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Effect of long-term naltrexone treatment on endocrine profile, clinical features, and insulin sensitivity in obese women with polycystic ovary syndrome

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Objective: Evaluation of clinical and endocrine effects of naltrexone administration in obese women with PCOS.

Design: Open, controlled, clinical study.

Setting: Department of Reproductive Medicine and Child Development, Section of Gynecology and Obstetrics, University of Pisa, Pisa, Italy.

Patient(s): Ten PCOS women were studied.

Intervention(s): Women were treated with naltrexone (50 mg/day) for 6 months.

Main Outcome Measure(s): Body mass index and the menstrual cyclicity during naltrexone treatment were assessed. Basal levels of LH, FSH, 17β-estradiol (E_2), 17-hydroxyprogesterone, total and free T, androstenedione, dehydroepiandrosterone sulfate, cortisol, sex hormone-binding globulin were evaluated before treatment and every 3 months. Progesterone levels were measured in the luteal phase during the sixth month. Gonadotropin response to GnRH administration (10 μg) and a 75-g oral glucose tolerance test were performed before and every 3 months.

Result(s): Body mass index significantly decreased from 29.94 $\pm$ 1.04 to 26.07 $\pm$ 0.81 during treatment. The menstrual cyclicity improved in 80% of PCOS women: the mean cycle length was 40–360 days before treatment and ranged between 25 and 120 days and 28–120 days after 3 and 6 months of treatment. Plasma levels of free T, androstenedione, dehydroepiandrosterone sulfate, and cortisol significantly decreased. Fasting glucose-to-insulin ratio improved in women with insulin resistance.

Conclusion(s): Naltrexone may have a beneficial effect on the clinical and endocrine–metabolic disturbances of obese PCOS women. Whether these effects are the consequences of weight loss or are due to changes in opioidergic tone is debatable. (Fertil Steril® 2002;77:936–44. ©2002 by American Society for Reproductive Medicine.)

Key Words: PCOS, opioid system, weight loss, naltrexone, insulin sensitivity

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The main features of polycystic ovary syndrome (PCOS) are chronic anovulation, elevated serum androgen concentrations, and inappropriate gonadotropin secretion (1). An augmented GnRH pulse frequency and an increased sensitivity of the pituitary to GnRH account for the abnormal gonadotropin levels (2). The β-endorphin is an important hypothalamic neurotrasmitter that inhibits GnRH release (3–5). Conflicting data are present in literature concerning a possible role of opioids in the abnormalities of gonadotropin secretion in PCOS (6–8). In fact, although some investigators have suggested that hypothalamic opioid activity may be decreased in patients with PCOS (6), Barnes and Lobo (7) do not sustain a specific role of opioids on gonadotropin secretion in PCOS women. Neverthless, Lanzone et al. (8) found that the inhibition of opioid activity lowers LH secretion in hyperinsulinemic PCOS women; this suggests that abnormalities of this system may exist in some groups of women with PCOS.

Opioids influence some metabolic functions. The isolation of β-endorphin in human

endocrine pancreas (9) and the findings that endorphins stimulate insulin and glucagon secretion in vivo (10) suggest a possible role for endogenous opiates in glycoregulation. The observation that both acute and short–chronic inhibition of the opioidergic system significantly decreases the insulinemic response to an oral glucose tolerance test (OGTT) in a group of hyperinsulinemic women with PCOS (11, 12), further suggests that endogenous opiates are involved in the control of insulin secretion in PCOS.

Obesity is frequently found in women with PCOS with a range of 30%–60% (13). Obesity exerts an additive synergic effect on the clinical and metabolic manifestations of PCOS (14). Weight reduction can lower androgen levels and restore ovulation in women with PCOS (15). Much evidence has accumulated indicating the involvement of opioid peptides in the control of appetite and in the pathogenesis of obesity: plasma levels of β-endorphin are increased in obese subjects, both in adults and adolescents (16) and this increase is not corrected by weight loss (17). Moreover, the administration of naloxone, an opiate receptor antagonist, is able to reduce food intake in both Prader Willi syndrome (18) and obese hirsute women (19).

Therefore, increasing evidence indicates that endogenous opiates are involved not only in the mechanism of gonadotropin release, but also in the control of many other clinical and metabolic features of PCOS. At the present time no data are available about the effects of a chronic inhibition of the opioid system in women with PCOS. The aim of this study was to evaluate the clinical, endocrine, and metabolic effects of a 6-month treatment with naltrexone, an inhibitor of the opioid system, in obese women with PCOS.

MATERIALS AND METHODS

Subjects

Ten women, aged 18–26 years, with PCOS were recruited for the study. The selected women were patients attending the Reproductive Endocrinology Clinic at the Department of Obstetrics and Gynecology of the University of Pisa.

None of the subjects had clinical signs of virilization, Cushing's syndrome, evidence of enzymatic adrenal deficiences, or drug-induced hyperandrogenism. None of the enrolled women had markedly elevated plasma androgen levels or a history compatible with an androgen-producing neoplasm. Moreover, all women had normal PRL levels (range 5–25 ng/mL). None of the subjects had received any hormonal treatment for at least 6 months before the study. Polycystic ovary syndrome was diagnosed by clinical findings of irregular menses (oligoamenorrhea/amenorrhea) and clinical signs of hyperandrogenism (acne and hirsutism), elevated plasma androgen levels at the upper limit of the normal range (androstenedione [A]: 0.25–3.02 ng/mL; total T: 0.1–0.8 ng/mL; free T: $<$3.6 pg/mL), and bilaterally normal or enlarged ovaries with the presence of at least 7 to 10 microcysts ($<$5 mm in diameter) on ultrasonography. A normal LH/FSH ratio was not considered an exclusion criteria. Obesity was defined as a body mass index (BMI) $>$25 kg/m^2 (normal range 19–25 kg/m^2).

