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Hyperinsulinemia is closely related to low urinary clearance of D-*chiro*-inositol in men with a wide range of insulin sensitivity

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Abstract

We have previously shown that women with polycystic ovary syndrome (PCOS) have increased urinary clearance of D-*chiro*-inositol (uCl_{DCI}), which was positively associated with hyperinsulinemia. The objective of this study was thus to determine if such relationship also exists in men with a large range of insulin sensitivity and levels. A cross-sectional study was performed on 11 brothers of women with PCOS and 21 control men. In this study, brothers served as a model of insulin resistance. We assessed uCl_{DCI}, urinary clearance of *myo*-inositol, and insulin levels with a standard 75-g oral glucose tolerance test, a 2-hour euglycemic-hyperinsulinemic clamp, and a 24-hour urine collection. Our results showed in all men together that low uCl_{DCI} was strongly associated ($P < .001$) with hyperinsulinemia, for which uCl_{DCI} was a significant predictor independent of other classic factors. Brothers were heavier than controls ($P = .02$), with increased glucose-stimulated glucose ($P < .001$) and insulin levels ($P < .001$) and reduced insulin sensitivity ($P = .001$). In this group, plasma DCI was increased by 3-fold ($P = .02$), with a 3-fold decrease in the uCl_{DCI} to urinary clearance of *myo*-inositol ratio, which was almost significant ($P = .07$). Low uCl_{DCI} is strongly associated with hyperinsulinemia in all men, and brothers of PCOS women who are more insulin resistant display increased plasma DCI and borderline decreased uCl_{DCI}. Thus, compensatory hyperinsulinemia might suppress renal clearance of DCI to increase plasma DCI levels and partially compensate for insulin resistance by improving DCI availability in men. The apparent discrepancy with PCOS women might be explained by higher insulin levels in men as compared with women and requires confirmation.

1. Introduction

Polycystic ovary syndrome (PCOS) is a very common disorder that affects 6% to 10% of women of reproductive age [1]. This syndrome is defined by anovulation, hyperandrogenism, and/or polycystic ovaries [2,3], and is the most common endocrinopathy in this age group in developed countries. However, in the past 15 years, it has been associated with higher risks of developing hypertension, dyslipidemia, impaired glucose tolerance, type 2 diabetes mellitus, metabolic syndrome, and cardiovascular diseases [1]. Cardiometabolic risks related to PCOS are indeed due to insulin resistance (IR) and/or hyperinsulinemia that seems to play a critical role in the pathogenesis of PCOS [4,5].

Some actions of insulin are performed by inositolphosphoglycan (IPG) mediators that are released by cells after stimulation by insulin [6,7]. It was found that a deficiency in a specific D-*chiro*-inositol–containing IPG (DCI-IPG) may contribute to IR in individuals with impaired glucose tolerance or type 2 diabetes mellitus [8,9]. We have shown that metformin may improve the action of insulin in obese women with PCOS in part by improving insulin-mediated release of the DCI-IPG mediator [10].

We have also demonstrated that insulin-resistant women with PCOS display increased urinary clearance of DCI (uCl_{DCI}) and decreased insulin-stimulated release of DCI-IPG as compared with controls. This higher uCl_{DCI} was correlated with IR and hyperinsulinemia in all women together [11]. Our group [12,13] and others [14] have also demonstrated that DCI oral supplementation enhances insulin sensitivity and improves clinical features of PCOS. Thus, we have hypothesized that, in

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women with or without PCOS, higher urine loss of DCI could decrease DCI plasma levels and availability to tissues, which will in turn reduce DCI incorporation into IPG and the release of DCI-IPG by insulin. This defect could therefore contribute to the IR of the syndrome.

