Effect of Pinitol Treatment on Insulin Action in Subjects With Insulin Resistance

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OBJECTIVE — Endogenous low-molecular-weight glycans containing pinitol (3-O-methyl- 

cello- inositol) and d-chiro-inositol are thought to mediate certain actions of insulin. We tested 

the hypothesis that oral administration of soybean-derived pinitol would improve insulin sen- 

sitivity in obese subjects (BMI = 36.6 kg/m2) with diet-treated type 2 diabetes or glucose intol- 

erance (HbA1c = 6.8%).

RESEARCH DESIGN AND METHODS — There were 22 subjects randomized to 

receive either pinitol 20 mg ⋅ kg−1 ⋅ day−1 (n = 12) or placebo (n = 10) in a 28-day double- 

blind trial.

RESULTS — No toxicity due to the pinitol was observed during the study. The sensitivity of 

glucose and lipid metabolism to insulin were assessed by measurement of whole-body glucose, 

palmitate, and glycerol kinetics during basal conditions and a hyperinsulinemic-euglycemic 

clamp. Metabolic measurements were made at baseline and again at the end of the study. Final 

plasma levels of pinitol were 48-fold (1.06 ± 0.15 vs. 0.02 ± 0.01 µmol/l, P < 0.0001) and 

d-chiro-inositol levels 14-fold (0.56 ± 0.08 vs. 0.04 ± 0.02 µmol/l, P < 0.0001) greater in the pini- 

tol group compared with placebo.

CONCLUSIONS — Four weeks of pinitol treatment did not alter baseline glucose produc- 
	dion, insulin-mediated glucose disposal, or rates of appearance of free fatty acids and glycerol 

in plasma. We conclude that plasma levels of both pinitol and d-chiro-inositol are very respon- 

sive to pinitol ingestion, but insulin sensitivity does not increase after pinitol treatment in 

individuals with obesity and mild type 2 diabetes.

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Insitol phosphoglycans are potentially important postreceptor mediators of 

insulin action (1,2). They are generated rapidly in response to physiological concen- 

trations of insulin and have insulin-like effects in vivo (1–3). The unusual inositol 

isomer, d-chiro-inositol (Fig. 1), has been identified in several putative insulin media- 

tor preparations and is found in trace amounts in tissues and body fluids (4,5). 

Pinitol (3-O-methyl-d-chiro-inositol) (Fig. 1) has also been identified in putative insulin 

mediator fractions that have hypoglycemic activity, and appears to act downstream in the 

insulin-signaling pathway to mimic the effects of insulin (6). Administration of 

d-chiro-inositol has been shown to lower blood glucose concentration in streptozo- 

tocin-induced diabetic rats and in normal rats given glucose (7). This isomer also 

increased the rate of glucose disappearance in insulin-resistant and hyperinsulinemic 

monkeys (7,8), suggesting that the inositol itself might improve glucose metabolism. 

Insulin resistance, a hallmark of type 2 diabetes and a characteristic feature of obe- 

sity and several other diseases, may represent the earliest metabolic abnormality in 

the transition from normal to impaired glu- 

cose tolerance (9,10). The role of d-chiro- 

inositol in insulin resistance has been investigated in patients with diabetes 

because chiro-inositol may represent an 

important new signaling mechanism (2). 

Its role as a mediator of insulin action has 

been investigated using both naturally iso- 

lated and chemically synthesized analog 

and by measurement of tissue and body 

fluid levels (2,11). Kennington et al. (11) 

showed low urinary excretion and low tis- 

sue levels of chiro-inositol in patients with 

type 2 diabetes compared with normal sub- 

jects. However, the analytical method used 

was criticized for its inadequate sensitivity 

and proneness to generation of artificially 

low values due to interference (12). A more 

recent study using a mass spectroscopic 

method with greatly improved sensitivity 

(13) found that urinary d-chiro-inositol lev- 

els were increased in diabetic subjects and 

were positively correlated with HbA1c levels. 

