Increased secretion of amylin in women with polycystic ovary syndrome

Summer James, M.D., Jennifer Moralez, M.D., and Manubai Nagamani, M.D.

Division of Reproductive Endocrinology, Department of Obstetrics and Gynecology, University of Texas Medical Branch, Galveston, Texas

Objective: To investigate amylin secretion in women with polycystic ovary syndrome (PCOS).

Design: Prospective, case–control study.

Setting: Academic institution.

Patient(s): Twenty women with PCOS and 10 with ovulatory cycles who matched for body mass index.

Intervention(s): An oral glucose tolerance test was performed, and glucose, insulin, and amylin levels were measured at fasting and after glucose ingestion. The area under the curve for insulin, amylin, and glucose was calculated. Ten women with PCOS were treated with metformin and 10 women with rosiglitazone for 6 months. Amylin levels were measured before and after treatment.

Result(s): Fasting amylin levels and amylin response to oral glucose were significantly increased in women with PCOS. At fasting, there was significant positive correlation between insulin and amylin levels both in women with PCOS and control subjects. After glucose ingestion, amylin response correlated with the glucose response in women with PCOS. Amylin levels decreased with metformin but not with rosiglitazone treatment.


Key Words: Amylin, PCOS, metformin, rosiglitazone

Amylin is a 37-amino-acid peptide hormone that is coproduced with insulin by the β cells of the pancreas (1, 2). Amylin is also referred to as islet amyloid peptide because it is the main component of the islet amyloid in patients with type 2 diabetes (3). Although insulin stimulates glucose disposal by increasing glucose utilization by peripheral tissues, amylin plays a role in glucose homeostasis by acting centrally to suppress glucagon secretion from the α cells, which in turn suppresses the release of endogenous glucose from the liver. Amylin also slows gastric emptying, thereby decreasing glucose absorption, centrally stimulating satiety, and reducing food intake. Amylin levels have previously been shown to be elevated in patients with obesity and with insulin resistance (4, 5). Amylin has been shown to be deficient in patients with diabetes. Amylin is thought to play a role in the development of diabetes by inducing apoptosis of β cells, which results in progressive glucose dysregulation (6, 7).

Polycystic ovary syndrome (PCOS) is one of the most common endocrine disorders, affecting 5%–10% of women of reproductive age (8, 9). They are at risk of developing type 2 diabetes, with 40% of women with PCOS progressing to impaired glucose tolerance or diabetes by age 40 years (10). To our knowledge, amylin levels in women with PCOS have not been investigated. The aim of this study was to evaluate amylin secretion in PCOS.

MATERIALS AND METHODS

Subjects

The study was performed at the University of Texas Medical Branch, a tertiary-care center in Galveston. The study was approved by the institutional review board of the University of Texas Medical Branch, and all women provided written, informed consent. The National Institutes of Health/National Institute of Child Health and Human Development criteria were used to make the diagnosis of PCOS (11). Twenty women with anovulatory cycles and evidence of hyperandrogenism were recruited for the study. Anovulation was established from menstrual history of oligomenorrhea (cycle length >45 days) or amenorrhea (cycle length >6 months). Clinical hyperandrogenism is defined as presence of hirsutism, acne, or signs of virilization. Hirsutism was assessed by Ferriman-Gallwey-Lorenzo scores, with patients having a score of >6 considered hirsute (12, 13). Patients with possible ovarian tumor (T >200 ng/dL) and nonclassic congenital adrenal hyperplasia (17α-hydroxyprogesterone levels >2 ng/mL) were excluded from the study. All women had polycystic ovaries (>12 cysts measuring 2–8 mm) on ultrasound examination. All women had normal TSH and PRL levels. Ten body mass index (BMI)-matched women who had
regular ovulatory cycles (midluteal P > 10 ng/mL) and no evidence of hyperandrogenism served as controls. Clinical characteristics of PCOS and control subjects are shown in Table 1.

Methods
After a high carbohydrate diet for 3 days, a 3-hour oral glucose tolerance test (OGTT) was performed in all PCOS and control subjects. Blood samples for glucose, insulin, and amylin were obtained while subjects were fasting and at 1, 2 and 3 hours after a 75-g oral glucose load. The area under the curve (AUC) for glucose, insulin, and amylin levels during the OGTT was calculated by the trapezoid method. To evaluate the effect of insulin-sensitizing medications on amylin secretion, fasting amylin levels were measured in 10 women with PCOS after treatment with metformin, 850 mg twice daily for 6 months, and 10 women treated with rosiglitazone, 4 mg daily for 6 months. Fasting amylin levels after treatment were compared with the levels before treatment.

