A single acute dose of pinitol from a naturally-occurring food ingredient decreases hyperglycaemia and circulating insulin levels in healthy subjects

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A limited amount of research suggests that oral ingestion of pinitol (3-0-methyl-0-chiro-inositol) positively influences glucose tolerance in humans. This study assessed the effects of different doses of pinitol supplementation on glucose tolerance, insulin sensitivity and plasma pinitol concentrations. Thirty healthy subjects underwent two one-day trials in which they consumed a nutritive beverage (Fruit Up®) containing 2.5, 4.0 or 6.0 g of pinitol and a corresponding placebo equivalent in both energy and carbohydrates. Blood samples were collected frequently over the 240-min test period. The pinitol-enriched beverage reduced serum glucose and insulin at 45 and 60 min, but only at a dose of 6.0 g. Plasma pinitol concentrations, maximum concentration and AUC increased according to the dose administered. The results show that a single dose of pinitol from a naturally-occurring food ingredient at the highest dose administered acutely influences indices of whole-body glucose tolerance and insulin sensitivity in healthy subjects.

1. Introduction

Inositol phosphoglycans (IPG) are important post-receptor mediators of insulin action (Larner, Brautigan, & Thorner, 2010; Larner et al., 1998) and contain several compounds, including 0-chiro-inositol, myo-inositol, galactosamine, glucosamine, and other residues. Of these compounds, myo-inositol is widely distributed in mammalian tissues, whereas 0-chiro-inositol is relatively uncommon (Larner et al., 1998). The 0-chiro-inositol-containing IPG – a secondary messenger in the mediation of insulin’s effect on peripheral glucose utilisation – activates glycogen synthase and pyruvate dehydrogenase (Larner, 2002; Larner et al., 2010; Ortmeyer, Bodkin, Hansen, & Larner, 1995; Saltiel, 1990). A potential dietary source of 0-chiro-inositol is pinitol (3-0-methyl-0-chiro-inositol), a methylated derivate of 0-chiro-inositol present at high concentrations in various legumes, such as carob and whole soybean. Pinitol exerts an insulin-like effect to improve glycaemic control, which suggests that there is a synergy between the two at submaximal concentrations, though this is not evident with glucose transporter (Bates, Jones, & Bailey, 2000).

The literature is inconclusive regarding the effects of pinitol supplementation on glycaemic control where most of the study populations were diabetic or had impaired glucose tolerance. Several long-term studies in humans (Kim et al., 2005, 2007, 2012) have demonstrated that using pinitol as a dietary supplement improves glycaemic control, whereas others did not find any effect despite an increase in plasma concentrations of pinitol (Campbell et al., 2004; Choi et al., 2011; Davis et al., 2000). In contrast with the abovementioned chronic studies, only two reports have evaluated the acute effects of oral pinitol supplementation on glycaemia response showing contradictory results. Whereas Kang, Kim, Yoon, Kim, and Cha (2006) showed a reduction in postprandial blood glucose, Stull, Wood, Thuyaft, and Campbell (2009) did not report
effects on glycaemic control despite the increase in plasma pinitol. Therefore, the purpose of this study was to assess the acute effects of increasing doses of pinitol on glucose tolerance and insulin sensitivity and to evaluate the absorption of pinitol after consumption of an oral nutritive pinitol-enriched beverage in healthy subjects.

2. Materials and methods

2.1. Subjects

Thirty-one healthy volunteers were recruited among healthy relatives of patients and clinical and laboratory staff to the Outpatient’s Department of the Endocrinology Service of the University Hospital Dr. Peset, between July 2011 and December 2011. The inclusion criteria for all subjects were age range of 18–65 years, body mass index of 20–30 kg/m² and clinically normal kidney function, liver function, heart function, protein status and haematological profile. Exclusion criteria were pregnancy or lactation, alteration of carbohydrate metabolism, fasting glycaemia ≥ 100 mg/dl on at least two previous occasions, diabetes, or medication known to interfere with glucose metabolism.