In women with polycystic ovary syndrome, 

which is characterized by menstrual distur- 

bances, obesity, hyperinsulinemia, and 

insulin resistance, oral administration of 

d-chiro-inositol for several weeks reduced 

insulin area during a glucose tolerance test, 

as well as basal plasma triglyceride levels 

(14). Glucose and d-chiro-inositol have also 

been shown to compete for uptake by spe- 

cific pathways defined in cultured cells (15). 

These results suggest that d-chiro-inositol 

might be useful in the treatment of predia- 

betic patients as well as patients with 

insulin-resistant diabetes.

Although in vivo production of chiro- 

inositol has been demonstrated (16), dietary 

intake of pinitol, a methylated derivative of 

d-chiro-inositol, represents the major meta- 

bolic source (17). Pinitol is found primarily 

in legumes and citrus fruits and constitutes 

1% of the dry weight of soy meal. Labora- 

tory animals consuming commercial rodent 

diets typically take in up to 160 mg ⋅ kg−1 ⋅ 

day−1 of d-chiro-inositol, mostly in the form 

of pinitol, contained in soy meal and alfalfa.
Subjects
A total of 22 obese subjects (12 men and 10 women) with a mean age of 51 ± 12 years and abnormal glucose tolerance participated. All subjects had a BMI > 30 kg/m² (mean 36.6 ± 4.9). Mean percent body fat was 42.0 ± 7.2% measured by dual energy X-ray absorption (Hologic, Bedford, MA). Eighteen subjects were classified as having diabetes based on a fasting plasma glucose concentration ≥ 7.0 mmol/l (126 mg/dl) or a 2-h postload 75-g plasma glucose concentration ≥ 11.1 mmol/l (200 mg/dl). Four subjects were found to have various degrees of glucose intolerance with either a 2-h postload plasma glucose concentration 7.8–11.1 mmol/l (140–200 mg/dl) or an intervening value >11.2 mmol/l (202 mg/dl) (18). No subjects had received drugs or insulin for the treatment of diabetes for at least 2 months before the study and none had active medical or surgical illnesses, hepatic or renal disease, a history of allergy to soy protein or soy products, or used any medications that affect insulin action. In all subjects, body weight was stable for 6 months before the study. Studies were performed at Washington University School of Medicine in St. Louis, Missouri and the University of California at San Francisco. The institutional review boards at each site approved the protocol and informed consent was obtained from each participant. Subjects were randomly assigned in a double-blind fashion to either the placebo or treatment group and treatment codes were kept in a central registry.

Materials
Pinitol containing 3% d-chiro-inositol was prepared from soybeans (Archer Daniels Midland, Decatur, IL) by molecular filtration of aqueous soy fractions followed by ion exchange chromatography to remove contaminating sugars (20). [6, 6-2H2]Glucose, [1,1,2,3,3-2H5]glycerol, [2-13C]glycerol, [1-13C]palmitic acid, and [2,2-2H2]palmitic acid were purchased from Isotec (Miamisburg, OH), and Cambridge Isotope Laboratories (Andover, MA). Albumin was obtained from Immuno-U.S. (Rochester, MI). Other reagents were from Sigma (St. Louis, MO).

Study protocol
All subjects participated in 5 visits (Fig. 2) and were monitored by questionnaire and routine blood tests for potential adverse effects. At visit 1, a comprehensive medical screening was performed, including routine blood tests and a glucose tolerance test as needed. At the second visit, a dietitian instructed subjects on the avoidance of legumes and citrus fruits but did not otherwise change their habitual diet. Subjects then began consuming a placebo drink consisting of 30 ml of saccharin-sweetened cherry Kool-Aid twice daily for a 1-week run-in period. After the run-in period was completed, subjects were admitted to the clinical research center in the evening (visit 3), and the following morning, substrate kinetics were measured during basal conditions and during a hyperinsulinemic-euglycemic clamp. Subjects then consumed a Kool-Aid drink 2 times per day containing either 20 mg · kg⁻¹ · day⁻¹ of pinitol or placebo for 4 weeks. A research diettian who had no contact with the subjects kept the identity of the coded solutions. The solution code was broken after the data analysis was completed. Pinitol has a slightly sweet flavor, but it could not be detected in the drink. Pinitol and placebo solutions were stored in the refrigerator and replaced at 2 weeks (visit 4); dietary counseling was also reinforced at this visit. After the 4-week treatment period, substrate kinetic studies and other measurements were repeated (visit 5). The final aliquot of the study drink was consumed just before the start of the second clamp study.

