Altered D-*Chiro*-Inositol Urinary Clearance in Women With Polycystic Ovary Syndrome

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OBJECTIVE — Evidence suggests that some actions of insulin are effected by inositolphosphoglycan (IPG) mediators. We hypothesize that a deficiency in D-*chiro*-inositol (DCI) and/or a DCI-containing IPG (DCI-IPG) may contribute to insulin resistance in humans.

RESEARCH DESIGN AND METHODS — To assess this possibility in polycystic ovary syndrome (PCOS), we determined insulin sensitivity (S_i by frequently sampled intravenous glucose tolerance test), plasma and urinary DCI and *myo*-inositol (MYO) levels (by gas chromatography/mass spectrometry), and the release of insulin and DCI-IPG during the oral glucose tolerance test (area under the curve [AUC]) in 23 women with PCOS and 26 normal women.

RESULTS — Women with PCOS were heavier than control subjects ($P = 0.002$ for BMI), but also had decreased S_i ($P < 0.001$) and increased $AUC_{insulin}$ ($P < 0.001$) compared with normal women, even when corrected for BMI. The urinary clearance of DCI (uCl_{DCI}) was increased almost sixfold in PCOS compared with normal women ($P = 0.001$), but not MYO clearance ($P = 0.10$). uCl_{DCI} correlated inversely with S_i when all women were analyzed together ($n = 49$, $r = -0.50$, $P < 0.001$) and was one of the three best independent parameters predicting S_i. Finally, the ratio of $AUC_{DCI\text{-}IPG}$ to $AUC_{insulin}$ was decreased threefold in women with PCOS ($P < 0.001$).

CONCLUSIONS — uCl_{DCI} is inversely correlated with insulin sensitivity in women and is a strong independent predictor of insulin resistance in multivariate models. PCOS, which is characterized by insulin resistance, is associated with a selective increase in uCl_{DCI} and impaired DCI-IPG release in response to insulin. These findings are consistent with a defect in tissue availability or utilization of DCI in PCOS that may contribute to the insulin resistance of the syndrome.

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Polycystic ovary syndrome (PCOS) is a prevalent but poorly understood disorder associated with significant adverse short- and long-term health consequences. It is defined by hyperandrogenism, chronic anovulation, and/or polycystic ovaries (1,2) and affects 6–10% of women of childbearing age (3–5). PCOS is the most common cause of anovulatory infertility in the U.S. and is associated with an increased risk of developing cancer, hypertension, dyslipidemia, impaired glucose tolerance or type 2 diabetes, and cardiovascular disease (3,4,6).

During the past decade, increasing evidence supports the central role of insulin resistance and/or compensatory hyperinsulinemia in the syndrome's pathogenesis (3,7,8). Obese and lean women with PCOS manifest insulin resistance independent of fat mass (3,9), and administration of insulin-sensitizing drugs, such as metformin (3), troglitazone (3), and D-*chiro*-inositol (DCI) (10–12), to both obese and lean women with the syndrome increases the frequency of ovulation and decreases circulating androgens.

Some actions of insulin may be affected by putative inositolphosphoglycan (IPG) mediators of insulin action (13,14), and evidence suggests that a deficiency in a specific DCI-containing inositolphosphoglycan (DCI-IPG) may contribute to insulin resistance in individuals with impaired glucose tolerance or type 2 diabetes (15,16). Several lines of evidence suggest that a deficiency in DCI-IPG contributes to insulin resistance in PCOS as well.

Our group (10,11) and others (12) have shown that oral administration of DCI to women with PCOS improves glucose tolerance while reducing insulin in both obese (10) and lean (11) women with PCOS and also decreases serum androgens and improves ovulatory function. The idea that a deficiency in DCI-IPG, related perhaps to an actual or functional deficiency of the precursor DCI, contributes to the insulin resistance of PCOS is further supported by evidence that administration of metformin to PCOS women enhances insulin-stimulated release of DCI-IPG (17).

Based on these findings, we hypothesized that a defect in an alternative insulin-signaling pathway, in which DCI-IPG acts as a mediator of insulin action, contributes to the pathophysiology of the insulin resistance of PCOS. We also hypothesized that women with PCOS would be characterized by abnormal metabolism of DCI, the precursor of DCI-IPG, and diminished insulin-stimulated release of the putative DCI-IPG mediator.

