OVULATORY AND METABOLIC EFFECTS OF D-*CHIRO*-INOSITOL IN THE POLYCYSTIC OVARY SYNDROME

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ABSTRACT

Background   Women with the polycystic ovary syndrome have insulin resistance and hyperinsulinemia, possibly because of a deficiency of a D-*chiro*-inositol–containing phosphoglycan that mediates the action of insulin. We hypothesized that the administration of D-*chiro*-inositol would replenish stores of the mediator and improve insulin sensitivity.

Methods   We measured steroids in serum and performed oral glucose-tolerance tests before and after the oral administration of 1200 mg of D-*chiro*-inositol or placebo once daily for six to eight weeks in 44 obese women with the polycystic ovary syndrome. The serum progesterone concentration was measured weekly to monitor for ovulation.

Results   In the 22 women given D-*chiro*-inositol, the mean (±SD) area under the plasma insulin curve after the oral administration of glucose decreased from 13,417±11,572 to 5158±6714 μU per milliliter per minute (81±69 to 31±40 nmol per liter per minute) (P=0.007; P=0.07 for the comparison of this change with the change in the placebo group); glucose tolerance did not change significantly. The serum free testosterone concentration in these 22 women decreased from 1.1±0.8 to 0.5±0.5 ng per deciliter (38±28 to 17±17 pmol per liter) (P=0.006 for the comparison with the change in the placebo group). The women's diastolic and systolic blood pressure decreased by 4 mm Hg (P<0.001 and P=0.05, respectively, for the comparisons with the changes in the placebo group), and their plasma triglyceride concentrations decreased from 184±88 to 110±61 mg per deciliter (2.1±0.2 to 1.2±0.1 mmol per liter) (P=0.002 for the comparison with the change in the placebo group). None of these variables changed appreciably in the placebo group. Nineteen of the 22 women who received D-*chiro*-inositol ovulated, as compared with 6 of the 22 women in the placebo group (P<0.001).

Conclusions   D-*Chiro*-inositol increases the action of insulin in patients with the polycystic ovary syndrome, thereby improving ovulatory function and decreasing serum androgen concentrations, blood pressure, and plasma triglyceride concentrations. (N Engl J Med 1999;340:1314-20.)

INSULIN resistance and compensatory hyperinsulinemia, which can occur in otherwise normal persons, are risk factors for type 2 diabetes mellitus,[1-4] dyslipidemia,[5-7] hypertension,[5-7] and atherosclerosis[5] — a constellation of findings termed syndrome X, or the metabolic syndrome.[5] Both are also prominent features of the polycystic ovary syndrome,[8-10] a disorder characterized by chronic anovulation and hyperandrogenism that affects approximately 6 percent of women of reproductive age.[11] In these women, hyperinsulinemia both inhibits ovulation[12] and stimulates ovarian testosterone production[13,14]; the women also have an increased incidence of impaired glucose tolerance or type 2 diabetes mellitus[10,15-17] and the other features of syndrome X.[18-22]

Some of the actions of insulin may involve low-molecular-weight inositol phosphoglycan mediators. When insulin binds to its receptor, mediators of this class are generated by hydrolysis of glycosylphosphatidylinositol lipids located at the outer leaflet of the cell membrane. Released mediators are then internalized and affect intracellular metabolic processes. Although different species have been identified, an inositol phosphoglycan molecule containing D-*chiro*-inositol and galactosamine is known to have a role in activating key enzymes that control the oxidative and nonoxidative metabolism of glucose.[23]

A deficiency of the D-*chiro*-inositol phosphoglycan mediator of the action of insulin may result in resistance to insulin. Insulin resistance has been linked to decreased urinary excretion of *chiro*-inositol (a component of the putative D-*chiro*-inositol phosphoglycan mediator) in primates,[24] in humans with impaired glucose tolerance[25] or type 2 diabetes mellitus,[26] and in nondiabetic first-degree relatives of persons with diabetes.[26] The amount of *chiro*-inositol in muscle is lower in subjects with type 2 diabetes mellitus than in normal subjects.[27,28] In a study of rats, administration of D-*chiro*-inositol decreased hyperglycemia in rats with diabetes and improved glucose tolerance in normal rats.[29] In a study of monkeys with varying degrees of insulin resistance,

D-*chiro*-inositol accelerated the disposal of glucose and decreased insulin secretion.[29] These observations suggest that the administration of D-*chiro*-inositol, which is then presumably used in the formation of the active D-*chiro*-inositol phosphoglycan mediator, may increase insulin sensitivity and improve the action of insulin in insulin-resistant subjects.

In this study we tested the hypothesis that D-*chiro*-inositol, by virtue of its ability to increase sensitivity to insulin, would have beneficial effects on ovulation and ovarian production of androgens in women with the polycystic ovary syndrome.

