FERTILITY AND STERILITY®
VOL. 81, NO. 1, JANUARY 2004
Copyright ©2004 American Society for Reproductive Medicine
Published by Elsevier Inc.
Printed on acid-free paper in U.S.A.

Metformin administration modulates and restores luteinizing hormone spontaneous episodic secretion and ovarian function in nonobese patients with polycystic ovary syndrome

Alessandro D. Genazzani, M.D., Ph.D.,[a] *Cesare Battaglia, M.D.,*[b] *Barbara Malavasi, M.D.,*[a] *Claudia Strucchi, M.D.,*[a] *Francesca Tortolani, M.D.,*[a] *and Ombretta Gamba, M.D.*[a]

Department of Obstetrics and Gynecology, University of Modena, Modena, Italy

Objective: To evaluate the effects of metformin administration on spontaneous LH episodic release in a group of nonobese polycystic ovary (PCOS) patients.

Design: Controlled clinical study.

Setting: PCOS patients in a clinical research environment.

Patient(s): Twenty nonobese PCOS patients were enrolled after informed consent.

Intervention(s): All patients underwent hormonal evaluations and a pulsatility study (sampling every 10 minutes for 4 hours) before and at the sixth month of therapy (metformin, 500 mg, p.o. b.i.d.). Ultrasound examinations and Ferriman-Gallwey scoring were also performed.

Main Outcome Measure(s): Measurements of plasma LH, FSH, estradiol (E_2), androstenedione (A), 17-hydroxy-progesterone (17-OHP), and testosterone (T), glucose, insulin, and C-peptide concentrations.

Result(s): After 6 months of metformin administration, the plasma LH, 17-OHP, A, and T levels and LH/FSH ratio were significantly reduced. Insulin sensitivity, expressed as the glucose-to-insulin ratio, was significantly improved under glucose load after 6 months of treatment. Spontaneous LH episodic release showed a significant reduction in pulse amplitude with no changes in pulse frequency. Menstrual cyclicity was restored in all amenorrheic and oligomenorrheic women. The ovarian volume and Ferriman-Gallwey scores also were significantly reduced.

Conclusion(s): Metformin administration improves reproductive axis functioning in hyperandrogenic nonobese PCOS patients. By acting on the ovary and restoring normal ovarian activity, metformin positively modulates the reproductive axis (namely GnRH–LH episodic release). (Fertil Steril® 2004;81:114–9. ©2004 by American Society for Reproductive Medicine.)

Key Words: Polycystic ovary syndrome, metformin, insulin, leuteinizing hormone, nonobese women

Received December 30, 2002; revised and accepted May 28, 2003.

Reprint requests: Alessandro Genazzani, M.D., Ph.D., Department of Obstetrics and Gynecology, University of Modena, Via del Pozzo 71, 41100 Modena, Italy (FAX: ++39-59-4224394; E-mail: algen@unimore.it).

[a] Department of Obstetrics and Gynecology, University of Modena, Modena, Italy.

[b] Department of Obstetrics and Gynecology, University of Bologna, Bologna, Italy.

0015-0282/04/$30.00
doi:10.1016/j.fertnstert.2003.05.020

Polycystic ovary syndrome (PCOS) is typically characterized by hyperandrogenism, anovulation, and metabolic disturbances in both obese and nonobese women (1–4). It has been recently observed that insulin resistance and hyperinsulinemia are relevant causal factors in inducing the endocrine disorders that are associated with PCOS (5–7). Such findings have provoked the development of specific therapeutic strategies intended to reduce the hyperinsulinemic condition. This confirmed the clinical observation that weight loss was effective in restoring menstrual cyclicity and/or ovulation in overweight PCOS patients, thanks to the reduction in fat mass and the increase in tissue insulin sensitivity.

