Hyperinsulinemia Is Associated With Menstrual Irregularity and Altered Serum Androgens in Pima Indian Women

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To determine whether hyperinsulinemia is associated with menstrual irregularity or hyperandrogenemia among Pima Indians, a population with a high prevalence of hyperinsulinemia, we retrospectively studied 20 hyperinsulinemic (higher insulin [HI]) and 20 relatively nonhyperinsulinemic (lower insulin [LI]) nondiabetic Pima women 18 to 45 years of age. Reproductive histories were obtained by review of medical records. Stored serum samples were used for measurement of total testosterone, androstenedione, and dehydroepiandrosterone sulfate (DHEAS) levels. Fifty percent (nine of 18) of HI women had irregular menses, as compared with none of the LI women (0 of 19, $P = .0004$). HI women were significantly more obese than LI women. Serum testosterone and androstenedione levels were similar in HI and LI women (median testosterone, 1.13 *v* 1.13 nmol/L, $P = .55$; median androstenedione, 3.79 *v* 3.26 nmol/L, $P = .90$). Serum DHEAS was lower in HI than in LI women (median, 2.85 *v* 4.55 μmol/L, $P < .01$). HI women with irregular menses had significantly higher testosterone levels than HI women with regular menses (median, 1.62 *v* 0.76, nmol/L, $P = .04$). Androstenedione and DHEAS levels were not different between these women. In conclusion, the association of obesity, hyperinsulinemia, irregular menstruation, and high testosterone concentration described in the polycystic ovarian syndrome (PCO) also occurs in Pima Indian women. Moreover, low concentrations of DHEAS are associated with hyperinsulinemia in these women.

THE OBSERVATION that hyperinsulinemia increases ovarian production of androgens has been used to explain the association between insulin resistance, hyperinsulinemia, hyperandrogenemia, and the polycystic ovarian syndrome (PCO).[1,2] Laboratory studies have demonstrated both increased production of androgens and increased responsiveness of ovarian androgen production to insulin in ovarian tissues isolated from women with PCO.[3-5] Clinical studies have yielded conflicting results with respect to androgen production in response to in vivo insulin infusion[6-10] or to stimulation of endogenous insulin secretion,[10-13] but some have suggested a direct effect of insulin on ovarian steroidogenesis.

If hyperinsulinemia stimulates ovarian androgen production, menstrual irregularities and hyperandrogenemia should occur frequently in hyperinsulinemic populations. However, no published study has so far addressed this question. Pima Indians are known for a high prevalence of non–insulin-dependent diabetes,[14] and nondiabetic Pima Indians are hyperinsulinemic compared with whites.[15] This study is a retrospective analysis of reproductive histories and of androgen concentrations from stored serum samples in a group of hyperinsulinemic and relatively nonhyperinsulinemic nondiabetic Pima women of childbearing age.

SUBJECTS AND METHODS

Subjects

Data were obtained from volunteers participating in a longitudinal study of risk factors for the development of non–insulin-dependent diabetes, performed in the clinical research unit of the National Institutes of Health in Phoenix, AZ.[16] Each subject stayed in the research unit for 8 to 15 days and underwent a physical examination including measurements of height and weight. On separate days, oral and intravenous (IV) glucose tolerance tests and hyperinsulinemic-euglycemic clamp studies were performed as previously described.[16]

The women included in this study were selected through their insulin concentrations, which were thought to be more relevant to the eventual ability to stimulate androgen production than the actual magnitude of insulin resistance. One hundred thirty-six nondiabetic women aged 18 to 45 years were selected. They were ranked according to the insulin area under the oral glucose tolerance curve obtained from determinations at 0, 30, 60, 120, and 180 minutes. The 20 women with the highest insulin area (range, 301,140 to 685,884 pmol/L × 180 min) and the 20 women with the lowest insulin area (range, 42,390 to 134,730 pmol/L × 180 min) were chosen for chart review of reproductive history and androgen measurements in stored serum samples.

Reproductive History

Each subject's medical record was reviewed, as was the detailed health questionnaire administered at the time of the clinical research unit study. The reviewer had no knowledge of the subject's insulin area. Specific information was obtained concerning gravidity, parity, menarche, menstrual history, and thyroid function. Oligomenorrhea was defined as either an interval of 2 or more months between menses or six or fewer menses per year. Amenorrhea was defined as an interval of 6 or more months between menses or two or fewer menses per year.

