Supplemental Methods

Liquid chromatography-mass spectroscopy (LC-MS). The LC-MS system consisted of a TSQ mass spectrometer coupled to a Shimadzu (Columbia, MD) HPLC system equipped with a CBM-20A system controller, an LC-20 AD pump, a SIL-20AC autosampler, a CTO-20A column oven, a DGU-20A5 degasser, and a FCV-11AL valve unit. The temperature of the autosampler was set at 4ºC during operation. Kisspeptin-10 and the internal standard (substance P, SP) were separated on a BetaBasic C_8 column (2.1x50 mm, 5 μm, Thermo Hypersil-Keystone, Bellefonte, PA) coupled with a BetaBasic C8 guard column (2.1x10 mm, 5 μm) under a gradient elution at a flow rate of 0.20 mL/min. Mobile phase A consisted of water/0.1% formic acid and mobile phase B consisted of acetonitrile/0.1% formic acid. The gradient was initiated from 5% B, increased to 25% B in 2 min, to 50% B in 8 min, to 70% B in 1 min, was held at 70% B for 1 min, then decreased to 5% B in 1 min, and further held at 5% B for 7 min before the next sample injection, for a total run time of 20 min. The samples and standards were eluted at 7.4 min and 6.3 min, respectively. The eluent was diverted to the waste in the first 3.5 min and the last 10 min to minimize matrix effect. The mass spectrometer was operated in the positive ESI mode with a helium pressure of 20 psi, a typical electrospray needle voltage of 4,900 V, a sheath nitrogen gas flow of 33 (arbitrary units), and a heated capillary temperature of 325ºC. Kisspeptin 112-121 and SP analyzed using multiple reaction monitor (MRM) mode with ion transitions at m/z 651.9>277.8 and relative collision energy of 23%, 674.5>253.8 and relative collision energy of 25%, and 571.0>300.9 and relative collision energy of 23%. The mass spectrometer was tuned to its optimum sensitivity by direct infusion of kisspeptin. All operations were controlled by Finnigan Xcalibur software on a Windows NT 4.0 system.

Sample preparation. Aliquots (20 μl) of kisspeptin in 50% acetonitrile (ACN)/water at concentrations ranging from 0.1 to 1000 ng/mL were mixed with 20 μL SP stock solution (1000 ng/mL). Then 200 μL human plasma was added to the above mixtures, mixed by vortex for 1~2 seconds, immediately followed by addition of 600 μL acetonitrile to precipitate plasma proteins and to prevent decomposition of kisspeptin. The supernatant was transferred to a borosilicate glass tube (12x75 mm, Fisher, Pittsburgh, PA) and dried under a mild stream of nitrogen. The residues were reconstituted in 100 μL solution of 5% ACN/0.1% formic acid (FA) and a 50 μL aliquot of the reconstituted solution was injected into the liquid chromatograph (LC) port coupled to the LC-MS.

Assay validation. Plasma samples for the standard curves were generated for a concentration range of 0.5-20 ng/mL for the low end and 20-1000 ng/mL for the high end concentrations. The within-run precision values were determined in six replicates for each of the quality control (QC) data points at concentrations of 0.5, 1, 5, 50 and 500 ng/mL of kisspeptin-10. The accuracy of the assay was determined by comparing the nominal concentrations with the corresponding calculated mean concentrations. The lower limit of quantitation (found to be 0.5 ng/mL) was defined by a signal-to-noise ratio of >1:10.

Supplemental Figure Legends

Figure 1. Representative calibration curves for quantitation of kisspeptin 112-121 by liquid chromatography-mass spectroscopy. **A**, curve for 0.5-20 ng/mL. **B**, curve for 20-1000 ng/mL. SP, substance P, used as an internal standard.

Figure 2. LH pulse profiles of subjects not shown in Figure 1. Arrows, time of kisspeptin administration; arrowheads, pulses.

