Increase in daily LH secretion in response to short-term calorie restriction in obese women with PCOS

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Van Dam, Eveline W. C. M., Ferdinand Roelfsma, Johannes D. Veldhuis, Frans M. Helmerhorst, Marijke Fröhlich, A. Edo Meinders, H. Michel J. Krans, and Hanno Pijl. Increase in daily LH secretion in response to short-term calorie restriction in obese women with PCOS. Am J Physiol Endocrinol Metab 282: E865–E872, 2002. First published December 11, 2001; 10.1152/ajpendo.00458.2001.—We hypothesized that short-term calorie restriction would blunt luteinizing hormone (LH) hypersecretion in obese women with polycystic ovary syndrome (PCOS) and thereby ameliorate the anovulatory endocrine milieu. To test this hypothesis, 15 obese patients with PCOS and nine age- and body mass index-matched healthy women underwent 24-h blood sampling to quantitate plasma LH, leptin, and insulin levels. PCOS subjects were prescribed a very low caloric liquid diet (4.2 MJ/day) for 7 days and were then resampled. Basal and pulsatile LH secretion was threefold higher in PCOS subjects, but plasma insulin and leptin levels were not different in the calorie-replete state. Contrary to expectation, calorie restriction enhanced basal and pulsatile LH secretion even further. As expected, plasma glucose, insulin, and leptin concentrations decreased by 18, 75, and 50%, respectively. Serum total testosterone concentration fell by 23%, whereas serum estrone, estradiol, sex hormone-binding globulin (SHBG), and androstenedione concentrations remained unchanged. Enhanced LH secretion in the presence of normal metabolic and hormonal adaptations to calorie restriction points to anomalous feedback control of pituitary LH release in PCOS.

leptin; insulin; dietary intervention; feedback regulation

SPONTANEOUS PULSATILE LUTEINIZING HORMONE (LH) and exogenous gonadotropin-releasing hormone (GnRH)-stimulated LH release are increased (the former as a result of augmented LH pulse frequency and amplitude), whereas follicle-stimulating hormone (FSH) secretion appears normal or reduced in patients with polycystic ovary syndrome (PCOS) compared with normal women studied in the follicular phase of the menstrual cycle (2, 7, 23, 46, 57, 58). The pathogenesis of LH hypersecretion in PCOS has not been elucidated but could include intrinsic defects in the hypothalamic control of GnRH pulse generation, accentuated pituitary responsiveness to GnRH stimulation, and/or altered hormonal feedback regulation of GnRH release or LH bioactivity (7, 19, 23, 57). Whatever the mechanism, the deleterious effects of LH hypersecretion on fertility are indisputable (for review see Ref. 21). Transgenic mice overexpressing LH are anovulatory and hyperandrogenic and exhibit polycystic ovaries (27, 36). Although a coexistent ovarian defect may contribute to anovulation in PCOS, it is conceivable that restoration of normal pulsatile LH secretion in obese PCOS patients would establish ovulatory cycles.

Weight loss is often accompanied by resumption of ovulation in obese women with PCOS (33). The mechanism underlying this response remains unclear. One consideration is that calorie restriction and/or the concomitant reduction of plasma insulin and/or leptin concentrations may be responsible. A negative energy balance dampens pulsatile LH secretion in various mammalian species, including humans (for review see Ref. 55). Diminished central nervous system glucose oxidation appears to play a role in the regulation of LH secretion in response to calorie restriction in the rat and sheep. In addition, caloric deprivation is associated with a marked decline in leptin output, which in the rodent plays a critical role in reproductive physiology. Leptin relays nutritional status information to the hypothalamic GnRH neuronal ensemble, which controls gonadotropin secretion (for review see Ref. 8). Thus exogenous leptin administration partially rescues hypogonadotropism induced by starvation in female rats and male mice (1, 29). Concomitantly, the hyperinsulinism of PCOS may modulate LH secretion.

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Therefore, relative hypoinsulinemia induced by calorie restriction may provide an additional mechanism to suppress LH secretion in women with PCOS when undergoing weight loss.