We have also found that brothers of women with PCOS, who were similar to control men for anthropometric measures, displayed decreased insulin sensitivity (measured by insulin-glucose clamp), increased insulin levels, decreased glucose tolerance, increased triglyceride levels, and increased levels of plasminogen activator inhibitor–1 (PAI-1) and factor VIII [15]. Except for triglycerides and PAI-1, all these differences remained significant after adjustments for age and body mass index (BMI). Brothers of PCOS women are thus characterized by IR, compensatory hyperinsulinemia, dysglycemia, and hypercoagulability, independently of obesity.

Based on these previous findings, we hypothesized that DCI metabolism also contributes to IR in men. To evaluate this hypothesis, we assessed 24-hour urinary clearance of DCI and *myo*-inositol (MYO), as well as insulin sensitivity and levels, in brothers of women with PCOS and in control men. In this study, brothers of PCOS women served as a model of IR, as previously shown [15], to widen the range of insulin sensitivity and levels and thus improve the capacity of our study to find a relationship.

2. Subjects and methods

2.1. Subjects

From 17 male siblings of women with PCOS and 28 control men recruited in a previous study [15], we included in this pilot research all subjects who accepted to collect their urine during 24 hours, that is, 11 brothers and 21 controls. Index women were diagnosed with PCOS following these criteria [2,3]: oligomenorrhea (≤8 menstrual periods in the preceding year) or confirmed anovulation, hyperandrogenemia (calculated free testosterone >50 pmol/L), and exclusion of secondary causes (normal serum prolactin, normal serum 17α-hydroxyprogesterone [16], and thyroid-function tests). All PCOS probands were treated at the Reproductive Endocrinology Clinic of the Centre hospitalier universitaire de Sherbrooke (CHUS). Because only 2 brothers came from the same family, there was no familial clustering in this study.

Subjects were all aged between 18 and 40 years, were not affected by impaired glucose tolerance or diabetes (evaluated by 75-g oral glucose tolerance testing, OGTT), and had acceptable health (no clinically significant pulmonary, cardiac, renal, hepatic, neurologic, psychiatric, infectious, neoplastic, and malignant disease) based on interview, medical history, physical examination, and laboratory tests. Men with current or past use within 2 months of medications known to affect insulin sensitivity were also excluded. Finally, healthy subjects must not have family history of a first-degree relative with PCOS. The study was approved by the Human Research Ethics Committee of the CHUS, and all participants gave their written informed consent.

2.2. Experimental protocol

All procedures were performed at the Centre de recherche clinique Etienne-Le Bel of the CHUS. During the screening visit, which was also the first study visit, after a 12-hour overnight fast, blood samples were taken to determine whether subjects were eligible (ie, basic blood count, electrolytes, creatinine, liver function tests, and basic urinalysis in the reference range for our laboratory). Complete medical and familial history was recorded; and physical examination was performed, including lean body mass by standing electrical bioimpedance (Tanita weight scale model TBF-300A, Arlington Heights, IL). Waist circumference (WC) was measured with a flexible tape midway between the last rib and iliac crest, at the end of a normal expiration. Blood pressure (BP) was recorded after a 5-minute rest period in the sitting position. Thereafter, a 2-hour 75-g OGTT was performed to assess glucose tolerance and glucose-stimulated insulin levels. During this test, blood samples were taken every 15 minutes to determine levels of serum glucose and insulin.

All subjects were asked to follow a mixed balanced diet at least 2 days before the second visit to limit carbohydrates intake (300 g/d) and normalize DCI intake because fruits and legumes contain a significant amount of DCI or its precursors [17]. During the 24 hours before the following visit, subjects collected their urine for inositol content.

At least 2 days after the first visit, following a 12-hour overnight fast, insulin sensitivity was directly evaluated using a 2-hour euglycemic-hyperinsulinemic clamp. A constant infusion of insulin at a rate of 40 mU/m^2 min was given with a variable infusion of 20% dextrose to maintain plasma glucose concentration to approximately 90 mg/dL (5 mmol/L). Total body carbohydrate metabolism (*M*-value, in micromoles per kilogram minute) was calculated as follows: glucose infusion rate during the last 30 minutes of the clamp (in micromoles per minute) divided by the subject's weight (in kilograms) [18]. Blood samples for plasma insulin levels were collected at baseline and every 10 minutes during the insulin infusion. A baseline fasting blood sample was also collected for inositol determination.