Hormone Assays
Plasma amylin levels were measured by the ELISA method using a kit purchased from Linco Research (St. Charles, MO). This amylin ELISA is a monoclonal antibody–based sandwich immunoassay for determining amylin levels in human plasma. The antibody used is specific, with <1% cross-reactivity with glucagon, glucagon-like peptide 1, insulin, adrenomedullin, calcitonin, and calcitonin gene-related peptide. The sensitivity of the assay is 1 pM. Intra-assay variation of the assay was 2%, and interassay variation was 6%. High and low controls were run with each assay, and assays were accepted only if the controls were within the expected range. Insulin, total T, DHEAS, and 17α-hydroxyprogesterone levels were measured by specific RIA with kits purchased from Diagnostic Systems Laboratories (Webster, TX). Plasma glucose levels were measured by the glucose oxidase technique.

Statistical Analysis
Statistical analysis was performed using Sigmastat software (SPSS, Chicago, IL). Hormone levels in women with PCOS and controls were compared by unpaired Student’s t-test. Whenever the normality test or the equal variance test failed, the Mann-Whitney rank sum test was used. Levels of amylin and insulin before and after treatment were compared by paired t-test. Correlation between amylin levels and various parameters of insulin resistance was determined by Pearson correlation coefficients. Data are presented as mean ± SE, and P < .05 was considered statistically significant.

RESULTS
Fasting amylin levels were significantly elevated in women with PCOS (12.1 ± 2.4 pM) compared with controls (7.7 ± 0.7 pM; P < .001). Amylin response (AUC) during the OGTT for women with PCOS (91.2 ± 10.8 pM) was also significantly (P < .001) higher than in control women (28.6 ± 5.4 pM) (Fig. 1). Fasting insulin levels in women with PCOS (23.2 ± 3.8 μU/mL) were significantly increased compared with the control group (7.4 ± 0.6 μU/mL; P < .001). The AUC for insulin levels during the OGTT in women with PCOS was also significantly higher than that of control women (476.5 ± 71.5 μU/mL vs. 154.3 ± 17.8 μU/mL; P < .001), whereas the glucose levels were normal, indicating that the women with PCOS had insulin resistance (Table 1).

There was a significant positive correlation between amylin and insulin levels at fasting (r = 0.586, P < .02) both in women with PCOS and controls, but amylin did not correlate with fasting glucose levels. However, after glucose ingestion, amylin levels positively correlated with the glucose levels (r = 0.674, P < .01) (Fig. 2) but not with insulin levels in women with PCOS. In control subjects, amylin response during OGTT did not correlate with either insulin or glucose. Metformin treatment of patients with PCOS and insulin resistance resulted in a decrease in amylin levels (P < .01), whereas there were no significant changes in amylin levels with rosiglitazone treatment (Fig. 3).

### TABLE 1
Clinical and hormonal characteristics of women with PCOS and controls.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>PCOS (n = 20)</th>
<th>Controls (n = 10)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (y)</td>
<td>27.5 ± 1.3</td>
<td>35.6 ± 1.8</td>
<td>&lt; .01</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>36.3 ± 1.9</td>
<td>35.7 ± 1.6</td>
<td>NS</td>
</tr>
<tr>
<td>Total T (ng/dL)</td>
<td>68.9 ± 5.2</td>
<td>36.5 ± 3.6</td>
<td>&lt; .004</td>
</tr>
<tr>
<td>DHEAS (ng/mL)</td>
<td>1,839.1 ± 175.7</td>
<td>850.2 ± 191.2</td>
<td>&lt; .02</td>
</tr>
<tr>
<td>Fasting insulin (μU/mL)</td>
<td>23.2 ± 3.8</td>
<td>7.4 ± 0.6</td>
<td>&lt; .001</td>
</tr>
<tr>
<td>Insulin (AUC)</td>
<td>476.5 ± 71.5</td>
<td>154.3 ± 17.8</td>
<td>&lt; .001</td>
</tr>
<tr>
<td>Fasting glucose (mg/dL)</td>
<td>88.0 ± 2.2</td>
<td>87.6 ± 2.3</td>
<td>NS</td>
</tr>
<tr>
<td>Glucose (AUC)</td>
<td>387.4 ± 17.0</td>
<td>314.0 ± 20.1</td>
<td>NS</td>
</tr>
</tbody>
</table>

Note: NS = not significant.

