

# Does postprandial hypersinsulinemia contribute to hyperandrogenism in patients with polycystic ovary syndrome?

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## Summary

Twenty patients with polycystic ovarian syndrome and with or without insulin resistance, and 20 healthy women (controls) underwent an oral glucose tolerance test, which resulted in a short duration but significant increase of serum insulin levels. Serum testosterone, androstenedione and dehydroepiandrosterone sulfate levels were estimated before and 180 minutes after administration of 75 gr. dextrose. Our results, three hours after dextrose administration, showed that: (1) serum testosterone levels decreased significantly, (2) serum androstenedione levels decreased but not significantly, and (3) serum dehydroepiandrosterone sulfate levels were not altered. The observation of decreased ovarian androgen levels after induced hyperinsulinemia is very interesting, the explanation, however, is quite difficult. This unexpected ovarian androgen response needs further investigation.

**Key words:** Polycystic ovary syndrome; Insulin; Androgens; Oral glucose tolerance test.

## Introduction

Polycystic ovarian syndrome (PCOS) is an incompletely understood disorder characterized by chronic anovulation with menstrual disturbances, hyperandrogenemia, and, frequently, obesity [1, 2, 3, 4, 5]. Hyperandrogenemia is the most sensitive biochemical marker of PCOS [3, 4, 5]. Insulin resistance and hyperinsulinemia are well recognized features in many women with this syndrome [6, 7, 8]. Although luteinizing hormones (LH) is known to stimulate androgen overproduction by the ovary, several investigators have theorized that hyperinsulinemia may also contribute to hyperandrogenemia in PCOS [9, 10].

The cellular mechanism(s) whereby insulin might exert its effects on androgen homeostasis in obese women with PCOS are unknown. Although insulin might crossreact with the insulin-like growth factor I (IGF-I) receptor and thereby activate postreceptor events [11], the serum insulin concentrations in the obese women with PCOS were not elevated sufficiently to activate IGF-I receptors. Striking insulin increases in the range required for IGF-I receptor activation are seen only in response to meals in women with PCOS. Indeed, one study has suggested that hyperinsulinemia after an oral glucose load can increase plasma androgen levels in hyperandrogenic women [12]. Thus, it is possible that postprandial hyperinsulinemia contributes to hyperandrogenism in patients with PCOS.

This study was performed to determine whether there are differences in basal androgen levels between patients with PCOS and with/or without insulin resistance, and whether there are differences in the effect of transient endogenous hyperinsulinemia on circulating androgen levels in women.

## Materials and Methods

Forty women, whose age ranged from 18 to 37 years, were included in this study. All women were recruited from the population of the Fertility and Family Planning Center of our Department. They were fully informed and gave their written consent for the investigation.

Women were divided into four groups according to the presence of PCOS, insulin resistance and normal or abnormal body mass index (BMI). Each group consisted of ten women. The first group (Group I) was comprised of patients with PCOS, insulin resistance and abnormal BMI (mean value $\pm$ SD 32.9 $\pm$ 3.4, range 27.7-39.0, normal value  $\sim$  24.9). The second group (Group II) was also, comprised of patients with PCOS, but without insulin resistance and with normal BMI (mean value $\pm$ SD 19.9 $\pm$ 0.8, range 19.1-21.5). In the other two groups healthy women were included to serve as controls. The third group (Group III) consisted of women with abnormal BMI (mean $\pm$ SD 32.5 $\pm$ 3.9, range 26.5-38.3), while the fourth group (Group IV) of women had normal BMI (mean $\pm$ SD 21.6 $\pm$ 1.8, range 19.0-23.5).

The diagnosis of PCOS was based on history, clinical examination and laboratory evaluation. History revealed that all patients had oligomenorrhea, acne and hirsutism in adolescence. Clinical examination showed that hirsutism ranged from 11 to 30 grades, according to the Ferriman-Gallwey scale [13] (Group I: mean $\pm$ SD 18.8 $\pm$ 3.5, range 11-23; Group II: 17.2 $\pm$ 3.2, 12-22; normal value  $\sim$  10). Attenuated 21-hydroxylase deficiency, Cushing's syndrome, androgen secreting tumors and hyperprolactinemia were excluded by appropriate tests.

