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Direct Relationship between Elevated Free Testosterone and Insulin Resistance in Hyperprolactinemic Women

Seong Yeon Kim, M.D., Yun Ah Sung, M.D., Kyung Soo Ko, M.D.
Bo Youn Cho, M.D., Hong Kyu Lee, M.D.
Chang-Soon Koh, M.D. and Hun Ki Min, M.D.

Department of Internal Medicine, Seoul National University College of Medicine, Seoul, Korea

Women with hyperprolactinemia have been reported to have hyperandrogenemia and/or insulin resistance. However, little is known about the association of hyperandrogenemia and insulin resistance in these women. To investigate whether hyperandrogenemia and/or insulin resistance occur in hyperprolactinemic women, and to assess the relationship between them, we measured basal androgen level and both glucose and insulin levels after oral glucose administration in 20 hyperprolactinemic women and 7 female control subjects. Free testosterone level was higher and estradiol level lower in hyperprolactinemic women than in control subjects ($p<0.05$), whereas dehydroepiandrosterone sulfate (DHEAS) and total testosterone levels were similar ($p>0.05$). Both fasting glucose and insulin levels didn' t differ in the two groups ($p>0.05$). However, both serum glucose and insulin levels, after a 75 g glucose load, were significantly increased in hyperprolactinemic women ($p=0.001$, $p<0.001$, respectively). In simple linear regression analysis in hyperprolactinemic women, only free testosterone level had a positive correlation with the incremental area under the insulin curve (insulin-IAU) ($r=0.47$, $p<0.05$). In multiple stepwise regression analysis, free testosterone level, mean blood pressure and DHEAS level were associated significantly with insulin-IAU (beta $=0.98$, $p<0.0001$; beta $=0.58$, $p=0.002$; beta $=-0.67$, $p=0.003$, respectively) These results suggest that insulin resistance is closely related to elevated free testosterone level in hyperprolactinemic women.

Key Words: *Hyperprolactinemia, Free testosterone, Insulin resistance*

INTRODUCTION

Elevation in serum prolactin has been associated with increased dehydroepiandrosterone (DHEA) or its sulfate (DHEAS) levels, suggesting that prolactin may play a role in adrenal androgen secretion[1~3]. Moreover hyperprolactinemia in women has been reported to be associated with multiple androgenic abnormalities as well as elevated adrenal androgens[4~6]. The association, however, has not been universal[7,8].

It has been suggested that prolactin is a diabetogenic hormone[9]. Various clinical studies in hyperprolactinemic women with or without pituitary tumors have revealed insulin resistance and hyperinsulinemia[10~14]. In these studies, impaired glucose tolerance was noted in some but not all women. By contrast, other investigators could not demonstrate a diabetogenic effect of prolactin either in man[15] or in animals[16]. Furthermore, the underlying mechanisms of prolactin-induced changes in insulin release and glucose homeostasis remain unclear.

Although insulin resistance or hyperandrogenemia has been commonly found in hyper-

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Address reprint requests to: Seong Yeon Kim, M. D., Department of Internal Medicine, Seoul National University, 28 Yongon-Dong, Chongno-gu, Seoul, 110-744, Korea

prolactinemic women, to our knowledge there are few data about the association of insulin resistance and hyperandrogenemia in these women. It has been reported previously that, in women with polycystic ovary syndrome, serum insulin levels are elevated and correlated with hyperandrogenemia[17~20]. Recently, androgens have been found to have divergent functional relationship with insulin level, according to the type of androgen secreted[21,22].

To determine if hyperandrogenemia or insulin resistance occurs in hyperprolactinemic women, basal androgen secretion and both glucose and insulin responses to oral glucose were evaluated in hyperprolactinemic women. The relationship between circulating androgen and insulin levels was also assessed