Based on history, oligoamenorrhea was reported by nine women (range of mestrual cycle 40–120 days) and only one woman had amenorrhea. Hirsutism was present in all women and two of them also had acne. Four (40%) women showed high plasma levels of total T, two (20%) had high levels of free T, and eight (80%) showed high concentrations of A. All the enrolled women had a BMI $>$25 kg/m^2 (29.94 $\pm$ 1.04 kg/m^2; range 25.4–36.1 kg/m^2; Table 1).

As control group for baseline comparisons, 10 age-matched healthy women, with 26- to 32-day regular menses and no clinical or biochemical evidence of androgen excess, were also studied (Table 1).

Study Design

The protocol was approved by the Ethics Committee of the University of Pisa, Pisa, Italy. After detailed information on the study had been provided and informed consent had been obtained, women with PCOS who agreed to take part in the study received 25 mg/day of naltrexone (Nalorex, DuPont Pharma, Weinheim, Germany) for 15 days and then they received 50 mg/day of the drug for 6 months. The changes in endocrine and metabolic features of the enrolled women with PCOS were assessed before and at 3-month intervals during naltrexone treatment. To compare the baseline values, the same endocrine and metabolic parameters were assessed in the healthy controls. All subjects were studied in the follicular phase of the menstrual cycle (4–9 days after the onset of spontaneous or progestin-induced menstrual bleeding). The following evaluations were carried out.

Clinical Assessment: Determination of Body Weight and Evaluation of Menstrual Cyclicity

Women were weighed in the morning after overnight fasting. This measurement was always performed by the same physician, and it was reported as BMI (in kilograms per square meter). The menstrual cycle length was calculated as the number of days between the onset of two consecutive menstrual bleedings. To assess the effects of treatment on ovulatory function, plasma P levels were measured in the luteal phase (day 21 of the menstrual cycle) of subjects experiencing regular menses during naltrexone treatment. Eight women accepted to undergo this additional assessment, which was carried out during month 6 of therapy. Ovulation was presumed to have occurred when plasma P levels exceeded 8 ng/mL.

Endocrine Assessment

At 8:00 A.M., after overnight fasting, a polyethylene catheter was inserted into an antecubital vein and two baseline

TABLE 1

Clinical and endocrinological[a] features of women participating in the study.

Subject	Age (y)	BMI[b]	LH-AUC[c]	Total T[b]	Free T[b]	A[b]	DHEAS[b]	SHBG[b]	G:I[b]
1	22	29.2	1728.5	0.70	1.70	4.00	2.60	5.50	14.4
2	18	25.9	897.0	0.67	3.20	2.00	1.10	4.70	4.4
3	22	31.8	2401.5	1.70	6.50	5.60	2.10	7.00	15.8
4	22	36.1	479.5	0.67	1.80	4.10	3.50	3.20	2.8
5	31	29.1	1787.0	1.00	3.80	4.70	2.50	7.40	4.4
6	21	29.4	2212.0	0.83	2.70	3.70	2.10	9.00	3.4
7	23	27.8	323.0	0.53	2.30	4.70	3.16	7.20	3.4
8	20	31.2	2153.0	0.36	1.30	3.70	1.00	9.15	4.4
9	26	25.4	2302.5	0.98	3.20	4.50	2.30	6.90	11.47
10	24	33.5	492.5	0.62	2.80	3.80	2.28	4.40	2.7
Mean ± SE	22.9 ± 1.13	29.94 ± 1.04[d]	1477.65 ± 265.10	0.80 ± 0.12[d]	2.93 ± 0.46[d]	4.08 ± 0.29[d]	2.26 ± 0.24[d]	6.44 ± 0.61[d]	7.56 ± 2.21
Controls	24.1 ± 1.07	22.8 ± 0.21	898.57 ± 81.19	0.35 ± 0.04	1.51 ± 0.21	1.77 ± 0.15	1.27 ± 0.13	17.07 ± 0.91	13.80 ± 1.6

[a] Blood samples for hormonal measurements have been collected during the follicular phase of the menstrual cycle (days 4–9).
[b] Normal values: BMI = 19–25 kg/m^2; total T = 0.1–0.8 ng/mL; free T = <3.6 ng/mL; A = 0.25–3.02 ng/mL; DHEAS = 1.2–3.6 μg/mL; SHBG = 13–26 ng/mL; fasting glucose:insulin (G:I) ratio = >4.5 mg/10^{-4} U.
[c] The basal nonstimulated secretion of LH calculated over the 2-hour period (mIU/mL).
[d] $P<.01$ vs. healthy controls.

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blood samples were collected 15 minutes apart for determination of plasma levels of gonadotropins (LH and FSH), P, E_2, androgens (total T, free T, A, dehydroepiandrosterone sulfate [DHEAS], and 17-hydroxyprogesterone [17-OHP]), cortisol (F), and sex hormone-binding globulin (SHBG). Thereafter, plasma samples for LH and FSH determination were collected in all women every 10 minutes for 2 hours from the same cannula kept patent by a saline infusion. After the last blood sample, a 10-μg i.v. bolus dose of GnRH was injected in the same cannula. To evaluate the FSH and LH response to exogenous GnRH, additional blood samples were collected after 15, 30, 45, 60, and 90 minutes after the i.v. injection.

On the following day an OGTT was performed at 8:00 A.M. after an overnight fast. Blood samples for insulin and glucose determination were collected basally and 30, 60, 90, and 120 minutes after ingestion of 75 g of glucose in 150 mL of water. All women were instructed to ingest a 300 g/day carbohydrate diet for 3 days before the OGTT.

During the study period, none of the patients with PCOS started a new diet. Each woman was asked to report side effects during the treatment. In addition, safety parameters (liver and renal function) were assessed before and at 3-month intervals during the study. Women were advised to use contraception barrier methods or an intrauterine device during the study to avoid conception.

