Free Androgen Index and Leptin Are the Most Prominent Endocrine Predictors of Ovarian Response during Clomiphene Citrate Induction of Ovulation in Normogonadotropic Oligoamenorrheic Infertility*

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ABSTRACT

We have previously demonstrated that obese hyperandrogenic amenorrheic women are less likely to ovulate after clomiphene citrate (CC) medication. The present study was designed to identify whether additional endocrine screening characteristics, all potentially involved in ovarian dysfunction in 182 normogonadotropic oligoamenorrheic infertile women, are associated with ovarian response, which may improve overall prediction of CC-resistant anovulation. Standardized endocrine screening took place before initiation of CC medication (50 mg/day; increasing doses up to 150 mg/day if required) from cycle days 3–7. Screening included serum assays for fasting insulin and glucose, insulin-like growth factor I (IGF-I), IGF-binding protein-1 (IGFBP-1), IGFBP-3, free IGF-I, inhibin B, leptin, and vascular endothelial growth factor. Forty-two women (22% of the total group) did not ovulate at the end of follow-up (a total number of 325 cycles were analyzed). Fasting serum insulin, insulin/glucose ratio, IGFBP-1, and leptin were all significantly different in univariate analyses ($P \leq 0.02$), comparing CC responders *vs.* nonresponders. Forward stepwise multivariate analyses in combination with factors reported earlier for prediction of patients remaining anovulatory after CC revealed a prediction model including 1) free androgen index (FAI = testosterone/sex hormone-binding globulin ratio), 2) cycle history (oligomenorrhea or amenorrhea), 3) leptin level, and 4) mean ovarian volume. These data suggest that decreased insulin sensitivity, hyperandrogenemia, and obesity, all associated with polycystic ovary syndrome, are prominent factors involved in ovarian dysfunction, preventing these ovaries from responding to stimulation by raised endogenous FSH levels due to CC medication. By using leptin instead of body mass index or waist to hip ratio, the previous model for prediction of patients remaining anovulatory after CC medication could be slightly improved (area under the curve from 0.82–0.85). This may indicate that leptin is more directly involved in ovarian dysfunction in these patients. The capability of insulin and IGFBP-1 to predict patients who remain anovulatory after CC disappears when FAI enters into the model due to a significant correlation between FAI and these endocrine parameters. This suggests that markers for insulin sensitivity (*e.g.* IGFBP-1 and insulin) are associated with abnormal ovarian function through its correlation with androgens, whereas leptin is directly involved in ovarian dysfunction. (*J Clin Endocrinol Metab* **85:** 676–682, 2000)

CLOMIPHENE CITRATE (CC) is considered to be the first line strategy for ovulation induction in normogonadotropic anovulatory infertility (WHO group 2) (1) since its clinical introduction almost 4 decades ago (2). This synthetic steroid, characterized by its mixed estrogenic and antiestrogenic properties, is still extensively used. Approximately 75% of treated patients will ovulate after CC

medication (3–5), and once ovulation is achieved, cumulative conception rates are 75% within six consecutive CC cycles (4, 6–8).

Polycystic ovary syndrome (PCOS), usually referred to as chronic hyperandrogenic anovulation (9), is a common endocrine disorder and represents a significant proportion of normogonadotropic anovulatory infertility (10). Etiological factors involved in ovarian dysfunction in this heterogeneous patient group have not yet been fully elucidated. Various investigators reported on the prominent role of locally produced growth factors, such as insulin-like growth factors (IGFs) (11–13) or vascular endothelial growth factor (VEGF) (14). Moreover, recent evidence suggests that decreased insulin sensitivity plays an important role in initiating ovarian dysfunction in these patients, possibly by stimulating thecal cell androgen production (15, 16). A significant proportion of patients with PCOS suffer from severe obesity (17, 18), and

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therefore, leptin, the hormone product of the obesity gene (19), has been the focus of intense investigation (20–23). It has been demonstrated that leptin can impair insulin action in hepatocytes (20). Although leptin does not appear to play a direct role in either hyperandrogenemia or hypersecretion of LH (23), abnormalities in its signaling may be involved in hypogonadotropic hypogonadism (24) and in certain cases of PCOS (21). Finally, inhibin B, which has been shown to be produced by healthy antral follicles under the control of FSH in the early follicular phase of the normal menstrual cycle (25), may serve as a marker for the severity of ovarian dysfunction in PCOS patients (26, 27).

