Calciotropic hormones, insulin resistance, and the polycystic ovary syndrome

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Objective: To investigate whether abnormalities in serum concentrations of 1,25-dihydroxyvitamin D [1,25(OH)2D], 25-hydroxyvitamin D [25(OH)D], parathyroid hormone (PTH), calcium, and phosphorus were associated with risk of polycystic ovary syndrome (PCOS) and obesity. The possible correlations of the calciotropic hormones with insulin resistance were also examined.

Design: Case-control study.

Setting: Department of Genetics, Royan Institute.

Patient(s): Eighty-five women with PCOS and 115 control women were recruited.

Intervention(s): None.

Main Outcome Measure(s): Serum levels of glucose, insulin, total calcium, phosphorus, PTH, 25(OH)D, and 1,25(OH)2D were measured in all 200 subjects.

Result(s): The presence of PCOS had age- and body mass index (BMI)–independent positive effects on serum phosphorus, PTH, 25(OH)D, and insulin concentrations as well as on insulin resistance. Furthermore, overweight/obese (BMI ≥ 25 kg/m2) women with PCOS had significantly decreased levels of 1,25(OH)2D and glucose compared with normal-weight (BMI < 25 kg/m2) women with PCOS. In women with PCOS, phosphorus was correlated negatively with insulin and insulin resistance and positively with 1,25(OH)2 D. In addition, in normal-weight patients, PTH correlated positively with insulin and insulin resistance.

Conclusion(s): It is possible that elevated levels of phosphorus and PTH in women with PCOS, at least in part, through their effects on insulin levels and insulin resistance, are involved in pathogenesis of the syndrome. (Fertil Steril® 2009;: : – : ©2009 by American Society for Reproductive Medicine.)

Key Words: Polycystic ovary syndrome, insulin resistance, phosphorus, parathyroid hormone, vitamin D

Polycystic ovary syndrome (PCOS) is one of the most common endocrinopathies, affecting approximately 6.5% of reproductive-age women (1, 2). In addition to hyperandrogenism, menstrual disturbance, and anovulatory infertility, PCOS patients demonstrate an increased prevalence of insulin resistance, hyperinsulinemia, and obesity (3–5). Obesity, insulin resistance, and hyperinsulinemia are associated with alterations in the hormones related to calcium homeostasis. It is known that obese individuals have lower serum levels of 25-hydroxyvitamin D [25(OH)D] and higher parathyroid hormone (PTH) concentrations than do nonobese control individuals (6–8). Moreover, circulating 25(OH)D levels correlate inversely with serum glucose level, insulin resistance, and prevalence of diabetes (9–13). Recently, it has been reported that even in women with PCOS, serum concentrations of 25(OH)D are inversely correlated with obesity and insulin resistance (14). In one report (15), a significantly higher serum Ca2+/Mg2+ ratio in women with PCOS compared with control women was found. Recent studies have shown associations between calciotropic hormones 25(OH)D and PTH with hirsutism and PCOS phenotypes; a negative association between serum 25(OH)D levels and hirsutism (16) and a positive association between serum concentrations of PTH and PCOS (7) have been found. Accordingly, the present study investigated whether abnormalities in serum concentrations of 1,25-dihydroxyvitamin D [1,25(OH)2D], 25(OH)D, PTH, calcium, and phosphorus were associated with risk of PCOS, insulin resistance, and obesity phenotypes. In addition, the possible relationships of the calciotropic hormones with serum insulin levels were evaluated.

MATERIALS AND METHODS

Participants

The experimental population consisted of 85 women with PCOS (age range 20–40 years) and 115 healthy women (age range 18–47 years) as control subjects reporting to the Royan Institute. Diagnosis of PCOS was based on the National Institute of Child Health and Human Development criteria. These criteria are: 1) the presence of menstrual dysfunction, i.e., oligomenorrhea (fewer than six menstrual
periods in the preceding year) or amenorrhea (absence of periods for more than 6 months); 2) clinical hyperandrogenism (i.e., hirsutism: Ferriman-Gallwey score >6) and/or hyperandrogenemia; and 3) the exclusion of related disorders such as nonclassic congenital adrenal hyperplasia, androgen secreting tumors, Cushing syndrome, and hyperprolactinemia (17).