We hypothesized that short-term calorie restriction would repress inappropriate LH secretion in obese women with PCOS and ameliorate the hyperandrogenic milieu, possibly in association with attendant normalization of leptin and insulin secretion. To test this hypothesis, we measured 24-h plasma concentrations of LH, leptin, and insulin and ovarian steroids in the basal condition and after 7 days of a very low calorie diet (VLCD) in obese women with PCOS and compared the results with those in a group of body mass index (BMI)-matched women with regular menstrual cycles.

SUBJECTS AND METHODS

Subjects

Fifteen obese women [mean age 29 (range: 20–38) yr; mean BMI 39 (range: 30–54) kg/m²] with the diagnosis of PCOS were studied. The PCOS women were recruited from the outpatient department for reproductive medicine, Leiden University Medical Center. The diagnosis of PCOS was based on the presence of infertility due to anovulation that was not secondary to a specific underlying disease of the pituitary, ovaries, or adrenal glands and with elevated serum levels of testosterone in the absence of hyperprolactinemia or thyroid disease. Late-onset adrenal hyperplasia was excluded by a morning plasma 17-hydroxyprogesterone level <10 nmol/l. Nine age- and BMI-matched women [mean age 34 (range: 20–40) yr, mean BMI 39 (range: 32–61) kg/m²] with a regular menstrual cycle comprised the control group. Volunteers were recruited through advertisements in local newspapers. None of the subjects used any neuroactive medications or hormones (including oral contraceptives) for ≥3 mo before the study. All women had stable body weight for ≥3 mo before the study. None of the subjects engaged in regular heavy physical exercise. The purpose, nature, and possible risks of the study were explained to all subjects, and written informed consent was obtained. The study protocol was approved by the ethics committee of the Leiden University Medical Center.

Study Design and Protocol

An- or oligo-ovulatory patients with PCOS were studied on a random day. Ovulation had not occurred before admission, as evidenced by evaluation of serum progesterone levels measured 20 days (range 9–45 days) before the study and on study days. Control women were studied in the early follicular phase of their menstrual cycle (days 2–5). All subjects were admitted to the Clinical Research Center of the Leiden University Medical Center at 0800 after a 10-h overnight fast. Women with PCOS and controls were instructed to follow a liquid diet [total of 8.3 MJ (1,970 kcal)/day] consisting of Modifast [2 MJ (471 kcal)/day in 3 equal proportions; macronutrient composition: 43% protein, 15% fat, 42% carbohydrate; Novartis Nutrition Benelux, Breda, the Netherlands] plus Nutridrink [6.3 MJ (1,500 kcal)/day in 1,000-ml servings; macronutrient composition: 13% protein, 39% fat, 48% carbohydrate; Nutricia, Zoetermeer, the Netherlands] for 3 days before admission. On the first study day, these food products were served as meals at 0900 (300 kcal of Nutridrink and 157 kcal of Modifast), 1400 (600 kcal of Nutridrink and 157 kcal of Modifast), and 1900 (600 kcal Nutridrink and 157 kcal of Modifast). Subjects were advised to refrain from drinking or drinking caffeinated beverages during the study. They were allowed to walk around inside the research center during the day. Lights were put out at 2300.

On the morning of admission, body weight, waist and hip circumference, and bioelectrical impedance were measured. A single venous blood sample was withdrawn ≥30 min after insertion of an intravenous canula at 0800 for baseline serum hormone measurements, including 17-hydroxyprogesterone, androstenedione, testosterone, estrone, estradiol, sex hormone-binding globulin (SHBG), FSH, and LH. At least 1 h after canulation, blood samples were drawn into heparinized tubes every 10 min for 24 h. LH concentrations were determined every 10 min, leptin every 20 min, and insulin and glucose 1.5 h after each meal. Samples were centrifuged within 1 h of sampling, and plasma was stored at −20°C until assay. Control women were studied according to the same protocol.