MATERIALS AND METHODS

20 hyperprolactinemic women, aged 22 to 57 years (mean age 33 years) with amenorrhea (or oligomenorrhea) and/or galactorrhea were compared with 7 female control subjects. Informed consent was obtained from all subjects. None of hyperprolactinemic women had any illness other than hyperprolactinemia and were on any medication at the time of study. 6 women had macroprolactinoma diagnosed by CT and plasma prolactin level. 14 women had microadenoma or were included in so-called idiopathic hyperprolactinemia. In the women with macroprolactinoma, thyroid hormone and cortisol secretion were normal, as determined by circulating thyroxine and TSH level, cortisol and ACTH levels and clinical status, but the cortisol and growth hormone responses to insulin-induced hypoglycemia were not observed in all women. One macroprolactinoma patient had previously undergone transsphenoidal surgery with persistent hyperprolactinemia (prolactin level: 185 ng/ml at the time of study) and was included in the microprolactinoma group. No differences were found in clinical data and hormonal levels between the women with microprolactinoma or idiopathic hyperprolactinemia and macroprolactinoma except for plasma prolactin level (data not shown).

Control subjects, aged 20 to 38 years (mean age 27 years) were recruited from medical and paramedical staff of our hospital. These women had regular ovulatory menses, had been on no medication and had no family history of diabetes mellitus. Blood samples for hormone measurement were collected in the early follicular phase of the menstrual cycle.

Venous blood was drawn after overnight fast, and serum glucose and insulin levels were measured 30, 60, 90 and 120 minutes after a 75 g oral glucose load. The sera were stored at -20 C until assayed. Glucose was measured by glucose oxidase method. Insulin, prolactin, 17β-estradiol, DHEAS, total testosterone and free testosterone were measured by specific radioimmunoassay using commercial kits without extraction and chromatographic isolation. Blood pressure was measured on both arms twice, with a mercury sphygmomanometer, with subjects sitting for at least 5 minutes. The mean of 4 readings was used in the analysis. Anthropometric measurements used in this study were body mass index (BMI; kg/m^2) and waist-to-hip circumference ratio (WHR; cm/cm). WHR, an index of body fat distribution, was determined by measurement with a tape of waist circumference at the umbilical level and hip circumference at the greater trochanter level of the femur.

Results are expressed as mean$\pm$S.D or mean$\pm$S.E.M. as appropriate. Statistical analysis was done, using an SPSS statistical package. The total area under the serum glucose and insulin profiles (area under curve) was calculated by the following formula: area under curve$=\{1/2$ (fasting value$+$120-minute value)$+30$-minute value$+60$-minute value$+90$-minute value$\}\times 30$ minutes. From this, we derived the incremental area under the curve (IAU), i.e., areas between the fasting value and the oral glucose tolerance value profile, because these provide measures of response unconfounded by changes in the fasting level. Between-group differences in mean values were compared using unpaired student's t-tests. The serum glucose and insulin responses in the two groups during oral glucose tolerance test were compared in a two-way analysis of variance. The simple and multiple regression analysis was performed according to standard methods. P values were one-tailed. A P value of less than 0.05 was regarded as significant.

RESULTS

The clinical and biochemical characteristics of all study subjects are given in Table 1. Hyperprolactinemic women were more obese, and had higher circulating levels of prolactin and testoster-

Table 1. Characteristics of the Study Subjects

	Control (n=7)	Hyperprolactinemia (n=20)
Age (years)	27±6	33±11
BMI (kg/m²)	21.4±1.9	24.5±3.1*
WHR (cm/cm)	0.73±0.06	0.81±0.05**
MBP (mmHg)	96±1	94±3
Prolactin (ng/ml)	19±7	1024±1921*
Estradiol (pg/ml)	64.1±29.9	37.9±29.1*
DHEAS (μg/dl)	185±97	234±133
TT (ng/ml)	0.29±0.22	0.30±0.24
FT (pg/ml)	0.68±0.37	1.26±0.83*

Values are mean±S.D.
BMI: body mass index
MBP: mean blood pressure
WHR: waist-to-hip circumference ratio
DHEAS: dehydroepiandrosterone sulfate
TT: total testosterone
FT: free testosterone
*: $p<0.05$ vs. Control
**: $p<0.01$ vs. Control

one and lower circulating level of estradiol than control subjects ($p<0.05$), whereas circulating levels of DHEAS and total testosterone were similar in the two groups ($p>0.05$). The serum glucose and insulin levels after a 75 g glucose challenge are shown in Fig. 1. During fasting, both serum glucose and insulin levels didn't differ in the two groups (79.3±13.6 vs 86.7±7.8 mg/dl; 17.9±11.3 vs 21.8±14.5 uU/ml). After 75 g oral glucose load, both glucose and insulin levels were significantly higher in the hyperprolactinemic women (two-way analysis of variance $p=0.001$, $p<0.001$, respectively).