Biological Measurements

Samples from the IV glucose tolerance tests, stored at −20°C, were available for measurements of androgen levels. For each of

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the 40 subjects, we used the samples of the 8- and 10-minute aliquot post–IV glucose infusion. Samples for each individual were pooled and assayed for total testosterone, androstenedione, and dehydroepiandrosterone sulfate (DHEAS) according to published methods.[17] Sex hormone–binding globulin, dehydroepiandrosterone, and free testosterone levels were not determined because of insufficient sample availability.

To control for possible desication resulting from long-term sample storage, the protein content of each pooled sample was estimated by the Lowry method.[18] Analyses were performed using both the androgen concentrations and the ratio of androgen to protein concentrations. Since the results were similar, only data obtained using androgen concentrations are shown.

Serum insulin concentrations were measured by radioimmunoassay[19] and plasma glucose concentrations by the glucose oxidase method. Rates of glucose uptake (M values) were calculated during the hyperinsulinemic-euglycemic clamp at two different insulin infusion rates, low-dose and high-dose (0.24 and 2.4 nmol/L/m^2 body surface area per minute), and were adjusted for estimated metabolic body size and steady-state plasma glucose concentration during the clamp, as described previously.[20]

Statistical Analysis

Groups were compared with the Mann-Whitney test for continuous variables and Fisher's Exact Test for categorical variables. In multivariate analyses, associations between menstrual patterns and androgen concentrations were tested by logistic regression.

RESULTS

From the chart review, menstrual histories were obtained for 37 of 40 subjects, gravidity and parity for 40, and age of menarche for 38. Thyroid functions were documented for 27 women, all of which were within normal limits.

Characterization of the study population by insulin concentration is shown in Table 1. Women with higher insulin concentrations (HI) had significantly higher body mass indexes (BMIs) and fasting and 2-hour glucose concentrations and were significantly more insulin-resistant as shown by the lower M values than women with lower insulin concentrations (LI). Age and gravidity were not significantly different between the two groups.

Table 1. Characteristics of Women in the LI and HI Groups

	Median [Q1-Q3]*		
	LI	HI	*P*
n	20	20	
Age (yr)	24.7 [22.5-29.3]	27.2 [23.2-32.0]	>.05
BMI (kg/m^2)	28.6 [24.2-37.2]	38.1 [34.2-43.0]	.002
Age at menarche (yr)	13 [12-13]	12 [12-13]	>.05
Gravidity	2 [0-4]	2 [0-4]	>.05
% irregular menses	0 (0/19)	50 (9/18)	.0004
Fasting glucose (mmol/L)	5.0 [4.8-5.5]	5.5 [5.2-6.1]	.003
2-hour glucose (mmol/L)	7.1 [5.8-8.2]	8.8 [7.2-10.2]	.006
Fasting insulin (pmol/L)	117 [102-162]	444 [360-576]	.0001
2-hour insulin (pmol/L)	579 [417-729]	2,280 [2,184-2,937]	.0001
Insulin clamp			
Low-dose M†	3.4 [2.7-4.2]	2.2 [2.0-2.5]	.001
High-dose M†	10.3 [8.7-10.8]	5.6 [5.1-7.6]	.001

*Q1-Q3 corresponds to the interquartile range.

†M values are expressed in mg/min · kg estimated metabolic body size.

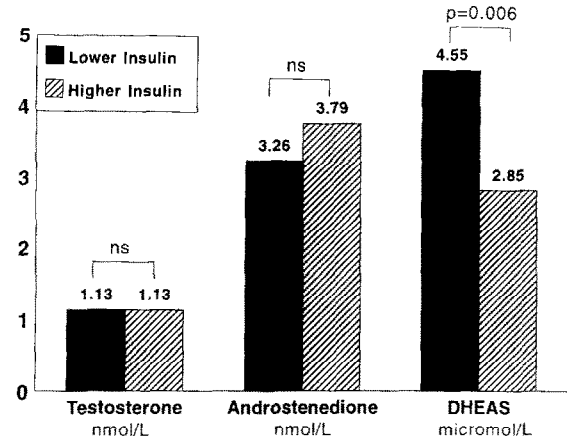

Fig 1. Median serum androgen concentrations in LI and HI groups.