Substrate kinetic studies
Subjects were admitted to the clinical research center in the evening before each substrate kinetic study for visits 3 and 5. A standard bedtime snack was consumed between 9:00 and 10:00 p.m. Two intravenous catheters were placed in forearm veins to infuse isotope tracers, insulin, and dextrose. A third intravenous catheter was inserted in a hand vein that was heated for arterialized blood sampling. After 8 h of fasting (−240 min), baseline blood biochemistries, hematology tests, background plasma isotope enrichment, and plasma inositol profiles were obtained. Glucose, glycerol, and palmitate kinetics were measured in the basal state (−240 to 0 min) and during a hyperinsulinemic-euglycemic clamp (0 to 240 min). The hyperinsulinemic clamp protocol was designed to increase serum insulin concentrations into the modestly hyperinsulinemic range (−200–250 pmol/l), at which level endogenous glucose production is not completely suppressed, so that effects on glucose production as well as disposal could be assessed. Infusions of [6,6-2H2]glucose (priming dose: 26.7 µmol/kg, constant infusion: 0.33 µmol · kg⁻¹ · min⁻¹), and either [2-13C]glycerol (San Francisco site), (constant infusion: 2.70 µmol · kg⁻¹ · min⁻¹) or [1-13C]glycerol (St. Louis site), (priming dose: 0.9 µmol/kg, constant infusion: 0.06 µmol · kg⁻¹ · min⁻¹) were started at time −240 min and infused
Effect of pinitol on insulin action throughout the experimental period. A higher glycerol infusion rate was used at the San Francisco site to measure gluconeogenesis in addition to glycerol rate of appearance (R_a) (data to be presented elsewhere). Labeled palmitate bound to albumin was infused (0.04 µmol · kg⁻¹ · min⁻¹) for 90 min before the end of the basal and clamp periods. During the hyperinsulinemic-euglycemic clamp (0–240 min), insulin was infused (20 mU · m⁻² · min⁻¹) along with a variable infusion of 20% dextrose to maintain the basal plasma glucose concentration obtained at time 0. Arterialized blood samples were obtained at 5 min intervals to determine plasma glucose concentration. The infusion rate of labeled glucose was also increased 2-fold during the clamp to maintain relatively constant plasma glucose enrichments. Plasma samples for substrate isotope tracer-to-tracee ratios were collected every 10 min during the final 30 min of the basal and clamp periods.

Analyses
Plasma inositol concentrations were determined by gas chromatography/mass spectrometry. [2H₃]Pinitol, [2H₆]racemic chiro-inositol, and [2H₆]myo-inositol were added to plasma as internal standards. The samples were then purified, derivatized with pentafluoropropionic anhydride, separated on a Chirasil-Val capillary column (Alltech, State College, PA), and analyzed in a negative ion chemical ionization mode on an HP 5988 mass spectrometer (Agilent Technologies, Palo Alto, CA) with methane as the reagent gas (13). Lipids and lipoproteins were quantitated by the Lipid Research Clinics methods (19).

Plasma samples were analyzed for glucose, glycerol, and palmitate enrichment by gas chromatography/mass spectrometry. A 250-µl plasma sample was added to 62.5 nmol heptadecanoic acid in 250 µl heptane and 10 nmol [2,13C]glycerol in 250 µl water (St. Louis site). The proteins were precipitated with 3-m1 cold acetone and centrifuged. Then, 3 ml hexane and 3 ml reagent grade water were added to the supernatant and the organic and aqueous layers were processed and separated. The aqueous phase was dried in 2 aliquots. One portion was derivatized to glucose pentaacetate and separated on a DB-1 column (J&W Scientific, Folsom, CA). Equilibrium glucose turnover was calculated for each infusion period and hepatic glucose production was calculated during the insulin infusion period by subtracting the unlabeled glucose infusion rate from the total glucose disappearance rate. For determination of plasma glycerol enrichment, 1:1 heptfluorobutyril anhydride:acetonitrile was added to the remaining portion of the dried aqueous phase. The derivatized glycerol was separated on an RTX-200 capillary column (Restek, Bellefonte, PA). The organic phase was treated with 250 µl of 0.2 mol/l sodium phosphate and 0.05 mol/l tetrabutylammonium hydrogen sulfate pH 9.0 buffer, then with 250 µl 10% methyl iodide in methylene chloride. The fatty acid methyl esters were extracted and purified by solid-phase extraction on 3-ml LC-Si columns (Supelco, Bellefonte, PA). They were separated on an RTX-200 column.