The control group came from healthy blood donors who were randomly selected from community volunteers. The control subjects had normal menstrual cycles and proven fertility, and none of them had clinical evidence of hyperandrogenism. Both patients and healthy control subjects were enrolled in the study between October and March 2006, and all of them were Iranian and genetically unrelated. None of the subjects was taking drugs known to affect calcium metabolism for at least 3 months before each study. All subjects provide informed consent to the study, and the Institutional Review Boards of the Royan Institute and the Ethical Committee of the Institute reviewed and approved the study. The body mass index (BMI) of each subject was calculated as weight/height-squared (kg/m²), and the 200 subjects were phenotyped based on the diagnosis of PCOS and BMI values as follows: normal-weight (BMI \(< 25 \text{ kg/m}^2\)) control subjects (n = 49); overweight/obese (BMI \(\geq 25 \text{ kg/m}^2\)) control subjects (n = 66); normal-weight women with PCOS (n = 29); and overweight/obese women with PCOS (n = 56).

Biochemical Measurements

Studies were performed between days 2 and 6 of a menstrual cycle in the control subjects or during a spontaneous bleeding episode or progestin-induced menstrual cycle in the PCOS patients. Blood samples were collected after an overnight fast and were immediately centrifuged, and sera were separated and frozen at \(-20^\circ\text{C}\) until assayed. In all 200 women, serum concentrations of glucose, insulin, total calcium, phosphorus, intact PTH (iPTH), 25(OH)D, and 1, 25(OH)₂ D were measured. Insulin resistance was measured by the homeostasis model assessment (HOMA-IR) method: [fasting insulin (μIU/mL) \times fasting glucose (mg/dL)]/405 (18).

Serum concentrations of the two vitamin D metabolites, 25(OH)D and 1,25(OH)₂ D, were determined by RIA method, and PTH concentrations were determined by an immunoradiometric assay (IRMA) (DRG Instruments, Marburg, Germany). Serum insulin levels were measured by IRMA (BioSource Europ, Nivelles, Belgium). Serum glucose, calcium, and phosphorus measurements were performed using standard methods (CinnaGen, Tehran, Iran). The intra- and interassay coefficients of variation, respectively, were 2.2% and 6.5% for insulin, 5.7% and 4.5% for PTH, 6.4% and 7.1% for 25(OH)D, and 12.4% and 13.8% for 1,25(OH)₂ D.

Statistical Methods

Unpaired Student t test, analysis of variance, or analysis of covariance were used to compare the different groups of women studied when appropriate. To examine the normality of distribution, the Kolmogorov-Smirnov goodness-of-fit test was used and, where necessary, log transformation was performed. Because the values of HOMA-IR, serum glucose, insulin, phosphorus, PTH, 25(OH)D, and 1,25(OH)₂ D were skewed, they were log transformed for the analysis and presented as the geometric mean (geometric standard error of the mean). Correlations between parameters were computed