The proportion of women with irregular menses was markedly different between HI and LI women for whom information was available (50% *v* 0% respectively, $P = .0004$). Five of the HI subjects with irregular menses had oligomenorrhea, whereas four had histories of both oligomenorrhea and amenorrhea.

Because the BMI was higher in the HI group than in the LI group, a subgroup comparison was performed that was restricted to HI women with a BMI less than the median and LI women with a BMI greater than the median. Median BMIs of the HI and LI subgroups were 34.2 and 37.2 kg/m^2, respectively (P = NS). Three of eight women with known menstrual histories in the HI subgroup had menstrual irregularities, as compared with none in the LI subgroup (Fisher's Exact Test, $P = .08$).

There were no significant differences in serum testosterone and androstenedione levels between HI and LI women (medians, 1.13 *v* 1.13 nmol/L, NS, and 3.79 *v* 3.26 nmol/L, NS, respectively); however, DHEAS was significantly lower in HI women (median, 4.55 *v* 2.85 μmol/L, $P < .01$; Fig 1).

Among HI women, testosterone concentrations were higher in women with irregular menses than in those with normal menses (1.62 *v* 0.76, $P = .05$; Fig 2). In these women, a high testosterone concentration (defined as a concentration exceeding the median for the group) was associated with a 3.5-fold increased risk of having irregular menses as compared with the low-testosterone group ($P = .03$; Fig 3). In contrast, a high BMI (defined as a value exceeding the median for the group) was associated with only a 1.6-fold increased risk of irregular menses ($P = .32$). After adjusting for age, BMI, and M value (either from high- or low-insulin clamp) by multiple logistic regression, a high testosterone level remained associated with a higher probability of irregular menses ($P = .04$). Neither androstenedione nor DHEAS was associated with irregular menses in the HI group.

DISCUSSION

Pima Indians are notable for high insulin concentrations and a high degree of insulin resistance.[15] Among the 40 Pima women of childbearing age selected, menstrual irregu-

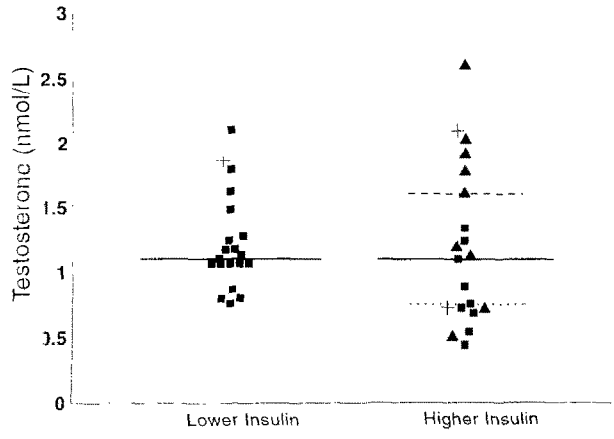

Fig 2. Testosterone concentrations in LI and HI groups in women with (■) normal menses, (▲) irregular menses, or (+) unknown status. (—) The median of each group. In the higher insulin group, (····) and (----) indicate the medians for women with regular menses and irregular menses, respectively.

larities were reported only in the HI women. The HI women were also more obese. Therefore, an association between hyperinsulinemia, obesity, and menstrual irregularities, as described in women with PCO,[1] also occurs in Pima women.

However, in this association the etiologic relationship remains unclear. Arguments supporting the hypothesis of hyperandrogenemia causing insulin resistance and hyperinsulinemia have been based on studies in hyperandrogenemic women in whom insulin resistance decreased after reduction of androgenemia by treatment with oral contraceptives or spironolactone.[21,22] However, these studies were constrained by imprecise measurements of insulin action and small sample size. Studies using more precise determinations of insulin sensitivity have shown that suppressing androgen levels with a gonadotropin-releasing hormone analog had no effect on insulin resistance in women with PCO.[23,24] Moreover, oophorectomy does not improve insulin sensitivity in women with PCO.[25]

More evidence supports the hypothesis that hyperinsulinemia increases ovarian androgen production. Insulin and insulin-like growth factor I can bind to granulosa and theca cells in vitro, stimulating cell growth and androgen production.[3,4] These effects may be synergistic with follicle-stimulating hormone or luteinizing hormone.[3,4] Ovarian stroma isolated from women with PCO show increased basal androgen release and increased responsiveness to insulin-stimulated androgen release compared with stroma isolated from normal women.[5] Insulin at high concentrations can bind to insulin-like growth factor I receptors and therefore still act on the ovary in the insulin-resistant state.[2]

