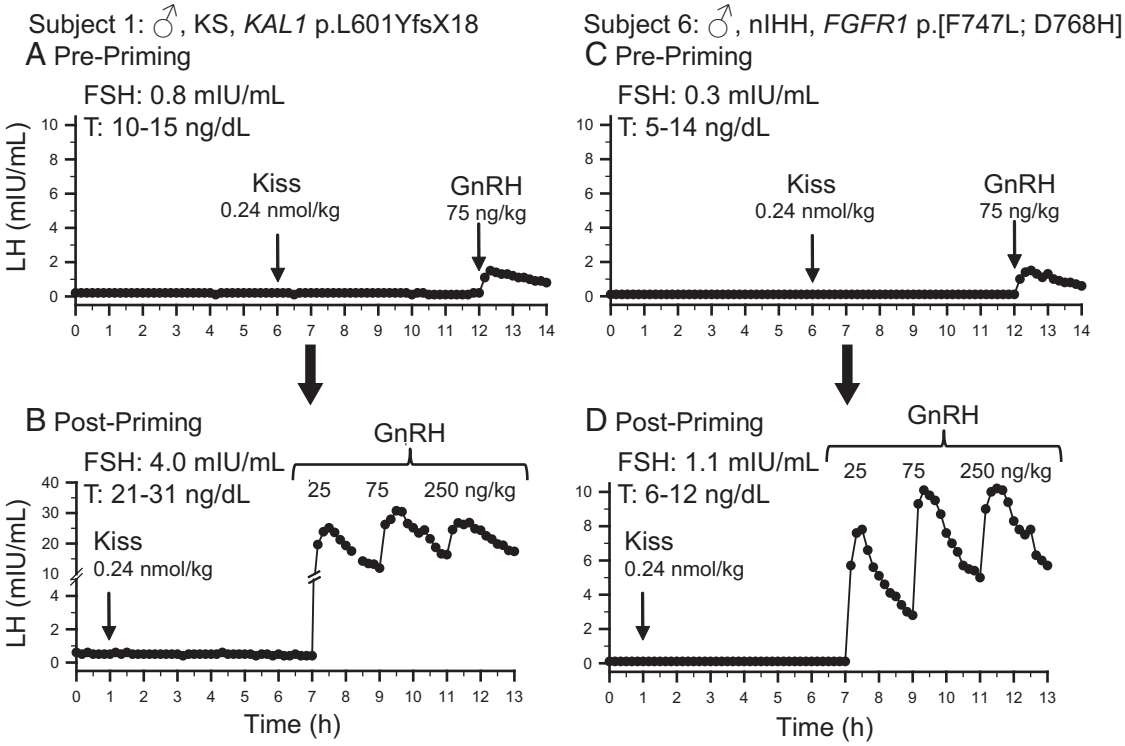


**Figure 2.** Baseline LH secretion and responses to kisspeptin and GnRH in two representative male (♂) subjects. The phenotype, Kallmann syndrome (KS) or normosmic IHH (nIHH), and genotype are noted for each subject. Results for subject 1 before and after priming with exogenous GnRH are shown in panels A and B, respectively. Similarly, results for subject 6 before and after priming are shown in panels C and D, respectively. Arrows indicate times of boluses. T, testosterone levels during the study. FSH values measured from first 2-h pool.

abiding hypogonadotropism was seen regardless of their baseline LH levels and their responses to exogenous GnRH (Figure 2, A and C and Supplemental Figure 1).

### "Post-priming": responses to kisspeptin and GnRH

Eight subjects underwent pituitary priming with exogenous pulsatile GnRH × 6 days. This resulted in successful priming in all subjects (except the subject with *GNRHR* mutations described below), with robust responses to boluses of GnRH administered during the second "post-priming" CRC admission. Because LH levels from a single GnRH bolus did not return to baseline before the next bolus was administered, it was only possible to quantify the LH response to the first GnRH dose of 25 ng/kg. The amplitude of LH pulses in response to this first bolus of GnRH was 7.7 ± 8.0 mIU/mL (mean ± SD).