METHODS

Subjects

We studied 44 women, 18 to 40 years of age, with the polycystic ovary syndrome, indicated by the presence of oligomenorrhea (eight or fewer menstrual periods in the previous year) and hyperandrogenism (high serum concentrations of free testosterone or hirsutism). They were recruited from the Hospital de Clinicas Caracas in Caracas, Venezuela. Other inclusion criteria were obesity, defined as a body-mass index (the weight in kilograms divided by the square of the height in meters) of more than 28, normal results on thyroid-function tests, and normal serum prolactin concentrations. Ultrasonography of the ovaries revealed polycystic ovaries in all the women,[30] but this condition was not an inclusion criterion. None of the women had diabetes mellitus, but 10 had impaired glucose tolerance, defined as a serum glucose concentration of at least 140 but less than 200 mg per deciliter (7.8 to 11.2 mmol per liter) two hours after the oral administration of 75 g of glucose.[31] None had taken any medications for at least two months. Thirty-two of the women were white, seven Hispanic, two Afro-Hispanic, two Arabic, and one Asian; four women had one child each, and the remainder had no children.

Twenty-two women were randomly assigned to receive D-*chiro*-inositol (INS-1, Insmed Pharmaceuticals, Richmond, Va.) and 22 to receive placebo in a double-blind trial. The randomization schedule was generated in blocks of four, and the drug and placebo were packaged at the same time and labeled according to subject number. The study was approved by the institutional review boards of the Hospital de Clinicas Caracas and Virginia Commonwealth University, and each woman gave written informed consent.

Design of the Study

At the time of entry into the study, all the women were in the equivalent of the follicular phase of the menstrual cycle, as documented by a serum progesterone concentration below 2.5 ng per milliliter (8.0 nmol per liter). The women came to the hospital after a 12-hour overnight fast, at which time their weight, height, waist-to-hip ratio, and blood pressure while supine were measured. Blood samples were drawn at 8:30, 8:45, and 9 a.m., and equal volumes of serum were pooled for the measurement of serum steroids and sex hormone–binding globulin. At 9 a.m., 75 g of dextrose (Glycolab, Relab Laboratory, Caracas, Venezuela) was given orally. Blood samples were collected after 30, 60, 90, and 120 minutes for the measurement of plasma glucose and insulin.

The women ate a light meal after the oral glucose-tolerance test and returned for a leuprolide stimulation test at 4 p.m. (described below). After this test, the women began to take 1200 mg of D-*chiro*-inositol or placebo orally once daily. They were instructed not to alter their usual eating habits, physical activity, or lifestyle during the study; they were also advised to refrain from sexual intercourse or to use a barrier method of contraception.

The women returned weekly for measurements of serum progesterone, and ovulation was presumed to have occurred if the

value exceeded 8 ng per milliliter (25 nmol per liter). They returned for the second study after six weeks, if they were confirmed to be in the follicular phase of the menstrual cycle by measurement of a low serum progesterone value (<2.5 ng per milliliter). All the studies performed at base line were repeated at this visit. Four women in the D-*chiro*-inositol group and four women in the placebo group had serum progesterone values in the postovulatory range after six weeks and therefore continued to receive the study treatment or placebo as assigned. Six of these women were studied during week 7 and two women (both treated with D-*chiro*-inositol) were studied during week 8, when their serum progesterone concentrations were low. No side effects were noted in the women in either group.

Leuprolide Stimulation Test

After base-line blood samples had been obtained at 4 p.m., leuprolide (Lupron, Abbott, Takeda, Japan) was administered subcutaneously at a dose of 10 µg per kilogram of body weight. Blood samples were collected 0.5, 1, 16, 20, and 24 hours after leuprolide administration for measurement of serum luteinizing hormone and 17α-hydroxyprogesterone concentrations. The women ate an evening meal but subsequently fasted until completion of the test. The average of the serum luteinizing hormone concentrations in the blood samples obtained after 0.5 and 1 hour was considered the early serum luteinizing hormone response, and the average of the values at 16, 20, and 24 hours was considered the late serum luteinizing hormone response. The serum 17α-hydroxyprogesterone concentration at 0 hours was the basal value, and the highest serum 17α-hydroxyprogesterone value after leuprolide administration was considered the peak value.

Assays

Blood samples were centrifuged as soon as they were obtained, and the serum or plasma was stored at −20°C until assayed. The plasma or serum hormones and sex hormone–binding globulin (measured as protein) were assayed as previously described.[12] The serum free testosterone concentration was calculated according to the method of Sodergard et al.[32] with use of the simultaneously measured serum albumin concentration. To avoid variation between assays, all the samples from an individual woman were analyzed in duplicate in a single assay for each hormone. The intraassay coefficient of variation was 5.5 percent for the plasma insulin assay, 1.6 percent for the serum luteinizing hormone assay, and less than 10 percent for all serum steroid hormone assays.

Plasma concentrations of cholesterol and triglycerides were measured at the Hospital de Clinicas Caracas, and plasma concentrations of high-density lipoprotein and low-density lipoprotein cholesterol were measured by Penn Medical (Washington, D.C.).