All women in the four groups had a laboratory evaluation which included ultrasound examination of the internal genitalia, as well as measurement of serum levels of testosterone (T), androstenedione ( $\Delta^4$ A) and dehydroepiandrosterone sulfate (DHEA-S). Ultrasound examination was performed on the fifth day and blood samples were collected on the sixth day of spon-

taneous or induced menstrual cycle. Three serum samples drawn at 30 minute intervals were mixed and used to determine the levels of androgens.

In all women, an oral glucose tolerance test (OGTT) was performed on day six of the cycle to identify the presence of insulin resistance. After an overnight fast, the patients were given a continuous intravenous infusion of normal saline, starting at 8 a.m. The basal blood samples were drawn thirty minutes apart, and immediately thereafter 75 grams of dextrose were administered per os, at time 0 (9 a.m.). Further blood samples were collected at 30 minute intervals for 180 minutes. Plasma glucose and serum androgen and insulin concentrations were measured before dextrose ingestion. After dextrose ingestion plasma glucose was assessed six times, at +30, +90, +120, +150 and +180 minutes, serum insulin three times, at +60, +120 and +180 minutes and serum androgens one time, at +180 minutes.

Resistance to insulin was evaluated in our study by measuring the baseline testing glucose and insulin serum levels, as well as the glucose and insulin responses following administration of dextrose during the OGTT. The women in Group I had higher than normal values of two or more of the parameters studied.

Serum glucose concentrations were determined by the enzymatic method (GOD/POD). Serum levels of insulin and androgens were measured by radioimmunoassay using commercial kits (Coat-A-count Insulin, Diagnostic Products Corporation, Testosterone Direct Radioimmunoassay Kit, Sorin, Biomedica, Gamma Coat [ $^{125}$ I] Androstenedione Radioimmunoassay Kit, Incstar Corporation, DHEA-S Direct Radioimmunoassay Solid Phase Coated Tubes, Zer Science Based Industries Ltd).

The unpaired student's t-test was used for statistical analysis of androgen, glucose and insulin basal serum levels. The same test was used for statistical analysis of glucose and insulin after calculation of the total area above baseline levels (0-180 minutes for the OGTT). The paired t-test was used for statistical analysis of androgen serum levels before and after OGTT.

## Results

The mean values and the standard error of serum glucose levels before and during the OGTT in all women studied are illustrated in Fig. 1. The mean fasting serum glucose value of Group I ( $106.0 \pm 38.7$  mg/dl) was higher than that of Group II ( $88.1 \pm 31.6$  mg/dl), of Group III ( $93.3 \pm 14.4$  mg/dl) and of Group IV ( $88.9 \pm 8.4$  mg/dl). However, the difference was not significant among the groups due to the great fluctuation observed in the values of each group. Statistical analysis after calculation of the total area above baseline levels (0 to 180 min) did not show any significant differences in the glucose levels among women in all groups.

Fig. 2 shows the mean values and the standard errors of serum insulin levels before and during the OGTT in women in all groups. No significant differences in the fasting serum insulin levels were noticed among Groups II, III and IV. However, the mean fasting insulin levels of Groups II, III and IV were significantly lower than those of Group I ( $p < 0.001$ ). The statistical analysis after calculation of the total area above baseline levels (0 to 180 min.) did not show any significant difference in the insulin levels among women in Groups II, III and IV, but these levels were significantly lower than those in Group I ( $p < 0.05$ ).

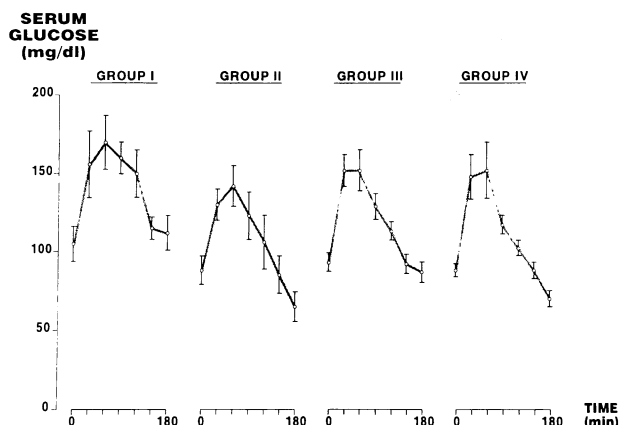


Figure 1. — Mean±SE glucose response during the OGTT (0', +30', +60', +90', +120', +150', +180') of all studied women (glucose value at time 0 represents the mean value of the three drawn blood samples before dextrose administration).