Because obesity influences insulin sensitivity, hyperprolactinemic women were divided into obese (BMI≥25) and lean (BMI<25) subgroups. As shown in Table 2, there were no significant differences in clinical data, hormone levels and both glucose and insulin responses to oral glucose between the two groups except for BMI and WHR. However insulin-IAU of the lean hyperprolactinemic women remained higher, compared with control subjects ($p<0.05$). Therefore all hyperprolactinemic women were pooled in the statistical analysis.

Simple and multiple regression were used to examine the relationship between insulin level as dependent variable and other independent variables. The results of simple linear regression are shown in Table 3. There was a significant linear

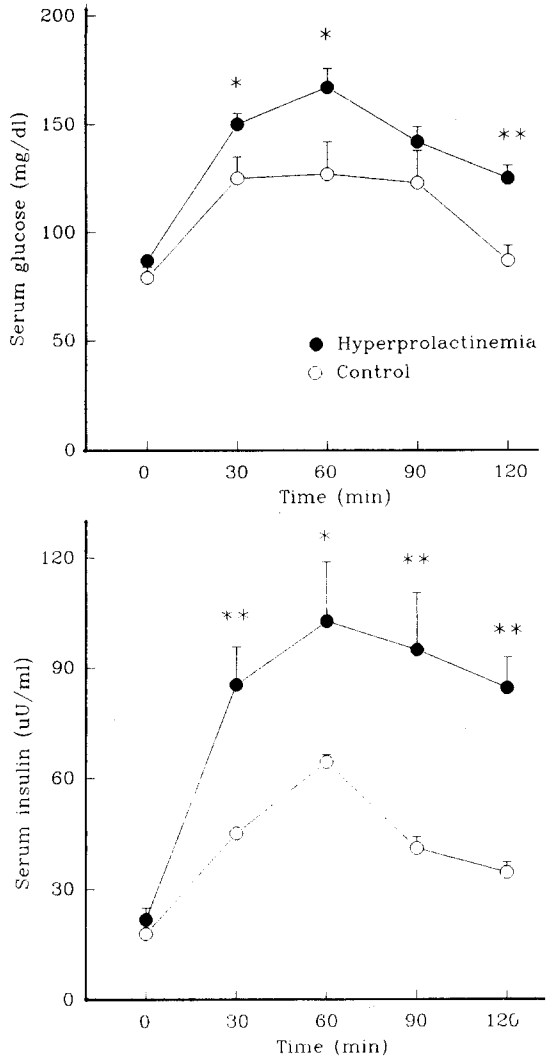

Fig. 1. *Response of serum glucose and insulin to 75 g glucose in patients with hyperprolactinemia (●) and control (○) subjects. Bar denotes 1 S.E.M.*
*: $p<0.05$ **$p<0.01$ vs. control*

correlation between insulin-IAU and free testosterone level ($r=0.47$, $p<0.05$). Table 4 shows the results of multiple stepwise regression for insulin level when all other independent variables were considered. Free testosterone level, mean blood pressure and DHEAS level were the major determinants of insulin-IAU (beta=0.98, $p=0.0001$; beta=0.58, $p=0.002$; beat=-0.67, $p=0.003$, respectively).

Table 2. Clinical and Biochemical Data of Patients with Hyperprolactinemia According to Body Mass Index

	Control (n=7)	BMI<25 (n=12)	BMI≥25 (n=8)
Age (years)	27±6	31±7	37±15
BMI (kg/m²)	21.4±1.9	22.5±1.8	27.5±2.0**
WHR (cm/cm)	0.73±0.06	0.79±0.04	0.84±0.03**
MBP (mmHg)	96±1	95±3	93±2
Prolactin (ng/ml)	19±7	926±1469	1170±2567
Fasting Serum Glucose (mg/dl)	79±14	84±5	90±10
Glucose-IAU (mg·min/dl)	4221±2499	6201±2140	7074±3858
Fasting Serum Insulin (μU/ml)	17.9±11.3	23.3±17.5	19.6±9.0
Insulin-IAU (μU·min/ml)	3154±1183	7455±5437*	7476±2270*
Estradiol (pg/ml)	64.1±29.9	41.0±31.4	32.2±25.9
DHEAS (μg/dl)	185±97	263±148	189±100
TT (ng/ml)	0.29±0.22	0.27±0.25	0.35±0.24
FT (pg/ml)	0.68±0.37	1.30±0.92	1.17±0.75