2.3. Laboratory assays

Blood samples were assayed by the biochemistry core laboratory of the CHUS, except for inositol levels. Levels of insulin during the OGTT and the clamp were quantified by double-antibody radioimmunoassay (Diagnostic Products, Los Angeles, CA). Plasma glucose levels during the second visit were assessed at bedside using a Beckman Coulter Glucose Analyser II (Beckman Coulter, Mississauga, Ontario, Canada).

Total testosterone, androstenedione, 17-OH-Pg, and dehydroepiandrosterone sulfate (DHEAS) were assayed by

radioimmunoassay (Diagnostic Systems Laboratories, Webster, TX). Sex hormone–binding globulin (SHBG) levels were determined by immunoradiometric assay (Diagnostic Products). Serum free testosterone was calculated by the method of Sodergard et al [19] using a serum albumin concentration of 4.0 g/dL. Estradiol, progesterone, follicle-stimulating hormone, luteinizing hormone, thyroid-stimulating hormone, prolactin, total cholesterol, triglycerides, and high-density lipoprotein cholesterol (HDL-C) concentrations were measured by chemiluminescent immunoassay on an automated ADVIA Centaur analyzer (Bayer HealthCare, Toronto, Ontario, Canada). Low-density lipoprotein cholesterol was calculated using the Friedewald equation [20]. Fibrinogen was assayed by the modified Clauss technique using Behring Coagulation System kits (BCS, Newark, DE). Plasminogen activator inhibitor–1 and factor VIII levels were determined by enzyme-linked immunosorbent assay (Diagnostica Stago, Asnières-sur-Seine, France). Inter- and intraassay coefficients of variation were less than 7.5% for insulin, less than 10% for total testosterone, and less than 8.5% for all other steroid hormones.

2.4. DCI and MYO analyses

To analyze blood and urine inositol content, gas chromatography and mass spectrometry (GC/MS) were used. [$^2H_6$]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 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 [21]. Twenty-four–hour urinary clearance was calculated by dividing 24-hour urinary excretion by plasma concentration.

myo-Inositol is another inositol that may be implicated in insulin sensitivity, based on recent evidences [22-25]. Its urinary clearance may also serve as control for general inositol urinary clearance. Thus, uCl_{DCI} can be corrected for overall inositol urinary clearance by assessing the ratio of DCI to MYO urinary clearances (uCl_{DCI}/uCl_{MYO} ratio).

2.5. Statistical analyses

Results not normally distributed were log transformed to normalize their distribution for all statistical analyses and are reported herein back transformed in their original units (geometric means with 95% confidence interval). Other results are reported as means ± standard error of the mean (SEM). *P* values not exceeding .05 were considered significant for all analyses, which were performed using JMP 4.0 software (SAS Institute, Cary, NC).

The response of glucose and insulin after the oral administration of glucose was analyzed by calculating the areas under the corresponding response curves (AUCs) by the trapezoidal rules using absolute values. The primary analyses of interest of this study were correlations between parameters of inositol metabolism and measures of insulin sensitivity or levels, which were performed using Pearson correlation tests. Continuous variables were compared between control men and brothers of PCOS women using 2-tailed unpaired Student *t* tests. Variable differences between groups were corrected for anthropometric discrepancies, ie, BMI, using multiple linear regression analyses. Differences were not further adjusted for WC and fat percentage (fat%) because of colinearity with BMI (Spearman $r = 0.93$ and 0.92, respectively). Significant results are reported in Table 1.

