Supplemental Methods

Kisspeptin-10 and -54 peptides:

Kisspeptin-54 peptide was prepared as previously described (24-27). Human sequence kisspeptin-10 was synthesised by Bachem Holding AG (Bubendorf, Switzerland), and purified by reverse-phase high performance liquid chromatography (HPLC). Electrospray mass spectroscopy and amino acid analysis confirmed identity of the peptide (lot number 3004009). The Limulus amoebocyte lysate test detected no endotoxin (Associates of Cape Cod, Liverpool, UK), and bacterial culture was sterile (Department of Microbiology, Hammersmith Hospital, London, UK), in samples of kisspeptin-10 peptide. Bioactivity of kisspeptin-10 and -54 peptides was confirmed by stimulation of LH release in female mice (Supplementary Figure 1). Vials of freeze-dried kisspeptin-10 and -54 were stored at -20^{O}C and reconstituted in 0.9% saline.

Collection, processing and analysis of blood samples

LH, FSH, E2 and total testosterone were measured using automated chemiluminescent immunoassays (Abbott Laboratories, Abbott Park, IL). Reference ranges for males were as follows: LH, 4–14 U/l; FSH, 1.5–8 U/l; testosterone, 10–28 nmol/l. Reference ranges for females were as follows: LH in iU/l , (2–10) follicular), 20–60 (midcycle), 4–14 (luteal); FSH in iU/l, 1.5–8 (follicular and luteal), 10-50 (mid-cycle); E2 in pmol/l, <300 (early follicular), 400-1500 (midcycle), 200-1000 (luteal). The respective intraassay and interassay coefficients of variation for each assay were: 4.1 and 2.7% (LH); 4.1 and 3.0% (FSH); 3.3 and 3.0% (E2); 4.2 and 2.8% (total testosterone). Analytical sensitivities were: 0.5iU/L (LH), 0.05iU/L (FSH); 37pmol/L (E2); 2nmol/L (total testosterone).

Plasma kisspeptin immunoreactivity (IR) was measured using an established radioimmunoassay (22-24). The antibody cross-reacted 100% with human kisspeptin-54, kisspeptin-14, and kisspeptin-10 and less than 0.01% with other related RF amide proteins, including prolactin-releasing peptide, RF amide-related peptide 1 (RFRP1), RFRP2, RFRP3, QRFP43, neuropeptide FF, and neuropeptide AF. The limit of detectability was 2pmol/l, and the intra- and interassay coefficients of variation were 8.3 and 10.2%, respectively. Serum and plasma samples were stored at -20^{O}C until analysis.

Supplemental Table 1: Inclusion criteria for healthy male and femal e volunteers recruited to study.

Inclusion Criteria	
Male and female subjects	
	age 18 to 40 years
	no clinical or biochemical evidence of hypogonadism
	normal thyroid function
	normal serum prolactin
	no therapeutic or recreational drug use
	no systemic disease co-morbidity
Female subjects	
	regular menstrual cycles
	no oral contraceptive pill therapy within the last year
	no clinical or biochemical evidence of polycystic ovarian syndrome

Supplemental Figure 1. Plasma LH following intraperitoneal injection of kisspeptin-10 and kisspeptin-54 to female mice.

In order to confirm bioactivity of kisspeptin-10, female C57BL/6 mice (weight 20-25g) were administered an intraperitoneal injection of saline, kisspeptin-10 (1 or 30nmol), or kisspeptin-54 (1 or 30nmol) (n=8 per group). Since kisspeptin-10 stimulates LH release throughout the estrous cycle (ROA 2008), animals were injected in different phases of the estrous cycle. Mice were killed after 20 minutes and blood was sampled for plasma LH, as previously described (25). Kisspeptin-54 was synthesised, purified and tested as previously described (22). Data is shown as mean +/- SEM. # $P<0.001$ vs. Saline; & $P<0.01$ vs. 1nmol kisspeptin-10. Intraperitoneal (ip) injection of 1nmol kisspeptin-10 resulted in a plasma LH level non-significantly higher than plasma LH following saline injection (mean plasma LH 20 minutes following ip injection: saline, 0.57 ± 0.70ng/ml; 1nmol kisspeptin-10, 1.82 ± 0.31 ng/ml, $P>0.05$ vs. saline). However 30nmol kisspeptin-10 significantly increased plasma LH compared with saline (mean plasma LH 20 minutes following ip injection: saline, 0.57 ± 0.70 ng/ml; 30nmol kisspeptin-10, 6.01 ± 0.74 ng/ml, $P<0.001$ vs. saline). Intraperitoneal injection of 1 or 30nmol/kg kisspeptin-54 significantly increased plasma LH compared with saline, to a level similar to plasma LH following injection of 30nmol kisspeptin-10 (mean plasma LH 20 minutes following ip injection: 30nmol kisspeptin-10 6.01 ± 0.74 ng/ml; 1nmol kisspeptin-54 5.93 ± 1.18 ng/ml, $P > 0.05$ vs. 30nmol kisspeptin-10; 30nmol kisspeptin-54 6.28 ± 0.88 ng/ml, $P > 0.05$ vs. 30nmol kisspeptin-10).

Supplemental Figure 1