Statistical Analysis

The results are reported as means ±SD. Fisher's exact test was used to analyze the differences in ovulation rates between the women who received D-*chiro*-inositol and those who received placebo. For the other variables, the results were analyzed by comparing the changes in values from base line to the end of the study in the D-*chiro*-inositol group with the corresponding changes in the placebo group. We tested the distribution of the changes in the two groups for normality with the Wilk–Shapiro test and then compared the changes with use of Student's two-tailed unpaired t-test or the Mann–Whitney rank-sum test. For differences between the groups that were of borderline significance, we also report the within-group comparisons, which were analyzed by Student's two-tailed paired t-test or the Wilcoxon signed-rank test.

The responses of plasma glucose and insulin to the oral administration of glucose and the responses of serum luteinizing hormone and 17α-hydroxyprogesterone after the administration of leuprolide were analyzed by calculating the areas under the response curves by the trapezoidal rule.

RESULTS

Base-Line Characteristics

The base-line clinical and biochemical characteristics of the women in the D-*chiro*-inositol and placebo groups were similar (Table 1 and Fig. 1 and 2). Six women in the D-*chiro*-inositol group had impaired glucose tolerance, as did four women in the placebo group.

Anthropometric Measurements and Plasma Lipid Concentrations

The body-mass index did not change in either group during the study, but the change in the waist-to-hip ratio in the D-*chiro*-inositol group was significantly different from that in the placebo group (P<0.001). In the D-*chiro*-inositol group, both the decrease in diastolic blood pressure, from 89±5 to 85±6 mm Hg, and the decrease in systolic blood pressure, from 130±7 to 126±7 mm Hg, differed significantly from the corresponding changes in the placebo group (P<0.001 and P=0.05, respectively).

In the D-*chiro*-inositol group, the plasma total cholesterol concentration decreased significantly (P=0.03), but this decrease did not differ significantly from the slight increase in the placebo group (P=0.08) (Table 1). In contrast, the decrease in the plasma triglyceride concentration in the D-*chiro*-inositol group was significantly greater than that in the placebo group (P=0.002) (Table 1). Plasma high-density and low-density lipoprotein cholesterol concentrations did not change significantly in either group.

Plasma Insulin and Glucose Profiles

There was no statistically significant difference between the decrease in the plasma insulin concentration during fasting in the D-*chiro*-inositol group and the small increase in the placebo group (Table 1). In the D-*chiro*-inositol group, the area under the plasma insulin curve after oral glucose administration decreased from 13,417±11,572 to 5158±6714 μU per milliliter per minute (81±69 to 31±40 nmol per liter per minute) (P=0.007), but this decrease did not differ significantly from that in the placebo group (P=0.07).

The plasma glucose concentration during fasting and the area under the plasma glucose curve did not change significantly in either the D-*chiro*-inositol or the placebo group. However, when the women with impaired glucose tolerance at base line were analyzed separately, the area under the plasma glucose curve decreased from 13,983±3625 to 10,945±2410 mg per deciliter per minute (783±203 to 613±135 mmol per liter per minute) in the D-*chiro*-inositol group (P=0.03), resulting in normal glucose-tolerance curves in these six women but did not change substantially in four women with impaired glucose tolerance in the placebo group (15,334± 1446 vs. 13,920±1272 mg per deciliter per minute [859±81 vs. 780±71 mmol per liter per minute]) (P=0.18).

Responses of Serum Luteinizing Hormone to Leuprolide

Changes in basal concentrations of serum luteinizing hormone did not differ between the D-*chiro*-inositol and placebo groups. In contrast, the decrease in the early response of serum luteinizing hormone to leuprolide in the D-*chiro*-inositol group, from 66±55 mIU per milliliter at base line to 30±15 mIU per milliliter after six to eight weeks of treatment, differed significantly from the change in the placebo group (P=0.05) (Fig. 1). Similarly, the decrease in the late response of serum luteinizing hormone to leuprolide in the D-*chiro*-inositol group, from 103±83 mIU per milliliter at base line to 49±29 mIU per milliliter at six to eight weeks, differed significantly from that in the placebo group (P=0.04).

Responses of Serum 17α-Hydroxyprogesterone to Leuprolide

The mean basal serum 17α-hydroxyprogesterone concentration did not change significantly with treatment in either the D-*chiro*-inositol or the placebo group (Fig. 2). In the D-*chiro*-inositol group, the decrease in the peak serum 17α-hydroxyprogesterone concentration after the administration of leuprolide, from 319±212 ng per deciliter at base line to 183±117 ng per deciliter after six to eight weeks (9.6±6.4 to 5.5±3.5 nmol per liter), differed significantly from that in the placebo group (P=0.05). The area under the serum 17α-hydroxyprogesterone curve also decreased, from 5538±3304 to 3103± 1765 ng per deciliter per hour (167±100 to 94±53 nmol per liter per hour), in the D-*chiro*-inositol group (P=0.006), but this change did not differ significantly from that in the placebo group (P=0.07).