To determine the best independent predictors of uCl_{DCI} in our population, we performed a manual stepwise regression analysis using all variables of Table 1 that were associated with uCl_{DCI} with a *P* value not exceeding .10, except for WC that was collinear with BMI. These variables were entered successively in the model (forward method) based on the next lowest *P* value, with all appropriate interactions. At each step, parameters that did not contribute significantly to the model were then successively excluded. Because fasting insulin levels

Table 1
Clinical and laboratory characteristics

Characteristics	Brothers (n = 11)	Controls (n = 21)	*P* value[c]
Age (y)	30.6 ± 2.5	30.3 ± 1.7	.91
BMI (kg/m^2)	30.1 ± 1.3	26.3 ± 0.9	.02
WC (cm)	99 ± 4	90 ± 3	.06
Fat% (%)	27.9 ± 2.2	21.8 ± 1.6	.03
Systolic BP (mm Hg)	135 ± 4	124 ± 3	.03
Diastolic BP (mm Hg)	79 ± 3	71 ± 2	.03
Calculated free testosterone (pmol/L)[a,b]	464 (369-585)	476 (403-563)	.86
DHEAS (μmol/L)[a]	7.5 (5.1-10.9)	6.6 (5.0-8.6)	.58
SHBG (nmol/L)	17.9 ± 3.0	25.8 ± 2.2	.04
Triglycerides (mmol/L)[a]	1.67 (1.07-2.61)	1.11 (0.80-1.53)	.14
HDL-C (mmol/L)	1.12 ± 0.09	1.24 ± 0.07	.28
Fibrinogen (g/L)	3.19 ± 0.19	2.86 ± 0.14	.16
PAI-1[a]	34 (23-50)	16 (12-22)	.005*
Factor VIII[a]	1.15 ± 0.07	1.08 ± 0.05	.38
Fasting glucose (mmol/L)	5.2 ± 0.2	4.8 ± 0.1	.05
$AUC_{glucose}$ (mmol min/L)[a]	1,002 ± 49	775 ± 34	<.001[†]
Fasting insulin (pmol/L)	68 (46-100)	53 (40-70)	.31
$AUC_{insulin}$ (μmol min/L)[a]	79 (58-108)	36 (29-45)	<.001[†]
M-value (μmol/kg min)	23.9 ± 4.3	42.3 ± 3.1	.001*

Mean ± SEM, except when specified otherwise. To convert values of free testosterone to nanograms per deciliter, divide by 34.7; DHEAS to micrograms per deciliter, divide by 0.027; SHBG to micrograms per deciliter, divide by 34.7; triglycerides to milligrams per deciliter, divide by 0.0113; cholesterol to milligrams per deciliter, divide by 0.0259; glucose to milligrams per deciliter, divide by 0.0556; and insulin to micro–international units per milliliter (in micro–international units minute per milliliter for $AUC_{insulin}$), divide by 6.945.

[a] Log transformed for analyses. Results are expressed as geometrical means with 95% confidence intervals.

[b] Free testosterone was calculated by the method of Sodergard et al [19].

[c] Two-tailed unpaired *t* tests.

* $P < .04$ after adjustment for BMI.

[†] $P < .005$ after adjustment for BMI.

were highly associated with uCl_{DCI}, we sought to determine if measures of inositol metabolism were associated with fasting insulin independently of all other traditional factors. Thus, we performed another stepwise analysis using the same variables plus all measures reported in Table 2, except for uCl_{DCI}/uCl_{MYO} ratio that was collinear with uCl_{DCI}. For this stepwise analysis, only interactions with a partial P value less than .01 were kept in the model to limit the total number of parameters analyzed. No more than 5 parameters were tested at each step for both stepwise analyses.

Finally, to appreciate the interaction between BMI and fasting insulin levels that was identified in the first stepwise analysis, subgroup analyses by BMI status were performed using BMI not exceeding 27.5 kg/m^2 or greater than 27.5 kg/m^2 (median of BMI for all subjects).