The study was conducted according to the guidelines laid down in the Declaration of Helsinki, and all procedures involving human subjects were approved by the hospital’s Ethics Committee. Written informed consent was obtained from all patients. One volunteer dropped out of the study for personal reasons, including lack of time and difficulties in attending the research clinic.

2.2. Study design

The study consisted of a randomised parallel trial with a three-arm design single-blinded placebo controlled and cross-over. The subjects were randomised by alternation method to belong to a group of low dose (n = 10), intermediate dose (n = 10) or high dose (n = 10), containing 2.5, 4.0 or 6.0 g of pinitol, respectively. Each subject was newly randomised (1:1) into pinitol-enriched beverage group or placebo beverage group and cross-over; in this way each subject completed two 1-day trials separated by a 1-week interval.

Participants received detailed written and oral instructions about the diet from an experienced dietician on the three days prior to initiation of the trial, including the precise amounts of food to be eaten and the quality of the food, according to the main food groups. The recommended diet consisted of 15–20% protein, 28–33% fat and 50–55% carbohydrate. Alcohol and drinks containing caffeine were restricted, as were naturally pinitol-enriched foods (e.g., soy or legumes). Adherence to the diet was monitored by means of 3-day food records (compiled on weekdays) and 24-h diet recall. Food intake was converted into energy and nutrients with the help of the software NutriPre v1.1.3. The diet recalls and records revealed no deviations from the guidelines during the course of the study. Subjects were encouraged to maintain their normal pattern of activity.

The pinitol-enriched beverage (prepared from the commercial natural food ingredient Fruit Up®) and placebo beverage were produced by Wild-Valencia SA (Spain) and packed in silver cans. Fruit Up® consists of a complex mixture of naturally-occurring soluble carbohydrates (including mono-, di-, oligo-saccharides and polyalcohols) and minor compounds (organic acids, minerals, amino-acids) derived from carob. Three doses of Fruit Up® (diluted with mineral water to a final volume of 330 ml) were evaluated, and were equivalent to an intake of 2.5, 4.0 and 6.0 g of pinitol. The placebo beverage contained equal amounts of non-polyol carbohydrates with similar macronutrient composition, glycaemic load ((glycaemic index + dietary carbohydrate content)/100) and total calories as those obtained through the enriched beverage, but excluding pinitol. Subjects were recommended to consume the beverage in a time interval of 5–10 min. Nutritional composition of the different doses of the pinitol-enriched product with respect to their placebos is shown in Table 1.

Anthropometrical parameters were evaluated as follows: weight was determined using electronic scales with an approximation of 0.1 kg; height was measured with a stadiometer with an approximation of 0.5 cm; BMI was calculated by dividing the weight in kilograms by the square of the height in metres; blood pressure was measured twice consecutively using a mercury sphygmomanometer; waist circumference was measured at the natural indentation between the 10th rib and the iliac crest using a metric tape with approximations of 0.5 cm.

2.3. Blood sampling

Each subject attended the Endocrinology Service first thing in the morning after 12-h overnight fasting. A catheter was inserted into an antecubital vein and a blood sample was collected at baseline (minute 0; while still fasting), and immediately after (within 5 min) consumption of the test product placebo or pinitol-enriched beverage. Blood samples were collected in Vacutainer serum separator tubes (BD, Franklin Lakes, NJ), for serum glucose and insulin analysis at 15, 30, 45, 60, 90 and 120 min. For plasma pinitol analysis blood samples were collected into Vacutainers containing lithium heparin at 60, 120, 180 and 240 min. All samples were centrifuged at 2000g for 15 min at 4 °C. Freshly separated serum was employed for determination of glucose, and the remaining aliquots of serum and plasma were dispensed into cryovials and then stored at −80 °C until they were assessed for circulating levels of insulin and pinitol, respectively.