We previously demonstrated that obese, hyperandrogenic, amenorrheic women are less likely to respond to stimulation by increasing endogenous FSH levels after CC compared to normal weight, oligomenorrheic women presenting with normal androgen concentrations (5). This approach may have clinical implications in terms of health economics, but may also provide information regarding factors involved in ovarian dysfunction in these women. We now report on the predictive power of additional endocrine screening of factors potentially involved in ovarian abnormalities in this patient population, including insulin sensitivity, the IGF system, leptin, inhibin B, and VEGF.

Materials and Methods

Subjects and study protocol

Between February 1993 and January 1998, 182 women attending our infertility unit presenting with 1) oligomenorrhea (bleeding intervals between 35 days and 6 months) or amenorrhea (bleeding interval >6 months), 2) serum FSH levels within normal limits (1–10 IU/L) (1, 28) and normal serum PRL and TSH levels, 3) spontaneous menses or positive bleeding response to progestagen withdrawal, 4) body mass index (BMI; weight divided by height squared) more than 18 kg/m^2, and 5) age between 19–40 ys were included in the present study. Study approval was obtained from the human subjects committee of the Dijkzigt Hospital/Erasmus University, and informed consent was obtained from all subjects.

Standardized initial clinical, sonographic, and endocrine screening took place before initiation of induction of ovulation with CC medication, as described previously (5). Clinical screening included age, infertility and cycle history, BMI, waist to hip ratio (WHR), previous medication, and/or surgery. Transvaginal sonographic screening included assessment of the ovarian stroma echogenicity (arbitrarily classified from one to three per ovary), ovarian volume (milliliters), and total number of follicles (both ovaries), as described previously (10, 29). Endocrine screening included serum assays for FSH, PRL, TSH, LH, estradiol, androstenedione, testosterone, and sex hormone-binding globulin (SHBG), as described previously (5). In addition, serum was assayed for fasting insulin and glucose, free and total IGF-I, IGF-binding protein-1 (IGFBP-1), IGFBP-3, inhibin B, leptin, and VEGF concentrations. Fasting venous blood samples were taken on a random day between 0800–1000 h before initiation of therapy. Blood samples were centrifuged within 2 h after withdrawal and stored at −20 C until assayed.

Hormone assays

Serum insulin levels were measured by immunoradiometric assay (IRMA; Biosource Technologies, Inc., Fleurus, Belgium), glucose levels by the hexokinase method (Gluco-quant, Roche Molecular Biochemicals, Mannheim, Germany) (30), and free IGF-I levels by a two-site IRMA (Active, Diagnostic Systems Laboratories, Inc., Webster, TX) direct assay. Serum was incubated in tubes and decanted, and the tubes were washed according to the manufacturer's instructions. Free IGF-I, bound to the tube, was detected by radiolabeled antibody as described previously (31). Serum IGF-I levels were mea-

sured using a two-site IRMA (Active, Diagnostic Systems Laboratories, Inc.), which includes an extraction step to separate the IGF-I from its binding protein in serum. IGFBP-1 and IGFBP-3 levels were assayed using a two-site IRMA (Active, Diagnostic Systems Laboratories, Inc.) assay as described previously (31). The IGFBP-3 samples were diluted 1:100 before assay. Dimeric inhibin B levels were measured using an immunoenzymometric assay (Serotec, Oxford, UK) (32), leptin levels using a RIA (by means of reagents supplied by Linco Research, Inc., St. Charles, MO) (33), and VEGF levels by enzyme immunoassay (Cytokit Red EIA kits, Peninsula Laboratories, Inc., College Park, MD) (34). Midluteal serum progesterone (P) levels were measured by RIA, as described previously (8). Intra- and interassay coefficients of variation were less than 6.1% and 9.9% for insulin, less than 1.0% and 1.9% for glucose, less than 10.3% and 10.7% for free IGF-I, less than 3.4% and 8.2% for IGF-I, less than 5.2% and 6.0% for IGFBP-1, less than 3.9% and 1.9% for IGFBP-3, less than 8% and 14% for inhibin B, less than 3.6% and 4.6% for leptin, and less than 7.8% and 12.2% for VEGF, respectively.