Despite this evidence for successful pituitary priming, subjects with abiding hypogonadotropism still failed to respond to kisspeptin 0.24 ng/kg iv (change in LH 0.1 ± 0.1 mIU/mL) (Figure 2, B and D and Supplemental Figure 1).

### "Post priming": a subject bearing *GNRHR* mutations serves as a negative control

One normosmic subject had compound heterozygous mutations in *GNRHR*: p.R262Q, which has been shown to be deleterious in functional studies (43), and p.L286P which was predicted to be deleterious in 4 of 4 prediction programs. Consistent with her genotype, despite priming with pulsatile GnRH, she failed to respond to exogenous GnRH and exogenous kisspeptin (Figure 3). Moreover, this patient's lack of response to kisspeptin suggests that kisspeptin does not trigger the release of the LH at the level of the pituitary.

### From pituitary gonadotrope priming to GnRH neuronal priming

To test the possibility that the GnRH neuronal network itself requires priming with repetitive exposure to kisspeptin, three subjects returned for a third visit to the CRC and received multiple boluses of kisspeptin. These patients were selected to participate in this additional arm of the protocol because they had evidence for an intact albeit enfeebled GnRH secretory program at baseline (measurable baseline LH levels and responsiveness to exogenous GnRH before formal priming). Thus, they did not require priming with the GnRH pulsatile pump. Despite these repeated and/or escalating doses of kisspeptin, no LH response to kisspeptin was seen (Figure 4).

### Exploring the sub-phenotype of hypogonadotropic reversal: response to kisspeptin

Subject #12 had previously been diagnosed with normosmic IHH based on low serum testosterone (16 ng/dL),

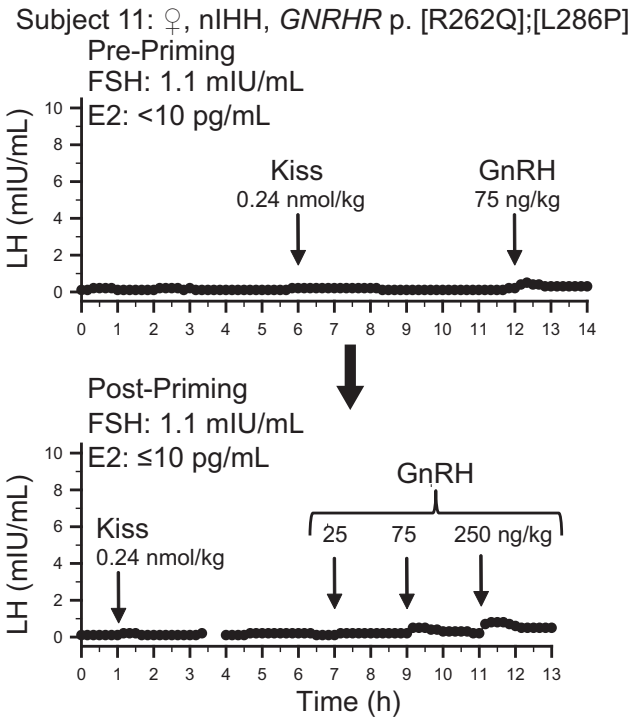


**Figure 3.** Baseline LH secretion and responses to kisspeptin and GnRH in a female (♀) subject with normosmic IHH (nIHH) and GnRH resistance due to the specified GNRHR mutations. Upper panel: results before priming with exogenous GnRH (pre-priming). Lower panel: results after priming with a failure to prime due to GnRH resistance (post-priming). Arrows indicate times of boluses. E2, estradiol levels during the study. FSH values measured from the first 2-h pool.

low gonadotropin levels, and small testicular volumes at age 19 (42). No protein-altering genetic mutations were found in 14 IHH genes. Over the next 4 years he demonstrated evidence for spontaneous activation of his hypothalamic-pituitary-gonadal cascade. By age 23, he had a testosterone level of 918 ng/dL and testicular volumes of 15 and 20 mL. During his initial admission to the CRC, he exhibited pulsatile LH secretion at baseline (2 pulses in 6 h, with amplitudes of 3.0 and 0.9 mIU/mL) (Figure 5).