Glucose concentrations were measured by means of enzymatic assay in a Beckman LX-20 autoanalyzer (Beckman Coulter, La Brea, CA). The intraseries coefficient of variation was <3.5% for all determinations. Concentration of the sample was calculated as the ratio of absorbance of sample to absorbance of standard multiplied by standard concentration (100 mg/dl) according to manufacturer’s instructions. For quality control, we used control sera to monitor the performance of the assay procedure. Insulin concentrations were determined by enzyme-linked immunosorbent assay (human insulin ELISA, Millipore Corporation, Billerica, MA) (intra-assay coefficient of variation <5%). The calibration curve was linear within the concentration range of 2–200 μM. Insulin resistance was estimated according to the homeostasis model assessment of insulin resistance (HOMA-IR) index (fasting insulin (μU/ml) x fasting glucose (mg/dl)/405); insulin secretion (early secretory responses to an oral glucose load) was estimated as Δln[insulin30-0]/ΔGlucone30 (Phillips, Clark, Hales, & Osmond, 1994).

Plasma pinitol concentrations were determined by gas chromatography/mass spectrometry. In short, 0.2 μg of internal standard allo-inositol (Sigma–Aldrich, St. Louis, MO) were added to 0.1 ml plasma samples and 0.1 ml zinc sulphate heptahydrate (10%) (Sigma–Aldrich) that were then saponified with 1 ml of sodium hydroxide (1 N) (Scharlau, Barcelona, Spain). Finally, 140 μl of the extract were derivatized with 260 μl of pyridine:hexamethyldisilazane:chlorotrimethylsilane (10:2:1) (Sigma–Aldrich) for 1 h at room temperature. The derivatised samples were analysed using electron impact capillary gas chromatography/mass spectrometry. Typical electron energy was 70 eV, with the ion source temperature maintained at 250 °C. The sample was separated using a fused-silica capillary column (HP-5MS, 5% phenyl methyl siloxane, 30 m × 0.25 mm, Agilent Technologies, Santa Clara, CA). The oven temperature was maintained at 100 °C for 1 min, then raised at a rate of 30 °C/min to 200 °C and maintained for 5 min, and then raised once again at a rate of 15 °C/min to a final temperature of 285 °C. The injector temperature was set at 300 °C. The calibration
The integrated area under the curve (AUC) for glucose, insulin (at 120 min) and pinitol (at 240 min) was calculated using the trapezoidal method (Tai, 1994).

2.4. Statistical analyses

Sample size was 10 subjects per group, in order to provide 80% statistical power and, thus, detect differences between the two paired groups in the values of the primary efficacy criterion (pos-partial glycaemia variation) equal to or greater than 5 mg/dl, assuming a common standard deviation of 5 mg/dl.

Statistical analysis was carried out with the statistics program SPSS version 17.0 (SPSS Inc., Chicago, IL). Data are expressed as mean and standard deviation (SD) in tables and standard error of the mean (SEM) in figures. Differences between-groups and within-group differences were analysed using two-factor repeated-measures analysis of variance (ANOVA) followed by paired Student’s t-test to compare placebo vs pinitol. Different pinitol doses were compared with a one-way analysis of variance (ANOVA) and post hoc Tukey test. Finally, the correlation between variables was determined using Spearman’s correlation coefficient. All tests used a confidence interval of 95% and differences were considered significant when p < 0.05.

3. Results

The present study analysed a total of 30 healthy voluntaries – 11 men and 19 women – with a mean age of 32.5 ± 7.0 years. Anthropometric data, blood pressure and analytical variables at baseline are shown in Table 2. The results showed no significant differences among the study groups when the population was divided into three groups according to the varying doses of pinitol administered. In relation to compliance with the dietetic recommendations, which was essential for a correct assessment of glycaemia, the minimum carbohydrate ingestion was adequate in all cases (data not shown). Acute pinitol ingestion was found to be well tolerated and no adverse events (including hypoglycaemic episodes) were reported for any of the three doses administered.