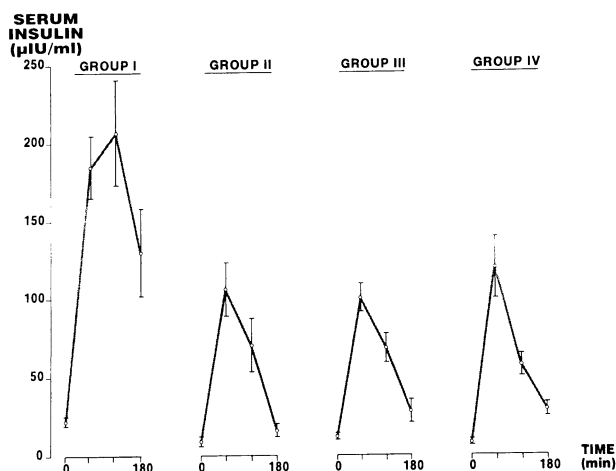


Figure 2. — Mean±SE insulin response during the OGTT (0', +60', +120', +180') of all studied women.

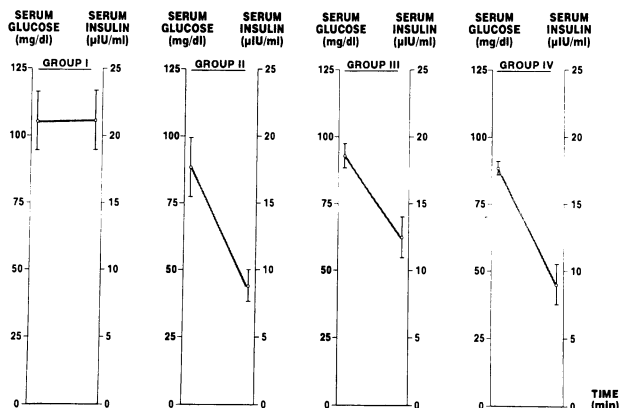


Figure 3. — Mean±SE fasting glucose and insulin serum levels of all studied women.

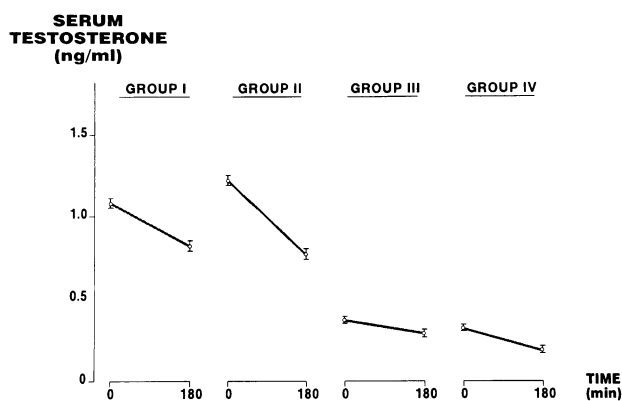


Figure 4. — Mean  $\pm$  SE testosterone serum levels before and 180 minutes after dextrose administration of all studied women.

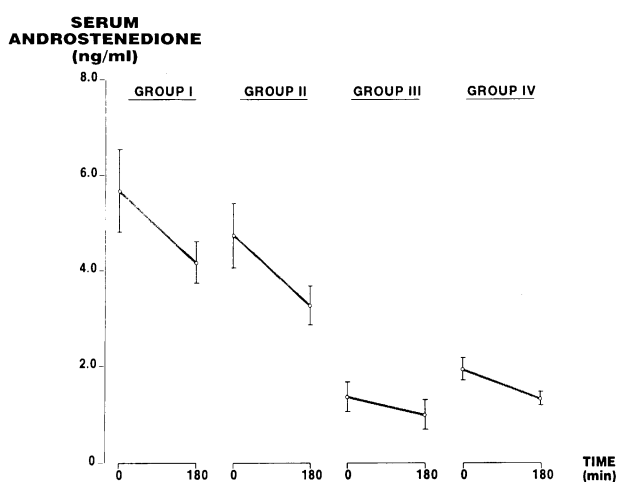


Figure 5. — Mean  $\pm$  SE androstenedione serum levels before and 180 minutes after dextrose administration of all studied women.