To assess these hypotheses in vivo, we studied women with PCOS and normal women and assessed circulating DCI and 24-h urinary clearance of DCI (uCl_{DCI}), release of insulin and DCI-IPG during an oral glucose tolerance test (OGTT), and insulin sensitivity by the minimal model technique. As a control, we also assessed the urinary clearance of *myo*-inositol (MYO), an inositol not believed to influence insulin sensitivity. The findings indi-

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Abbreviations: $AUC_{DCI\text{-}PGI}$, area under the bioactivity curve for DCI-IPG during OGTT; $AUC_{insulin}$, area under the insulin curve during OGTT; DCI, D-*chiro*-inositol; DCI-IPG, DCI-containing inositolphosphoglycan; IPG, inositolphosphoglycan; MYO, *myo*-inositol; OGTT, oral glucose tolerance test; PCOS, polycystic ovary syndrome; uCl_{DCI}, urinary clearance of D-*chiro*-inositol; WHR, waist-to-hip ratio.

A table elsewhere in this issue shows conventional and Système International (SI) units and conversion factors for many substances.

cate a marked alteration in DCI urinary clearance and deficient insulin-stimulated DCI-IPG release in obese women with PCOS.

RESEARCH DESIGN AND METHODS

— A total of 23 women with PCOS and 26 normal control subjects were evaluated at the General Clinical Research Center of the Virginia Commonwealth University Health System. PCOS was defined by oligomenorrhea (eight or fewer menstrual periods in the previous year) and hyperandrogenemia (elevated serum total or free testosterone concentration); hyperprolactinemia, thyroid dysfunction, and late-onset adrenal hyperplasia were excluded by the appropriate tests (1,2). Women were 18–40 years old and none had diabetes or took oral contraceptives or any medication known to affect insulin sensitivity for at least 2 months before study.

Normal women had regular menstrual cycles, normal androgen levels, and normal glucose tolerance and did not have any history of gestational diabetes or family history of a first-degree relative with diabetes. They were free of disorders that have been linked to insulin resistance, such as hypertension or dyslipidemia. The study has been approved by the institutional review board of Virginia Commonwealth University, and each woman gave written informed consent.

Study protocol

Because DCI may be ingested as part of a diet high in legumes or fruits, the women were given instructions to follow a balanced mixed diet for at least 3 days before the start of the study. PCOS women were studied during the equivalent of the follicular phase of the menstrual cycle, as documented by a serum progesterone ≤2 ng/ml. Normal women were studied during the mid-follicular phase of the menstrual cycle (days 5–9), which most closely approximates the hormonal milieu of anovulatory women with PCOS.

On the first day, fasting baseline laboratories and a 2-h OGTT with 75 g dextrose were performed. During the OGTT, blood samples were collected every 15 min for determination of serum glucose and insulin concentrations, and serum DCI-IPG bioactivity was determined every 30 min. Women were then instructed to collect all their urine for the next 24 h.

On the second day, after a 12-h overnight fast, insulin sensitivity was measured by the frequently sampled intravenous glucose tolerance test as described by Bergman and colleagues (18–20). At time zero, 300 mg/kg dextrose was administered intravenously and 0.03 units/kg insulin was administered intravenously 20 min later. A total of 27 blood samples for determination of insulin and glucose were collected over the 3-h duration of the protocol. Data were analyzed with the Minimal Model Identification Software (MINIMOD, version 3.0, 1994) (21), which yields quantitative determination of tissue insulin sensitivity (S_i).

Laboratory assays

Blood samples were centrifuged immediately, and sera were stored at −70°C until assayed. All hormones were assayed as previously described (22–24). Serum free testosterone was calculated by the method of Sodergard et al. (25) using a serum albumin concentration of 4.0 g/dl (40 g/l). To avoid interassay variation, all samples were analyzed in duplicate in a single assay for each hormone. The intra-assay coefficient of variation (CV) for the insulin was 5.5% and was <10% for all steroid hormone assays.

DCI and MYO analyses

Plasma and urinary inositol concentrations were determined by gas chromatography and mass spectrometry. [$^2H_6$]racemic *chiro*-inositol and [$^2H_6$]*myo*-inositol were added to plasma or urine as internal standards. The samples were then purified, derivatized with pentafluoropropionic anhydride, separated on a Chirasil-Val capillary column (Alltech, State College, PA), and analyzed in a negative ion chemical ionization mode on an HP 5973 mass spectrometer (Agilent Technologies, Palo Alto, CA) with methane as the reagent gas, as previously reported (26). The 24-h urinary clearance was calculated by dividing 24-h urinary excretion by plasma concentration.