3. Results

3.1. Clinical and laboratory characteristics (Table 1)

Eleven brothers of women with PCOS and 21 healthy control men were studied. All were white French Canadian or of European origin. The 2 groups were comparable for age; but brothers were heavier (P = .02), with increased WC, although this was not significant (P = .06), and higher total fat% (P = .03) and diastolic and systolic BPs (Ps = .03 for both).

Regarding sex steroids, free testosterone concentrations were similar between groups; but SHBG levels were reduced by 30% in brothers of PCOS women (P = .04). No significant differences were found between groups for the levels of triglycerides, HDL-C, factor VIII, and fibrinogen. However, PAI-1 levels were more than doubled in brothers as compared with controls (P = .005). This difference remained significant after correction for BMI (P = .037). Fasting levels of glucose were borderline increased in brothers (P = .05). Fasting insulin levels were not significantly different between groups, but brothers displayed significantly higher glucose-stimulated insulin ($AUC_{insulin}$) and glucose ($AUC_{glucose}$) levels (Ps < .001 for both), even after adjustment for anthropometric variables (P = .003 and .004, respectively). Finally, insulin sensitivity (M-value) was reduced by 43% in brothers as compared with controls (P = .001) and remained significantly decreased after correction (P = .014).

Table 2
Levels and 24-hour urinary excretion of inositols

Characteristics	Brothers (n = 11)	Controls (n = 21)	P value[b]
Plasma DCI (μmol/L)[a]	0.73 (0.32-1.67)	0.22 (0.12-0.40)	.02
DCI excretion (μmol/d)[a]	4.0 (0.9-17.0)	2.6 (0.9-7.3)	.61
DCI clearance (mL/min)[a]	3.8 (0.9-16.3)	8.0 (2.8-23.1)	.40
Plasma MYO (μmol/L)[a]	22.4 (18.4-27.4)	22.5 (19.5-26.0)	.97
MYO excretion (μmol/d)[a]	121 (68-216)	85 (56-130)	.33
MYO clearance (mL/min)[a]	4.9 (2.6-9.1)	3.0 (1.9-4.7)	.21
Cl_{DCI}/Cl_{MYO} ratio[a]	0.78 (0.26-2.33)	2.67 (1.20-5.91)	.07

Mean ± SEM, except when specified otherwise.

[a] Log transformed for analyses. Results are expressed as geometrical means with 95% confidence intervals.

[b] Two-tailed unpaired t tests.

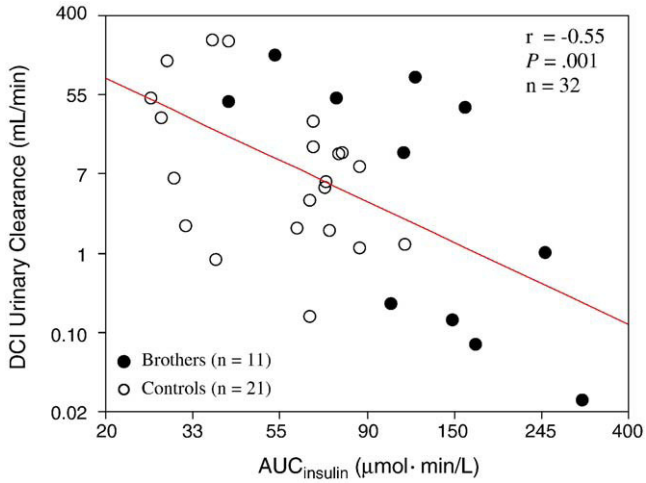

Fig. 1. Linear correlation between 24-hour uCl_{DCI} and the AUC during OGTT. Open circles correspond to healthy control men; and closed circles, to brothers of PCOS women.

3.2. Plasma levels and urinary excretion of DCI and MYO (Table 2)

The levels of plasma DCI were more than 3 times higher in the group of brothers (P = .02), but this difference was no longer significant after correction for BMI. Although uCl_{DCI} was decreased by more than 2-fold in brothers and the uCl_{DCI}/uCl_{MYO} ratio was decreased by more than 3-fold, they did not reach statistical significance because of the high variability of these measurements. Finally, plasma MYO levels and urinary clearance were not significantly different between groups.