Serum Sex-Steroid Concentrations

The administration of D-*chiro*-inositol was associated with a decrease in the serum testosterone concentration and an increase in the serum sex hormone–binding globulin concentration (Table 1); both changes differed significantly from those in the placebo group (P=0.003 for both comparisons). This change resulted in a 55 percent decrease in the serum free testosterone concentration in the D-*chiro*-inositol group, from 1.1±0.8 to 0.5±0.5 ng per deciliter (38±28 to 17±17 pmol per liter), which differed significantly from that in the placebo group (P=0.006). The 47 percent decrease in serum dehydroepiandrosterone sulfate in the D-*chiro*-inositol group differed significantly from that in the placebo group (P=0.001) (Table 1). The serum concentrations of the other steroids did not change substantially in either group.

TABLE 1. CHARACTERISTICS OF 44 WOMEN WITH THE POLYCYSTIC OVARY SYNDROME AT BASE LINE AND AFTER THE ADMINISTRATION OF D-*CHIRO*-INOSITOL OR PLACEBO FOR SIX TO EIGHT WEEKS.*

CHARACTERISTIC	D-*CHIRO*-INOSITOL GROUP (N=22)		PLACEBO GROUP (N=22)	
	BASE LINE	AFTER D-*CHIRO*-INOSITOL	BASE LINE	AFTER PLACEBO
Age (yr)	29±6	—	26±5	—
Body-mass index	31.3±2.4	31.5±2.4	31.0±2.2	31.0±2.2
Waist-to-hip ratio	0.86±0.05	0.84±0.06†	0.84±0.08	0.85±0.08
Blood pressure (mm Hg)				
Systolic	130±7	126±7‡	131±13	128±6
Diastolic	89±5	85±6†	87±6	89±5
Plasma cholesterol (mg/dl)				
Total	209±45	192±58§	200±36	201±39
High-density lipoprotein	36±11	38±8	36±9	38±8
Low-density lipoprotein	124±36	124±7	127±35	126±27
Plasma triglycerides (mg/dl)	184±88	110±61¶	136±71	130±63
Plasma insulin during fasting (μU/ml)	35±40	22±21	38±51	42±52
Area under the plasma insulin curve (μU/ml/min)	13,417±11,572	5158±6714‖	11,371±8027	9210±7840
Plasma glucose during fasting (mg/dl)	86±12	90±19	95±21	95±24
Area under the plasma glucose curve (mg/dl/min)	13,796±2591	12,656±4316	14,115±2462	14,014±3089
Serum progesterone (ng/ml)	0.7±0.4	0.6±0.2	0.8±0.4	0.7±0.2
Serum testosterone (ng/dl)	90±47	61±33**	80±43	79±39
Serum free testosterone (ng/dl)	1.1±0.8	0.5±0.5††	0.8±0.4	0.8±0.4
Serum androstenedione (ng/dl)	201±69	173±50	180±51	186±53
Serum 17β-estradiol (ng/dl)	8.8±4.0	8.9±4.4	9.6±4.1	10.8±9.4
Serum dehydroepiandrosterone sulfate (μg/dl)	519±229	274±91‡‡	459±177	421±179
Serum sex hormone–binding globulin (μg/dl)	2.5±1.0	4.8±2.2**	2.6±0.9	2.8±0.9

*Values are means ±SD. To convert the values for cholesterol to millimoles per liter, multiply by 0.026. To convert the values for triglycerides to millimoles per liter, multiply by 0.11. To convert the values for insulin to picomoles per liter, multiply by 6.0. To convert the values for glucose to millimoles per liter, multiply by 0.056. To convert the values for progesterone to nanomoles per liter, multiply by 3.18. To convert the values for testosterone to picomoles per liter, multiply by 34.7. To convert the values for androstenedione to picomoles per liter, multiply by 34.9. To convert the values for 17β-estradiol to picomoles per liter, multiply by 36.7. To convert the values for dehydroepiandrosterone sulfate to micromoles per liter, multiply by 0.027. To convert the values for sex hormone–binding globulin to nanomoles per liter, multiply by 34.7.

†P<0.001 for the comparison with the change in the placebo group.

‡P=0.05 for the comparison with the change in the placebo group.

§P=0.03 for the comparison with the base-line value in the D-*chiro*-inositol group, and P=0.08 for the comparison with the change in the placebo group.

¶P=0.002 for the comparison with the change in the placebo group.

‖P=0.007 for the comparison with the base-line value in the D-*chiro*-inositol group, and P=0.07 for the comparison with the change in the placebo group.