Values are mean±S.D.
BMI: body mass index
MBP: mean blood pressure
WHR: waist-to-hip circumference ratio
Glucose-IAU: incremental area under the glucose curve
Insulin-IAU: incremental area under the insulin curve
DHEAS: dehydroepiandrosterone sulfate
TT: total testosterone
FT: free testosterone
*$p<0.05$ vs. Control
**: $p<0.05$ vs. Control and BMI<25

Table 3. Correlation Coefficients of the Insulin Level with Other Variables in Patients with Hyperprolactinemia (n=20)

	Fasting Serum Insulin	Insulin-IAU
Age	-0.21	0.23
BMI	-0.18	0.19
WHR	0.14	0.14
MBP	0.01	0.30
Prolactin	0.13	0.09
Estradiol	-0.32	-0.05
DHEAS	-0.03	-0.09
TT	-0.23	0.00
FT	0.01	0.47*

BMI: body mass index
MBP: mean blood pressure
WHR: waist-to-hip circumference ratio
Insulin-IAU: incremental area under the insulin curve
DHEAS: dehydroepiandrosterone sulfate
TT: total testosterone
FT: free testosterone
*: $p<0.05$

Table 4. Multiple Stepwise Regression Analysis for the Insulin Level in Patients with Hyperprolactinemia (n=20)

	Beta	T	P value
Fasting Serum Insulin Level			
No Variable Included			
Incremental Area under the Insulin Curve			
1. Free Testosterone	0.98	5.24	0.0001
2. Mean Blood Pressure	0.58	3.78	0.0021
3. Dehydroepiandrosterone Sulfate	-0.67	-3.64	0.0027

DISCUSSION

The present study demonstrated the close association between elevated free testosterone and insulin resistance in hyperprolactinemic women.

In accordance with the results of other studies[7,8], our hyperprolactinemic women showed normal serum testosterone level. However, contrary to most[1,2,4,6], although not all[7,8], reports, hyperprolactinemic women had normal DHEAS level, a main adrenal androgen (Table 1). Although we have no complete explanation for this observed discrepancy, it is possibly due to the small sample size of the present study and the different analytic techniques. The observation of an elevated serum free (unbound) testosterone in hyperprolactinemic women confirms previous reports[4~6]. Glickman et al[4] and Lobo et al[6] found the serum free testosterone level to be increased in hyperprolactinemic women and suggested that elevated free testosterone level may be the consequences of the decrease in the sex hormone-binding globulin, which may result from diminished estrogen levels or direct inhibitory effect of prolactin on the sex hormone-binding globulin in the liver. We did not measure the sex hormone-binding globulin, but serum estradiol level was significantly lower in hyperprolactinemic women in the present study (Table 1). Considering that the circulating levels of androgens represent a balance between the production rate and the metabolic clearance rate, these two parameters must be investigated sys-

tematically in order to better define their kinetics in hyperprolactinemic women.