After baseline study, women with PCOS were prescribed a very low calorie diet (VLCD, Modifast, 2 MJ [471 kcal]/day in 3 equal proportions; see beginning of this section for macronutrient composition) for 7 days and instructed not to change their physical activity level. Compliance with the dietary prescription was assessed by dietary history, and the participants were asked to make note of any unusual physical activity. On the 7th day, the aforementioned sampling study was repeated. Modifast was served at 0900, 1400, and 1900. Control women were studied only once.

Body Composition

The percentage of body fat was measured by bioelectrical impedance analysis (Bodystat, Douglas, Isle of Man, UK) before each 24-h hormone profile. The impedance measurements were obtained on the morning of the study after an overnight fast, after the subjects had voided, and while they were resting in bed. The waist-to-hip ratio was calculated as waist circumference (measured halfway between the lower costal margin and the iliac crest) divided by hip circumference (measured at the maximum circumference of the hip with the subjects in a standing position).

Assays

Androstenedione, testosterone, and 17-hydroxyprogesterone were measured with coated-tube radioimmunoassays (RIA) and progesterone with solid-phase RIA (Diagnostic Products, Los Angeles, CA). Estrone and SHBG were measured with a coated-tube RIA and an immunoradiometric assay (IRMA), respectively, of Spectria (Espoo, Finland). Estradiol was determined with an RIA (Diagnostic Systems Laboratory, Webster, TX). FSH was measured with a time-resolved immunofluorometric assay (TR-IFMA; Wallac, Turku, Finland). Insulin was measured with IRMA (Bio-source, Nivelles, Belgium). Glucose was measured with a fully automated chemical analyzer (Hitachi 747, Hitachi, Japan).

Leptin was determined by RIA (Linco Research, St. Louis, MO) with an interassay coefficient of variation (CV) of 3.9–7.2%, and LH was measured with a TR-IFMA (Wallac) with a CV of 5.5–6.7%.

Analytical Techniques

Cluster. A largely model-free computerized peak detection algorithm was used to quantify statistically significant LH
and leptin pulses in relation to dose-dependent measurement error (51). A 2 × 1 test cluster size was used to test for rises and falls in the data and t-statistics of 2.0 for significant upward and downward slopes of LH and leptin peaks. Cluster analysis was validated to quantify pulsatile properties of leptin release (25) and of LH (18) in earlier studies.

**Deconvolution analysis.** A wave form-independent deconvolution analysis was used (pulse) to calculate the mean and nadir 24-h LH secretion (52). The biexponential kinetics allowed for a rapid-component LH decay rate (half-life) of 18 min, a slower decay rate of 90 min, and proportionate (slow/total) decay of 0.37, as reported earlier (48).

**Cosinor analysis.** Diurnal rhythmicity of serum leptin concentrations was appraised by cosinor analysis as described previously (49, 50). Cosinor analysis simply entails trigonometric regression of a 1,440-min cosine function on the full 24-h serum hormone concentration vs. time profile.

**Statistical analysis.** Data are expressed as means ± SE unless otherwise indicated. Differences within and between groups were assessed using Student’s t-tests (paired or unpaired when appropriate). Data were log transformed before analysis if necessary. Computations were performed using SYSTAT 9 and/or SPSS 9 for Windows (SPSS, Chicago, IL). P < 0.05 was considered statistically significant.

**RESULTS**

**Basal Plasma Hormone Levels in PCOS vs. Control Subjects**

The clinical, hormonal, and metabolic characteristics of the subjects are shown in Table 1. Biochemical hyperandrogenism in PCOS women was corroborated by increased plasma concentrations of androstenedione and testosterone compared with the controls. Plasma estrone and estradiol levels were also increased in PCOS women. The plasma SHBG and FSH concentrations tended to be lower in PCOS subjects compared with controls (P = nonsignificant). Fasting plasma insulin and glucose concentrations were not significantly different in the two groups.