DCI-IPG insulin mediator bioactivity assay

Blood samples were centrifuged immediately, and sera were stored at −70°C until processed. The DCI-IPG mediator was isolated from serum as previously described (17). To date, it has not been possible to measure the content of extracted DCI-IPG because its structure and exact mass are unknown, and no specific antibody suitable for an immunoassay has been developed. Therefore, DCI-IPG mediator bioactivity was determined using the specific activation of phosphate dehydrogenase phosphatase, as previously validated in women with PCOS and described in detail (17). The interassay CV of the bioassay was 17.4%. The phosphate dehydrogenase activity intra-assay CV was 6.7%. CVs of the entire method (extraction and assay) were 10.7 and 8.5%, respectively, for the absolute values of basal and peak DCI-IPG bioactivity.

To adjust for variation in basal phosphate dehydrogenase activity from one assay to the other, and therefore from subject to subject, the water-blank activity was subtracted from the bioactivity of DCI-IPG released into serum during OGTT, which was then expressed as the percentage of its bioactivity at baseline (0 min).

Statistical analysis

We analyzed the response of serum insulin concentrations and relative bioactivities of DCI-IPG to the oral administration of glucose by calculating the areas under the respective response curves by the trapezoidal rule. Results not normally distributed, based on the normal quintile plot, were log-transformed for all statistical analyses and reported back-transformed in their original units. All results are reported as means, or geometric means for log-transformed variables, with 95% CIs. P values <0.05 were considered significant. All analyses were performed using JMP 4.0 software (SAS Institute, Cary, NC).

Variable comparisons between groups were made with the Student's two-tailed t test, and equalities of variances were tested with the Brown-Forsythe test. For comparisons with unequal variances, P values of Welch ANOVA tests were reported, as indicated. Correlation analysis was performed using Pearson's correlation test. Correction for BMI and/or S_i was performed with multiple linear regression analyses.

We also analyzed the effect of weight on the relationship between PCOS status and DCI urinary clearance by two-way ANOVA. For this analysis, the dependant variable was DCI urinary clearance and the independent variables were PCOS status, BMI status, and the interaction of both. BMI status was categorized as obese (BMI ≥30 kg/m^2) and nonobese (BMI <30 kg/m^2) women.

Three stepwise multiple linear regression analyses were performed. For the first one, the dependant variable was DCI urinary clearance, the exposition variable

Table 1 —*Clinical and biochemical characteristics of women with PCOS and normal control women*

	PCOS subjects	Normal subjects	*P* value (*t* test)
n	23	26	—
Age (years)	31 (28–33)	29 (26–31)	0.30*
BMI (kg/m^2)†	33.9 (29.9–38.5)	25.6 (22.7–28.9)	0.002
WHR	0.81 (0.78–0.84)	0.76 (0.73–0.78)	0.006
Systolic blood pressure (mmHg)	119 (113–125)	109 (104–115)	0.02
Diastolic blood pressure (mmHg)	70 (66–73)	64 (61–67)	0.03
Total testosterone (ng/dl)†	71 (58–87)	39 (32–47)	<0.001*‡
Free testosterone calculation (ng/dl)†	0.79 (0.59–1.06)	0.24 (0.19–0.32)	<0.001‡
Fasting insulin (μIU/ml)†	4.9 (3.5–6.8)	3.0 (2.2–4.1)	0.047*
$AUC_{insulin}$ (μIU · min^{-1} · ml^{-1})†	5,479 (4,206–7,140)	2,312 (1,803–2,965)	<0.001§
S_i (by FSIVGTT)†	2.3 (1.4–3.8)	12.5 (7.7–20.2)	<0.001§
Plasma DCI (μmol/l)†	0.10 (0.07–0.16)	0.19 (0.13–0.28)	0.035
24-h urinary DCI (μmol/day)†	2.3 (1.1–4.9)	0.7 (0.4–1.5)	0.043*
Urinary clearance of DCI (ml/min)†	15.3 (7.7–30.5)	2.7 (1.4–5.1)	0.001*§
Plasma MYO (μmol/l)	20.6 (18.8–22.4)	21.2 (19.5–23.0)	0.62
24-h urinary MYO (μmol/day)†	98 (66–144)	65 (45–94)	0.13
Urinary clearance of MYO (ml/min)†	3.4 (2.3–4.9)	2.2 (1.5–3.1)	0.10
$AUC_{DCI\text{-}IPG}$ (%/min)†	12,492 (11,212–13,919)	14,820 (13,386–16,408)	0.025
$AUC_{DCI\text{-}IPG}/AUC_{insulin}$†	2.3 (1.7–3.0)	6.4 (4.9–8.4)	<0.001§‖