In recent years, several studies have demonstrated the efficacy of insulin-sensitizing compounds such as metformin in reducing PCOS-associated hyperinsulinemia and in correcting most of the endocrine and metabolic abnormalities found in women with PCOS (1, 2, 8). Among the available compounds, metformin, a water-soluble oral biguanide, enhances insulin

sensitivity in the liver, where it inhibits hepatic glucose production, and in the muscle tissue, where it improves the glucose uptake and use (8, 9). Metformin is well tolerated as it does not cause episodes of hypoglycemia (10) and has no direct effect on insulin secretion by the pancreatic cells (11).

The metformin-induced decrease in hyperinsulinemia underlies significant modifications in ovarian response to gonadotropin stimulation (3, 4, 7, 8, 12–15). The aim of the study is to evaluate the specific changes induced by long-term metformin administration on the spontaneous episodic release of LH in a group of nonobese hyperandrogenic PCOS patients.

MATERIALS AND METHODS

Patients

Twenty women with PCOS aged 20 to 28 years were recruited for this study. These patients were selected from among the patient population attending the Gynecological Endocrinology Center at the University of Modena according to the following criteria: presence of micropolycystic ovaries at ultrasound; plasma androstenedione levels equal to or above the normal laboratory range (3 ng/100 mL; conversion factor to pmol/L: 34.92) and LH/FSH >2.5; absence of enzymatic adrenal deficiency and/or other endocrine disease; hirsutism and/or acne, from grade mild to severe; normal prolactin (PRL) levels (range: 5 to 25 ng/mL); no hormonal treatment for at least 6 months before the study; and body mass index (BMI) below 25.

All patients were from 5% to 15% above their ideal body weight; the mean BMI was 22.5 ± 1.1. The glucose-to-insulin ratio, in baseline conditions, was >4.5 in all patients. Twelve patients were amenorrheic, and eight were oligomenorrheic (menstrual cycle every 50 days or more).

Patients were treated with metformin (Metforal; Guidotti, Pisa, Italy), 500 mg, p.o. b.i.d., 20 to 30 minutes before lunch and dinner, for 6 months. All patients underwent a pulsatility study on day 7 of the menstrual cycle if eumenorrheic or oligomenorrheic and on day 7 of the first menstrual cycle occurring after the fifth month of treatment. The pulsatility study for LH and FSH determinations was carried out for 4 hours, sampling every 10 minutes. On the same day, plasma estradiol (E_2), androstenedione (A), 17-hydroxy-progesterone (17-OHP), and testosterone (T) levels were determined. The following day all patients underwent an oral glucose tolerance test (OGTT) for insulin, glucose, and C-peptide determinations, sampling 15 minutes before, and 30, 60, 90, 120, and 240 minutes after the oral consumption of 100 grams of glucose. Insulin sensitivity was then computed as the glucose-to-insulin ratio, because this ratio has been shown to be a good index of insulin sensitivity in women with PCOS (16, 17).

Vaginal ultrasound and the Ferriman-Gallwey score were performed before treatment and after 6 months of treatment. The study protocol was approved by the Human Investigation Committee of the University of Modena.

Assay

All samples from each woman were assayed in duplicate in the same assay. Plasma LH and FSH concentrations were determined using a previously described immunofluorimetric assay (IFMA) (18, 19). The sensitivity of the assay expressed as the minimal detectable dose was 0.1 IU/mL. The cross reactivities with free and β subunits of LH, FSH, and TSH were less than 2% (18). Intra-assay and interassay coefficients of variation were 4.9 and 7.4%, respectively.

Plasma E_2, 17-OHP, A, and T were determined by radioimmunoassay (Radim; Pomezia, Rome, Italy) as previously described (20). Based on two quality control samples, the average intra-assay and interassay coefficients of variation were 4.0% and 9.7%, respectively.

Plasma insulin was determined using an immunoradiometric assay (Biosource Europa S.A., Nivelles, Belgium). Based on two quality control samples, the average intra-assay and interassay coefficients of variation were 4.5% and 11.7%, respectively.