<table>
<thead>
<tr>
<th>Variable</th>
<th>Control (n = 115)</th>
<th>PCOS (n = 85)</th>
<th>P₁ (^a)</th>
<th>P₂ (^b)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yrs)</td>
<td>29.83 (0.67)</td>
<td>28.60 (0.50)</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>26.32 (0.45)</td>
<td>27.64 (0.52)</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>HoMA-IR (^c)</td>
<td>2.91 (1.12)</td>
<td>4.03 (1.09)</td>
<td>.021</td>
<td>.027</td>
</tr>
<tr>
<td>Glucose (^c) (mg/dL)</td>
<td>92.05 (1.02)</td>
<td>90.79 (1.02)</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Insulin (^c) (μIU/mL)</td>
<td>12.83 (1.11)</td>
<td>18.03 (1.09)</td>
<td>.011</td>
<td>.017</td>
</tr>
<tr>
<td>Calcium (mg/dL)</td>
<td>9.41 (0.98)</td>
<td>9.67 (0.11)</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Phosphorus (^c) (mg/dL)</td>
<td>3.19 (1.02)</td>
<td>3.58 (1.08)</td>
<td>.002</td>
<td>.002</td>
</tr>
<tr>
<td>iPTH (^c) (pg/mL)</td>
<td>16.19 (1.08)</td>
<td>22.32 (1.10)</td>
<td>.010</td>
<td>.016</td>
</tr>
<tr>
<td>25(OH)D (^c) (ng/mL)</td>
<td>19.40 (1.13)</td>
<td>29.32 (1.10)</td>
<td>.008</td>
<td>.013</td>
</tr>
<tr>
<td>1,25(OH)₂ D (^c) (pg/mL)</td>
<td>30.26 (1.10)</td>
<td>32.74 (1.07)</td>
<td>NS</td>
<td>NS</td>
</tr>
</tbody>
</table>

\(\text{Note: Values are mean (SE). Values for HoMA-IR and serum glucose, insulin, phosphorus, PTH, 25(OH)D, and 1,25(OH)₂ D are geometric mean (geometric SEM). 1,25(OH)₂ D = 1,25-dihydroxyvitamin D; 25(OH)D = 25-hydroxyvitamin D; BMI = body mass index; HoMA-IR = homeostasis model assessment insulin resistance; iPTH = intact parathyroid hormone; NS = not significant.} \)

\(^a\) Unadjusted P values.

\(^b\) Adjusted P values for age and BMI.

\(^c\) Significance was tested on log-transformed values.

by using Pearson correlation coefficients. Independent relationships were carried out using partial correlation analysis. Data were analyzed using SPSS software (version 15.0; SPSS, Chicago, IL). In all of the statistical tests, the difference was considered to be significant at $P < .05$. 

RESULTS

The clinical and endocrine characteristics of the women studied and their statistical significance are summarized in Table 1. The presence of PCOS had significant positive effects on serum phosphorus, PTH, 25(OH)D, and insulin concentrations as well as on HOMA-IR (Figs. 1A–1C). The effects of PCOS on these variables remained significant after adjustment for age and BMI. Furthermore, the increases of PTH and 25(OH)D in women with PCOS were independent of calcium, phosphorus, and 1,25(OH)₂D as well as of each other’s serum levels. In addition, the positive effect of PCOS on serum levels of phosphorus remained significant after adjustment for calcium, PTH, 25(OH)D, 1,25(OH)₂D, and insulin.

Selected characteristics of the study population by PCOS diagnosis and BMI status are compared in Table 2. Overweight/obese women with PCOS had significantly decreased levels of 1,25(OH)₂D ($P = .035$) and glucose ($P = .044$) compared with normal-weight women with PCOS (Fig. 1D). These differences remained significant after adjustment for age, calcium, phosphorus, PTH, 25(OH)D, and insulin.

As shown in Figure 2, calculation of the Pearson coefficients showed that in women with PCOS, the concentrations of phosphorus were negatively correlated with insulin ($r = -0.370; \ P < .001$) and HOMA-IR ($r = -0.352; \ P = .001$) and positively with 1,25(OH)₂D ($r = 0.216; \ P = .047$). Also, partial correlation analysis indicated that these correlations were age-, BMI-, calcium-, PTH-, 25(OH)D-, and insulin-independent. In the normal-weight patients, PTH correlated positively with insulin ($r = 0.530; \ P = .003$) and HOMA-IR ($r = 0.509; \ P = .005$). These correlations remained significant after adjusting for age, calcium, phosphorus, 25(OH)D, 1,25(OH)₂D, and insulin. Furthermore, in these women 1,25(OH)₂D correlated positively with age ($r = 0.462; \ P = .012$), insulin ($r = 0.404; \ P = .030$), and HOMA-IR ($r = 0.377; \ P = .048$), but the two latter correlations were age dependent.