Hormone Determination

After collection, blood samples were centrifuged and plasma was immediately frozen at −20°C until assay. Plasma LH and FSH were measured by a specific time-resolved fluoroimmunoassay method (Delfia-Pharmacia, Milan, Italy). For LH determination the intra-assay and interassay coefficients of variation (CV) ranged from 3.7% to 4.5% and from 2.4% to 3.8%, respectively. For FSH determination the intra-assay and interassay CV ranged from 3% to 4% and from 3.7% to 4.3%, respectively. Plasma levels of E_2 and P were measured with RIA kits (Radim, Pomezia, Italy). The intra-assay and interassay CV were 3.3% and 4.1% for E_2 and 6.4% and 9.2% for P. Prolactin determination was performed by an immunoradiometric assay with a magnetic solid phase.

The concentrations of A and free T were measured with RIA techniques (Radim). The intra- and inter-assay CV were <4.8% and 7.8% for T and 4.3% and 7% for A. Plasma total T levels were measured by RIA kits (Immunotech, Marseille, France). The intra-assay CV ranged from 7.2% to 11% for values ranging from 0.5 to 1.4 ng/mL. The interassay CV for total T determination ranged from 11.9% to 15% for values ranging from 0.7 to 1.6 ng/mL.

Plasma 17-OHP, DHEAS, and F determinations were performed with RIA kits (Radim). The intra-assay and interassay CV were 3.6% and 7.3% for F, 6.2% and 8.3% for DHEAS, and 7.2% and 9.3% for 17-OHP. Insulin and SHBG determinations were performed by immunoradiometric assay (Orion Diagnostica, Expoo, Finland for SHBG and Biosource Europa S.A., Nivelles, Belgium for insulin). The intra-assay CV for the insulin determination ranged from 4.5% and 2.1% for values ranging from 6.6 to 53 μU/mL. The interassay CV ranged from 12.2% to 4.7% for insulin values ranging from 6.6 to 53 μU/mL. The intra-assay CV for the SHBG determination ranged from 4.2% to 1.8% for values ranging from 2.16 to 14.7 ng/mL, whereas the inter-

TABLE 2

Plasma hormone concentrations,[a] fasting glucose:insulin ratio (G:I), and BMI before and during administration of naltrexone (50 mg/d).

Variable	Baseline[b]	3rd month[b]	6th month[b]	*P* value
BMI (kg/m^2)	29.94 ± 1.04	27.00 ± 0.92[d]	26.07 ± 0.81[d]	.0001
G:I ratio (mg/10^{-4} U)	7.56 ± 2.21	6.41 ± 0.85	7.40 ± 0.68	.79
LH-AUC (mIU/mL × 120 min)[c]	3142.77 ± 691.45	1927.17 ± 541.68[d]	1756.02 ± 427.64[d]	.05
E_2 (pg/mL)	58.3 ± 9.71	106.5 ± 19.75[d]	116.0 ± 19.73[d]	.008
Total T (ng/mL)	0.80 ± 0.12	0.67 ± 0.11	0.58 ± 0.09	.07
Free T (ng/mL)	2.93 ± 0.46	1.92 ± 0.19	1.29 ± 0.17[d]	.003
A (ng/mL)	4.08 ± 0.29	2.46 ± 0.28[d]	2.01 ± 0.32[d]	.0001
DHEAS (μg/mL)	2.26 ± 0.24	1.61 ± 0.17[d]	1.33 ± 0.19[d]	.0001
17-OHP (ng/mL)	1.36 ± 0.18	1.29 ± 0.24	1.23 ± 0.22	.68
F (ng/mL)	193.27 ± 20.15	163.16 ± 21.60	152.44 ± 22.18[d]	.008
SHBG (ng/mL)	6.44 ± 0.61	6.59 ± 0.56	6.2 ± 0.54	.56

[a] Blood samples for hormonal measurements have been collected during the follicular phase of the menstrual cycle (days 4–9).
[b] Values are expressed as means ± SE.
[c] LH secretion after GnRH bolus (10 μg i.v.). The values are reported as area under curve (LH-AUC).
[d] vs. baseline values.

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assay CV ranged from 1.58% to 8.33% for values ranging from 7.13 to 67.2 ng/mL. Glucose levels were determined by the glucose oxydase technique.

Statistical Analysis

All results are expressed as mean ± SE. The basal secretion of gonadotropins tested during a 2-hour period and their response to GnRH were expressed as area under curve (AUC). The AUC was calculated by the trapezoidal rule and expressed as milliunits per milliliter × 120 and milliunits per milliliter × 90 minutes, respectively. Insulin and glucose concentrations after glucose ingestion were also expressed as AUC, milligram per deciliter × 120 minutes for glucose and microunit per milliliter × 120 minutes for insulin. Insulin resistance was estimated using the ratio of simultaneous steady-state (fasting) insulin and glucose measurement. This ratio (G:I) has been shown to correlate with more formal dynamic and steady-state measurements of insulin resistance in women with PCOS (20). As previously described by Legro et al. (20), we considered a fasting G:I ratio value <4.5 as abnormal.

The difference between women with PCOS and healthy controls was analyzed by Student's *t*-test for unpaired data. In women with PCOS, the changes in body weight, metabolic and basal hormone levels after 3 and 6 months of naltrexone treatment were statistically tested by using the analysis of variance (ANOVA) for repeated measures. After ANOVA, the Scheffe's procedure was used making post hoc comparisons. Simple linear regression and correlation analysis were also performed to assess a relationship between insulin levels and androgen concentrations. Statistical significance was set at $P<.05$, two-sided.