Plasma C-peptide concentrations were determined using a chemiluminescence assay (DBC Immulite One, Los Angeles, CA). Based on two quality control samples, the average intra-assay and interassay coefficients of variation were 4.5% and 8.2%, respectively.

Pulse Detection and Statistical Analysis

Time series of LH were first evaluated separately to estimate the random measurement error on the duplicates, using the program PREDETEC.WK1 (21, 22). Then secretory episodes on each time series were identified using the program DETECT (21–23), with $P=.01$ (1%) for the nominal false-positive rate. At the nominal $P=.01$ (1%) for false-positive errors, DETECT showed a consistent specificity, as previously reported elsewhere (23), because the false-positive rate observed from the data of the plasma pool of each patient assayed together with the time series was not statistically different from 1%.

We also tested for statistically significant differences among the groups, after analysis of variance (one-way ANOVA), using Student's *t*-test for paired and unpaired data, as appropriate. Data are expressed as mean ± SEM.

RESULTS

Hormonal parameters of the patients are reported in Table 1 (mean ± SEM), which shows the statistically significant reduction of the LH to FSH ratio, LH, 17-OHP, T, and A plasma levels after 6 months of metformin administration. Insulin and C-peptide plasma levels did not show any change during metformin administration in baseline conditions.

When the area under curve (AUC) of OGTT was evaluated, a significantly lower insulin AUC was observed during

TABLE 1

Hormonal characteristics of the patients (n = 20) under study before and during metformin treatment.

Characteristic	Baseline	With metformin
BMI	22.5 ± 1.1	21.7 ± 1.4
LH (mIU/mL)	15 ± 2.3	8.8 ± 1.8[a]
FSH (mIU/mL)	4.4 ± 0.5	4.5 ± 0.6
E_2 (pg/mL)	75.1 ± 15.6	54.2 ± 9.8
17-OHP (ng/mL)	2.5 ± 0.3	1.7 ± 0.3[a]
A (ng/100 mL)	386.5 ± 35.2	141.7 ± 26.6[a]
T (ng/100 mL)	65.5 ± 7.5	45.0 ± 5.5[a]
LH/FSH	3.5 ± 0.5	1.9 ± 0.2[a]
Glucose/insulin	9.2 ± 1.8	9.1 ± 1.2
Insulin (μU/mL)	12.0 ± 1.8	12.4 ± 2.4
C-peptide (μg/L)	2.7 ± 0.5	2.9 ± 0.6

[a] $P<.05$.

Genazzani. Metformin restores episodic LH secretion in PCOS. Fertil Steril 2004.

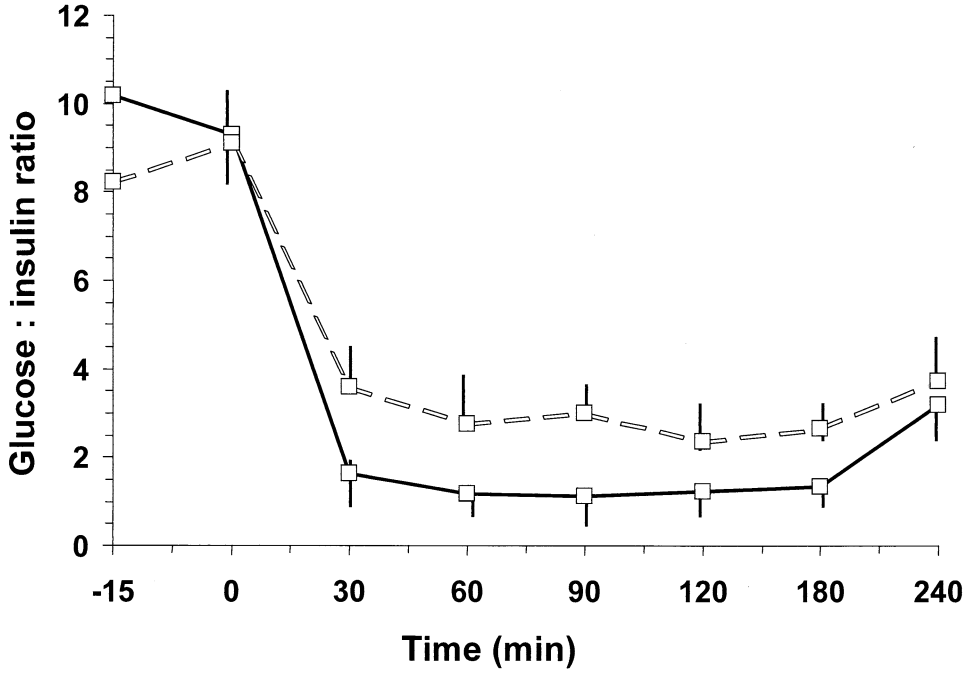
metformin administration (before metformin: 18,477 ± 245; and during metformin: 13,420 ± 185 U/mL/240 minutes, $P<.05$), but no statistically significant change was observed for AUC of C-peptide (before: 1,516.5 ± 95; and during: 1,393.5 ± 110 g/L/240 minutes). In baseline conditions the glucose-to-insulin ratio was not different under metformin administration (before: 9.2 ± 1.8; during: 9.1 ± 1.2) (see Table 1). However, the ratio was evaluated all along the OGTT curve, it was found to be significantly increased, thus confirming that sensitivity to insulin was improved under metformin administration (Fig. 1).