CC ovulation induction protocol

CC was administered at a daily dose of 50 mg (which was increased to 100 and 150 mg in subsequent cycles in the case of absent ovarian response) from cycle days 3–7 after initiation of a spontaneous or progestin-induced withdrawal bleeding, as described previously (5). Ovulation after CC treatment was assessed by midluteal serum P levels above 25 nmol/L combined with transvaginal sonographic monitoring of follicle growth until visualization of a preovulatory follicle (mean diameter, >18 mm) and subsequent disappearance or biphasic basal body temperature charts, as indicated previously (5). If ovulation occurred, the CC dose remained unaltered during subsequent cycles. The duration of follow-up for all patients included in the study was at least three treatment cycles. First ovulation after CC was used as the end point. Responders were defined as patients who ovulated after CC, independent of the dose administered. The number of treatment cycles and the CC dose in which first ovulation occurred were recorded. Clomiphene-resistant anovulation (CRA) was defined as patients who did not ovulate despite receiving maximum treatment doses of 150 mg/day.

Data analysis

$P = 0.05$ was chosen as threshold level for statistical significance in univariate analyses. For comparison of initial screening parameters of CC responders and nonresponders, we used the Mann-Whitney U test and Wilcoxon rank sum W test. The univariate relation with response to CC was assessed using logistic regression analysis. The following initial serum parameters were used in the multivariate analyses (forward stepwise selection of variables, $P_{entry} < 0.10$): fasting serum insulin and glucose, IGF-I, IGFBP-1, IGFBP-3, free IGF-I, inhibin B, leptin, and VEGF in combination with parameters of the previous model for prediction of patients remaining anovulatory after CC treatment, including FAI, cycle history (oligomenorrhea vs. amenorrhea), BMI, and mean ovarian volume (5). The area under the receiver operating characteristics curve (AUC) was used to assess the discriminative ability of the tests, as described previously (5). Data were analyzed using the commercially available software package SPSS, Inc. (Chicago, IL).

Results

From the total number of 182 patients fulfilling the in/exclusion criteria and treated with CC medication, 131 (72%) suffered from primary infertility, 43 (24%) from amenorrhea, and the remaining 139 (76%) had oligomenorrhea. Eighty-two (45%) women were obese (BMI, ≥26), 113 (62%) patients presented with an elevated FAI (≥4.0), and 67 (37%) patients presented with hyperandrogenemia (testosterone, ≥3.2 nmol/L; and/or androstenedione, ≥16.3 nmol/L) (10, 35). In 134 patients (74%) polycystic ovaries (mean ovarian volume, ≥10.8 mL; and/or mean follicle number per ovary, ≥10) (10, 29) were diagnosed. Finally, 93 patients (51%) presented with elevated serum LH levels (≥7.0 IU/L) (10, 35). From the total

TABLE 1. Endocrine findings (mean ± SD) upon initial screening of 182 normogonadotropic oligoamenorrheic infertile women[a] and separately for patients who did (CC responders) or did not (CRA) ovulate after CC induction of ovulation