RESULTS

Clinical Results

At the dosage used, naltrexone treatment is safe as demonstrated by the absence of adverse side effects such as astenia, nausea, and headache. The biochemical parameters of liver and renal function were unaffected during the entire treatment period.

Every woman lost weight (range 2–19 kg) during treatment. From values of 29.94 ± 1.04 at baseline, BMI significantly ($P<.01$) decreased to 27.0 ± 0.92 and of 26.07 ± 0.81 after 3 and 6 months, respectively (Table 2).

At the same time, eight women (80%) showed a significant improvement in menstrual cyclicity. The cycle length of these women ranged between 40 and 90 days before treatment, whereas at the end of the observation period, the average of cycle length was 29.37 ± 0.62 days (range 28–33 days). Figure 1 shows the menstrual cycle length in women with PCOS after 3 and 6 months of naltrexone treatment. In subjects experiencing regular menses, this improvement was observed within 45 days after naltrexone intake. In six of the women with regular menses during the treatment, plasma P levels were indicative of ovulation at the sixth month of naltrexone administration. Naltrexone did not induce any changes in the menstrual cycles of two women.

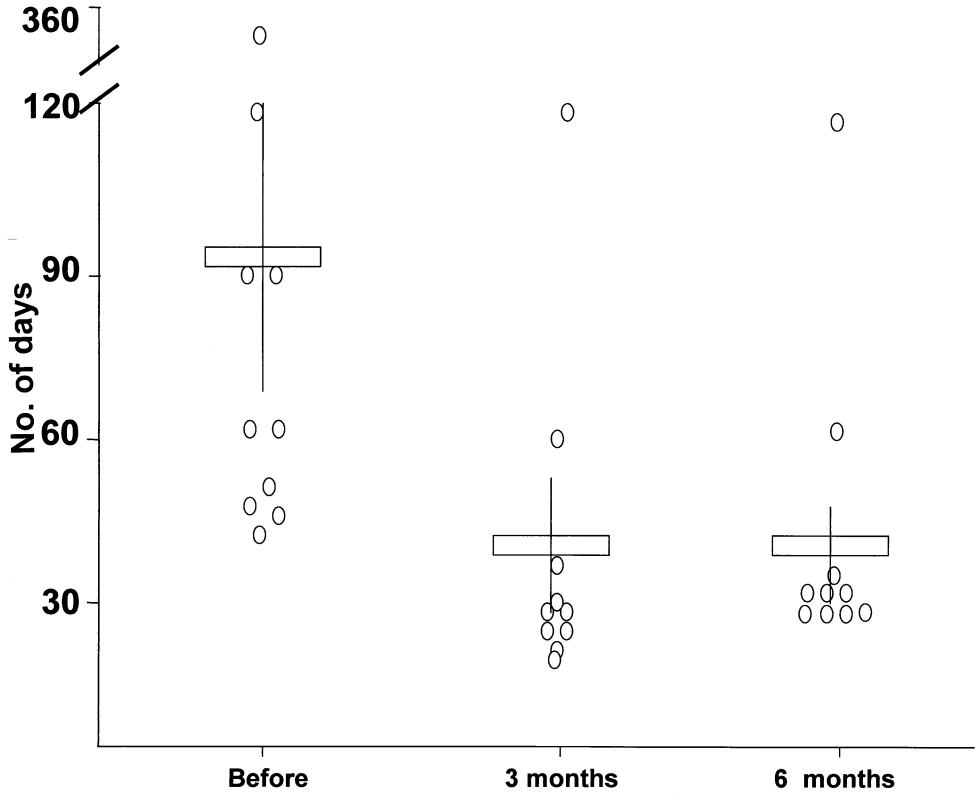
Endocrine Study

Gonadotropin Secretion

Before naltrexone administration, basal LH and FSH were within the normal range for the follicular phase of the menstrual cycle (LH: 9.91 ± 1.81 mU/mL [5–15 mU/mL];

FIGURE 1

Mean (±SE) and individual menstrual cycle length (*open circle*) before and after 3 and 6 months of naltrexone treatment (50 mg/day).



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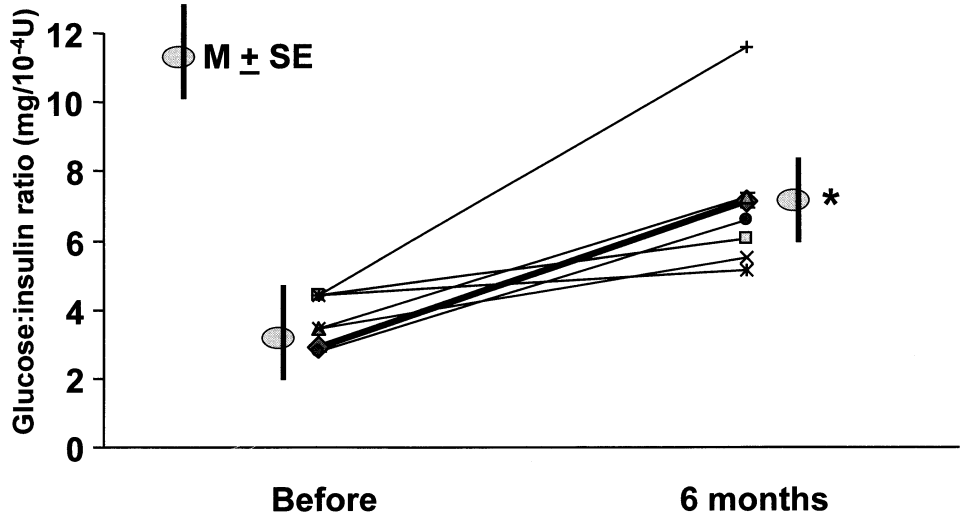
FSH: 5.19 ± 0.43 mIU/mL [4–12 mIU/mL]) and there were no significant differences in LH and FSH plasma levels between women with PCOS and control group (LH: 9.91 ± 1.81 mIU/mL vs. 6.62 ± 1.22 mIU/mL and FSH: 5.19 ± 0.43 mIU/mL vs. 5.85 ± 0.42 mIU/mL). A slight, but not significant, decrease in plasma LH levels was observed during the study in women with PCOS (7.57 ± 1.44 mIU/mL and 5.71 ± 0.94 mIU/mL after 3 and 6 months, respectively). Basal plasma levels of FSH were not different from those obtained at the end of the observation period (4.98 ± 0.51 mIU/mL and 4.93 ± 0.43 mIU/mL after 3 and 6 months, respectively).