Statistical methods
Differences between and within groups before and after treatment were assessed by paired Student's t test or by repeated-measures analysis of variance using the Statistical Analysis System (Cary, NC). It was estimated that 24 subjects would be needed to detect whether pinitol treatment caused a 20% change in insulin sensitivity, with respect to glucose or lipid kinetics, with a power of 0.80 at a significance level of 0.05.

RESULTS — Pinitol was well tolerated by all subjects and no subjective complaints were reported. In addition, there were no adverse changes in baseline blood

Figure 2—Study protocol. DEXA is a dual energy X-ray absorption determination of percent body fat.
biochemistry or hematology profiles, creatinine phosphokinase, or liver enzymes.

Pinitol therapy increased plasma pinitol concentration 48-fold and d-chiro-inositol 14-fold. Plasma pinitol concentration was 1.06 ± 0.15 µmol/l in the pinitol group and 0.02 ± 0.02 µmol/l in the placebo group (P < 0.0001). The d-chiro-inositol values were 0.56 ± 0.08 and 0.04 ± 0.02 µmol/l, respectively (P < 0.0001), in the 2 groups. If kinetic properties of the 2 substances are similar, these values suggest that 33% of the pinitol was converted to d-chiro-inositol. Table 1 shows fasting plasma glucose, HbA1c, insulin, free fatty acid, and lipoprotein concentrations before and after the 28-day study period. No statistical differences were detected in these values (P > 0.1).

There was no effect of pinitol treatment on basal glucose or lipid kinetics (Table 2). Insulin infusion raised serum insulin concentrations to ~220-250 pmol/l and caused a marked decrease in palmitate $R_{p}$ (by 53%) and glycerol $R_{g}$ (by 31%), but did not significantly affect glucose metabolism. At baseline, endogenous glucose production was lower in the pinitol group compared with the placebo group (2.88 ± 0.66 and 4.52 ± 1.15, respectively) (Table 2), but the differences did not approach statistical significance (P = 0.21). There was no effect of pinitol or placebo treatment on insulin-mediated glucose disposal or production during the clamp.

**Table 1—Study end points**

<table>
<thead>
<tr>
<th></th>
<th>Pinitol group</th>
<th>Placebo group</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fasting glucose (mmol/l)</td>
<td>7.6 ± 0.7</td>
<td>7.8 ± 0.9</td>
<td></td>
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<tr>
<td>Fasting insulin (pmol/l)</td>
<td>102 ± 14</td>
<td>83 ± 13</td>
<td>0.10</td>
</tr>
<tr>
<td>Clamp insulin (pmol/l)</td>
<td>245 ± 22</td>
<td>217 ± 25</td>
<td>0.97</td>
</tr>
<tr>
<td>HbA1c (%)</td>
<td>6.8 ± 0.4</td>
<td>6.8 ± 0.5</td>
<td>0.28</td>
</tr>
<tr>
<td>Clamp plasma free fatty acids (µmol/ml)</td>
<td>0.20 ± 0.03</td>
<td>0.22 ± 0.03</td>
<td>0.41</td>
</tr>
<tr>
<td>Basal plasma free fatty acids (µmol/ml)</td>
<td>0.49 ± 0.04</td>
<td>0.49 ± 0.04</td>
<td></td>
</tr>
<tr>
<td>Total cholesterol (mmol/l)</td>
<td>5.28 ± 0.26</td>
<td>4.97 ± 0.39</td>
<td>0.10</td>
</tr>
<tr>
<td>LDL cholesterol (mmol/l)</td>
<td>3.26 ± 0.28</td>
<td>3.10 ± 0.34</td>
<td></td>
</tr>
<tr>
<td>HDL cholesterol (mmol/l)</td>
<td>0.85 ± 0.05</td>
<td>0.81 ± 0.04</td>
<td>0.27</td>
</tr>
<tr>
<td>Total triglycerides (mmol/l)</td>
<td>2.59 ± 0.26</td>
<td>2.31 ± 0.25</td>
<td>0.34</td>
</tr>
</tbody>
</table>