3.3. Correlations between insulin sensitivity or levels and DCI clearance (Fig. 1)

Using Pearson correlation tests, it was determined that insulin sensitivity (M-value) was not significantly associated with uCl_{DCI} in all men together (P = .09), as well as within each group. However, uCl_{DCI} was significantly and highly correlated with insulin levels for all men and within each group. Urinary clearance of DCI was negatively associated with $AUC_{insulin}$ ($r = -0.55$, $P = .001$) (Fig. 1) and, to the same degree, with fasting insulin levels ($r = -0.56$, $P < .001$). These associations tended to be more pronounced in brothers, but there was no significant interaction between group status and $AUC_{insulin}$. Therefore, these correlations were not driven by the effects in only one of the groups. Urinary clearance of DCI was correlated both with $AUC_{insulin}$ ($r = -0.70$, $P = .02$) and fasting insulin levels ($r = -0.65$, $P = .03$) in brothers as well as in controls ($AUC_{insulin}$: $r = -0.46$, $P = .04$; fasting insulin levels: $r = -0.52$, $P = .02$).

Similar negative associations were also found between insulinemia and the uCl_{DCI}/uCl_{MYO} ratio. For all men, the ratio was significantly associated both with $AUC_{insulin}$ ($r = -0.52$, $P = .001$) and fasting insulin levels ($r = -0.51$, $P = .003$). The ratio was also associated with insulinemia in brothers ($AUC_{insulin}$: $r = -0.68$, $P = .02$; fasting insulin: $r = -0.58$, $P = .06$) and control men ($AUC_{insulin}$: $r = -0.21$, $P = .36$; fasting insulin: $r = -0.48$, $P = .03$).

3.4. Stepwise multivariate analyses

To determine the factors that were best independently associated with DCI clearance, the following variables were considered for manual forward stepwise analysis, with all possible associations, in this order: fasting insulin ($P = .001$), $AUC_{insulin}$ ($P = .001$), SHBG ($P = .04$), WC ($P = .06$), diastolic BP ($P = .07$), BMI ($P = .09$), and *M*-value ($P = .09$). This analysis revealed that the best model to predict uCl_{DCI}, with an R^2 of 48.7% and a *P* value less than .001, included fasting insulin (negative correlation, partial $P = .001$) and the interaction between fasting insulin and BMI (partial $P = .005$).

To illustrate the interaction between BMI and fasting insulin in this model, all men were classified as overweight (BMI $\geq$27.5 kg/m^2) or nonoverweight (BMI <27.5 kg/m^2) based on the median of BMI. This subgroup analysis showed that the association between fasting insulin and uCl_{DCI} was stronger in overweight men ($r = -0.66$, $P = .006$, n = 16) as compared with nonoverweight men ($r = -0.54$, $P = .03$, n = 16).

Given the magnitude of the association between uCl_{DCI} and fasting insulin, a second stepwise analysis was performed to determine if measures of inositol metabolism were significant predictors of insulinemia independently of typical factors. For this analysis, the following predictors were considered: DCI clearance ($P < .001$), fat% ($P = .004$), WC ($P = .004$), BMI ($P = .005$), triglycerides ($P = .001$), fibrinogen ($P = .02$), diastolic and systolic BP ($P = .03$ and .04, respectively), MYO clearance ($P = .04$), and fasting glucose ($P = .05$). After stepwise analysis, the best model to predict hyperinsulinemia (fasting insulin), with an R^2 of 44.0% and a *P* value less than .001, included uCl_{DCI} (partial $P = .004$) and fat% (partial $P = .02$). It should be noted that fat% is colinear with BMI and WC ($r = 0.92$ and 0.95, respectively), such that fat% could be replaced by any of these factors in the model with similar effects.