When spontaneous episodic LH release was considered, statistically significant changes of the intrinsic characteristics were noted (Table 2) (Fig. 2). In fact, integrated LH levels (computed as mean levels of the pulsatility study) were significantly reduced under metformin administration. Although no changes occurred in LH pulse frequency, the LH pulse amplitude was significantly reduced (from 5.7 ± 0.5 to 3.4 ± 0.5 mIU/mL, $P<.05$). Statistically significant modifications were also observed in terms of menstrual cyclicity. In 10 out of 12 amenorrheic patients, a normal menstrual cycle was recovered within the third month of therapy, with a mean menstrual cycle of 29.5 ± 2.5 days, and two were oligomenorrheic (cycle every 35 to 50 days). Among the eight initially oligomenorrheic patients (cycles every 40 to 60 days), two women remained that way; the other six women recovered a normal menstrual cycle (30.5 ± 2.0 days) (Table 3).

The Ferriman-Gallwey score as well as the ovarian volume showed statistically significant decreases after 6 months of metformin therapy (Ferriman-Gallwey score: before: 22.3

FIGURE 1

The ratio of glucose to insulin, an index of sensitivity to insulin, was significantly improved by metformin administration. Before metformin: —. During metformin: - - -. ** $P<.01$.



Genazzani. Metformin restores episodic LH secretion in PCOS. Fertil Steril 2004.

TABLE 2

LH pulsatile characteristics of patients under study.

	Baseline			With metformin		
	Mean (mIU/mL)	No. of peaks/4 h	Amplitude (mIU/mL)	Mean (mIU/mL)	No. of peaks/4 h	Amplitude (mIU/mL)
PCOS (n = 20)	10.4 ± 1.7	3.5 ± 0.4	5.7 ± 0.5	5.6 ± 1.0[a]	3.5 ± 0.3	3.4 ± 0.5[a]

[a] $P<.05$.

Genazzani. Metformin restores episodic LH secretion in PCOS. Fertil Steril 2004.

± 1.7; during: 14.0 ± 0.8, $P<.01$; and ovarian volume before: 12.0 ± 0.8 mL; during: 7.5 ± 1.0 mL, $P<.05$).