	Overall group (n = 182)	CC responder (n = 142; 78%)	CRA (n = 40; 22%)	P value[b]
Insulin (mU/L)	14.7 ± 10.4	13.6 ± 9.5	19.0 ± 13.0	0.009
Glucose (mmol/L)	4.3 ± 1.6	4.2 ± 1.0	4.8 ± 2.9	—[c]
Insulin/glucose ratio	3.6 ± 2.5	3.4 ± 2.3	4.4 ± 3.0	0.05
Inhibin B (ng/L)	153 ± 102	156 ± 103	149 ± 99	—
IGF-I (ng/mL)	237 ± 97	242 ± 99	218 ± 83	—
Free IGF-I (ng/mL)	2.8 ± 1.7	2.8 ± 1.6	3.0 ± 2.0	—
IGFBP-1 (ng/mL)	24.2 ± 18.1	26.4 ± 18.5	16.7 ± 14.5	0.001
IGFBP-3 (ng/mL)	3297 ± 693	3320 ± 714	3240 ± 622	—
Leptin (ng/mL)	21.6 ± 17	19.3 ± 15	30.4 ± 20	0.003
VEGF (ng/mL)	4.8 ± 2.9	4.9 ± 2.9	4.3 ± 3.1	—

[a] Remaining characteristics: age, 28 ± 4.5 yr; BMI, 26.5 ± 6 kg/m^2; T, 2.4 ± 0.9 nmol/L; FAI, 6.2 ± 4.2; LH, 7.9 ± 4.4 IU/L; FSH, 4.5 ± 4.6 IU/L; E$_2$, 258 ± 174 pmol/L; and mean ovarian volume, 9.8 ± 4.2 mL.

[b] Comparison of CC responders *vs.* CRA (by Mann-Whitney U test).

[c] Not significant.

group of patients, 40 (22%) women remained anovulatory despite the maximum CC dose. A total number of 325 CC cycles were analyzed (205 ovulatory and 120 anovulatory cycles).

Endocrine screening characteristics of the overall study group and separately for patients who did or did not ovulate after CC medication are presented in Table 1. Fasting insulin level, insulin/glucose ratio, and serum leptin levels were significantly higher and IGFBP-1 levels significantly lower in CRA patients. *P* values after univariate analysis with logistic regression analyses, odds ratio, 95% confidence interval, and the receiver operating characteristics AUC of additional initial endocrine screening parameters are depicted in Table 2. Again, serum insulin, insulin/glucose ratio, IGFBP-1, and leptin levels were significantly different between patients who did *vs.* those who did not ovulate after CC treatment. Results of multivariate analyses for prediction of patients remaining anovulatory after CC induction of ovulation are depicted in Table 3. Using a forward stepwise selection procedure, various significant predictors during univariate analysis (such as insulin, IGFBP-1, and BMI) disappear, and four variables were eventually selected: 1) FAI, 2) cycle history (oligomenorrhea *vs.* amenorrhea), 3) leptin, and 4) mean ovarian volume. The association of FAI, leptin, and insulin with other endocrine and clinical features was analyzed in more detail. Correlations between FAI and BMI, leptin, insulin, and IGFBP-1 are depicted in Fig. 1. Correlations between initial serum leptin levels and BMI, WHR, and insulin are shown in Fig. 2. Correlations between initial serum insulin levels and BMI, SHBG, and IGFBP-1 are shown in Fig. 3. For all figures, individual correlations are shown separately for CC responders and nonresponders. With the exception of leptin, correlations between all other factors depicted in the figures were significantly stronger in CC nonresponders than in responders (data not shown).

Discussion

The present prospective follow-up study was designed to evaluate whether initial screening of insulin sensitivity, the IGF system, leptin, inhibin B, or VEGF could predict the ovarian response to CC medication in normogonadotropic oligomenorrheic infertile women. We recently reported (5)

TABLE 2. Univariate logistic regression analyses, odds ratio, 95% confidence interval, and area under the ROC curve of endocrine screening parameters in 182 normogonadotropic oligoamenorrheic infertile women for the prediction of patients remaining anovulatory after CC induction of ovulation