**Daily Plasma LH, Leptin, and Insulin Concentrations in PCOS vs. Control Subjects**

Women with PCOS had higher mean plasma LH concentrations than controls. On the basis of cluster analysis, women with PCOS had significantly higher nadir LH concentrations and an increased number of LH pulses over 24 h. Pulse area was not significantly different between PCOS and control subjects. It was interesting to note that pulse configuration appeared to be changed in PCOS women: maximal LH pulse amplitude was significantly higher, whereas pulse width was smaller. Deconvolution analysis revealed that both basal (nonpulsatile) and total LH secretion values were increased approximately threefold in PCOS women compared with controls (Table 2).

The plasma leptin concentration profiles were similar in PCOS and control subjects. Mean concentration and pulse area were not different between groups. The number of leptin peaks was slightly reduced in PCOS women. Mesor, amplitude, and acrophase of circadian rhythmicity were similar (Table 3).

Twenty-four-hour mean plasma insulin concentrations were not different in PCOS compared with control subjects (Table 1).

**Effects of Calorie Restriction**

Seven days of calorie restriction induced a small but significant reduction of body weight and percentage of body fat in PCOS women. The change of body weight, although small, was highly significant, because all 15 subjects lost (a little) weight during caloric deprivation. Fasting glucose concentrations decreased considerably, and basal plasma leptin and insulin concentrations were almost halved (Table 1). Basal estradiol, estrone, SHBG, and androstenedione levels did not change in response to calorie restriction. In contrast, serum total testosterone levels were significantly reduced (Table 1).

| Table 1. Clinical, endocrine, and metabolic characteristics before and after short-term calorie restriction of obese women with PCOS and obese control women |
|---|---|---|---|---|---|---|
| Age, yr | PCOS Before (A) | 29.1 ± 1.3 | PCOS After (B) | 29.1 ± 1.3 | Controls (C) | 34.1 ± 2.4 | P (A vs. B) | 0.09 | P (A vs. C) | 0.823 |
| Weight, kg | 113.3 ± 4.4 | 112.3 ± 4.3 | 113.7 ± 8.8 | <10−6 | BMI, kg/m² | 39.2 ± 1.7 | 38.2 ± 1.7 | 40.1 ± 3.5 | <10−6 | 0.803 |
| Body fat, % | 46.4 ± 1.4 | 44.8 ± 1.6 | 49.2 ± 2.0 | 0.0023 | 17-Hydroxyprogesterone, nmol/l | 4.3 ± 0.5 | 3.5 ± 0.4 | 2.2 ± 0.2 | 0.360 | 0.007 |
| Androstenedione, nmol/l | 10.4 ± 1.2 | 9.3 ± 1.0 | 5.1 ± 1.2 | 0.166 | Testosterone, nmol/l | 2.6 ± 0.4 | 2.0 ± 0.2 | 0.6 ± 0.1 | 0.045 | 0.0002 |
| SHBG, nmol/l | 22.3 ± 3.5 | 25 ± 3.5 | 27.2 ± 3 | 0.248 | Estrone, pmol/l | 248 ± 23 | 258 ± 26 | 127 ± 23 | 0.684 | 0.002 |
| Estradiol, pmol/l | 167 ± 20 | 151 ± 17 | 98 ± 10 | 0.192 | FSH, U/l | 5.4 ± 0.3 | 5.6 ± 0.3 | 6.3 ± 0.4 | 0.464 | 0.093 |
| Fasting glucose, mmol/l | 5.6 ± 0.2 | 4.6 ± 0.14 | 5.4 ± 0.1 | 0.000046 | 0.682 |
| Fasting insulin, pmol/l | 154 ± 20 | 91 ± 13 | 141 ± 31 | 0.00071 | 0.711 |
| Mean insulin, pmol/l | 601 ± 101 | 144 ± 17 | 424 ± 80 | 0.00013 | 0.235 |

Data are given means ± SE. Within- and between-group differences were calculated with the two-tailed Student’s t-test for paired and unpaired samples when appropriate. Data were log transformed before statistical analysis if necessary. PCOS, polycystic ovary syndrome; BMI, body mass index; SHBG, sex hormone-binding globulin; FSH, follicle-stimulating hormone.
Cluster analysis compared with controls matched for age and BMI

Table 3.