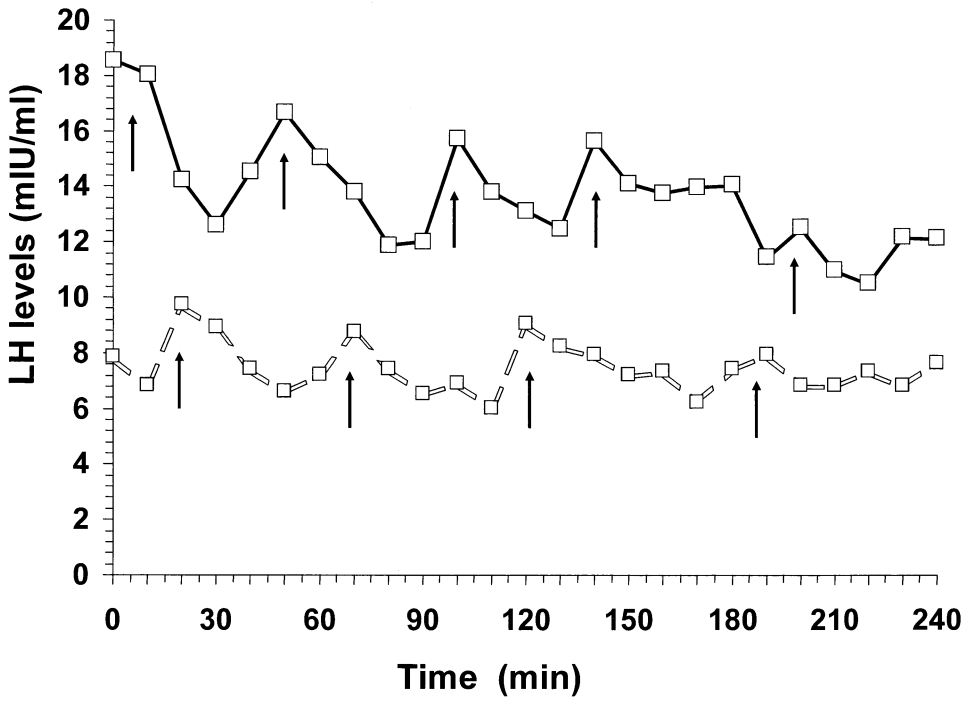
DISCUSSION

To gain more insight into novel therapeutic strategies for PCOS, we explored the effects of an insulin-sensitizing (metformin) therapy on LH pulsatile release. Our study demonstrated that statistically significant modifications of both LH spontaneous episodic secretion and menstrual cyclicity could be obtained with metformin therapy in a group of nonobese PCOS patients.

In agreement with previous reports (8, 13, 24), our study shows that metformin administration results in a reduction in 17-OHP, A, and T plasma levels in PCOS patients, with a statistically significant reduction in LH plasma levels. This result is in agreement with Velazquez et al. (15), who demonstrated a reduction in LH plasma levels in a group of PCOS patients undergoing metformin treatment, but it dif-

FIGURE 2

Episodic LH spontaneous secretion in one PCOS patient under study shows that, after 6 months of treatment, LH pulses were significantly reduced in amplitude with no change in pulse frequency. *Arrows* indicate statistically significant LH pulses detected by the program DETECT. Before metformin: —. During metformin: - - -.



Genazzani. Metformin restores episodic LH secretion in PCOS. Fertil Steril 2004.

TABLE 3

Menstrual cycles of patients during metformin treatment.

Menstrual cycle before treatment	Menstrual cyclicity after 6 months of treatment		
	Amenorrhea	Oligomenorrhea	Eumenorrhea
Amenorrhea (n = 12)	0	2	10
Oligomenorrhea (n = 8)	0	2	6

Genazzani. Metformin restores episodic LH secretion in PCOS. Fertil Steril 2004.

fers from previous reports that did not observe changes in LH plasma levels (13, 24, 25). This difference probably depends on the fact that our study was carried out for a longer interval (6 months). According to our data, the reduction in LH plasma levels is mainly related to the presence of a modification of its secretory pattern.

It is well known that PCOS patients have a distinctive, abnormal LH pulsatile secretion, with normal (sometimes higher) pulse frequency and higher pulse amplitude (20, 26). Our study showed that after 6 months of metformin administration the LH pulse amplitude was significantly reduced and similar to that of normal eumenorrheic women (27). This supports the hypothesis that reduced LH pulse amplitude is the result of a modulation exerted by a metformin-induced change(s) in the ovarian function and/or in the tissue sensitivity to insulin or growth factors on GnRH discharge.