After receiving a bolus of exogenous kisspeptin, this subject demonstrated a robust response with an LH pulse amplitude of 3.7 mIU/mL (Figure 5).

Given the robust nature of this subject's endogenous GnRH pulse generator, it was possible that the administration of kisspeptin in this initial study coincided with the nadir of an endogenous GnRH pulse. This subject therefore returned for a second study during which he received multiple boluses of kisspeptin. Each of these boluses induced an LH pulse (Figure 5). Based on the subject's endogenous pulse frequency, it is improbable that all five kisspeptin boluses coincided with endogenous LH pulses ($P = .001$).

## Discussion

Contrary to our hypothesis that exogenous kisspeptin administration could be used to distinguish patients with an absent GnRH neuronal complement from those with a reduced or impaired neuronal network, all subjects with abiding IHH failed to respond to kisspeptin, regardless of their genotype. In contrast, one subject who had undergone reversal of his hypogonadotropism demonstrated robust responses to kisspeptin.

The range of genotypes represented in this study provides an important dimension to the interpretation of the findings. Subject 1 carried a hemizygous frameshift mutation in *KAL1*; nearly all patients with *KAL1* mutations exhibit lifelong hypogonadotropism attributed to abnormal GnRH neuronal migration (44). Consistent with a lack of GnRH neurons, subject 1 had no response to kisspeptin. Another subject, subject 11, carried compound heterozygous mutations in *GNRHR*. Consistent with a lack of GnRH signaling in the pituitary, she also exhibited no response to kisspeptin. In contrast to the these two individuals, several other subjects (subjects 4, 6, 9, 10) carried mutations in genes characterized by variable

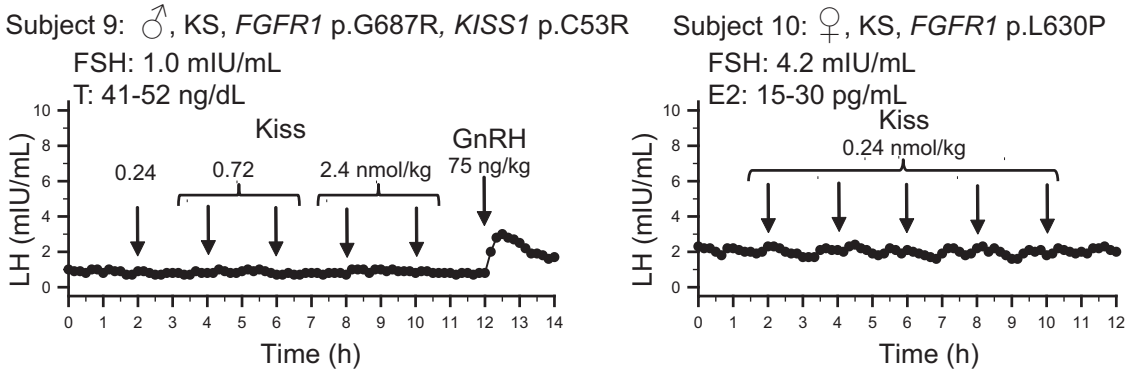


**Figure 4.** Responses to multiple boluses kisspeptin in a male (♂) and a female (♀) subject with Kallmann syndrome (KS) and evidence of endogenous GnRH neuronal activity. Arrows indicate times of boluses. Note escalating doses of kisspeptin provided to subject 9. T, testosterone; E2, estradiol levels during the study. FSH values measured from the first 2-h pool.