Data are means ± SEM and are reported before (pre) and after (post) 28 days of pinitol or placebo feeding. Clamp insulin is the average plasma insulin concentration during the last 2 h of the hyperinsulinemic-euglycemic clamp study. The P values indicate the significance in the listed variables between the groups at the end of the 28-day study period.

**Table 2—Substrate kinetic studies**

<table>
<thead>
<tr>
<th></th>
<th>Pinitol group</th>
<th>Placebo group</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Basal</td>
<td></td>
<td></td>
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<tr>
<td>Palmitate $R_{p}$ (µmol · kg⁻¹ · min⁻¹)</td>
<td>1.78 ± 0.22</td>
<td>1.69 ± 0.21</td>
<td>0.60</td>
</tr>
<tr>
<td>Glycerol $R_{g}$ (µmol · kg⁻¹ · min⁻¹)</td>
<td>2.42 ± 0.27</td>
<td>2.49 ± 0.35</td>
<td>0.20</td>
</tr>
<tr>
<td>Glucose $R_{g}$ (µmol · kg⁻¹ · min⁻¹)</td>
<td>10.3 ± 0.8</td>
<td>11.7 ± 2.1</td>
<td>0.97</td>
</tr>
<tr>
<td>Clamp</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Palmitate $R_{p}$ (µmol · kg⁻¹ · min⁻¹)</td>
<td>0.82 ± 0.15</td>
<td>0.84 ± 0.09</td>
<td>0.94</td>
</tr>
<tr>
<td>Glycerol $R_{g}$ (µmol · kg⁻¹ · min⁻¹)</td>
<td>1.66 ± 0.23</td>
<td>1.70 ± 0.38</td>
<td></td>
</tr>
<tr>
<td>Glucose $R_{g}$ (µmol · kg⁻¹ · min⁻¹)</td>
<td>12.4 ± 0.42</td>
<td>11.2 ± 1.1</td>
<td>0.10</td>
</tr>
<tr>
<td>Endogenous glucose production (µmol · kg⁻¹ · min⁻¹)</td>
<td>2.88 ± 0.66</td>
<td>4.52 ± 1.15</td>
<td>0.57</td>
</tr>
</tbody>
</table>

Data are means ± SEM. Palmitate, glycerol, and glucose kinetics were measured before (pre) and after (post) 28 days of pinitol or placebo treatment. The P values indicate the significance between the groups in the listed variables at the end of the 28-day study period.

**CONCLUSIONS**—Our results show that dietary intake is an important determinant of plasma pinitol and d-chiro-inositol concentrations and is capable of markedly increasing plasma levels. Basal mean plasma d-chiro-inositol concentration was 0.04 µmol/l, which is less than the value (0.1 µmol/l) found previously in both normal subjects and those with type 2 diabetes (13). This lower concentration is consistent with the restriction of dietary legumes and citrus fruits that was imposed on study subjects. After the 28-day study period, the rise in fasting pinitol and d-chiro-inositol levels in the pinitol group suggests that conversion of pinitol to d-chiro-inositol took place in vivo since our dietary pinitol treatment contained only 3% free d-chiro-inositol. Therefore, humans may possess a pinitol demethylase. Further work is needed to study the metabolism of pinitol in normal and diabetic subjects.

