REPRODUCTIVE ENDOCRINOLOGY AND INFERTILITY

Serum leptin levels and reproductive function during the menstrual cycle

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OBJECTIVE: The purpose of this study was to investigate the role of leptin on reproductive hormones and ovulation.

STUDY DESIGN: The BioCycle Study (2005-2007) followed 259 healthy premenopausal women not using hormonal contraceptives for ≤2 menstrual cycles (n = 509 cycles). Serum leptin, estradiol, progesterone, luteinizing hormone (LH), follicle-stimulating hormone, and testosterone were measured ≤8 times per cycle. The association of time-varying leptin and reproductive hormones over the cycle was estimated with the use of linear mixed models that were adjusted for percent body fat and age with inverse probability weighting for time-varying physical activity, caloric intake, and other reproductive hormones. The odds ratio for sporadic anovulation (n = 42 cycles) was estimated with the use of generalized linear models that were adjusted for percent body fat and age.

RESULTS: Geometric mean serum leptin levels increased from menses to the late luteal phase (16.7-20.4 ng/mL; $P < .01$), with a mid-cycle peak (21.7 ng/mL) at the time of the LH surge ($P < .01$). A 10% higher leptin level across the menstrual cycle was associated with higher estradiol levels (2.2%; 95% CI, 1.5—3.0), luteal progesterone levels (2.1%; 95% CI, 0.5—3.7), ovulatory LH levels (1.2%; 95% CI, 0—2.3), testosterone levels (0.6%; 95% CI, 0.3—0.9), and lower follicle-stimulating hormone levels (—0.7%; 95% CI, —1.1 to —0.4). Leptin at the time of the expected LH surge was moderately inversely associated with sporadic anovulation (per log increase in leptin; adjusted odds ratio, 0.58; 95% CI, 0.28—1.22).

CONCLUSION: The association that was observed between leptin level and reproductive function points to a possible relationship between serum leptin level and enhanced fertility.

Key words: anovulation, leptin, menstrual cycle, reproductive hormone

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Leptin, a product of the *LEP* gene, is known widely to regulate appetite and energy expenditure.[1] Its involvement in the reproductive system was first suspected in 1949 when leptin homozygous recessive female mice were observed to be not only obese but sterile.[2] Future research that demonstrated that the administration of recombinant leptin to these mice restored fertility led researchers to theorize that leptin served as a signal of adequate fat deposition, allowing for the energy-intensive reproduction system to function appropriately.[3,4] Recent studies on the administration of recombinant leptin to women with lipodystrophy (ie, leptin deficiency) have also demonstrated restored menstrual cycle regularity and fertility.[5,6] Despite the clear involvement of leptin in the female reproductive system, its relationship to reproductive hormone production, menstrual cycle characteristics, and ovarian function remains unclear.

The role of leptin on menstrual cycle regulation was first suggested more than a decade ago by researchers who found that leptin levels varied across the menstrual cycle while remaining stable for men and postmenopausal women over a 28-day period.[7] Subsequently, a number of studies have found that either serum leptin increases from the follicular to the luteal phase (in a cyclic fashion) or shows no trend across the menstrual cycle.[7-22] Limitations of previous work included the small number of women studied, the limited number of serum samples that were collected over the cycle, and unverified menstrual cycle phase

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determination. Furthermore, associations between leptin and reproductive hormones have been identified primarily by statistical correlations, without further consideration for factors such as diet, physical activity, and other hormone levels, which may have resulted in bias. In addition, because adipose tissue is a source of both leptin and estradiol production,[23] adjustment for adiposity is critical for understanding leptin's effect on reproductive hormones outside of the influence of body fat and could help inform future clinical interventions.

The primary objective of our study was to describe leptin levels across the menstrual cycle among a cohort of premenopausal women. Our secondary objectives were to examine the associations between leptin and reproductive hormones (including estradiol, progesterone, luteinizing hormone [LH], follicle-stimulating hormone [FSH], and testosterone), menstrual cycle characteristics and the odds of sporadic anovulation. The results of our study are important for understanding the role of leptin on reproduction and fertility.