In women with PCOS, plasma FSH concentrations determined in samples taken during a 2-hour period were similar to values of control group (FSH AUC: 689.35 ± 68.77 mIU/mL vs. 771.51 ± 52.24 mIU/mL) and they showed no significant changes during the study (FSH AUC: 689.35 ± 68.77 mIU/mL, 661.65 ± 76.63 mIU/mL, and 691.55 ± 46.78 mIU/mL before and at 3-month intervals). The basal nonstimulated secretion of LH calculated during the 2-hour period in women with PCOS was slightly but not significantly higher than controls (LH AUC: 1,477.65 ± 265.10 mIU/mL vs. 898.57 ± 81.19 mIU/mL; Table 1). During treatment the LH AUC values showed a progressive, although not significant, decrease after 3 and 6 months of naltrexone treatment (1,477.65 ± 265.10 mIU/mL, 1,051.6 ± 251.51 mIU/mL, and 1,004.15 ± 145.24 mIU/mL, before and after 3 and 6 months).

The LH concentrations obtained in response to the GnRH bolus were significantly ($P<.05$) higher in women with PCOS than in controls (3,142.77 ± 691.45 mIU/mL vs. 1,670.59 ± 70.91 mIU/mL). During naltrexone treatment the LH responses to GnRH administration were significantly ($P<.05$) lower than those obtained before the treatment (3,142.77 ± 691.45 mIU/mL, 1,927.17 ± 541.68 mIU/mL, and 1,756.02 ± 427.64 mIU/mL before treatment and after 3 and 6 months, respectively; Table 2). The FSH response to GnRH was similar in healthy controls and in patients with PCOS (746.07 mIU/mL ± 61.09 vs. 755.48 mIU/mL ± 146.78 mIU/mL) . Moreover, the opioid antagonist treatment did not modify the FSH response to GnRH in women with PCOS (755.48 ± 146.78 mIU/mL, 638.9 ± 97.55

FIGURE 2

Individual fasting glucose:insulin ratio before and after 6 months of naltrexone treatment in seven women with PCOS and insulin resistance (G:I <4.5). Mean values (±SE) are also reported. *P<.01 vs. baseline values.



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mIU/mL, and 612.67 ± 78.01 mIU/mL before and after 3 and 6 months).

Before naltrexone treatment there were no significant differences in plasma E_2 levels between women with PCOS and controls (58.3 ± 9.71 pg/mL in PCOS and 72.5 ± 6.39 pg/mL in healthy controls). A significant (P<.05) increase in plasma E_2 levels was observed throughout the study period (58.3 ± 9.71 pg/mL vs. 106.5 ± 19.75 pg/mL after 3 months and 116.0 ± 19.73 pg/mL after 6 months) (Table 2).

Androgen Secretion

The basal concentrations of total and free T, A, 17-OHP, and DHEAS were significantly (P<.01) higher in women with PCOS than in controls (Table 1). Plasma SHBG levels in women with PCOS were significantly (P<.01) lower than healthy controls (Table 1). Before treatment, T and 17-OHP levels were correlated to insulin levels in women with PCOS (R = 0.62 for T, P<.05, and R = 0.73 for 17-OHP).

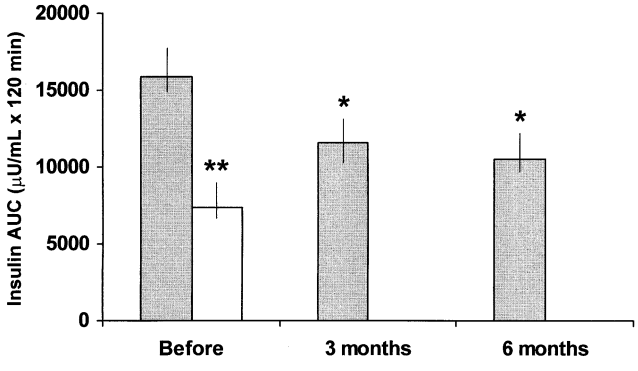
A significant (P<.01) decrease in plasma A levels was observed throughout the treatment period (4.08 ± 0.29 ng/mL before treatment vs. 2.46 ± 0.28 and 2.01 ± 0.32 ng/mL after 3 and 6 months, respectively) (Table 2). Plasma free T levels significantly (P<.01) decreased from baseline values of 2.93 ± 0.46 pg/mL to 1.92 ± 0.19 pg/mL and to 1.29 ± 0.17 pg/mL after 3 and 6 months of treatment, respectively (Table 2). Plasma levels of DHEAS significantly (P<.01) decreased from 2.26 ± 0.24 μg/mL to 1.61 ± 0.17 μg/mL and to 1.33 ± 0.19 μg/mL after 3 and 6 months, respectively (Table 2). Plasma levels of F significantly (P<.05) decreased from 193.27 ± 20.15 ng/mL to 163.16 ± 21.60 ng/mL and to 152.44 ± 22.18 ng/mL after 3 and 6 months of naltrexone treatment, respectively (Table 2). A slight, but not significant, decrease in plasma total T was observed during naltrexone treatment (0.80 ± 0.12 ng/mL before treatment and 0.67 ± 0.11 and 0.58 ± 0.09 ng/mL after 3 and 6 months of naltrexone, respectively; Table 2). The decrease in these androgens did not correlate with changes in insulin levels. No significant changes in plasma levels of 17-OHP and SHBG were observed (Table 2).