<table>
<thead>
<tr>
<th></th>
<th>PCOS Before (A)</th>
<th>PCOS After (B)</th>
<th>Controls (C)</th>
<th>P (A vs. B)</th>
<th>P (A vs. C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean, U/l</td>
<td>6.22 ± 0.68</td>
<td>7.22 ± 0.71</td>
<td>2.70 ± 0.24</td>
<td>0.0009</td>
<td>&lt;0.0005</td>
</tr>
<tr>
<td>Integrated area, U/l^-1·min^-1</td>
<td>8,950 ± 980</td>
<td>10,390 ± 1020</td>
<td>3,880 ± 340</td>
<td>0.0098</td>
<td>0.001</td>
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<tr>
<td>Pulse number, no/24 h</td>
<td>21.0 ± 0.6</td>
<td>20.0 ± 0.9</td>
<td>15.0 ± 1.0</td>
<td>0.24</td>
<td>0.0005</td>
</tr>
<tr>
<td>Pulse width, min</td>
<td>41 ± 1.6</td>
<td>45 ± 2.9</td>
<td>71 ± 7.0</td>
<td>0.30</td>
<td>&lt;0.0005</td>
</tr>
<tr>
<td>Pulse height, U/l</td>
<td>7.17 ± 0.75</td>
<td>8.53 ± 0.83</td>
<td>3.56 ± 0.26</td>
<td>0.0048</td>
<td>0.0014</td>
</tr>
<tr>
<td>Pulse area, U/l^-1·min^-1</td>
<td>46.2 ± 4.4</td>
<td>71.8 ± 13.8</td>
<td>57.1 ± 8.3</td>
<td>0.063</td>
<td>0.21</td>
</tr>
<tr>
<td>Pulse amplitude, U/l</td>
<td>1.6 ± 0.15</td>
<td>2.22 ± 0.29</td>
<td>1.25 ± 0.11</td>
<td>0.02</td>
<td>0.12</td>
</tr>
<tr>
<td>Pulse nadir, U/l</td>
<td>5.34 ± 0.59</td>
<td>6.04 ± 0.61</td>
<td>2.16 ± 0.22</td>
<td>0.021</td>
<td>0.001</td>
</tr>
<tr>
<td>Deconvolution analysis</td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Basal secretion, U/l^-1·day^-1</td>
<td>94.7 ± 10.8</td>
<td>110 ± 11.3</td>
<td>33.4 ± 3.6</td>
<td>0.016</td>
<td>&lt;0.0005</td>
</tr>
<tr>
<td>Total secretion, U/l^-1·day^-1</td>
<td>140 ± 15.3</td>
<td>163 ± 16.2</td>
<td>57 ± 5.1</td>
<td>0.008</td>
<td>0.001</td>
</tr>
</tbody>
</table>

Data are given as means ± SE. Within- and between-group differences were calculated with the two-tailed Student's t-test for paired and unpaired samples when appropriate. Data were log transformed before statistical analysis if necessary. LH, luteinizing hormone.

The 24-h plasma LH secretory profiles of two representative women with PCOS before and after calorie restriction and the profiles of two control subjects are displayed in Fig. 1. The 24-h mean plasma LH concentration and integrated LH area increased significantly after calorie restriction. This change was brought about by the conjoint influence of an increased pulse nadir concentration and increased pulse amplitude, whereas pulse duration and frequency were unaffected. Model-free deconvolution analysis revealed that both basal and total LH secretion increased significantly in response to calorie restriction by 17 and 16%, respectively (Table 2).

The 24-h mean plasma insulin concentration decreased by ~75% in response to calorie restriction (Table 1). The 24-h plasma leptin profiles of two women with PCOS before and after calorie restriction and the profiles of two control subjects are displayed in Fig. 2. Mean plasma leptin levels decreased by 50%, which was the result of considerably reduced leptin pulse amplitude and nadir concentrations. The number of leptin peaks over 24 h was not affected by calorie restriction (Table 3).