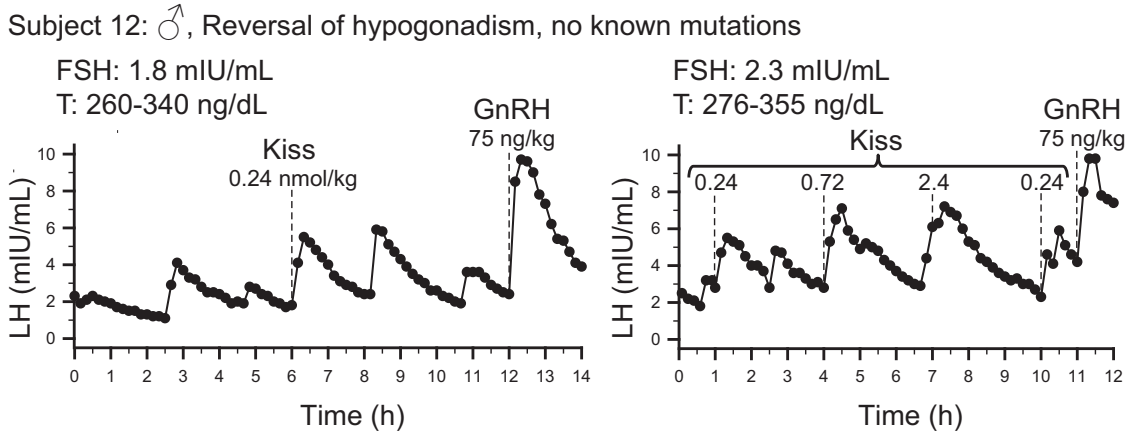


**Figure 5.** Intact responses to kisspeptin in a male (♂) subject with reversal of IHH. Both studies were performed without pituitary priming. The left panel shows the single bolus protocol and demonstrates the presence of endogenous pulsatile LH secretion and a response to kisspeptin. The right panel shows the study performed with multiple kisspeptin boluses and demonstrates consistent pulses of LH secretion in response to kisspeptin. Dashed lines mark the times kisspeptin or GnRH were given. T, testosterone levels during the study. FSH values measured from the first 2-h pool.

expressivity and incomplete penetrance (*PROKR2* or *FGFR1*) but importantly, none of these subjects responded to kisspeptin. Hypomorphic and knockout mouse models for these genes have revealed that mice with mutations in these genes still have an intact or only partially attenuated GnRH neuronal complement in the hypothalamus (45–47). By extrapolation, it is possible that patients bearing heterozygous mutations in *PROKR2* or *FGFR1* also retain some GnRH neurons. Indeed, several of the subjects in this study (subjects 7–10, two of whom have heterozygous *FGFR1* mutations) had evidence of some endogenous GnRH secretion, though not enough to sustain normal reproductive function. Thus, our results demonstrate that this residual GnRH neuronal complement is not sufficient to confer responsiveness to exogenous kisspeptin stimulation at a dose that evinces a robust response in healthy men and luteal phase women. It is important to note that patients bearing mutations in the neurokinin B pathway have been previously reported to be responsive to exogenous kisspeptin (48). However, those patients received a continuous infusion of kisspeptin as opposed to the single boluses administered in this study. Moreover, the magnitude of the LH response was quite modest, with LH and sex steroids remaining below the normal range and far below the magnitude of the response observed in healthy men undergoing a similar protocol, who achieved LH approximately two times higher than the upper limit of the normal range (30). Thus, in patients with abiding IHH, there is no clinically meaningful response to kisspeptin.