James, Increased amylin in polycystic ovary syndrome. Fertil Steril 2010.
DISCUSSION

Our data show that women with PCOS and insulin resistance have increased fasting amylin levels and an exaggerated amylin response to oral glucose. We are not aware of any previous studies on amylin levels in PCOS. However, our results are in agreement with previous studies that showed increased amylin levels in obese and prediabetic patients with insulin resistance (4, 14, 15). In our present study, amylin levels positively correlated with insulin levels at fasting. This correlation indicates coregulation of secretion of these two hormones from b cells at fasting state. Amylin is colocated with insulin in pancreatic b cells. Previous studies show that amylin is cosecreted with insulin in constant molar ratio in normal men (16). After glucose ingestion, we observed a lack of correlation between amylin and insulin levels, indicating that there is dissociation of amylin from insulin secretion. A similar effect of glucose on amylin secretion has been reported in animal models of insulin resistance. Gedulin et al. (17) observed increased amylin levels and dissociation of amylin secretion from insulin secretion when the pancreases from insulin-resistant diabetic fatty Zucker rats were infused with glucose. Because we found a significant positive correlation between glucose and amylin, glucose seems to be the major stimulus for amylin release. In women with PCOS, amylin probably plays an important role in glucose homeostasis during the postprandial period but only a minimal role in the fasting state. Lack of correlation between amylin and insulin after oral glucose could also be due to the difference in the clearance rate of these hormones. Amylin clearance has been found to be similar to that of C-peptide and much slower than that of insulin, indicating that the commonly used molar insulin/amylin ratio may not reflect the correct relationship of secretion of these two peptides (18).

Another important observation in our study is that metformin treatment in women with PCOS and insulin resistance resulted in a decrease in amylin levels. Metformin, a second-generation biguanide antihyperglycemic agent, is commonly used in the treatment of insulin resistance in women with PCOS. Metformin treatment improves insulin resistance and lowers insulin levels (19–21). However, it has recently been shown that metformin may also have a direct effect on b cells to reduce insulin secretion (22). A reduction in secretion of insulin from b cells might be associated with a reduction in amylin secretion as well. We did not observe any significant changes in amylin levels in women treated with rosiglitazone, even though insulin levels were decreased. It is possible that lack of suppression could be due to the lower dose of 4 mg daily that we used. Higher doses may be effective in suppressing the amylin levels, and further studies are needed to investigate this possibility. We used lower dosage because we excluded patients who had overt diabetes or impaired glucose tolerance. Our results on amylin levels after insulin-sensitizing agents are in agreement with previous animal studies. In transgenic mice expressing the gene for human islet amyloid polypeptide (hIAPP), fasting plasma
They comorbidities. Hyperamylinemia has been shown to and elevated amylin may contribute to the development of diabetes (24). The accumulation of amyloid deposition is worsens to extensive amyloid deposition in overt type 2 diabetes, islet amyloid deposition in the pancreas starts before the onset of fasting hyperglycemia and progressively worsens to extensive amyloid deposition in overt type 2 diabetes (24). The accumulation of amyloid deposition is associated with progressive β cell loss by apoptosis, as well as progressively impaired insulin secretion and glucose metabolism (25). We are not aware of any studies on pancreatic amyloidosis in women with PCOS. Because women with PCOS are at high risk for development of diabetes, high levels of amylin may contribute to their progression to diabetes. Beyond their risk for diabetes, women with PCOS also have risk of developing dyslipidemia and hypertension (26, 27), and elevated amylin may contribute to the development of these comorbidities. Hyperamylinemia has been shown to alter lipoprotein metabolism and result in elevated triglycerides (28). Hypertension afflicts 40% of perimenopausal women with a history of PCOS and insulin resistance. Amylin has been implicated in essential hypertension (29) through activation of components of the renin–angiotensin–aldosterone system (30). Women with PCOS have been shown to have elevated serum aldosterone levels (31). In addition to insulin, hyperamylinemia may also contribute to the risks of diabetes, hypertension, and hyperlipidemia in patients with PCOS.

It is possible that amylin also has an effect on ovarian function. Amylin receptors consist of the calcitonin receptor coupled to one of three receptor activity–modifying proteins (1). The calcitonin receptor gene is expressed in human ovary (32). Receptor activity–modifying protein-2 expression has been demonstrated specifically in the human corpus luteum (33). Given that amylin receptors are expressed in the ovary, and amylin levels are elevated in women with PCOS, the role of amylin in ovarian dysfunction needs further study.

In conclusion, we have shown for the first time that women with PCOS have increased amylin levels, and they have an exaggerated amylin response during an oral glucose tolerance test. Amylin is thought to play a role in the development of diabetes, hypertension, hyperlipidemia, and other problems in non-PCOS patients. If these findings can be extrapolated to women with PCOS, hyperamylinemia may contribute to their high risk for developing type 2 diabetes and its comorbidities. Because amylin receptors are present in the ovarian tissue, amylin may play a role in ovarian dysfunction in PCOS. Amylin levels decreased with metformin but not rosiglitazone and the clinical significance of this finding needs further investigation. Future studies are needed to determine the role that amylin plays in metabolic abnormalities and ovarian dysfunction in PCOS.

REFERENCES