DISCUSSION

This study was carried out to test the hypothesis that calorie restriction blunts pituitary LH hypersecretion and ameliorates the abnormal metabolic (leptin, insulin, and glucose) and endocrine milieu in obese patients with PCOS. This could explicate the beneficial effects of weight loss on ovulation and fertility in this disorder. Surprisingly, short-term calorie restriction augmented daily LH secretion while suppressing plasma insulin, glucose, and leptin concentrations. Specifically, 7 days of calorie restriction enhanced both basal and pulsatile LH release (through stimulation of LH pulse amplitude without affecting pulse frequency) in PCOS. On the basis of our and other findings in the healthy female of normal weight subjected to calorie restriction, the present data suggest that the gonadotropin response to a negative energy balance is fundamentally different in (obese) women with PCOS. For example, 5 days of calorie restriction significantly attenuated LH pulse frequency and amplitude in normal women studied in the early and late follicular phase of the menstrual cycle (26, 32), as observed in many other.

Table 2. LH pulse patterns in women with PCOS before and after short-term calorie restriction compared with controls matched for age and BMI

<table>
<thead>
<tr>
<th></th>
<th>PCOS Before (A)</th>
<th>PCOS After (B)</th>
<th>Controls (C)</th>
<th>P (A vs. B)</th>
<th>P (A vs. C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean, U/l</td>
<td>42 ± 5.0</td>
<td>23.7 ± 3.5</td>
<td>45 ± 5.7</td>
<td>&lt;0.0005</td>
<td>0.71</td>
</tr>
<tr>
<td>Integrated area, U/l^-1·min^-1</td>
<td>58,700 ± 7,600</td>
<td>34,100 ± 5,100</td>
<td>60,100 ± 6,500</td>
<td>&lt;0.0005</td>
<td>0.90</td>
</tr>
<tr>
<td>Pulse number, no/24 h</td>
<td>8.8 ± 0.8</td>
<td>9.5 ± 0.7</td>
<td>11.4 ± 0.9</td>
<td>0.42</td>
<td>0.05</td>
</tr>
<tr>
<td>Pulse width, min</td>
<td>104 ± 11</td>
<td>95 ± 6</td>
<td>84 ± 9</td>
<td>0.38</td>
<td>0.21</td>
</tr>
<tr>
<td>Pulse height, U/l</td>
<td>47.4 ± 5.1</td>
<td>27.0 ± 5.1</td>
<td>51.3 ± 6.6</td>
<td>&lt;10^-6</td>
<td>0.648</td>
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<tr>
<td>Pulse area, U/l^-1·min^-1</td>
<td>800 ± 130</td>
<td>350 ± 43</td>
<td>725 ± 87</td>
<td>0.004</td>
<td>0.68</td>
</tr>
<tr>
<td>Pulse amplitude, U/l</td>
<td>11.4 ± 1.3</td>
<td>5.9 ± 0.9</td>
<td>15.5 ± 3.6</td>
<td>0.0005</td>
<td>0.22</td>
</tr>
<tr>
<td>Pulse nadir, U/l</td>
<td>39.4 ± 4.1</td>
<td>21.0 ± 12.3</td>
<td>35.9 ± 3.9</td>
<td>0.0005</td>
<td>0.58</td>
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<td>Cosinor analysis</td>
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</tr>
<tr>
<td>Mesor, U/l</td>
<td>42.1 ± 5.1</td>
<td>24.3 ± 3.5</td>
<td>40.3 ± 3.5</td>
<td>&lt;0.0005</td>
<td>0.82</td>
</tr>
<tr>
<td>Amplitude, U/l</td>
<td>8.3 ± 1.0</td>
<td>3.6 ± 0.8</td>
<td>9.8 ± 1.5</td>
<td>&lt;0.0005</td>
<td>0.39</td>
</tr>
<tr>
<td>Relative amplitude, %</td>
<td>20.7 ± 1.7</td>
<td>15.5 ± 3.3</td>
<td>24 ± 2.4</td>
<td>0.12</td>
<td>0.26</td>
</tr>
<tr>
<td>Acrophase, clock time ± min</td>
<td>0116 ± 12</td>
<td>0200 ± 16</td>
<td>0116 ± 28</td>
<td>0.026</td>
<td>0.33</td>
</tr>
</tbody>
</table>