4. Discussion

To determine if men display a significant relationship between DCI metabolism and IR or levels, as previously shown in women with or without PCOS [11], plasma levels and 24-hour urinary clearances of DCI and MYO were assessed in men with a wide range of IR or levels, that is, PCOS brothers and control men. Baseline characteristics showed that brothers were heavier and fatter with higher BPs. They were also characterized by dyscoagulability, decreased glucose tolerance, hyperinsulinemia, and IR; and these differences persisted after correction for BMI (that was colinear with WC and fat%). These findings are comparable with the results of the entire cohort that were previously reported by our group [15]. Thus, we have identified a population of nondiabetic young men who were more insulin resistant and hyperinsulinemic than expected for their adiposity.

The PCOS brothers were also found to have more than 3-fold higher plasma DCI levels than controls ($P = .02$), with a more than 3-fold decrease in the ratio of urinary DCI to MYO clearances, which tended to be significant ($P = .06$). There was no significant change in other DCI and MYO parameters. Thus, these results suggest that hyperinsulinemic brothers of women with PCOS might be characterized by a specific decrease in DCI urinary clearance compared with MYO clearance.

An even more important and significant finding of our study is a strong negative association between insulinemia and uCl_{DCI}, both crude and corrected for MYO clearance using the uCl_{DCI}/uCl_{MYO} ratio. Interestingly, 50% of the variability of uCl_{DCI} was explained by insulin levels and its interaction with weight. Moreover, the best independent predictors of hyperinsulinemia in our study were low uCl_{DCI} and high fat% (or BMI or WC), which explained 44% of fasting insulin level variability. Thus, uCl_{DCI} was more strongly associated with hyperinsulinemia than some classic factors, such as BP, fibrinogen, and fasting glucose. Altogether, these findings suggest a strong interrelation between hyperinsulinemia and low DCI urinary clearance in men with or without a PCOS first-degree relative.

The results of this study also demonstrated that there was a significant modifying effect of weight on the relationship between insulin levels and uCl_{DCI}, such that hyperinsulinemia was more strongly related to low uCl_{DCI} in overweight than in nonoverweight men. Moreover, this interaction and fasting insulin levels were the 2 best independent predictors of uCl_{DCI} in all men. Indeed, only about half of the variability of uCl_{DCI} was not explained by these parameters. Thus, in men, there is a strong dependence of DCI urinary clearance on insulin level, which is synergistically increased by the development of obesity.

In addition to our previously published studies in women with PCOS [11,23], a total of 5 studies assessed DCI urinary excretion in various conditions associated with IR and compensatory hyperinsulinemia, that is, diabetes, impaired glucose tolerance, or familial history of type 2 diabetes mellitus [9,21,26,27]. Most of these studies reported decreased urinary excretion of DCI in conditions associated with IR [9,26,27], as opposed to increased DCI excretion found in subjects with type 2 diabetes mellitus by Ostlund et al [21] and in our populations of PCOS women [11,23]. Studies from Ostlund et al [21] and Campell et al [28] used the same GC/MS inositol assay methodology as ours. Other studies relied on methods requiring quantitative recovery

through several purification steps, without an internal recovery standard [9,26]. This may have introduced measurements errors, as previously discussed [21,29], and could explain in part discrepancies in study results.

Urinary excretion depends on renal handling of inositols as well as plasma levels, which are quite variable, whereas urinary clearance reflects only renal metabolism of DCI. Competition between renal excretion of glucose and DCI also confounds interpretation of the studies with diabetic subjects [21]. Indeed, Osltund et al [21] found that DCI urinary excretion was highly correlated with plasma and urinary glucose levels in diabetic subjects and that insulin therapy for poorly controlled diabetic subjects reduced uCl_{DCI} by 63% and increased plasma DCI by 8.8-fold. Thus, to assess the relationship of insulin or IR with DCI metabolism, it is preferable to assess DCI urinary clearance in groups with normal glucose control using GC/MS inositol assay methodology with appropriate deuterated internal standards, as performed in this study and our previously published articles in PCOS [11,23].