**P=0.003 for the comparison with the change in the placebo group.

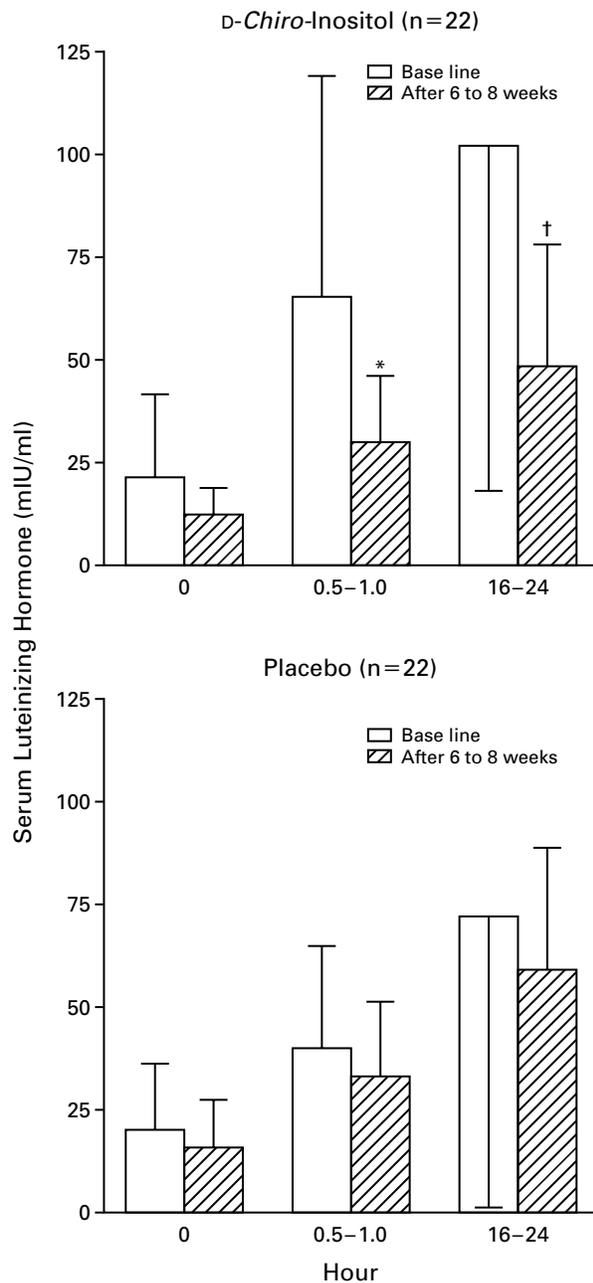††P=0.006 for the comparison with the change in the placebo group.

‡‡P=0.001 for the comparison with the change in the placebo group.

Ovulation

Nineteen of the 22 women in the D-*chiro*-inositol group (86 percent) ovulated during treatment with D-*chiro*-inositol, as compared with only 6 of the 22 women (27 percent) in the placebo group (P<0.001). The mean peak serum progesterone concentration in the 19 women in the D-*chiro*-inositol group who ovulated was 12.4±1.7 ng per milliliter (39.4±5.4 nmol per liter). Figure 3 shows the number of women in the D-*chiro*-inositol and placebo groups who had serum progesterone concentrations that exceeded 8 ng per milliliter (indicative of ovulation) in weeks 2 through 8.

DISCUSSION

In nonhuman primates[24] and in humans,[25-28] insulin resistance may be related to a deficiency in a putative D-*chiro*-inositol–containing phosphoglycan mediator of insulin action. The aim of this study was to determine whether the oral administration of D-*chiro*-inositol would improve insulin sensitivity in a group of insulin-resistant women with the polycystic ovary syndrome. The polycystic ovary syndrome was selected as the model for insulin resistance because it is known that increasing insulin sensitivity in women with this disorder results in improved ovulatory function and decreased serum androgen con-

D-*Chiro*-Inositol (n=22)

Placebo (n=22)

Figure 1. Serum Luteinizing Hormone Concentrations in Women with the Polycystic Ovary Syndrome at Base Line and after the Administration of D-*Chiro*-Inositol or Placebo for Six to Eight Weeks.

Serum luteinizing hormone was measured before and early and late after stimulation with leuprolide (10 μg per kilogram). Values are means ±SD. The asterisk indicates P=0.05 for the comparison of the change in the D-*chiro*-inositol group with the change in the placebo group. The dagger indicates P=0.04 for the comparison of the change in the D-*chiro*-inositol group with the change in the placebo group.

D-*Chiro*-Inositol (n=22)   Placebo (n=22)
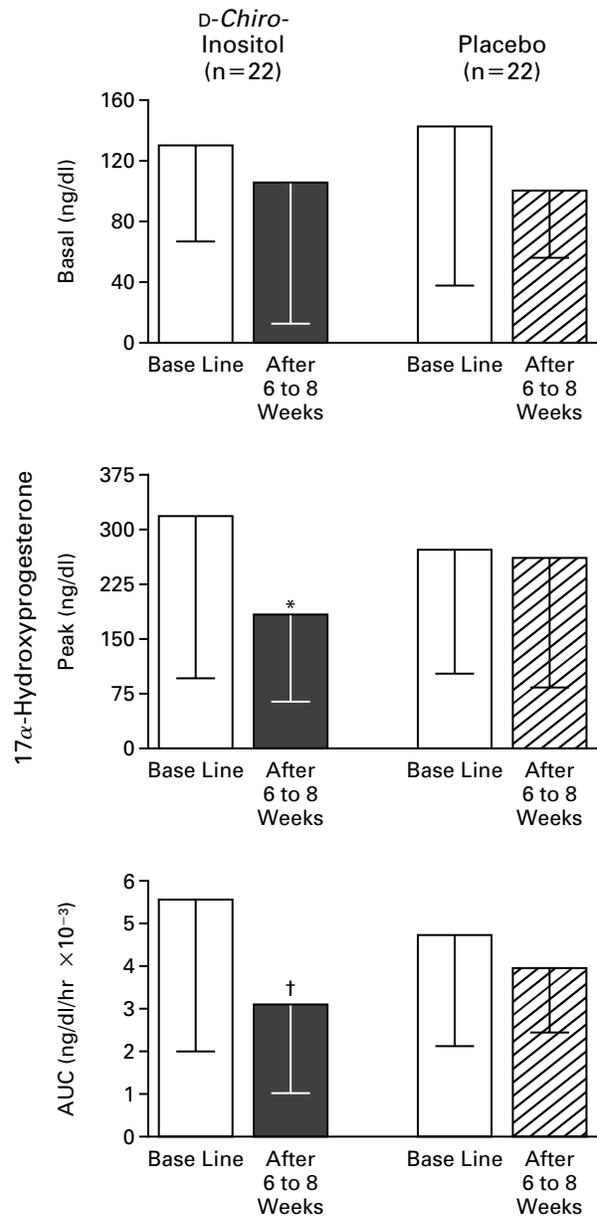
Figure 2. Serum 17α-Hydroxyprogesterone Concentrations in Women with the Polycystic Ovary Syndrome at Base Line and after the Administration of D-*Chiro*-Inositol or Placebo for Six to Eight Weeks.