Figure 3. Correlation analyses. Amplitude of the kisspeptin-induced pulse as a function of the amplitude of the previous endogenous pulse (**A**), of the previous inter-pulse interval (**B**), or of the ambient testosterone concentration (**C**). Amplitude of the first endogenous pulse following the kisspeptin-induced pulse as a function of the amplitude of the kisspeptin-induced pulse (**D**) or of the previous inter-pulse interval (**E**). **F**, schematic illustrating pulse intervals.

Figure 1

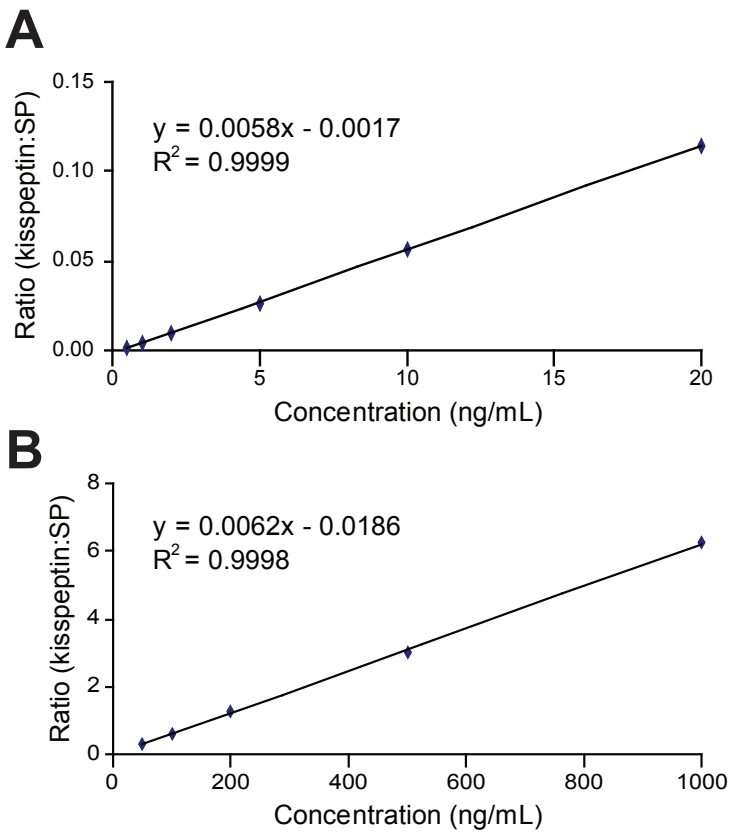

A
0.15
0.10
0.05
0.00
Ratio (kisspeptin:SP)
y = 0.0058x - 0.0017
R2 = 0.9999
0
5
10
15
20
Concentration (ng/mL)
B
8
6
4
2
0
Ratio (kisspeptin:SP)
y = 0.0062x - 0.0186
R2 = 0.9998
0
200
400
600
800
1000
Concentration (ng/mL)