DISCUSSION

The results of the present study not only demonstrated a significant direct association of serum PTH, phosphorus, and 25(OH)D with PCOS, but also suggested a significant
negative correlation between serum levels of phosphorus with insulin and insulin resistance in women with PCOS and a significant positive correlation between PTH with serum insulin and insulin resistance in the normal-weight patients. The present data also indicated that overweight/obese women with PCOS had significantly decreased levels of 1,25(OH)2D and glucose compared with normal-weight women with PCOS.

These data are in agreement with results obtained by Panidis et al. (7) showing a significantly higher serum levels of PTH in women with PCOS compared with control women. They also demonstrated that PTH probably is linked to PCOS-associated hyperandrogenemia by means of BMI-independent mechanisms. Furthermore, Panidis et al. observed an overall trend for increased vitamin D levels in the syndrome. In the present study, women with PCOS had significantly increased concentrations of 25(OH)D compared with control women, which to our knowledge has not been reported previously. The increases of PTH and 25(OH)D levels observed in PCOS seem to be independent of each other’s serum levels, and increased PTH concentrations can not be attributed to relative deficits in bioavailable vitamin D. On the other hand, in an earlier report, PCOS-associated signs of hyperandrogenism and menstrual disturbances were attenuated after administration of high doses of vitamin D (19). It is likely that 25(OH)D levels increase to compensate for insulin resistance (20).

Our findings are consistent with earlier studies (9, 21, 22) that suggested possible interactions between insulin resistance and PTH and vitamin D levels. In the present study, age-, BMI-, calcium-, phosphorus-, 25(OH)D-, and 1,25(OH)2D-independent positive correlations between PTH concentrations and insulin levels and insulin resistance in normal-weight women with PCOS were found, which to our knowledge have not been reported previously. On the other hand, earlier studies have demonstrated positive correlations between total serum calcium with insulin levels and insulin resistance (23) and fasting serum glucose (20, 23) in the larger healthy population. Furthermore, insulin secretion is a calcium-dependent process (24, 25) and 1,25(OH)2D likely acts on insulin secretion by the significant rise in intracellular ionic calcium levels following 1,25(OH)2D-stimulated secretion of insulin by islet cells (26, 27). In addition, calciotropic hormone 1,25(OH)2D, by binding to its receptor, stimulates the expression of insulin receptor gene (28, 29).

Another interesting finding which has not been reported previously was that in women with PCOS, serum levels of phosphorus were negatively correlated with insulin and insulin resistance and positively with 1,25(OH)2D, independent of age, BMI, calcium, PTH, and 25(OH)D. In earlier studies, a direct effect of phosphorus on PTH (30, 31) and 1,25(OH)2D production have been demonstrated (32). Furthermore, hyperphosphatemia leads to a decrease in serum levels of calcium and to altered synthesis and secretion of PTH (33).

Earlier studies have also shown that estrogens and androgens directly or indirectly affect vitamin D signaling pathways (34) and vice versa (35–39). 1,25-Dihydroxyvitamin D stimulates activity of aromatase, an enzyme involved in conversion of androgens to estrogens in granulosa cells, human prostate cancer cells (35), and osteoblasts (36), down-regulates estrogen receptor abundance and suppresses estrogen actions in human breast cancer cells (37), and up-regulates androgen receptor gene expression in prostate cancer cells (38, 39). Thus, vitamin D, through its influences on balance between androgens with estrogens, may have a role in pathophysiologic mechanisms of PCOS.