Metabolic Pattern

The fasting G:I was significantly (P<.05) lower in women with PCOS than in healthy controls (7.56 ± 2.21 vs. 13.80 ± 1.66; Table 1). Naltrexone treatment does not seem to influence insulin sensitivity in all women with PCOS. In fact, the fasting G:I ratio was not modified by naltrexone administration (7.56 ± 2.21 at baseline, 6.41 ± 0.85 and 7.40 ± 0.68 after 3 and 6 months; Table 2). However, when subjects were divided according to their G:I ratio (<4.5 or >4.5), in seven women with PCOS with G:I <4.5, the G:I ratio significantly (P<.01) increased after 3 and 6 months of treatment (from 3.68 ± 0.27 at baseline to 6.10 ± 1.08 and to 7.02 ± 0.82 after 3 and 6 months, respectively; Fig. 2). These subjects experienced the greatest weight loss (8–19 kg). In the three women with a G:I >4.5 in which insulin sensitivity did not improve, the weight loss during the entire period of observation was 2, 5, and 6 kg. Moreover, in the

FIGURE 3

Mean (±SE) of basal insulin levels after glucose load in healthy controls (*open bar*) and in seven women with PCOS and insulin resistance (G:I <4.5; *shaded bar*). Women with PCOS were studied before and during naltrexone treatment. Values are expressed as area under the curve (insulin AUC). *$P<.05$ patients with PCOS during treatment vs. baseline values. **$P<.001$ controls vs. patients with PCOS at baseline.



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seven women with PCOS with a G:I <4.5, basal plasma insulin levels significantly ($P<.05$) decreased from 22.80 ± 1.13 μU/mL before treatment to 15.81 ± 3.79 and 13.81 ± 1.82 μU/mL after 3 and 6 months of naltrexone intake, respectively.

The stimulated plasma insulin levels after OGTT were 7,326.35 ± 902.407 μU/mL × 120 minutes in controls and they were significantly lower than in all women with PCOS (13,072.95 ± 2,106.29 μU/mL × 120 minutes, $P<.05$) including women with PCOS with G:I <4.5 (15,920.35 ± 2,192.53 μU/mL × 120 minutes, $P<.001$; Fig. 3). A significant ($P<.05$) decrease in the stimulated plasma insulin levels after OGTT in women with PCOS with G:I <4.5 was observed. In fact, the insulin AUC, from basal values of 15,920.35 ± 2,192.53 μU/mL before treatment, reached values of 11,546.57 ± 1,175.77 after 3 months and 10,500.81 ± 1,988.28 μU/mL after 6 months of naltrexone (Fig. 3).

DISCUSSION

Many studies (11, 12, 20–22) indicate that endogenous opioid peptides are involved in the endocrine–metabolic imbalance of PCOS. Results of the present study seem to suggest that the chronic blockade of the opioid tone with naltrexone, a long-acting opioid antagonist, decreases the LH release from the pituitary gland in response to GnRH in women with PCOS, thus reducing the overall LH secretion. Unfortunately, the lack of a control group treated with naltrexone in our study does not allow any conclusion about the existence of a specific influence of the opioid tone on LH secretion in PCOS. However, Lanzone et al. (8, 23), by using the same dose of naltrexone for a shorter period in healthy women and women with PCOS, demonstrated that a derangement of opioid tone exists only in hyperinsulinemic women with PCOS. Because of the majority of women included in our study show insulin resistance, our results can be considered in line with results of Lanzone et al. (8, 23) who evidenced that a derangement of the opioid tone may be related in some way to the enhanced LH response to endogenous GnRH. Moreover, the effect of opioids seems to be selectively exerted on LH and not on FSH secretion. The administration of naltrexone did not modify basal FSH levels or FSH response to GnRH.

Although these findings support the evidence that opioids influence gonadotropin response to GnRH, they also question the belief that hypothalamic opioid activity is low in women with PCOS. If a low endogenous opioid tone is presumed to be responsible for the higher LH in women with PCOS (6), it is unclear why the blockade of opioids, which suppresses gonadotropin secretion, can reduce LH levels.

Experimental in vivo (24) and in vitro (25) data demostrated that in some conditions β-endorphin may stimulate LH secretion by acting directly on the pituitary gland. The administration of naloxone (an opioid antagonist) to ovariectomized E_2-treated rats reduces the number of pituitary GnRH receptors (26). Vice-versa, morphine increases the number of these receptors (27). The decrease of LH response to GnRH during naltrexone treatment might suggest that in these patients opioid tone influences the sensitivity of gonadotrop cells to GnRH.

From a clinical point of view, the most interesting result is the improvement in menstrual cyclicity. Eighty percent of the studied women, all with severe oligoamennorhea at baseline, experienced a normalization of their menstrual cycles. In addition, in six of the eight women who experienced regular menses after 6 months of naltrexone treatment, plasma P levels were indicative of ovulation. This improvement was not evidenced in other previous studies, probably because of the different duration of treatment. In this study only two women did not respond to naltrexone treatment in terms of menstrual cyclicity.

The limited number of subjects receiving the active drug did not allow statistically significant differences between responders and nonresponders. Whether this effect on menstrual cycle has to be ascribed to the central effect of naltrexone on LH secretion or to other actions of naltrexone is difficult to state. A decrease in appetite is a well-known effect of naltrexone (28, 29). All the studied women decreased their body weight from 2 to 19 kg.