Univariate analyses	Odds ratio (95% CI[a])	P[b] value	AUC[c]
Insulin (mU/L)	1.05 (1.01–1.08)	**0.005**	0.64
Glucose (mmol/L)	1.21 (0.97–1.50)	0.07	0.51
Insulin/glucose ratio	1.16 (1.01–1.32)	**0.03**	0.61
Inhibin B (ng/L)	0.99 (0.99–1.00)	0.5	0.53
IGF-I (ng/mL)	0.99 (0.99–1.00)	0.2	0.56
Free IGF-I (ng/mL)	1.06 (0.86–1.30)	0.3	0.51
IGFBP-1 (ng/mL)	0.96 (0.93–0.98)	**0.01**	0.67
IGFBP-3 (ng/mL)	0.99 (0.99–1.00)	0.6	0.54
Leptin (ng/mL)	1.03 (1.01–1.06)	**0.0003**	0.70
VEGF (ng/mL)	0.92 (0.79–1.07)	0.4	0.58

[a] Confidence interval.

[b] Univariate logistic regression analyses with likelihood ratio test (**Bold** = $P < 0.05$).

[c] Area under the ROC curve. (Bold = $P < 0.05$).

that obese, hyperandrogenic, amenorrheic women, a complex of signs and symptoms frequently referred to as PCOS (9), are more likely to remain anovulatory after CC medication. As a first step we focused on screening of the most pertinent clinical, endocrine, and sonographic characteristics of these patients (5). Further endocrine parameters potentially related to ovarian dysfunction in these women are the focus of the present study. Decreased insulin sensitivity/hyperinsulinemia or augmented free IGF-I may be involved in the pathophysiology of this heterogeneous syndrome by directly stimulating thecal cell androgen production (13, 15, 16). Serum leptin levels were also assessed in these patients, as it has been suggested recently that this is a reliable endocrine marker for obesity. Abnormalities in its signaling pathway may be involved in hypogonadotropic hypogonadism (24) and eventually in certain cases of PCOS (21, 22). In addition, leptin may directly modulate insulin activity in obese individuals (20). Inhibin B is produced by healthy early antral follicles under the control of FSH in the early follicular phase of the menstrual cycle (25) and may therefore be used as a marker for the severity of ovarian abnormalities (*i.e.* the sensitivity to FSH and assessment of the number of healthy, rather than atretic, follicles in polycystic ovaries) and the

TABLE 3. Forward stepwise multivariate analyses of parameters of the prediction model plus additional endocrine screening for prediction of normogonadotropic oligoamenorrheic patients remaining anovulatory during clomiphene citrate induction of ovulation

Analyses:	Univariate[a]	Multivariate			
Steps:	0	1	2	3	4[b]
Screening parameters					
a) Prediction model[c]					
FAI[d]	<0.0001	In model	In model	In model	In model
BMI (kg/m^2)	0.0003	0.03	0.04	0.46	0.46
Mean ovarian vol (mL)	0.007	0.12	0.13	0.06	In model
Cycle history[e]	0.0003	0.0001	In model	In model	In model
b) Additional endocrine parameters[f]					
Insulin (mU/L)	0.005	0.32	0.45	0.93	0.98
Insulin/glucose ratio	0.03	0.66	0.79	0.64	0.56
IGFBP-1 (ng/mL)	0.01	0.82	0.71	0.74	0.64
Leptin (ng/mL)	0.0003	0.01	0.009	In model	In model

[a] Step 0, Comparison of CC responders *vs.* CRA (univariate logistic regression analyses).

[b] Final model, Area under the ROC curve, 0.85; multivariate odds ratio (95% CI) of FAI, 1.19 (1.07–1.32); of cycle history, 0.16 (0.05–0.42); of mean ovarian volume, 1.11 (0.99–1.24); and of leptin, 1.04 (1.01–1.06). This means that, for instance, a 1-U increase in a given patient's serum leptin level gives her a 4% higher probability to be a CRA.

[c] As published previously (5).

[d] FAI = T $\times$ 100/SHBG.

[e] Amenorrhea *vs.* oligomenorrhea.