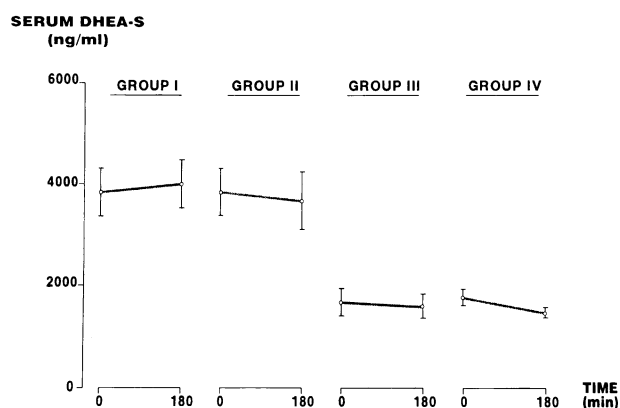


Figure 6. — Mean  $\pm$  SE dehydroepiandrosterone sulfate serum levels before and 180 minutes after dextrose administration of all studied women.

The baseline fasting glucose and insulin levels, which were determined by the same serum sample of all women studied, are displayed in Fig. 3. Higher levels of insulin were needed to maintain the glucose status in Group I than in Groups II, III and IV, as is indicated by the mean values.

Fig. 4 shows the mean values and the standard error of serum T levels before and 180 minutes after dextrose administration in women in all groups. There were no significant differences in serum testosterone levels between Groups I and II and between Groups III and IV, neither in time 0' nor 180 minutes after dextrose administration. However, serum testosterone levels were statistically significantly lower in Groups III and IV than those in Group I ( $p < 0.001$  and  $p < 0.001$ ) and in Group II ( $p < 0.001$  and  $p < 0.001$ ). Furthermore, three hours after dextrose administration serum T levels decreased significantly in all groups (Group I:  $p < 0.01$ ; Group II:  $p < 0.01$ ; Group III:  $p < 0.05$ ; Group IV:  $p < 0.001$ ).

Fig. 5 shows the mean values and the standard errors of serum  $\Delta^4$ A before and 180 minutes after dextrose administration in women in all groups. There were no significant differences in serum  $\Delta^4$ A levels between Groups I and II and between Groups III and IV neither in time 0' nor 180 minutes after dextrose administration. However, serum  $\Delta^4$ A levels were statistically significantly lower in Groups III and IV than those in Group I ( $p < 0.001$  and  $p < 0.001$ ) and in Group II ( $p < 0.001$  and  $p < 0.005$ ). Furthermore, three hours after dextrose administration serum  $\Delta^4$ A levels decreased; the decrease, however, was not significant.

The mean values and the standard errors of DHEA-S before and 180 minutes after dextrose ingestion in women in all groups are displayed in Fig. 6. There were no significant differences in serum DHEA-S levels between Groups I and II and between Groups III and IV neither in time 0' nor 180 minutes after dextrose ingestion. However, serum DHEA-S levels were statistically significantly lower in Groups III and IV than those in Group I ( $p < 0.001$  and  $p < 0.001$ ) and in Group II ( $p < 0.01$  and  $p < 0.001$ ). Three hours after dextrose ingestion serum DHEA-S levels were unaltered in all groups.

## Discussion

Our results, which are in agreement with those of other authors [6, 14], appear to indicate that serum basal androgen levels did not differ significantly between women in Groups I and II (Fig. 4, 5, 6), although women in Group I presented insulin resistance (Fig. 3). The fact that women in Groups I and II presented similar serum LH and FSH levels [15] suggests that LH is the major regulator of androgen secretion in PCOS, whereas hyperinsulinemia, may play a permissive role. This suggestion is strongly supported by the observation that gonadal steroid secretion can be ablated in hyperinsulinemic women with PCOS with a long-acting GnRH analogue [16].

The findings of our study support the view that hyperinsulinemia occurring after a meal does not stimulate

gonadal androgen production. Furthermore, the observation of decreased ovarian androgen levels following an acute increase of endogenous insulin levels during the OGTT is very interesting, the explanation, however, is quite difficult. The decrease of androgen levels is in disagreement with the findings of other authors [12, 17], although recent studies seem to present results similar to ours [14, 18]. The decrease in serum ovarian androgen levels may result from circadian variation of androgens [19]. It is possible that transient increases in insulin levels after an OGTT decrease androgen levels, whereas more prolonged insulin infusion increases androgen levels [14]. It is also possible that increased levels of other gut hormones associated with oral glucose load decrease androgen levels [14].

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