In vivo, prolonged insulin infusion at high concentrations performed in normal or hyperandrogenic women[6-8] has led to different results depending on the androgen considered. Androstenedione concentrations increased during insulin infusion,[6,7] whereas testosterone concentrations did not change[6,8] or decreased in hyperandrogenic women.[7] Insulin infusion at more physiologic concentrations showed either no response of testosterone[9,10] and androstenedione[10] or an increase in androstenedione concentrations.[9] Stimulation of endogenous insulin by glucose[10-13] or tolbutamide[11] did not lead to an increase in androstenedione or testosterone concentrations except in one study.[12] Using a different approach, Nestler et al[26] have shown that decreasing insulin with diazoxide decreases the testosterone concentration, but has no effect on androstenedione concentrations. The disparity in results among studies may arise in part from the fact that circulating androstenedione in women is of both ovarian and adrenal origin. Taken together, these studies suggest that prolonged insulin infusion can increase androstenedione concentrations, whereas decreasing insulin concentrations can decrease testosterone concentrations.

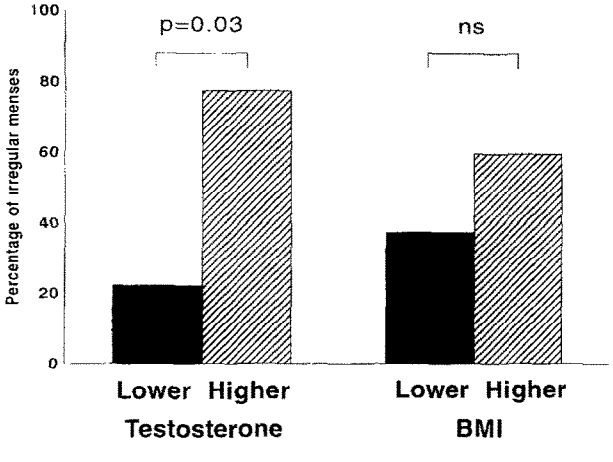

Fig 3. Percentage of women with irregular menses in HI women as a function of testosterone concentrations (< or > the median for the group) and BMI (< or > the median for the group).

In the present study, testosterone and androstenedione concentrations were similar in HI and LI women; however, women in the HI group were also more obese. Therefore, since obesity is associated with lower concentrations of sex hormone–binding globulin,[27] it is possible that HI women had higher free-testosterone concentrations than LI women, despite comparable total testosterone concentrations. We were unable to measure sex hormone–binding globulin levels in the stored serum specimens, so we could not test this hypothesis directly.

Among HI women, higher testosterone concentrations but not obesity were significantly associated with irregular menses. The mean testosterone concentration of HI women with irregular menses was similar to the mean reported previously in obese PCO women.[28] Thus, the high prevalence of irregular menses in obese HI Pima women may be related to hypertestosteronemia secondary to stimulation of ovarian androgen production by insulin.

Alternatively, obesity per se may have produced insulin resistance, alterations in androgen metabolism, and menstrual irregularities.[29-32] Arguing against this is the fact that among subgroups of similar BMI, menstrual irregularities tended to be more frequent in HI than in LI women. In addition, in the HI group, only women with menstrual irregularities had elevated testosterone levels. Not all obese women had menstrual irregularities and high testosterone concentrations. This suggests that there are additional

susceptibility factors, possibly genetically determined,[33,34] for the development of reproductive disturbances in obese HI women.

The significantly lower DHEAS concentration in HI versus LI women is consistent with most of the results in the literature showing a decrease of DHEAS after either infusion of exogenous insulin[8-10] or stimulation of endogenous insulin secretion.[10,12] The mechanism of the relation between DHEAS and insulin concentrations is not yet completely understood, but recent data from in vivo studies suggest that insulin may cause decreased adrenal DHEAS production by inhibition of adrenal 17,20-lyase activity.[35]

In summary, menstrual irregularities occur frequently among obese HI Pima women and are associated with higher concentrations of total testosterone. Hyperinsulinemia is also associated with lower concentrations of DHEAS.

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