In fact, the hyperandrogenic milieu of PCOS results in an abnormal neuroendocrine modulation of the hypothalamus-pituitary function, which is responsible for the increase of pituitary sensitivity to GnRH stimulation. This results in higher LH secretory episodes in response to each spontaneous GnRH secretory burst (3, 28–31). The exaggerated GnRH-induced LH response is reduced by the metformin-induced modulation on citP450 activity. This modulation reduces androgen production and is also enforced by the specific changes in IGF-I and IGFBP-I production (25). On this basis it could be argued that lower hyperandrogenic tone results in a reduction in the amount of GnRH released with each secretory burst from the hypothalamus, thus resulting in a lower LH pulse amplitude. The reduced LH plasma levels observed under metformin administration are the result of such a decreased pulse amplitude.

It is of great interest that the patients we studied were nonobese women with normal insulin sensitivity in baseline conditions, with a fasting glucose-to-insulin ratio above the cut-off limit of 4.5 (considered normal) (17). However, when an OGTT was performed, all the patients showed an exaggerated insulin response to the glucose load, with significant reduction (below 4.5) in the glucose-to-insulin ratio throughout the glucose load. After 6 months of metformin treatment, neither fasting insulin plasma levels nor the insulin sensitivity changed, in agreement to previous studies (16). Such an observation demonstrates that metformin mainly acts on the abnormal insulin response to glucose load (or feeding) (16). Moreover, our data show that when the OGTT was performed under metformin treatment, the glucose-induced hyperinsulinemia was significantly reduced with a statistically significant increase of the insulin sensitivity (i.e., glucose-to-insulin ratio), as demonstrated by the statistically significant reduction in the insulin AUC. The lack of changes in C-peptide AUC strongly sustains the hypothesis of a modification in insulin sensitivity and/or in its clearance rate dynamics rather than in the insulin secretory rate. A higher change in the insulin sensitivity probably may be achieved with a higher daily dosage of metformin.

Most peculiarly, our patients showed the greater response to the metformin treatment in terms of menstrual cyclicity. After 6 months of treatment, all the amenorrheic patients except two and all oligomenorrheic patients but two had recovered normal menstrual cycles. These data are in agreement with previous reports (2, 24, 32) that described a significant restoration of the menstrual cyclicity in PCOS patients after 4 to 6 months of therapy. The normal occurrence of menstrual cycles is mainly related to a better ovarian function. Our observations are similar to those reported by De Leo et al. (32), who described an improvement in ovulatory function in PCOS patients given metformin and undergoing induction of ovulation. This indicates that insulin (or metformin modulation) plays a pivotal role in the direct endocrine and paracrine control of the ovaries and of LH secretion from the pituitary (32). Together with the clinical improvements of the menstrual cyclicity, the 17-OHP, A, and T plasma levels were significantly reduced, in agreement with previous reports (13, 15, 16, 25). Our data are also in agreement with those reported by Ibanez et al. (1), who showed significant changes in androgen plasma levels using a higher metformin dosage.

Finally, it is important to mention the reduction of both the ovarian volume and the Ferriman-Gallwey score. These did not decrease as much as when oral contraceptives and/or GnRH-analogue were administered (30, 31) but clearly support the efficacy of metformin in modulating the ovarian activity, in particular follicular development and ovarian steroidogenesis.

Our data demonstrate that hyperandrogenic PCOS patients show statistically significant improvements of the ovarian function when treated with metformin, independent of their BMI. The restoration of a normal LH episodic secretion is mainly related to the significant metformin-induced changes in the ovarian function in terms of reduced androgen secretion and reduced pituitary response to the endogenous (hypothalamic) GnRH secretory bursts. Our data support that the insulin-sensitizing agent (metformin) improves ovarian control/modulation and menstrual cyclic-

ity through the normalization of the gonadotropin pulsatile release.