Weight loss may be the principal mechanism through which normal menstrual cyclicity was attained. Clark et al. (30) found that 12 of 13 subjects who had lost weight during

a 6-month program of regular exercise in conjunction with diet, resumed ovulation. In our study the improvement of menstrual cyclicity occurred concomitantly with significant changes in body weight. One cannot rule out that a change in body weight may be involved. According to this hypothesis, menstrual cyclicity might improve as a consequence of the effect of naltrexone on body weight. It is, however, also true that menstrual cyclicity was attained in too short a time period to be attributed to the effects of a change in body weight. In fact, the subjects who improved their menstrual cyclicity during naltrexone treatment had their first menstrual cycle within a short time (45 days) from the onset of drug ingestion. Moreover, the two women who did not improve their menstrual cyclicity, both decreased their body weight as well (2 and 8 kg).

It is then possible that explanations for the improvement of menstrual cycle length and ovulation may include factors other than weight loss alone.

The excess of body fat in patients with this syndrome is associated with hyperandrogenism and other metabolic disturbances, such as insulin resistance and hyperinsulinemia. Naltrexone reduces hyperinsulinemia in all women with PCOS with a decreased insulin sensitivity (G:I <4.5). Several studies (11, 12, 20–22) demonstrated that both acute and short-term inhibition of the opioid tone produced a partial reduction in hyperinsulinemia in hyperandrogenic–hyperinsulinemic women. Fulghesu et al. (22) reported that naltrexone reduces hyperinsulinemia by acting on hepatic insulin clearance only, without affecting β-cell secretion and insulin sensitivity as C-peptide levels and total body glucose utilization do not change after 6 weeks of treatment.

In this study we did not evaluate the effect of a chronic opioid blockade on peripheral insulin sensitivity by performing sophisticated and cost-intensive studies such as a clamp study. We evaluated the fasting G:I ratio that was proposed (20) as a useful test for identifying women with PCOS with insulin resistance.

The changes in G:I ratio suggest that long-term naltrexone treatment also affects insulin sensitivity. When the opioid tone is chronically inhibited for 6 months, an increase in the insulin sensitivity occurs as demonstrated by a change in fasting G:I ratio in women with PCOS with insulin resistance. Thus, the changes in the insulin resistance might be related not only to the effect on insulin metabolism, but also to an improvement in insulin sensitivity. However, one cannot exclude the effect of weight loss on insulin function. Insulin sensitivity and glucose uptake have been demonstrated to increase after a reduction in BMI obtained with a hypocaloric regimen only (31, 32).

On the basis of our results this last effect seems to take longer to become evident; no change in insulin resistance was evidenced after 3 months of observation despite weight loss. On this basis, we can suppose that, although the acute response to naloxone acts chiefly on hepatic insulin clearance, the chronic administration of naltrexone may also lead to an improvement in insulin sensitivity in women with PCOS with insulin resistance through changes in body weight. Why insulin sensitivity did not improve in the three women with a pretreatment G:I ratio of >4.5 despite their weight loss (2, 5, 6 kg) is not clear.

Plasma concentrations of A, free T, DHEAS, and F decreased during the 6 months of observation. An unexpected finding is the lack of changes in SHBG despite the decrease in BMI and free T and the increase in estrogens. The decrease in LH secretion might only partially account for the decrease of some androgens. Although insulin may affect adrenal androgens and F production (33), reduction of insulin by different methods (e.g., diazoxide, octreotide, or weight loss) does not always change the circulating levels of DHEAS and A (34, 35). Therefore, it is likely that the effect of insulin is minor. This may justify why neither F nor the decrease of any of these androgens was correlated to the decrease in insulin levels in the present study. Because several factors may be implicated in the adrenal androgen excess in PCOS, it is difficult to determine a specific cause leading to the decrease in androgens.

In conclusion, although the sample of women treated is too small to draw definite conclusions, our data seem to suggest clinical benefits obtained from the opioid blockage with an antagonist such as naltrexone on the endocrine–metabolic disturbance in women with PCOS. Whether these beneficial effects are mediated by the effect of naltrexone on the opioid system or are the consequence of a decrease in body weight observed during treatment cannot be established at the present time. Other studies performed on a greater number of subjects with the inclusion of a control group might help to clarify this issue.

References

1. Goldzieher JW. Polycystic ovarian disease. Fertil Steril 1981;35:371–94.
2. Waldstreicher J, Santoro NF, Hall JE, Filicori M, Crowley WF Jr. Hyperfunction of the hypothalamic-pituitary axis in women with polycystic ovarian disease: indirect evidence of a partial gonadotroph desensitization. J Clin Endocrinol Metab 1988;66:165–72.
3. Quigley ME, Yen SSC. The role of endogenous opiates on LH secretion during the menstrual cycle. J Clin Endocrinol Metab 1980;51:179–81.
4. Snowden EU, Khan-Dawood FS, Dawood MY. The effect of naloxone on endogenous opioid regulation of pituitary gonadotropins and prolactin during the menstrual cycle. J Clin Endocrinol Metab 1984;59: 298–302.
5. Genazzani AD, Petraglia F, Gastaldi M, Volpogni C, Gamba O, Genazzani AR. Naltrexone treatment restores menstrual cycles in patients with weight loss-related amenorrhea. Fertil Steril 1995;64:951–6.
6. Cumming DC, Reid RL, Quigley ME, Rebar RW, Yen SSC. Evidence for decreased endogenous dopamine and opioid inhibitory influences on LH secretion in polycystic ovary syndrome. Clin Endocrinol (Oxf) 1984;20:643–8.
7. Barnes RB, Lobo RA. Central opioid activity in polycystic ovary syndrome with and without dopaminergic modulation. J Clin Endocrinol Metab 1985;61:779–82.
8. Lanzone A, Fulghesu AM, Cucinelli F, Ciampelli R, Caruso A, Mancuso S. Evidence of a distinct derangement of opioid tone in hyperinsulinemic patients with polycystic ovarian syndrome: relationship with insulin and luteinizing hormone secretion. J Clin Endocrinol Metab 1995;80:3501–6.
9. Bruni J, Watkins W, Yen SSC. β-Endorphin in the human pancreas. J Clin Endocrinol Metab 1979;49:649–51.