Serum 17α-hydroxyprogesterone was measured before and after stimulation with leuprolide (10 μg per kilogram). Values are means −SD. To convert the values for 17α-hydroxyprogesterone to picomoles per liter, multiply by 30.2. The asterisk indicates P=0.05 for the comparison of the change in the D-*chiro*-inositol group with the change in the placebo group. The dagger indicates P=0.006 for the comparison with the baseline value in the D-*chiro*-inositol group, and P=0.07 for the comparison of the change in the D-*chiro*-inositol group with the change in the placebo group. AUC denotes area under the curve.
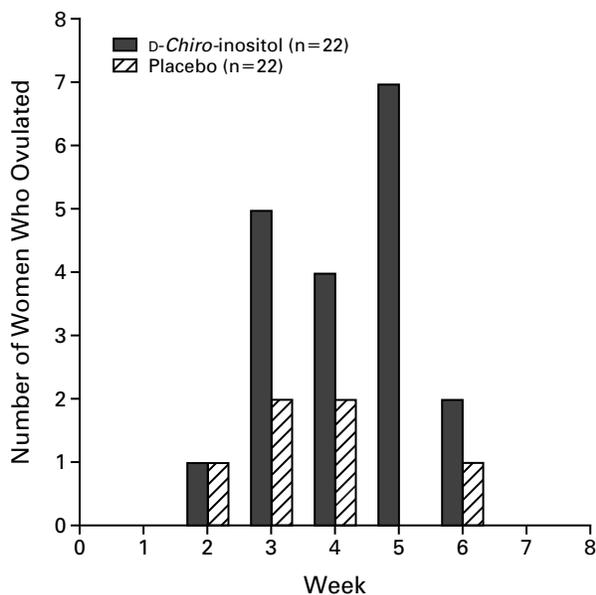
Figure 3. Number of Women with the Polycystic Ovary Syndrome Who Ovulated during the Administration of D-*Chiro*-Inositol or Placebo for Six to Eight Weeks.

Ovulation was indicated by a serum progesterone concentration greater than 8 ng per milliliter. After eight weeks, 19 women in the D-*chiro*-inositol group had ovulated, as compared with only 6 women in the placebo group (P<0.001).

centrations.[12-14] Moreover, the polycystic ovary syndrome is associated with several other metabolic abnormalities that are also probably linked to insulin resistance; they include glucose intolerance,[10,15-17] dyslipidemia,[20-22] and hypertension.[18,20]

We found that the administration of D-*chiro*-inositol to women with the polycystic ovary syndrome decreased the insulin response to orally administered glucose. Although its cause was not assessed directly in our study, this decrease was most likely due to an improvement in peripheral insulin sensitivity.[24-26,28,29] In the women who had impaired glucose tolerance at base line, glucose tolerance improved. Simultaneously with the reduction in insulin secretion, women who received D-*chiro*-inositol had a striking improvement in ovulatory function: 86 percent of these women ovulated, as compared with only 27 percent of the women who received placebo. Serum androgen concentrations also decreased in the women who received D-*chiro*-inositol, as did ovarian androgen production, as reflected by a decreased 17α-hydroxyprogesterone response to leuprolide. These findings are consistent with an improvement in insulin sensitivity, as demonstrated previously with drugs such as metformin[12,14,33-37] and troglitazone.[38,39] The women who received D-*chiro*-inositol also had decreases in both systolic and diastolic blood pressure and plasma triglyceride concentrations.