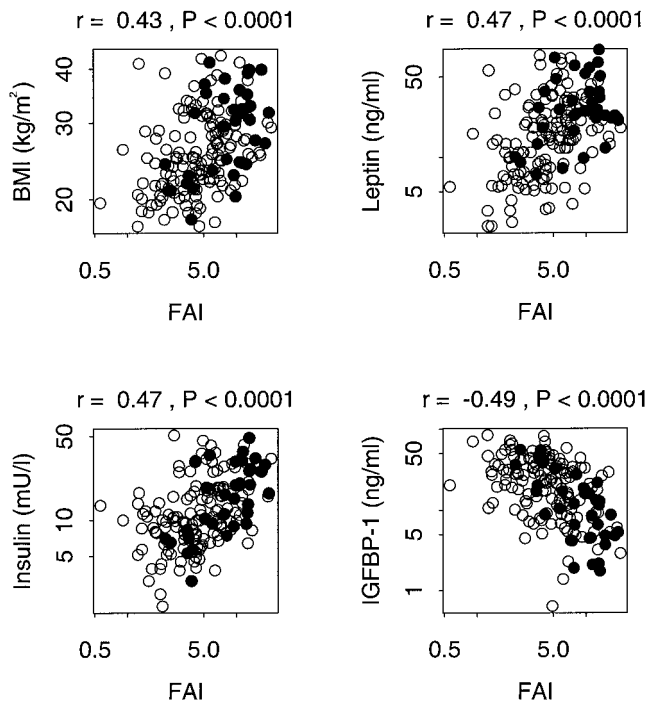[f] Only parameters that are significantly different (see Table 2) are included.

FIG. 1. Correlations between FAI (testosterone $\times$ 100/SHBG) and BMI, serum leptin, insulin, and IGFBP-1 in 182 normogonadotropic oligoamenorrheic infertile patients using a scatter plot. *Open circles* represent patients who ovulated after CC treatment, and *closed circles* represent CC nonresponders.

likelihood of ovaries to respond to ovulation induction (27). Finally, VEGF levels were assessed in these patients, as recent reports suggest that this protein may serve as a marker for changes in ovarian stroma of PCOS patients (14, 34).