This is the first study to measure changes in plasma pinitol and d-chiro-inositol levels after dietary treatment in humans. Negative ion chemical ionization mass spectrometry was especially useful for determining these levels because it is sensitive to sub-picomolar amounts, and when combined with appropriate deuterated internal standards, represents a definitive assay. The specificity of our measurements was also improved by separation of d-chiro-inositol from the L-chiro-inositol enantiomer by chiral gas chromatography.
Effect of pinitol on insulin action

since occasional samples contain large amounts of the latter.

Defective metabolism of D-chiro-inositol may be one of the causes for impaired insulin action and insulin resistance in type 2 diabetes. Inositol phosphoglycans are generated from membrane glycosylphosphatidylinositol (GPI) phospholipids and/or GPI proteins as a result of insulin receptor binding (20,21). They are transported into their target cells, by an unknown mechanism, where they modulate various kinases and phosphatases (10,20). Although inositol phosphoglycans have been shown to have insulin-like effects (1,4), the precise role in the insulin-signaling pathway is still unknown (20–22). A deficiency of D-chiro-inositol in the pathogenesis of insulin resistance is supported by animal studies (3,4,7) and measurement of reduced chiro-inositol tissue levels in diabetic subjects (5,11,13). These findings could be related to insulin and glucose levels or some unidentified impairment and cause a deficiency of D-chiro-inositol in diabetic tissues (11,13).

Nestler et al. (14) recently demonstrated increased insulin action in women with polycystic ovary syndrome treated with D-chiro-inositol. No change was seen in fasting plasma insulin concentration, although 6 women normalized their glucose tolerance curves as a result of treatment (14). These data suggest that inositol supplementation can improve insulin sensitivity and cause beneficial changes in either production or catabolism of plasma triglyceride rich lipoproteins.

In contrast to the report of Nestler et al. (14), results of the present study do not support a beneficial effect of inositol therapy on insulin sensitivity in diabetic subjects. There are several possible explanations for the differences observed. Most of our subjects had frank diabetes and were more obese (BM 36.5 vs. 31.2 kg/m²) and less hyperinsulinemic (112 vs. 206 pmol/l) than the women with polycystic ovary syndrome (14). Because post-receptor binding defects are a primary cause of insulin resistance (9), it is possible that our subjects were more resistant to the effects of inositol phosphoglycan insulin mediators as well as to insulin itself. In this case, the site of resistance would be in glycan receptor molecules or even later parts of the insulin-signaling pathway. Increased urinary losses of D-chiro-inositol have been related to urine and plasma glucose concentrations and HbA₁c (13), and in vitro studies have shown that D-chiro-inositol transport may be decreased in the presence of hyperglycemia (15). These interactions would have been absent in the non-diabetic polycystic ovary syndrome patient. Our sample size was designed to detect changes in insulin sensitivity exceeding 20%; a larger sample may have detected smaller changes in the parameters studied. In fact, the pinitol-treated group showed a trend of decreasing fasting glucose and total cholesterol that did not reach statistical significance (P = 0.10, Table 1). Our study also used pinitol instead of free D-chiro-inositol. Although pinitol was converted to D-chiro-inositol in vivo, our estimated conversion rate of 33% may not be sufficient for a measurable metabolic effect if conversion is important. There are no data available on conversion of pinitol to D-chiro-inositol in normal subjects and plasma inositols were not measured in the women with polycystic ovary syndrome (14).

An additional consideration is the underlying defect of insulin action in the populations studied. Insulin resistance is not necessarily an inherent feature of the polycystic ovary syndrome, whereas increased insulin secretion has been described in many such women (23,24). Thus, the primary effect of D-chiro-inositol in polycystic ovary syndrome may be to reduce the hypersecretion of insulin rather than to improve insulin sensitivity. If this is true, then the effect of D-chiro-inositol and pinitol needs to be evaluated in additional hyperinsulinemic subjects.

In summary, the results of the present study indicate that although 4 weeks of oral pinitol supplementation in people with insulin resistance caused marked increases in plasma pinitol and D-chiro-inositol concentrations, it did not alter basal glucose and lipid kinetics or the effect of insulin on glucose and lipid metabolism. Additional research is needed to define the physiological and potential therapeutic effects of pinitol and D-chiro-inositol administration.

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References