METHODS

Study population

The BioCycle Study (2005-2007) was a prospective cohort study of 259 regularly menstruating, healthy premenopausal women from western New York who were observed over 1 (n = 9) or 2 (n = 250) menstrual cycles. Women were not eligible for the study if they were using oral contraceptives or medications for a chronic medical condition, had been pregnant or breastfeeding within the past 6 months, had been diagnosed with a menstrual or ovulatory disorder, or self-reported their body mass index (BMI) as <18 or >35 kg/m^2 at screening. Additional information about the study population is described in more detail elsewhere.[24] The University at Buffalo Health Sciences Institutional Review Board approved the study and served as the institutional review board that was designated by the National Institutes of Health for this study under a reliance agreement. All participants provided written informed consent.

Measures

Leptin and reproductive hormones

Women provided morning fasting blood samples up to 8 times per cycle. Fertility monitors (Clearblue Easy Fertility Monitor; Inverness Medical, Waltham, MA) were used to time mid-cycle visits; the remaining visits were scheduled according to an algorithm that considered each woman's typical cycle length.[25] Consequently, blood samples were collected during the following expected phases of the menstrual cycle: menses, the middle and late follicular phase, LH surge, ovulation, and the early, mid, and late luteal phase. Most women adhered to the study protocol; 94% of them provided blood samples for at least 7 visits per cycle. Blood samples were processed according to standard protocols and frozen at −80°C within 90 minutes of phlebotomy.[24] Frozen sera were later shipped on dry ice to analytical laboratories. Samples from each participant were measured within a single run to limit analytical variability.

Leptin concentration was measured in multiple batches by immunoassay with the use of the Mercodia Leptin ELISA (Mercodia AB, Uppsala, Sweden) at the Advanced Research and Diagnostics Laboratory, University of Minnesota, Minneapolis, MN. No values were below the lower limit of detection for this assay (0.05 ng/mL). Select batches of measurements were recalibrated post-assay by a calibration curve that was estimated from all the calibration data.[26] The maximum interassay coefficient of variation was 10.2% after recalibration.

Estradiol, progesterone, LH, and FSH concentrations were measured by solid-phase competitive chemiluminescent enzymatic immunoassays on the Immulite 2000 analyzer (Siemens Medical Solutions Diagnostics, Deerfield, IL) at Kaleida Laboratories in Buffalo, NY. Total testosterone was measured by liquid chromatography/tandem mass spectrometry with the use of the Shimadzu Prominence Liquid Chromatogram (Shimadzu Corporation, Kyoto, Japan) with a tandem mass spectrometer (AB Sciex 5500; AB Sciex, Framingham, MA) at the Advanced Research and Diagnostics Laboratory in Minneapolis, MN. Increased sensitivity was achieved by using 100% acetonitrile mobile phase B as the solvent gradient elution and adding a low standard of 4 ng/dL. The maximum coefficient of variation for each assay was <10% for estradiol, <14% for progesterone, <4% for LH and FSH, and <7% for testosterone. Values falling below the lower limit of detection for each assay were rare (<3%) and were replaced with values equal to the lower limit of detection divided by the square root of 2.[27] All hormone measurements, including leptin, were transformed logarithmically for the analysis.

Sporadic anovulation and menstrual cycle characteristics

Sporadic anovulatory cycles were defined as cycles with a peak progesterone concentration of ≤5 ng/mL and no observed serum LH peak among samples that were collected during the later cycle visits (n = 42 cycles).[28] In a sensitivity analysis, a subgroup of anovulatory cycles with peak progesterone concentrations of ≤3 ng/mL (n = 28 cycles) were examined as an alternative definition of anovulation. Menstrual cycle characteristics (menstrual cycle length, menses length, and total blood loss during menses) were determined from daily diaries that documented bleeding days and blood loss with the use of validated pictograms.[29] Follicular and luteal phase lengths were determined based on the expected date of ovulation with the use of information from the fertility monitors and serum hormone levels.[30]

Hormone realignment

To correct for any residual errors in blood collection timing, hormone measurements were realigned within ovulatory cycles according to an algorithm based on the day of the serum LH peak.[31] This realignment affected 70% of ovulatory cycles, with 42% of realignments because of an LH peak that was detected at the visits adjacent to the expected LH surge visit. All hormone measurements were realigned together for a given cycle. Realignment procedures sometimes