Unexpectedly, our findings were in the opposite directions to our hypothesis and the findings we previously reported in American nondiabetic women with or without PCOS [11]. In these women, we found a significant correlation between hyperinsulinemia, as well as IR, with high uCl_{DCI}, not low! In our French Canadian men with or without PCOS heredity, correlations were stronger with insulin levels than insulin sensitivity, as opposed to American women, and were considerably stronger than those in American women, with or without PCOS (for $AUC_{insulin}$, $r = -0.55$ and $+0.36$, respectively). Finally, insulin levels were almost twice as high in our men compared with American women ($AUC_{insulin}$ = 6.8 and 3.5 mIU min/mL, respectively). Of note, we recently reported that Greek women with or without PCOS display high uCl_{DCI} in association with hyperinsulinemia, independently of obesity [23]. This last study confirmed our previous findings in a population with a different ethnic background. Insulin levels were also similarly low in Greek ($AUC_{insulin}$ = 3.1 mIU min/mL) as in American women.

Therefore, the observed sex discrepancy regarding DCI metabolism may be explained by sex dimorphism due to genetic or hormonal factors. Indeed, urinary DCI excretion was higher in a healthy population of older men as compared with older women [28], using the same methodology as our studies. The discrepancy may also arise from diet differences, or it could be explained solely by the modifying effects of different insulin levels. Indeed, it is possible that high uCl_{DCI} contributes to IR only when insulin levels are below some threshold, as in American women. Above this renal threshold, insulin might progressively decrease renal clearance of DCI to retain more plasma DCI and improve DCI availability for the generation of DCI-IPG. This, in turn, would partially compensate for IR. At higher insulin levels, this direct adaptive effect of insulin would mask the intrinsic variability of uCl_{DCI}, which is no longer positively associated with IR and insulin levels. This hypothesis could explain the stronger relationship between hyperinsulinemia and uCl_{DCI} observed in our male subjects as compared with American women, as well as the greater dependence of uCl_{DCI} on insulin levels that we found in men.

Despite highly significant and consistent results, based on different types of analyses, that is, crude, correlation, and multivariate analyses, our study has some limits. First, the number of brothers of PCOS women is relatively small. Type-2 errors are therefore possible for nonsignificant differences between groups. Furthermore, this is a fair number of subjects considering that we performed OGTTs and complex clamp techniques in healthy young volunteers, especially for PCOS brothers who were recruited from a highly selected population. Moreover, because we ascertained normal distribution, significant differences are valid and unlikely due to chance. However, we acknowledge that our provocative findings merit confirmation with another larger study. Second, cases and controls were not matched for adiposity (BMI for example), such that an effect of obesity cannot be completely excluded. However, the primary analyses of interest of our study were correlations in the entire group, not comparisons between groups. Third, because only PCOS brothers and healthy men were studied, inference to men with compensatory hyperinsulinemia of other origins would be only speculative. Finally, the cross-sectional design of this study does not allow to determine if significant correlations reflect causative links or only associations.

In conclusion, results of the present pilot study demonstrated that urinary clearance of DCI is inversely associated with hyperinsulinemia in all men. Moreover, when corrected for MYO clearance, uCl_{DCI} tends to be reduced in brothers of PCOS women with increased plasma DCI levels. It was also found that weight synergistically increases the relationship between hyperinsulinemia and low uCl_{DCI} and that men uCl_{DCI} is dependent by 50% on hyperinsulinemia (and its interaction with weight). Thus, in men, hyperinsulinemia due to IR might suppress renal clearance of DCI to increase plasma DCI levels and partially compensate for IR by improving DCI availability. The apparent discrepancy with previous findings in women, with or without PCOS, may be explained by higher insulin levels in men as compared with women and requires confirmation.

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