There are several possible explanations for why patients with abiding hypogonadotropism fail to respond to boluses of kisspeptin. First, it is possible that mutations exist in *KISS1R*, which encodes the kisspeptin receptor; while we screened for coding mutations in *KISS1R*, regulatory or intronic mutations would have escaped detection. Second, GnRH neurons may require "priming" with repeated exposure to kisspeptin to exhibit robust responses. However, our attempt to perform priming with the administration of multiple kisspeptin boluses failed to effect a GnRH-induced LH response. In addition, *Kiss1*$^{-/-}$ mice respond to their first exposure to kisspeptin (49), as do prepubertal rhesus monkeys (50), further arguing against the need for GnRH neuronal priming. Third, in mice, 5–40% of GnRH neurons do not respond to kisspeptin (51) and some GnRH neurons do not express *Kiss1r* (52), raising the possibility that different subsets of GnRH neurons may be differentially affected in IHH. In other words, congenital hypogonadotropism might preferentially affect those GnRH neurons that respond to kisspeptin. Finally, GnRH neurons form a complex network; kisspeptin may be able to signal at kisspeptin receptors on specific GnRH neurons, but other factors may prevent a coordinated response such that there is no significant LH secretion from the pituitary. Whatever the factors are that contribute to the lack of a response to exogenous administration of kisspeptin, they seem to supersede the specific genetic signature of each subject (with the exception of possible undetected regulatory mutations in *KISS1R*).

The fact that follicular phase women, who have physiologically low estradiol levels, demonstrate minimal responses to kisspeptin (28, 31, 53) suggests that sex steroids may play a role in modulating responsiveness to this neuropeptide. However, robust responses to exogenous kisspeptin have been observed in women with hypothalamic amenorrhea (low estradiol levels) (54, 55), in contrast to the lack of response to kisspeptin in IHH subjects observed in this study. Similarly, robust responses to kisspeptin are

seen in animal models of GnRH deficiency akin to hypothalamic amenorrhea, such as rats undergoing food deprivation and mice with leptin pathway mutations (56, 57). While sex steroids can modify kisspeptin responsiveness (50, 53), both IHH and HA subjects were studied in the hypogonadal state, raising the need to consider other factors that might account for the differences in their ability to respond to kisspeptin. Most patients with HA undergo a normal "minipuberty" of infancy and normal timing of sexual maturation. Hypothetically, the physiology underlying these developmental milestones may itself change the hypothalamic architecture regulating GnRH release in a permanent way, so that the ability to respond to kisspeptin is retained even in the face of stressors such as excessive exercise or caloric deprivation.

In contrast to the subjects with abiding IHH, one subject who underwent reversal of IHH responded robustly to kisspeptin. Although it is impossible to know from these studies when this patient acquired kisspeptin responsiveness, it appears reasonable to conclude that the activation of the hypothalamic-pituitary-gonadal cascade that characterizes the reversal state in this patient appears to be mediated through kisspeptin signaling. Prospective research examining kisspeptin responsiveness in IHH patients before and after reversal will determine whether kisspeptin responsiveness exists in these patients prior to reversal or is acquired during the process of reversal.

The strengths of this study include the use of patients with a rare but prismatic disease model that speaks directly to GnRH secretory function, incorporation of contemporary genetic testing, q10 min blood sampling to capture any pulsatile activity if present, and pituitary priming with exogenous pulsatile GnRH. Limitations of this study include the inability to study a patient with homozygous mutations in *KISS1*, as such patients are extremely rare (58). Kisspeptin was given as IV boluses to mimic the reported physiologic pulsatile secretion of kisspeptin. It remains possible that even higher doses or infusions of kisspeptin may elicit responses in IHH patients, though the physiologic significance of such findings would be unclear.

In summary, patients with congenital hypogonadotropism do not respond to a dose of kisspeptin that, when given to men and women in the luteal phase, stimulates a physiologic GnRH-induced LH response. This study raises the possibility that even if GnRH neurons reach the hypothalamus in patients with IHH, their functional integrity is compromised. In contrast, in a patient who underwent reversal of his hypogonadotropism, the capacity of GnRH neurons to respond to kisspeptin appears to be intact. Additional research is needed to determine whether reversal is caused by the acquisition of the ability to respond to kisspeptin.

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