Acknowledgments: The authors thank Patrizia Monteleone, M.D., for her editorial assistance and Michele Luisi, M.D., for his constructive criticisms.

References

1. Ibanez L, Valls C, Ferrer A, Ong K, Dunger DB, De Zengher F. Additive effects of insulin-sensitizing and anti-androgen treatment in young, non obese women with hyperinsulinism, hyperandrogenism, dyslipidemia and anovulation. J Clin Endocrinol Metab 2002;87:2870–4.
2. Ibanez L, Valls C, Ferrer A, Marcos MV, Rodriguez Hierro F, De Zengher F. Sensitization to insulin induces ovulation in nonobese adolescents with anovulatory hyperandrogenism. J Clin Endocrinol Metab 2001;86:3595–8.
3. Taylor AE. Polycystic ovary syndrome. Endocrinol Metab North Am 1998;27:877–902.
4. Dunaif A. Insulin resistance and the polycystic ovary syndrome: mechanism and implications for pathogenesis. Endocr Rev 1997;18:774–800.
5. Lanzone A, Fulghesu AM, Fortini A, Cutillo G, Cucinelli F, Di Simone N, et al. Long-term naltrexone treatment reduces the exaggerated insulin secretion in patients with polycystic ovary disease. Obstet Gynecol 1993;82:191–6.
6. Frank S. Polycystic ovary syndrome. N Engl J Med 1995;333:853–61.
7. Nestler JE, Strauss JF. Insulin as an effector of human ovarian and adrenal steroid metabolism. Endocrinol Metab Clin North Am 1991; 20:807–23.
8. Nardo LG, Rai R. Metformin therapy in the management of polycystic ovary syndrome: endocrine, metabolic and reproductive effects. Gynecol Endocrinol 2001;15:373–80.
9. Barbieri RL, Makris A, Randall RW, Daniels G, Kistner RW, Ryan KJ. Insulin stimulates androgen accumulation in incubations of ovarian stroma obtained from women with hyperandrogenism. J Clin Endocrinol Metab 1986;62:904–10.
10. Heaney D, Majid A, Junor B. Bicarbonate haemodialysis as a treatment of metformin overdose. Nephrol Dial Transplant 1997;12:1046–7.
11. DeFronzo RA, Goodman AM. Efficacy of metformin in patients with non-insulin-dependent diabetes mellitus. The Multicenter Metformin Study Group. N Engl J Med 1995;333:541–9.
12. Dunn CJ, Peters DH. Metformin. A review of its pharmacological properties and therapeutic uses in non-insulin-dependent diabetes mellitus. Drugs 1997;49:721–49.
13. La Marca A, Egbe TO, Morgante G, Paglia T, Ciani A, De Leo V. Metformin treatment reduces ovarian cytochrome P-450c17 response to human chorionic gonadotrophin in women with insulin resistance-related polycystic ovary syndrome. Hum Reprod 2000;15:21–3.
14. Vendermolen DT, Ratts VS, Evans WS, Stovall DW, Kauma SW, Nestler JE. Metformin increases the ovulatory rate and pregnancy rate from clomiphene citrate in patients with polycystic ovary syndrome who are resistant to clomiphene citrate alone. Fertil Steril 2001;75: 310–5.
15. Velazquez EM, Mendoza S, Hamer T, Sosa F, Glueck CJ. Metformin therapy in polycystic ovary syndrome reduces hyperinsulinemia, insulin resistance, hyperandrogenism and systolic blood pressure, while facilitating normal menses and pregnancy. Metabolism 1994;43:647–54.
16. Kolodziejczyk B, Duleba AJ, Spaczynski RZ, Pawelczyk L. Metformin therapy decreases hyperandrogenism and hyperinsulinemia in women with polycystic ovary syndrome. Fertil Steril 2000;73:1149–54.
17. Legro RS, Finegood D, Dunaif 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.