Data are given as means ± SE. Within- and between-group differences were calculated with the two-tailed Student's t-test for paired and unpaired samples when appropriate. Data were log transformed before statistical analysis if necessary.
mammalian species (55). Several endocrine and metabolic cues that are affected by caloric intake (i.e., glucose, leptin, insulin, and steroid hormones) are known to modulate LH secretion (1, 15, 41, 42). Also, calorie restriction seems to enhance the negative feedback actions of estradiol on pulsatile GnRH and LH secretion (30, 41), possibly by altering estrogen receptivity in the hypothalamus (24). However, none of these adaptations in the normal female can explain our observations in PCOS women.

In the present study, plasma estrone, estradiol, SHBG, and androstenedione concentrations in women with PCOS were not affected by short-term calorie restriction. Therefore, withdrawal of estrogen negative feedback cannot readily explain the observed increase in daily basal and pulsatile LH secretion. In contrast, the serum total testosterone concentration fell by 23%, possibly mediated by a reduction of insulin's effects on ovarian androgen production (35). Overwhelming evidence in men (38, 53) and at least some data in eugonadal women (42) indicate that androgens blunt pituitary LH release in healthy humans. Thus one may argue that the reduction of circulating plasma testosterone is likely to mediate the increase of LH secretion that we observed. However, several studies have suggested that androgens exert a paradoxical effect on gonadotropin secretion in women with PCOS. In particular, (sub)chronic blockade of androgen receptors by flutamide appears to blunt LH pulsatility in PCOS (10, 39), which supports the thesis that hyperandrogenemia impairs estrogen and progesterone negative feedback restraint of LH release in these patients (17, 34) and in experimental animal models of the syndrome (14). In this scenario, a reduction of testosterone concentrations would be expected to reduce pulsatile LH secretion in women with PCOS by enhancing hypothalamic feedback sensitivity to estrogens/progesterone. Thus the precise role of androgens in the feedback control of LH release in women remains to be established, in particular in women with PCOS. It is, therefore, uncertain whether the diminution of circulating testosterone in response to calorie restriction contributes to the increase of LH secretion that we observed.

Leptin appears to play a role in the regulation of gonadotropin secretion in response to calorie restriction in normal females, at least in the experimental animal. Leptin-deficient mice are infertile and hypogonadotropic (44). Leptin replacement restores fertility and gonadotropin secretion in these animals (3, 9) through stimulation of the GnRH pulse generator (13, 56). Furthermore, in fertile rats and mice, exogenous leptin administration can partially reverse the hypogonadotropic effect of fasting (1, 29). Because plasma leptin concentrations fell, as expected, during calorie restriction and weight loss in the present study, the data may point to a central defect of leptin feedback signaling to the gonadotropic axis in women with PCOS.

Plasma glucose levels decreased in response to the VLCD, indicating reduced availability of glucose for