Data are means (95% CIs) unless otherwise indicated. To convert values for total testosterone to nanomoles per liter, multiply by 0.0347; to convert values for free testosterone to picomoles per liter, multiply by 34.7; and to convert values for insulin to picomoles per liter, multiply by 6.9. Note that only significant partial *P* values (‡, §, or ‖) after correction for BMI and/or S_i are reported (multiple linear regression analyses). FSIVGGT, frequently sampled intravenous glucose tolerance test. *Unequal variance *t* test. †Geometric means. ‡$P < 0.05$ when corrected for BMI and S_i. §$P < 0.05$ when corrected for BMI. ‖$P < 0.05$ when corrected for S_i.

was group status, and the potential confounding variables were BMI, waist-to-hip ratio (WHR) circumferences, total testosterone, and S_i. These confounders were selected based on scientific literature and because they were significantly associated with the dependant variable in univariate analyses. To assess the effect of these potential confounders, they were entered successively in the model, based on next highest partial *F* test (forward method). All possible interactions were tested with each new variables added into the model.

For the second stepwise regression analysis, the dependant variable was S_i, the variable of interest was DCI urinary clearance, and the potential confounding variables were group status, BMI, WHR, and total testosterone. These confounders were also significantly associated with the dependant variable in univariate analyses. The same forward stepwise method was used, and all possible interactions were tested.

For the third stepwise regression analysis, the best model to predict S_i was assessed using all variables of Table 1. These variables, and all interactions found with the first two stepwise regression analyses, were entered successively in the model (forward method). At each step, parameters that did not contribute significantly to the model (partial $P < 0.05$) were then excluded.

RESULTS

Clinical and biochemical characteristics

Women with PCOS did not differ significantly from normal control women with respect to age. However, their BMI, WHR, systolic blood pressure, and diastolic blood pressure were significantly higher ($P \leq 0.03$) (Table 1).

Total and calculated free testosterone levels were significantly higher in women with PCOS than in control women ($P < 0.001$), even after correction for BMI and S_i ($P = 0.006$ and $P < 0.001$, respectively). Furthermore, fasting insulin levels ($P = 0.047$) and areas under the insulin curves during OGTT ($AUC_{insulin}$) ($P < 0.001$) were higher in PCOS women than in normal control subjects, and S_i values were significantly lower ($P < 0.001$). $AUC_{insulin}$ and S_i remained significantly different between groups after correction for BMI ($P = 0.001$ and $P = 0.002$, respectively).

Plasma and urine concentrations and urinary clearance of DCI and MYO

Plasma concentrations of DCI were significantly lower in PCOS women compared with normal control subjects ($P = 0.035$), and 24-h urinary excretion of DCI was significantly higher ($P = 0.043$) (Table 1). Consequently, uCl_{DCI} was greater than fivefold higher in PCOS women, which was highly significant ($P < 0.001$). This statistical significance persisted even after correction for BMI ($P = 0.015$). In contrast to the findings with DCI, MYO parameters were not altered in women with PCOS. There were no significant differences between groups for plasma MYO concentrations, 24-h urinary excretion of MYO, and urinary clearance of MYO ($P \geq 0.10$).