**TABLE 2**

<table>
<thead>
<tr>
<th>Variable</th>
<th>Control</th>
<th></th>
<th>PCOS</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Normal</td>
<td>Overweight/obese</td>
<td>Normal</td>
<td>Overweight/obese</td>
</tr>
<tr>
<td></td>
<td>weight</td>
<td>(n = 49)</td>
<td>weight</td>
<td>(n = 29)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(n = 66)</td>
<td>(n = 56)</td>
<td></td>
</tr>
<tr>
<td>Age (yrs)</td>
<td>28.61(0.86)</td>
<td>30.73(0.98)</td>
<td>NS</td>
<td>28.52(0.83)</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>22.58(0.24)</td>
<td>29.10(0.54)</td>
<td>—</td>
<td>23.06(0.29)</td>
</tr>
<tr>
<td>HoMA-IRa</td>
<td>2.61(1.19)</td>
<td>3.16(1.15)</td>
<td>NS</td>
<td>3.95(1.20)</td>
</tr>
<tr>
<td>Glucose[mg/dL]</td>
<td>90.22(1.04)</td>
<td>93.42(1.03)</td>
<td>NS</td>
<td>95.69(1.04)</td>
</tr>
<tr>
<td>Insulin[µIU/mL]</td>
<td>11.74(1.19)</td>
<td>13.71(1.14)</td>
<td>NS</td>
<td>16.77(1.19)</td>
</tr>
<tr>
<td>Calcium (mg/dL)</td>
<td>9.25(0.15)</td>
<td>9.52(0.13)</td>
<td>NS</td>
<td>9.66(0.18)</td>
</tr>
<tr>
<td>Phosphorus[mg/dL]</td>
<td>3.40(1.09)</td>
<td>3.22(1.07)</td>
<td>NS</td>
<td>3.74(1.10)</td>
</tr>
<tr>
<td>iPTH[pg/mL]</td>
<td>14.80(1.13)</td>
<td>17.31(1.11)</td>
<td>NS</td>
<td>20.39(1.17)</td>
</tr>
<tr>
<td>25(OH)Da[ng/mL]</td>
<td>16.41(1.19)</td>
<td>21.97(1.18)</td>
<td>NS</td>
<td>23.57(1.20)</td>
</tr>
<tr>
<td>1,25(OH)2Da[pg/mL]</td>
<td>29.42(1.14)</td>
<td>30.90(1.15)</td>
<td>NS</td>
<td>39.80(1.11)</td>
</tr>
</tbody>
</table>

Note: Values are mean (SE). Values for HOMA–IR and serum glucose, insulin, phosphorus, iPTH, 25(OH)D, and 1,25(OH)2D are geometric mean (geometric SEM). Abbreviations as in Table 1.

a Significance was tested on log-transformed values.

Another possible pathway by which vitamin D may influence PCOS risk is through apoptosis. Although the physiologic effects of vitamin D in the ovary are poorly understood, some experimental studies have shown that 1,25(OH)2D and calcium might be necessary for normal ovarian folliculogenesis. It is possible that loss of the apoptotic mechanism or overexpression of antiapoptotic factors and down-regulation of apoptotic effectors in PCOS leads to the ovarian polycystic appearance (40–42). On the other hand, 1,25(OH)2D–vitamin D receptor complex was found to promote and/or prevent apoptosis as required for normal tissue development (43). In addition, regarding the role of calcium in oocyte maturation (44), it has been suggested that disordered calcium regulation may in part be responsible for disorders of oocyte development such as PCOS (19).

We agree with others (7, 45, 46) who have suggested a negative relationship between serum concentration of 1,25(OH)2D and adiposity. It is likely that reduction of serum 1,25(OH)2D levels in overweight/obese women with PCOS,
at least in part, results from their decreased secretion of growth hormone (47), because growth hormone significantly increases renal 1α-hydroxylase expression and serum 1,25(OH)2D concentrations (48).

In conclusion, the present data suggest that it is possible that elevated levels of phosphorus and PTH in women with PCOS, at least in part, through their effects on insulin levels and insulin resistance, are involved in pathogenesis of the syndrome. Furthermore, a review of earlier studies shows that there are some other probable pathways by which these hormones could affect PCOS risk. However, studies in other populations are warranted to confirm our findings.

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REFERENCES


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