Because of technical obstacles, it has not been possible to determine whether the skeletal muscles of women with the polycystic ovary syndrome are deficient in the putative D-*chiro*-inositol phosphoglycan mediator, but evidence suggests that this is the case in patients with type 2 diabetes mellitus.[27,28] A deficiency in this mediator could result from a defect in an epimerase-type enzyme responsible for the intracellular conversion of *myo*-inositol to *chiro*-inositol[40,41] or, alternatively, from accelerated catabolism of D-*chiro*-inositol before renal filtration. If either was the case, then exogenous administration of D-*chiro*-inositol would replenish diminished intracellular D-*chiro*-inositol stores and restore the D-*chiro*-inositol phosphoglycan content in skeletal muscle and other insulin target tissues to normal levels.

We conclude that D-*chiro*-inositol improves ovulatory function and several metabolic abnormalities related to insulin resistance in women with the polycystic ovary syndrome. These observations also suggest that D-*chiro*-inositol could be used to treat the polycystic ovary syndrome and that D-*chiro*-inositol may prove useful in the treatment of other disorders that are pathophysiologically related to insulin resistance.

REFERENCES

1. Eriksson J, Franssila-Kallunki A, Ekstrand A, et al. Early metabolic defects in persons at increased risk for non-insulin-dependent diabetes mellitus. N Engl J Med 1989;321:337-43.
2. Ho LT, Chang ZY, Want JT, et al. Insulin insensitivity in offspring of parents with type 2 diabetes mellitus. Diabet Med 1990;7:31-4.
3. Lillioja S, Mott DM, Spraul M, et al. Insulin resistance and insulin secretory dysfunction as precursors of non-insulin-dependent diabetes mellitus: prospective studies of Pima Indians. N Engl J Med 1993;329:1988-92.
4. Warram JH, Martin BC, Krolewski AS, Soeldner JS, Kahn CR. Slow glucose removal rate and hyperinsulinemia precede the development of type II diabetes in the offspring of diabetic parents. Ann Intern Med 1990;113:909-15.
5. Reaven GM. Banting Lecture 1988: role of insulin resistance in human disease. Diabetes 1988;37:1595-607.
6. *Idem.* Insulin resistance, hyperinsulinemia, hypertriglyceridemia, and hypertension: parallels between human disease and rodent models. Diabetes Care 1991;14:195-202.
7. Zavaroni I, Bonora E, Pagliara M, et al. Risk factors for coronary artery disease in healthy persons with hyperinsulinemia and normal glucose tolerance. N Engl J Med 1989;320:702-6.
8. Dunaif A, Graf M, Mandeli J, Laumas V, Dobrjansky A. Characterization of groups of hyperandrogenic women with acanthosis nigricans, impaired glucose tolerance, and/or hyperinsulinemia. J Clin Endocrinol Metab 1987;65:499-507.
9. Dunaif A, Segal KR, Futterweit W, Dobrjansky A. Profound peripheral insulin resistance, independent of obesity, in polycystic ovary syndrome. Diabetes 1989;38:1165-74.
10. Ehrmann DA, Sturis J, Byrne MM, Karrison T, Rosenfield RL, Polonsky KS. Insulin secretory defects in polycystic ovary syndrome: relationship to insulin sensitivity and family history of non-insulin-dependent diabetes mellitus. J Clin Invest 1995;96:520-7.
11. Franks S. Polycystic ovary syndrome. N Engl J Med 1995;333:853-61. [Erratum, N Engl J Med 1995;333:1435.]
12. Nestler JE, Jakubowicz DJ, Evans WS, Pasquali R. Effects of metformin on spontaneous and clomiphene-induced ovulation in the polycystic ovary syndrome. N Engl J Med 1998;338:1876-80.

13. Nestler JE, Jakubowicz DJ. Lean women with polycystic ovary syndrome respond to insulin reduction with decreases in ovarian P450c17α activity and serum androgens. J Clin Endocrinol Metab 1997;82:4075-9.

14. *Idem.* Decreases in ovarian cytochrome P450c17α activity and serum free testosterone after reduction of insulin secretion in polycystic ovary syndrome. N Engl J Med 1996;335:617-23.

15. Dahlgren E, Johansson S, Lindstedt G, et al. Women with polycystic ovary syndrome wedge resected in 1956 to 1965: a long-term follow-up focusing on natural history and circulating hormones. Fertil Steril 1992; 57:505-13.

16. Ehrmann DA, Barnes RB, Rosenfield RL, Cavaghan MK, Imperial J. Prevalence of impaired glucose tolerance and diabetes in women with polycystic ovary syndrome. Diabetes Care 1999;22:141-6.

17. Legro RS, Finegood D, Dunaif A. A fasting glucose to insulin ratio is a useful measure of insulin sensitivity in women with polycystic ovary syndrome. J Clin Endocrinol Metab 1998;83:2694-8.

18. Björntorp P. The android woman — a risky condition. J Intern Med 1996;239:105-10.

19. Dahlgren E, Janson PO, Johansson S, Lapidus L, Odén A. Polycystic ovary syndrome and risk for myocardial infarction: evaluated from a risk factor model based on a prospective population study of women. Acta Obstet Gynecol Scand 1992;71:599-604.

20. Rebuffe-Scrive M, Cullberg G, Lundberg P-A, Lindstedt G, Björntorp P. Anthropometric variables and metabolism in polycystic ovarian disease. Horm Metab Res 1989;21:391-7.