The data were also analyzed with two-way ANOVA, which confirmed a significant difference in uCl_{DCI} between groups independently of obesity ($P = 0.006$), a significant difference in uCl_{DCI} between obese and lean women independently of PCOS status ($P = 0.02$), and a significant interaction between PCOS status and obesity ($P = 0.005$). To illustrate this interaction, a subgroup analysis was performed and showed that uCl_{DCI} was significantly increased by 14-fold in obese PCOS women (BMI ≥30 kg/m^2) compared with obese control subjects (31.8 [13.1–77.2] vs. 2.2 [0.7–7.3], $P < 0.001$), even after correction for S_i and total testosterone (partial $P = 0.003$). No association was found in nonobese

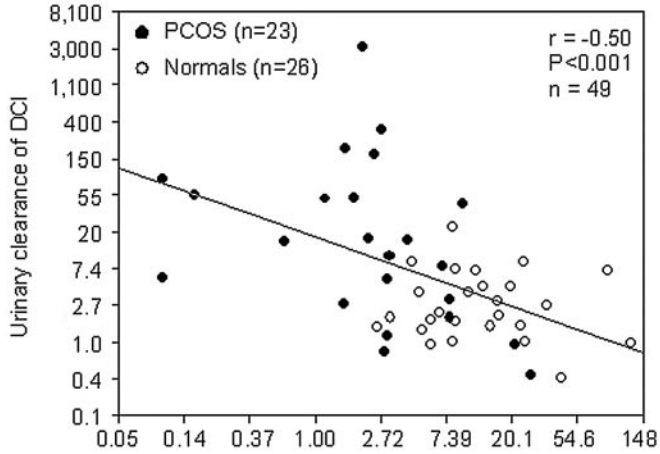

Figure 1—*Correlation between urinary clearance of DCI and S_i when women with PCOS and normal women were analyzed together. Results are represented back-transformed in their original units, on log scales.*

women (2.9 [1.1–7.3] vs. 2.9 [1.6–5.3], $P = 0.97$).

Serum DCI-IPG bioactivity profiles

Areas under the bioactivity curves for DCI-IPG during OGTT ($AUC_{DCI\text{-}IPG}$) were significantly lower in PCOS women than in normal control subjects ($P = 0.025$) (Table 1). Because insulin released during OGTT was much higher in PCOS women than in normal control subjects ($AUC_{insulin}$ = 5,479 μIU · min^{-1} · ml^{-1} [37.8 nmol · min^{-1} · l^{-1}] vs. 2,312 μIU · min^{-1} · ml^{-1} [16.0 nmol · min^{-1} · l^{-1}], respectively), the ratio of $AUC_{DCI\text{-}IPG}$ to $AUC_{insulin}$ was decreased almost threefold in PCOS ($P < 0.001$).

Correlation between insulin sensitivity and DCI clearance

When all women were included, there was an inverse correlation between S_i and uCl_{DCI}, which was relatively strong ($r = -0.50$) and highly significant ($P < 0.001$) (Fig. 1). Correlation between S_i and uCl_{DCI} showed a trend in PCOS women ($n = 23$, $r = -0.39$, $P = 0.07$), but not in control subjects ($P = 0.63$). These results might be related to the limited numbers of women in each group.

Stepwise multivariate analyses

The association between group status and uCl_{DCI} was no longer significant in multivariate analysis when the effect of group status (partial $P = 0.14$) was adjusted successively for BMI, S_i, total testosterone levels, and WHR (adjusted $R^2 = 0.49$ and $P < 0.001$ for model). However, significant interactions were found between BMI and group status (partial $P < 0.001$) and between BMI and total testosterone levels (partial $P = 0.02$). This first interaction has already been detailed above.

The highly significant association between uCl_{DCI} and S_i identified in the entire group by univariate analysis (Fig. 1) persisted in multivariate analysis when the effect of uCl_{DCI} (partial $P = 0.002$) was adjusted successively for total testosterone levels, WHR, BMI, and group status (adjusted $R^2 = 0.55$ and $P < 0.001$ for model). No interaction was present between BMI and uCl_{DCI}, which means that the relationship between uCl_{DCI} and S_i was not conditional to obesity status. A significant interaction was found between total testosterone levels and uCl_{DCI} (partial $P = 0.04$), which means that total testosterone was significantly associated with S_i only in women with high uCl_{DCI} (≥3.9 ml/min, which is the median of uCl_{DCI}) ($r = -0.60$, $P = 0.002$, $n = 24$). This association in subgroup analysis was stronger after correction for BMI (partial $P < 0.001$). No association between total testosterone and S_i was seen in women with a uCl_{DCI} <3.9 ml/min ($P = 0.51$, $n = 24$), even after correction for BMI.