Fig. 1. Serum luteinizing hormone (LH) levels (U/l) in 2 obese women with polycystic ovaries (PCO) before and after calorie restriction and in 2 equally obese controls.
oxidation in the brain. In the sheep (6, 45), rat (28, 41), hamster (5, 24, 37), and monkey (14), the latter change reduced GnRH-dependent LH pulsatility. Insulin concentrations also fell (see below), as expected, in response to caloric deficiency. Recent studies in healthy lambs indicate that insulin can stimulate LH secretion through central mechanisms (6, 45), as inferred earlier in the rat (11) and human (40). Insulin also facilitates LH-stimulated ovarian steroidogenesis in PCOS (40). Conversely, profound insulinopenia, as observed in uncontrolled insulin-deficient diabetes mellitus, results in relative hypogonadism. Moreover, diminution of hyperinsulinemia by insulin-sensitizing agents appears extremely effective as a tool to ameliorate the neuroendocrine milieu and induce ovulation in women with PCOS (16, 22, 31). Indeed, treatment with insulin sensitizers leads to concomitant reductions of plasma insulin and LH concentrations in these patients (16, 22, 31). Thus hyperinsulinemia in PCOS may contribute to elevated LH, androgen, and estrogen production in this syndrome. The reduction in insulin concentrations as observed here in the face of calorie restriction may thus contribute to the significant fall in total serum testosterone concentrations. However, the simultaneous increase of pulsatile LH secretion seems paradoxical and remains unexplained. Whether analogous responses to fasting or calorie restriction would occur in nonobese patients with PCOS is not yet known.

LH pulse frequency is high in lean and obese women with PCOS. In contrast, increased BMI and fat mass are associated with attenuation of LH pulse amplitude in PCOS (2, 46). Our data in obese PCOS patients corroborate these inferences inasmuch as LH pulse frequency, but not LH pulse amplitude, was elevated. Whereas the mechanisms by which obesity affects LH secretion in women with PCOS remain to be elucidated, the present results show that calorie restriction enhances LH pulse amplitude (whereas LH pulse frequency remains unaffected) in obese PCOS patients. It is therefore tempting to speculate that the “obesity factor” responsible for attenuation of LH pulse amplitude in these women is a nutritional factor related to increased caloric intake.

There is reason to assume that the increase of plasma LH levels in response to calorie restriction in obese women with PCOS is not a feature of their obesity but rather a consequence of the syndrome itself. Although the majority of studies investigating the effects of caloric deprivation on gonadotropin secretion have been performed in normal-weight humans, a number of studies indicate that calorie restriction does not increase LH plasma levels in obese women with regular menstrual cycles (12, 47, 54). Indeed, 3 wk of fasting did not change pulsatile LH plasma concentration patterns in massively obese women with regular menses (12), 4 wk of a VLCD reduced the plasma LH concentration in a single-morning blood sample in obese premenopausal women (47), and a mean weight loss of 8 kg induced a small (nonsignificant) decline of plasma LH in a large cohort of obese adolescent girls (54). Moreover, short-term fasting did not change pulsatile LH plasma levels in obese postmenopausal women (4), and weight loss did not affect average 24-h LH concentrations in massively obese men (43). These data suggest that the suppressive effect of calorie re-

Fig. 2. Serum leptin levels (µg/l) in 2 obese women with PCOS before and after calorie restriction and in 2 equally obese controls.

A

PCO before caloric restriction

PCO after caloric restriction

Controls

B

Serum Leptin (µg/l)

Time (clock hours)
striction on LH release is less explicit in obese than in normal-weight humans, perhaps because the maintenance of reproductive function is determined by a threshold level of body fat reserve rather than by caloric deprivation per se (20).

It is relevant to emphasize that the present results do not shed light on the mechanisms through which longer-term calorie restriction and weight loss restore ovulatory cycles in many women with PCOS. A significant reduction of body weight may ameliorate the metabolic and endocrine milieu in these patients, despite the fact that short-term calorie restriction does not seem to have these effects.

In summary, short-term calorie restriction does not ameliorate gonadotropin hypersecretion in obese women with PCOS, leaving the beneficial effect of long-term weight loss unexplained. In contrast, the present data document paradoxical gonadotropin responsiveness to calorie restriction, which might result from anomalous feedback impact of various endocrine and metabolic cues on pituitary LH release. This observation supports the concept of a defective GnRH pulse generator in PCOS, which is primary or driven by (unknown) peripheral cues. In addition, the data may offer an explanation for the attenuation of LH pulse amplitude in obese compared with normal-weight women with PCOS.

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