Figure 2

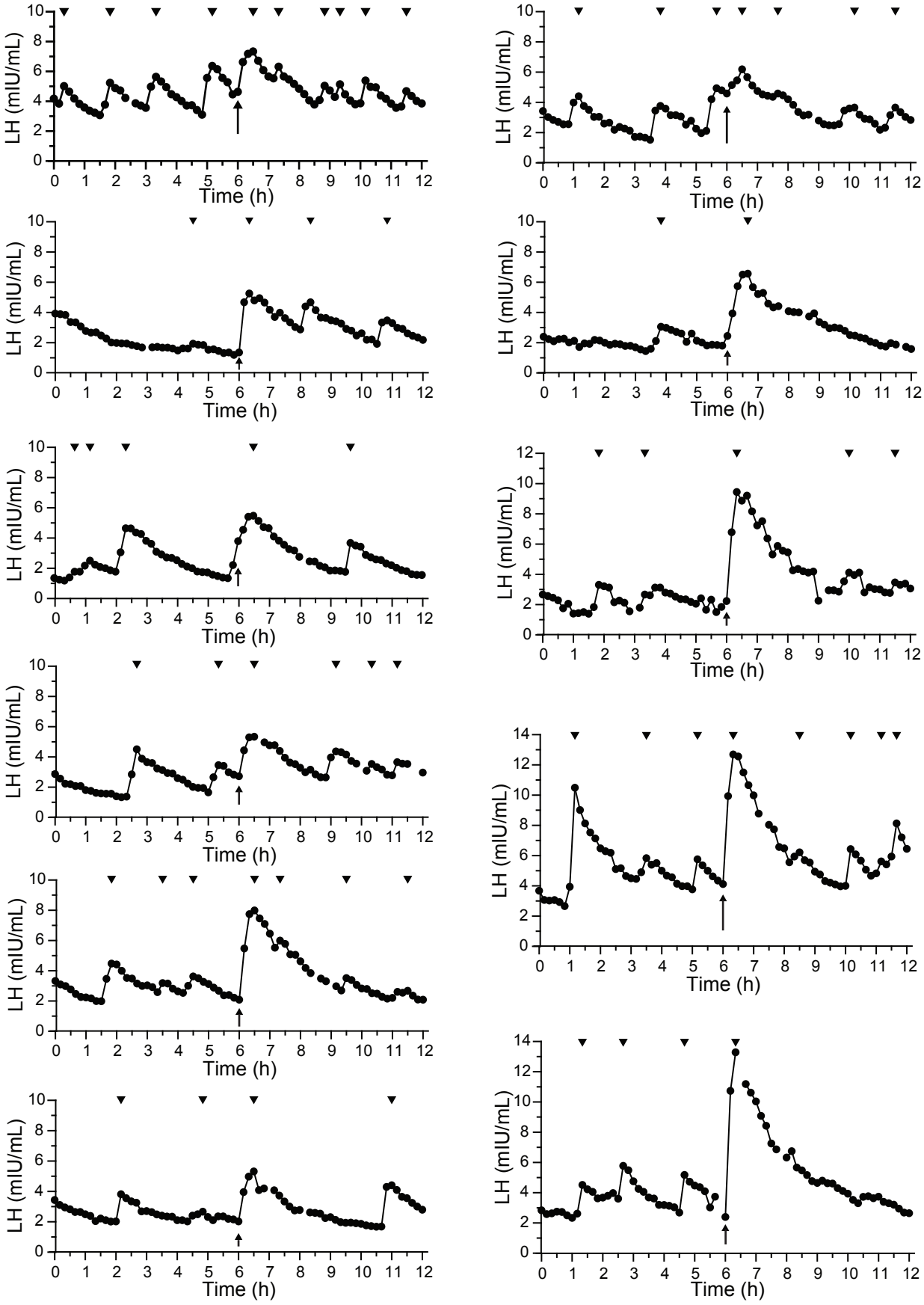
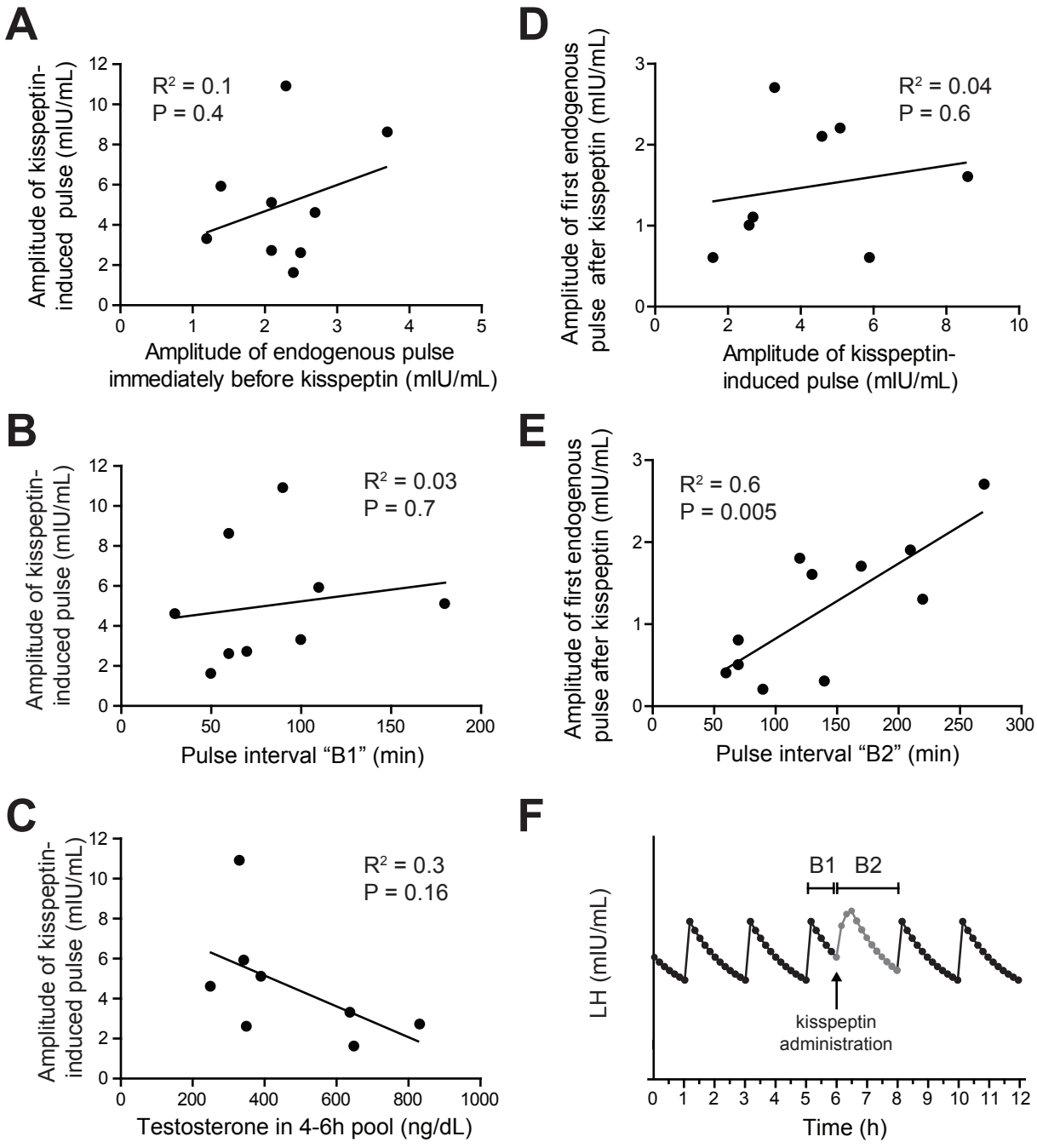


Figure 3


A
Amplitude of kisspeptin-induced pulse (mIU/mL)
R² = 0.1
P = 0.4
Amplitude of endogenous pulse immediately before kisspeptin (mIU/mL)
B
Amplitude of kisspeptin-induced pulse (mIU/mL)
R² = 0.03
P = 0.7
Pulse interval "B1" (min)
C
Amplitude of kisspeptin-induced pulse (mIU/mL)
R² = 0.3
P = 0.16
Testosterone in 4-6h pool (ng/dL)
D
Amplitude of first endogenous pulse after kisspeptin (mIU/mL)
R² = 0.04
P = 0.6
Amplitude of kisspeptin-induced pulse (mIU/mL)
E
Amplitude of first endogenous pulse after kisspeptin (mIU/mL)
R² = 0.6
P = 0.005
Pulse interval "B2" (min)
F
LH (mIU/mL)
B1
B2
kisspeptin administration
Time (h)