The best model to predict insulin sensitivity when all variables shown in Table 1 and the three interactions found above were considered was $S_i = \alpha + \beta_1 \cdot AUC_{insulin} + \beta_2 \cdot uCl_{DCI} + \beta_3 \cdot (uCl_{DCI} \times \text{total testosterone})$ (adjusted $R^2 = 0.73$, $P < 0.001$). The three parameters significantly and independently predicting S_i were therefore $AUC_{insulin}$ (negative association, partial $P < 0.001$), uCl_{DCI} (negative association, partial $P = 0.01$), and the interaction between uCl_{DCI} and total testosterone (partial $P = 0.001$). This interaction has already been explained above.

CONCLUSIONS — The aim of this study was to test the hypothesis that women with PCOS would exhibit abnormal metabolism of DCI and deficient insulin-stimulated release of DCI-IPG, which would correlate with their decreased sensitivity to insulin. Indeed, we found that women with PCOS, when compared with normal control women, had a greater than fivefold increase in uCl_{DCI} and a circulating concentration of DCI that was reduced by half. These abnormalities persisted even when corrected for differences in BMI between the PCOS and normal women and were confirmed with two-way ANOVA. Furthermore, insulin sensitivity correlated inversely and robustly with uCl_{DCI}, and the abnormal uCl_{DCI} in women with PCOS was accompanied by diminished insulin-stimulated release of the putative DCI-IPG mediator of insulin action. To serve as an internal control, the parameters of a different inositol, MYO, were also assessed. The levels and urinary clearance of MYO did not differ between PCOS women and normal women, confirming a defect specifically involving DCI in PCOS.

When the results were subjected to a two-way ANOVA and multivariate analyses, PCOS was significantly associated with increased uCl_{DCI} only in obese women, which was independent of total testosterone and S_i. Furthermore, uCl_{DCI} remained strongly associated with insulin resistance after correction for potential confounders, and the association between S_i and uCl_{DCI} was not conditional to obesity status. When assessing all conventional predictors of insulin sensitivity and parameters of DCI, increased uCl_{DCI} and the interaction between uCl_{DCI} and total testosterone were the best independent predictors of insulin resistance, along with hyperinsulinemia ($AUC_{insulin}$). Only 27% of the variability of S_i was not explained by these three parameters, indicating that few important variables were missing from our model. These findings strongly suggest a contribution of abnormal handling of DCI to the insulin resistance of PCOS.

Notably, group status (i.e., having PCOS or not) was not a predictor of insulin sensitivity independent of uCl_{DCI}, which supports the hypothesis that PCOS women develop insulin resistance in part because of impaired DCI metabolism.

Moreover, insulin resistance was independently associated with testosterone levels only in women with high uCl_{DCI}, which suggests a role of insulin resistance in the development of hyperandrogenemia when a defect in DCI metabolism is present.

Three different studies (16,26–28) have assessed DCI metabolism in normal and diabetic subjects. Urinary DCI excretion was remarkably heterogeneous among normal subjects of different populations, ranging from 2.1 to 96.0 μmol/day. Normal subjects in our study had a mean 24-h urinary excretion of DCI of 3.7 μmol/day (Table 1 reports geometric means), which was comparable to the 2.1 μmol/day reported by Ostlund et al. (26) using the identical gas chromatography/mass spectrometry inositol assay methodology. Other studies relied on methods requiring quantitative recovery through several purification steps and did not use an internal recovery standard (16,27), which may have introduced error into the measurements, as discussed elsewhere (26). Variation in obesity status in the studied populations might also contribute in part to these differences (29).

In contrast to our findings of increased uCl_{DCI} in PCOS, some of these studies (16,26–28) reported decreased urinary excretion of DCI in various conditions associated with insulin resistance, such as diabetes, impaired glucose tolerance, and familial history of diabetes. Ostlund et al. (26), however, reported an increased uCl_{DCI} in type 2 diabetic subjects, comparable in magnitude to our findings in PCOS women (mean uCl_{DCI} = 145 and 182 ml/min, respectively) (Table 1 reports geometric means). As noted above, analytical differences may be responsible for the difference between our findings and the findings of Ostlund et al. (26) compared with those of others (16,27,28). In addition, competition between renal excretion of glucose and *chiro*-inositol confounds interpretation of the studies of diabetic individuals, especially if diabetes was not well controlled.