18. Genazzani AD, Petraglia F, Fabbri G, Monzani A, Montanini V, Genazzani AR. Evidence of luteinizing hormone secretion in hypothalamic amenorrhea associated with weight loss. Fertil Steril 1990;54:222–6.
19. Genazzani AD, Petraglia F, Benatti R, Montanini V, Algeri I, Volpe A, et al. Luteinizing hormone (LH) secretory burst duration is independent from LH, prolactin or gonadal steroid plasma levels in amenorrheic women. J Clin Endocrinol Metab 1991;72:1220–5.
20. Genazzani AD, Petraglia F, Pianazzi F, Volpogni C, Genazzani AR. The concomitant release of androstenedione with cortisol and luteinizing hormone pulsatile releases distinguishes adrenal from ovarian hyperandrogenism. Gynecol Endocrinol 1993;7:33–41.
21. Oerter KE, Guardabasso V, Rodabrd D. Detection and characterization of peaks and estimation of instantaneous secretory rate for episodic pulsatile hormone secretion. Comp Biomed Res 1986;19:170–91.
22. Genazzani AD. Application of peak-detection programs to clinical data. In: Guardabasso V, Rodbard D, Forti G, eds. Computers in endocrinology: recent advances. New York: Raven, 1990:71–82.
23. Genazzani AD, Rodbard D. Use of the receiver operating characteristic curve to evaluate sensitivity, specificity, and accuracy of methods for detection of peaks in hormone time series. Acta Endocrinol (Copenh) 1991;124:295–306.
24. Morin-Papunen LC, Koivunen RM, Ruokonen A, Martikainen HN. Metformin therapy improves the menstrual pattern with minimal endocrine metabolic effects in women with polycystic ovary syndrome. Fertil Steril 1998;69:691–6.
25. De Leo V, La Marca A, Orvieto R, Morgante G. Effect of metformin on insulin-like growth factor (IGF) I and IGF-binding protein I in polycystic ovary syndrome. J Clin Endocrinol Metab 2000;85:1598–600.
26. Venturoli S, Porcu E, Fabbri R, Magrini O, Gammi L, Paradisi R, et al. Episodic pulsatile secretion of FSH, LH, prolactin, oestradiol, oestrone, and LH circadian variations in polycystic ovary syndrome. Clin Endocrinol 1988;28:93–107.
27. Genazzani AD, Petraglia F, Volpogni C, D'Ambrogio G, Facchinetti F, Genazzani AR. FSH secretory pattern and degree of concordance with LH in amenorrheic, fertile and postmenopausal women. Am J Physiol 1993;264:E776–81.
28. Adams J, Polson DW, Franks S. Prevalence of polycystic ovaries in women with anovulation and idiopathic hirsutism. BMJ 1986;293: 355–9.
29. Clayton RN, Ogden V, Hodgkinson J, Worswick L, Rodin DA, Dyer S, et al. How common are polycystic ovaries in normal women and what is their significance for the fertility of the population? Clin Endocrinol (Oxf) 1992;37:127–34.
30. Genazzani AD, Petraglia F, Battaglia C, Gamba O, Volpe A, Genazzani AR. A long-term treatment with gonadotropin-releasing hormone agonist plus a low-dose oral contraceptive improves the recovery of the ovulatory function in patients with polycystic ovary syndrome. Fertil Steril 1997;67:463–8.
31. Genazzani AD, Battaglia C, Gamba O, Petraglia F, Malavasi B, Genazzani AR. The use of a combined regimen of GnRH agonist plus a low-dose oral contraceptive improves the spontaneous pulsatile LH secretory characteristics in patients with polycystic ovary disease after discontinuation of treatment. J Assist Reprod Gen 2000;17:269–75.
32. De Leo V, La Marca A, Ditto A, Morgante G, Cianci A. Effects of metformin on gonadotropin-induced ovulation in women with polycystic ovary syndrome. Fertil Steril 1999;72:282–5.