The present study demonstrates that serum insulin, insulin/glucose ratio, and leptin levels are significantly higher and, in contrast, IGFBP-1 levels are lower in patients who remain anovulatory after CC. When a multivariate analysis for the prediction of CRA is applied, the capacity of serum insulin, insulin/glucose ratio, and IGFBP-1 levels to predict CRA is eliminated when FAI enters into the model due to a strong correlation between these parameters and FAI. In contrast, leptin levels enter into the model in the third step. These results can be interpreted as follows. Insulin is significantly higher in CRA patients. An association between decreased insulin sensitivity and hyperandrogenism is well known (36, 37). Signal transduction via the insulin receptor has been demonstrated to be frequently abnormal in PCOS (38). Moreover, IGF-I as well as insulin have been shown to augment LH-induced androgen biosynthesis by cultured thecal cells (38). As insulin binds to the structurally similar type 1 IGF receptor, it has been postulated that high levels of circulating insulin stimulate thecal cell androgen production through the type 1 IGF receptors (36, 37). However, this seems to be the case only in extreme hyperinsulinism, such as in patients suffering from leprechaunism. Moreover, insulin and BMI are the major determining factors of circulating IGFBP-1 levels in both normoovulatory obese and PCOS women. Insulin reduces circulating SHBG (39–41) and IGFBP-1 levels by inhibiting the production of these proteins in the liver (39, 41). Apparently, the liver is excluded from reduced sensitivity for insulin stimulation. Accordingly, it has been proposed that IGFBP-1 levels may be used as a simple marker for insulin resistance (48). Several researchers indicated lower circulating levels of IGFBP-1 (39, 50, 51), but not IGFBP-3 (51), in PCOS patients compared to normal cycling women (33–35, 48). Moreover, insulin may also inhibit IGFBP-1 production in ovarian granulosa cells, by acting through its own receptor (37). Circulating serum SHBG concentrations are significantly reduced in obese normoovulatory women (39) and in oligoamenorrheic CC-resistant infertile patients (5). Moreover, an increase in circulating serum SHBG concentration has been reported in ovulatory patients after CC medication (42) as well as in women with PCOS using insulin-sensitizing agents (43, 44). An insufficient or absent rise in circulating serum SHBG concentrations (with
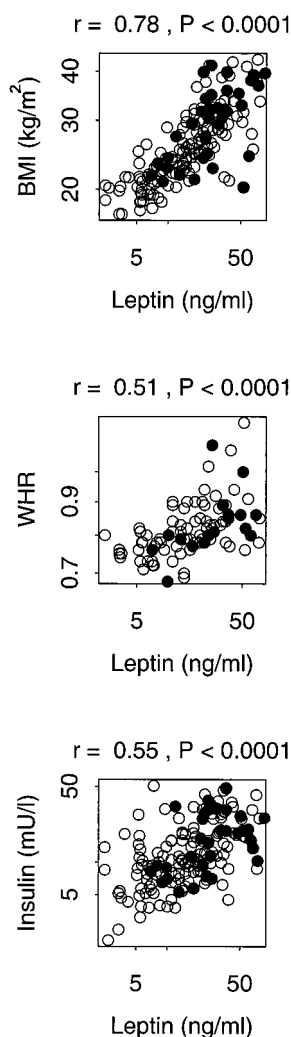
FIG. 2. Correlations between serum leptin and BMI, WHR, and serum insulin in 182 normogonadotropic oligomenorrheic infertile patients using a scatter plot. *Open circles* represent patients who ovulated after CC treatment, and *closed circles* represent CC non-responders.
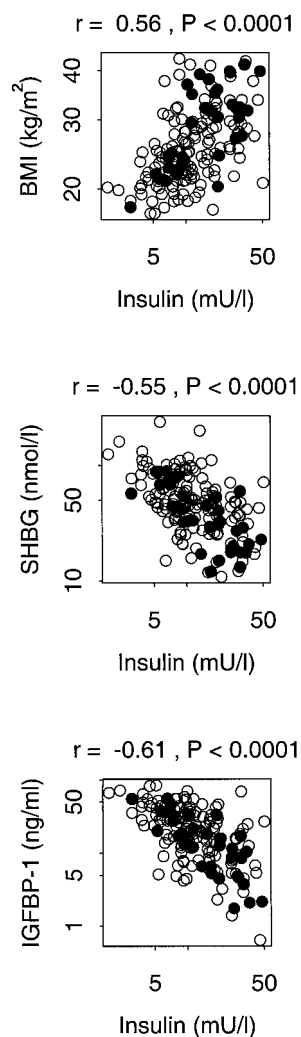

FIG. 3. Correlations between serum insulin and BMI, SHBG, and serum IGFBP-1 in 182 normogonadotropic oligomenorrheic infertile patients using a scatter plot. *Open circles* represent patients who ovulated after CC treatment, and *closed circles* represent CC nonresponders.

resulting high free androgen levels) after CC medication could be held responsible for CC unresponsiveness.

In the present study total and free IGF-I and IGFBP-3 levels are comparable between CC responders and nonresponders. No differences in IGFBP-3 levels were previously noted in follicular fluid from PCOS ovaries compared with androgen-dominant and estrogen-dominant follicles from normo-ovulatory women (45, 46). In contrast, IGFBP-1 levels are significantly lower in patients remaining anovulatory after CC medication. As a consequence of decreased hepatic production of serum IGFBP-1 levels, a greater proportion of IGF-I is biologically active (47). Taking into account that serum IGFBP-1 levels increase (48) and IGF-I levels decrease (42) in oligoamenorrheic women after CC medication, it may be suggested that an insufficient rise in serum IGFBP-1 levels after CC medication is involved in the follicle growth arrest in these patients. No difference could be observed in pre-treatment free IGF-I levels comparing responders *vs.* CRA patients. Overall, FAI is the most significant factor to predict

ovarian response. It appears that the prediction of insulin and IGFBP-1 completely depends on its association with FAI, in line with recent compelling molecular evidence for intrinsic abnormalities in the androgen synthesis pathway in PCOS thecal cells (49). It is likely that decreased IGFBP-1 is an excellent marker of hyperinsulinemia and that it does not affect the endocrine IGF system's effect on follicle growth.