In the present study, hyperprolactinemic women exhibited augmented serum glucose and insulin responses to oral glucose in spite of normal fasting serum glucose and insulin levels, compared with control subjects (Fig. 1). These results are in agreement with those of several reports[10~13]. Only one hyperprolactinemic woman fulfilled the criteria of impaired glucose tolerance[23]. In two other studies[11,12], fasting hyperinsulinemia as well as increased insulin response to oral glucose have been also noted. In one of the investigations, the insulin response was reduced after administration of bromocryptine, a known antagonist of prolactin secretion[10]. The present study comprised a heterogeneous group of hyperprolactinemic women, where the diagnosis of macroprolactinoma was included and weight which is known to seriously effect insulin levels[24,25] was not contolled. When these factors were taken into consideration, hyperprolactinemic women still showed a significantly higher insulin response to oral glucose than control subjects (Table 2). These results indicate that hyperprolactinemia has a direct correlation with elevated insulin level, independent of body weight. In contrast, Hagen et al[15] have reported that a centrally-acting dopamine antagonist, sulpiride-induced hyperprolactinemia in normal men, did not change blood glucose, plasma immunoreactive insulin level, hepatic insulin removal and glucose utilization during basal conditions or after i.v. glucose administration. The discrepancy between the report of Hagen et al[15] and the other reports including the present study[10~14] could be due to the transient and less pronounced hyperprolactinemia induced in the normal men in his study. Whereas, we and other investigators studied women with pituitary tumors. The underlying mechanism of hyperinsulinemia in women with chronic prolactin excess was not pursued in the present study. Various experimental and clinical studies suggest that severe hyperprolactinemia is associated with a diminished sensitivity of peripheral tissue to insulin[9~11,14]. In fact, our findings of hyperinsulinemia in the presence of mild glucose intolerance reflect insulin resistance. Schernthaner et al[11] reported that hyperprolactinemic women had impaired insulin receptor binding in vitro, which might decrease insulin effectiveness in vivo. The existence of in vivo insulin resistance in severe hyperprolactinemia was also supported by the findings obtained in hyperglycemic clamp study showing diminished peripheral insulin sensitivity[14].

We could not demonstrate the relationship between insulin-IAU and DHEAS in hyperprolactinemic woment using simple linear regression (Table 3). However, when multiple stepwise regression was used to compensate for correlation of other independent variables, a negative correlation between them emerged (Table 4). This finding is consistent with the report of Schriok et al[21] who described insulin-IAU was negatively related to DHEAS in women with elevated DHEAS, using multiple stepwise regression. Despite some inconsistency in results, negative correlations between DHEAS and insulin level have been observed with total testosterone exhibiting the opposite trend in hyperandrogenic females[17~19,21,22]. We found free testosterone level, not total testosterone, to be correlated significantly with insulin-IAU (Table 3, 4). The present study is the first one that shows the evidence for the association of free testosterone level and insulin resistance in hyperprolactinemic women. However no correlation of body mass index and insulin resistance was found. Furthermore insulin levels were similar between obese and nonobese hyperprolactinemic women (Table 2). It is well-known that weight is a major determinant of insulin resistance[24,25]. Shoupe et al[19] and Givens et al[26] found the highly significant correlation of insulin-IAU and free testosterone or body mass and suggested that both obesity and free testosterone level are important in the development of insulin resistance in hyperandrogenic women. The reasons for this discrepancy are not readily apparent but may relate to the differences in the study subjects (hyperprolactinemic women vs. other hyperandrogenic women including polycystic ovary syndrome) and the small sample size of the present study.

While the association of insulin level and free testosterone do not prove cause and effect, our findings that there is a positive correlation between insulin and free testosterone suggest either a role of hyperandrogenemia in the pathogenesis of insulin resistance or implicate the reverse. Several investigators have provided the evidence to suggest that hyperandrogenemia can induce insulin resistance[27,28]. On the contrary, it has been reported recently that the elevated insulin levels can enhance ovarian steroidogenesis[29]. Moreover, insulin resistance has been found to persist in women with polycysic ovary syndrome after sur-

gery or treatment, which results in normalization of androgen[20]. Further studies are needed to define the true role that hyperandrogenemia plays in insulin resistance in hyperprolactinemic women. We didn't investigate the changes of insulin and free testosterone levels after treatment of hyperprolactinemia.

The positive correlation of mean blood pressure with insulin-IAU in the present study was unexpected. This cannot be explained. There have been several epidemiologic and clinical data showing that insulin resistance and compensatory hyperinsulinemia are directly and possibly even causally related to hypertension[30]. The hyperprolactinemic women in the present study, however, had normal blood pressure.

In conclusion, the results of the present study indicate that hyperprolactinemia is associated with elevated free testosterone level and an insulin resistance state, and suggest that free teststerone and DHEAS may exert opposite effects on insulin action in hyperprolactinemic women.

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