10. Cozzolino D, Sessa G, Salvatore T, Sasso FC, Giuliano D, Lefebvre PJ, et al. The involvementof the opioid system in human obesity: a study in normal weight relatives of obese people. J Clin Endocrinol Metab 1996;81:713–8.
11. Lanzone A, Fulghesu AM, Fortini A, Cutillo G, Cucinelli F, Di Simone N, et al. Effect of opiate receptor blockade on the insulin response to oral glucose load in polycystic ovarian disease. Human Reprod 1991; 6:1043–9.
12. Fulghesu AM, Lanzone A, Cuccinelli F, Caruso G, Mancuso S. Long-term naltrexone treatment reduces the exagerated insulin secretion in patients with polycystic ovary disease. Obstet Gynecol 1993;82:191–7.
13. Franks S. Polycystic ovary syndrome: a changing perspective. Clin Endocrinol (Oxf) 1989;31:87–120.
14. Franks S. Polycystic ovary syndrome. N Engl J Med 1995;333:853–61.
15. Bates GW, Whitworth NS. Effect of body weight reduction on plasma androgens in obese, infertile women. Fertil Steril 1982;38:406–9.
16. Genazzani AR, Facchinetti F, Petraglia F, Pintor C, Rivier C. Hyperendorphinemia in obese children and adolescent. J Clin Endocrinol Metab 1986;62:36–40.
17. Pasquali R, Cantobelli S, Casimiri F, Bartoluffi L, Boschi S, Capelli M, et al. The role of the opioid peptides in the development of hyperinsulinemia in obese women with abdominal fat distribution. Metabolism 1992;41:763–7.
18. Kyriakides M, Silverstone T, Jeffcoate W, Laurence B. Effects of naltrexone on hyperfagia in the Prader-Willi syndrome. Lancet 1980; 1:876–7.
19. Atkinson RL. Naloxone decreases food intake in obese humans. J Clin Endocrinol Metab 1982;55:196–8.
20. Legro RS, Finegood D, Dunaif A. A fasting glucose to insulin ratio is a useful measure of insulin sensitivity in women with polycystic ovary syndrome. J Clin Endocrinol Metab 1998;83:2694–8.
21. Ciampelli M, Fulghesu AM, Guido M, Murgia F, Muzj G, Belosi C, et al. Opioid blockade effect on insulin β-cells secretory pattern in plycycstic ovary syndrome. Hormone Res 1998;49:263–8.
22. Fulghesu AM, Ciampelli M, Guido M, Murgia F, Caruso A, Mancuso S, et al. Role of opioid tone in the pathophysiology of hyperinsulinemia and insulin resistance in polycystic ovarian disease. Metabolism 1998; 47:158–62.
23. Lanzone A, Apa R, Fulghesu AM, Cutillo G, Caruso A, Mancuso S. Long-term naltrexone treatment normalizes the pituitary response to gonadotropin-releasing hormone in polycystic ovary syndrome. Fertil Steril 1993;59:734–7.
24. May PB, Mittler JC, Ertel NH. Enkephalins and pituitary hormone release. Modification of responsiveness to LHRH. Horm Res 1979;10: 57–63.
25. Petraglia F, Di Meo G, de Leo V, Nappi C, Facchinetti F, Genazzani AR. Plasma β-endorphin levels in anovulatory states. Changes after treatments for induction of ovulation. Fertil Steril 1984;45:185–90.
26. Barkan AL, Regiani S, Duncan JA, Papavasilious S, Marshall JC. Opioids modulate pituitary receptors for gonadotropin-relaesing hormone. Endocrinology 1983;112:387–9.
27. Barkan AL, Duncan JA, Shiff M, Papavasilious S, Garcia-Rodriguez A, Kelch RP, et al. Opioid and neurotrasmitter regulation of pituitary gonadotropin-releasing hormone (GnRH) receptors in the ovariectomized estradiol-treated rats: role of altered GnRH secretion. Endocrinology 1985;116:1003–10.
28. McKay DL, Kenney N, Edens NK, Williams RH, Woods SC. Intracerebroventricular β-endorphin increases food intake of rats. Life Sci 1981;29:1429–34.
29. Trenchard E, Silvestone T. Naloxone reduces the food intake of normal human volunteers. Appetite 1983;4:43–50.
30. Clark AM, Ledger W, Galletly C, Tomlinson L, Blaney F, Wang X, et al. Weight loss in significant improvement in pregnancy and ovulation rates in anovulatory obese women. Hum Reprod 1995;10:2705–12.
31. Futterweit W. Polycystic ovary syndrome: clinical perspectives and management. Obstet Gynecol Surv 1999;54:403–13.
32. Hoeger K. Obesity and weight loss in polycystic ovary syndrome. Obstet Gynecol Clin North Am 2001;28:85–97.
33. Lanzone A, Fulghesu AM, Guido M, Fortini A, Caruso A, Mancuso S. Differential androgen response to adrenocorticotropin hormone stimulation in polycystic ovary syndrome: relation with insulin secretion. Fertil Steril 1992;58:296–301.
34. Nestler JE, Barlascini CO, Matt DW. Suppression of serum insulin by diazoxide reduces serum testosterone levels in obese women with polycystic ovary syndrome. J Clin Endocrinol Metab 1989;68:1027–32.
35. Holte J, Bergh T, Gennarelli G, Wide L. The independent effect of polycystic ovary syndrome and obesity on serum concentrations of gonadotropins and sex steroid hormones in premenopausal women. Clin Endocrinol 1994;41:473–81.