In our study, bioactive DCI-IPG released during an OGTT was decreased significantly in PCOS women compared with normal women, and the percent release of bioactive DCI-IPG per unit of insulin ($AUC_{DCI\text{-}IPG}/AUC_{insulin}$ ratio) was reduced by threefold in PCOS women. Because it is assumed that DCI-IPG release is coupled with the release of insulin during an OGTT (30), the ratio of $AUC_{DCI\text{-}IPG}$-to-$AUC_{insulin}$ is likely a more accurate measure of the competence of insulin-mediated DCI-IPG release than $AUC_{DCI\text{-}IPG}$ alone.

DCI-IPG release has been reported to be diminished in other insulin-resistant states as well. Shashkin et al. (30) reported that the relative increase in the bioactivity of DCI-IPG during an OGTT is abolished in obese men with type 2 diabetes compared with healthy men. Furthermore, concentrations of DCI in IPG preparations from muscle biopsies obtained during euglycemic-hyperinsulinemic clamp studies were increased sixfold during the clamp in normal subjects, as opposed to undetectable levels throughout the clamp in patients with type 2 diabetes (16). Finally, a recent study showed that metformin might improve the action of insulin in obese women with PCOS in part by improving insulin-mediated release of the DCI-IPG mediator (17).

Only a small amount of DCI is consumed in the diet, and it is unlikely that a dietary deficiency could substantially alter circulating levels of DCI. The increased renal clearance of DCI in women with PCOS most likely reflects a reduced renal threshold for DCI, as supported by the observed 50% reduction in circulating DCI. Nonetheless, other mechanisms are also possible, such as an abnormality in tissue/cellular uptake of DCI and/or intracellular processing of DCI. However, these latter mechanisms should be associated with a normal or increased circulating level of DCI, which we did not observe. Finally, it is possible that more than one metabolic abnormality is present simultaneously in PCOS.

Our findings of a markedly increased renal clearance of DCI in women with PCOS, which correlates inversely with insulin sensitivity, is consistent with two possible scenarios. One is that the defect in renal clearance of DCI is primary and causes insulin resistance. The other is that insulin resistance, or more likely the hyperinsulinemia ensuing therefrom, induces a defect that increases the renal clearance of DCI. It is also possible that both scenarios are valid and present in PCOS.

Specifically, we propose that, in a woman with PCOS, an initial genetic or environmental "insult" causing insulin resistance leads to a compensatory hyperinsulinemia. Hyperinsulinemia then induces a defect that increases renal clearance of DCI, and this leads to a reduction in circulating DCI and its availability to tissues. The consequence is an intracellular deficiency of DCI and, ultimately, of the DCI-IPG mediator of insulin action. Diminished release of DCI-IPG in response to stimulation by insulin results in a further decrease in insulin sensitivity (i.e., aggravation of insulin resistance). Hence, a "vicious cycle" is initiated whereby insulin resistance is amplified in PCOS through the induction of a defect in renal clearance of DCI. Although well supported by our data, this hypothesis suffers from the lack of direct evidence for a link between increased DCI urinary clearance and impaired DCI-IPG generation in our study. This is because it is difficult to precisely assess the DCI-IPG pathway in vivo.

Despite interesting and highly significant results, even in multivariate analyses, our study has some limits. First, multiple linear regression models built for these analyses were not tested with another population sample to assess their stability. Such validation in another population would be of great interest. Second, because only PCOS and normal women were studied, inference to other populations would be only speculative.

In summary, our findings indicate that PCOS is associated with a selective increase in uCl_{DCI} in obese women, which is accompanied by a decrease in insulin-stimulated release of the DCI-IPG mediator during an OGTT. Furthermore, uCl_{DCI} is inversely correlated with insulin sensitivity in women, and this correlation is independent from all other parameters studied. Urinary clearance of DCI was also one of only three variables that best predicted insulin sensitivity in our study.

In conclusion, these findings are consistent with a defect in tissue availability or utilization of DCI in PCOS that may contribute to the insulin resistance of the syndrome by decreasing cellular availability of the putative DCI-IPG mediator of insulin action. These findings offer a potential mechanism for previous studies (10–12), which reported that oral administration of DCI to women with PCOS improved glucose tolerance and exerted other salutary effects consistent with an enhancement of insulin sensitivity. Whether abnormalities of DCI metabolism and DCI-IPG are specific to PCOS or relevant to other disorders characterized by insulin resistance is unknown.

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