The present study demonstrates a significant correlation between leptin levels and BMI and WHR, which is in agreement with previous reports (21–23). Serum leptin levels were significantly higher in CC nonresponders and enter into the model (instead of BMI) in multivariate analyses to predict CRA. The AUC of the previously developed prediction model (including FAI, BMI, cycle history, and mean ovarian volume) of 0.82 could be slightly improved to 0.85 by the inclusion of leptin rather than BMI. Due to the limited improvement of the AUC and the fact that information such as weight and height of the patient is easy to collect, one may prefer to use BMI to predict the probability of CRA in clinical

practice. Clearly, the end point for clinical practice should be pregnancy rather than ovulation. It has been demonstrated that leptin can directly impair insulin action in hepatocytes (20). In addition, leptin directly inhibits IGF-I augmentation of FSH-stimulated estradiol production (50) as well as LH-stimulated androgen synthesis *in vitro* (50). *In vivo* observations, however, would argue against a negative correlation between obesity/elevated leptin levels and androgens. High leptin levels could interfere with the ability of the dominant follicle to produce estradiol, both by inhibiting the production of androgen substrate and by decreasing the aromatizing capacity of granulosa cells (51). Moreover, changes in estradiol and P during the human menstrual cycle may participate in the control of leptin production (52). Caloric restriction, exercise, and weight-reducing diets may reduce leptin levels (53); result in normalization of insulin sensitivity, gonadotropins, and androgen metabolism (54); and lead to spontaneous ovulatory cycles in up to 50% of women (55).

Initial serum inhibin B as well as serum VEGF levels were similar comparing CC responders and nonresponders. These endocrine parameters had no predictive value for ovarian response to CC medication. The results of this study suggest that circulating inhibin B does not correspond to the number of healthy follicles present in polycystic ovaries, characterized by follicle maturation arrest and atresia of a proportion of follicles. This is in contrast with the conclusion of a recent report focusing on a relatively small group of patients (27). Recent observations suggest that increased intraovarian concentrations of VEGF may be related to increased stroma echogenicity and vascularity and increased secretion and pulsatility of LH (34). We have not been able to confirm that VEGF levels may represent the magnitude of ovarian dysfunction in these women.

The impact of markers for insulin sensitivity, the IGF system, leptin, inhibin B, and VEGF on the prediction of conception after ovulatory CC cycles has also been analyzed. None of these parameters predicts the probability of conceiving in women who ovulated after CC treatment (data not shown). This stresses again the concept previously put forward (8) that follicle growth and oocyte quality are differentially regulated.

In conclusion, the present longitudinal follow-up study suggests that FAI, as a marker for the severity of hyperandrogenemia, is the most significant endocrine marker of ovarian dysfunction and for the prediction of ovarian response after CC medication. Initial serum leptin insulin, and IGFBP-1 levels are factors in univariate analyses predicting patients who will remain anovulatory after CC. The capability of serum insulin and IGFBP-1 to predict CRA disappears when FAI enters into the model due to a significant correlation between FAI and these endocrine parameters. Finally, leptin is a better marker to predict ovarian response to CC medication compared to BMI. For the assessment of markers useful for clinical screening in patients, the present observations predicting ovarian response should be combined with factors predicting pregnancies in ovulatory CC women. Moreover, the question arises of whether factors involved in ovarian response or conception after CC medication may also predict individual responses to exogenous gonadotropins in patients who fail to ovulate or conceive after CC.

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