21. Wild RA, Alaupovic P, Parker IJ. Lipid and apolipoprotein abnormalities in hirsute women. I. The association with insulin resistance. Am J Obstet Gynecol 1992;166:1191-7. [Erratum, Am J Obstet Gynecol 1992;167: 575.]

22. Wild RA. Obesity, lipids, cardiovascular risk, and androgen excess. Am J Med 1995;98:Suppl 1A:27S-32S.

23. Larner J. Multiple pathways in insulin signalling — fitting the covalent and allosteric puzzle pieces together. Endocr J 1994;2:167-71.

24. Ortmeyer HK, Bodkin NL, Lilley K, Larner J, Hansen BC. Chiro-inositol deficiency and insulin resistance. I. Urinary excretion rate of chiroinositol is directly associated with insulin resistance in spontaneously diabetic rhesus monkeys. Endocrinology 1993;132:640-5.

25. Suzuki S, Kawasaki H, Satoh Y, et al. Urinary chiro-inositol excretion is an index marker of insulin sensitivity in Japanese type II diabetes. Diabetes Care 1994;17:1465-8.

26. Craig JW, Larner J, Asplin CM. Chiro-inositol deficiency and insulin resistance. In: Draznin B, LeRoith D, eds. Molecular biology of diabetes. Part 2. Totowa, N.J.: Humana Press, 1994:343-62.

27. Kennington AS, Hill CR, Craig J, et al. Low urinary *chiro*-inositol excretion in non-insulin-dependent diabetes mellitus. N Engl J Med 1990; 323:373-8.

28. Asplin I, Galasko G, Larner J. Chiro-inositol deficiency and insulin resistance: a comparison of the chiro-inositol- and the myo-inositol-containing insulin mediators isolated from urine, hemodialysate, and muscle of control and type II diabetic subjects. Proc Natl Acad Sci U S A 1993;90: 5924-8.

29. Ortmeyer HK, Huang LC, Zhang L, Hansen BC, Larner J. Chiro-inositol deficiency and insulin resistance. II. Acute effects of D-chiroinositol administration in streptozotocin-diabetic rats, normal rats given a glucose load, and spontaneously insulin-resistant rhesus monkeys. Endocrinology 1993;132:646-51.

30. Yeh HC, Futterweit W, Thornton JC. Polycystic ovarian disease: US features in 104 patients. Radiology 1987;163:111-6.

31. Report of the Expert Committee on the Diagnosis and Classification of Diabetes Mellitus. Diabetes Care 1997;20:1183-97.

32. Sodergard R, Backstrom T, Shanbhag V, Carstensen H. Calculation of free and bound fractions of testosterone and estradiol-17β to human plasma proteins at body temperature. J Steroid Biochem 1982;16:801-10.

33. Diamanti-Kandarakis E, Kouli C, Tsianateli T, Bergiele A. Therapeutic effects of metformin on insulin resistance and hyperandrogenism in polycystic ovary syndrome. Eur J Endocrinol 1998;138:269-74.

34. Morin-Papunen LC, Koivunen RM, Ruokonen A, Martikainen HK. Metformin therapy improves the menstrual pattern with minimal endocrine and metabolic effects in women with polycystic ovary syndrome. Fertil Steril 1998;69:691-6.

35. Velazquez E, Acosta A, Mendoza SG. Menstrual cyclicity after metformin therapy in polycystic ovary syndrome. Obstet Gynecol 1997;90: 392-5.

36. Velazquez EM, Mendoza S, Hamer T, Sosa F, Glueck CJ. Metformin therapy in polycystic ovary syndrome reduces hyperinsulinemia, insulin resistance, hyperandrogenemia, and systolic blood pressure, while facilitating normal menses and pregnancy. Metabolism 1994;43:647-54.

37. Velazquez EM, Mendoza SG, Wang P, Glueck CJ. Metformin therapy is associated with a decrease in plasma plasminogen activator inhibitor-1, lipoprotein(a), and immunoreactive insulin levels in patients with the polycystic ovary syndrome. Metabolism 1997;46:454-7.

38. Dunaif A, Scott D, Finegood D, Quintana B, Whitcomb R. The insulin-sensitizing agent troglitazone improves metabolic and reproductive abnormalities in the polycystic ovary syndrome. J Clin Endocrinol Metab 1996;81:3299-306.

39. Ehrmann DA, Schneider DJ, Sobel BE, et al. Troglitazone improves defects in insulin action, insulin secretion, ovarian steroidogenesis, and fibrinolysis in women with polycystic ovary syndrome. J Clin Endocrinol Metab 1997;82:2108-16.

40. Pak Y, Huang LC, Lilley KJ, Larner J. In vivo conversion of [3H]myoinositol to [3H]chiroinositol in rat tissues. J Biol Chem 1992;267:16904-10.

41. Pak Y, Paule CR, Bao YD, Huang LC, Larner J. Insulin stimulates the biosynthesis of chiro-inositol-containing phospholipids in a rat fibroblast line expressing the human insulin receptor. Proc Natl Acad Sci U S A 1993; 90:7759-63.