After oral ingestion of the nutritive beverage (pinitol-enriched or placebo), serum glucose and insulin concentrations were determined at 0, 15, 30, 45, 60, 90 and 120 min in a subset of 10 subjects for each dose of pinitol administered (2.5, 4.0 and 6.0 g). During the test period, mean glucose and insulin concentrations did not vary between the pinitol and placebo groups at either low or intermediate doses. However, the highest dose of pinitol produced a significant reduction in glucose and insulin levels at 45 and 60 min (Figs. 1 and 2, respectively). In this way, only the 6.0 g dose of pinitol reduced the integrated AUC for glucose and insulin with respect to its placebo (13261 ± 1416 vs 12532 ± 1675 mg dl⁻¹ min for glucose and 2005 ± 842 vs 1730 ± 644 μU ml⁻¹ min for insulin in placebo and pinitol-enriched beverages, respectively), although significant differences were only observed between insulin levels.

When evaluating glucose levels according to the dose–response curves for the placebo beverage, an increase in glucose levels was detected with the highest dose, which corresponded with the higher levels of sucrose in the product’s composition. However, non-significant differences in glucose levels were found when the different doses of pinitol were compared throughout the test period (data not shown).

The association between the increase in glucose levels and the increase in insulin secretion levels at 30 min – a surrogate for decreased secretory response that has been shown to be a significant predictor of type 2 diabetes (Haffner, Miettinen, Gaskill, & Stern, 1995) – versus glucose at 30 min is represented in Fig. 3. A negative correlation was observed in the placebo group between ΔInsulin30/ΔGlucose30 min and glucose at 30 min (r = -0.530, p = 0.004) (Fig. 3, panel A). Conversely, this correlation was not present in the group receiving the pinitol-enriched beverage (r = -0.256, p = 0.207) (Fig. 3, panel B).

Fasting plasma pinitol concentrations were below the level of detection at baseline. After ingestion of the placebo beverage, plasma pinitol remained undetected throughout the 240-min test period. However, 60 min after oral consumption of the pinitol-enriched beverage, plasma pinitol concentrations became detectable for all doses and increased over the 240-min test period (Fig. 4).

Standardised pharmacokinetic parameters of pinitol for each dose administered are shown in Table 3. The maximum/peak concentration (Cmax) and AUC0–240 min of pinitol increased with the dose of pinitol. Nevertheless, statistically significant differences were found only between the effects of low and high doses (2.5 and 6.0 g), whereas those of the intermediate dose (4.0 g) did not differ from those of the other two doses. Furthermore, the standardised Cmax and AUC0–240 min per gram of pinitol ingested (Cmax/dose and AUC0–240 min/dose) decreased significantly as the dose of pinitol increased.

4. Discussion

The present study shows that consumption of a pinitol-enriched beverage, containing a dose of 6.0 g, reduces the increase in glycaemia and insulinaemia provoked by oral carbohydrate over-

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**Table 1**

Nutritional composition of the main soluble carbohydrates contained in different Fruit Up® beverage administered in the study.

<table>
<thead>
<tr>
<th>Carbohydrate</th>
<th>Low dose (2.5 g)</th>
<th>Intermediate dose (4.0 g)</th>
<th>High dose (6.0 g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pinitol</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Placebo</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fruit Up</td>
<td>38.6</td>
<td>61.7</td>
<td>92.5</td>
</tr>
<tr>
<td>Pinitol</td>
<td>2.5</td>
<td>4.0</td>
<td>6.0</td>
</tr>
<tr>
<td>Sugars</td>
<td>21.2</td>
<td>34.0</td>
<td>51.0</td>
</tr>
<tr>
<td>Glucose</td>
<td>3.09</td>
<td>5.09</td>
<td>8.85</td>
</tr>
<tr>
<td>Fructose</td>
<td>2.86</td>
<td>4.57</td>
<td>6.85</td>
</tr>
<tr>
<td>Sucrose</td>
<td>14.7</td>
<td>23.5</td>
<td>35.3</td>
</tr>
<tr>
<td>Oligosaccharides</td>
<td>1.46</td>
<td>2.34</td>
<td>3.53</td>
</tr>
<tr>
<td>Total calorie</td>
<td>96.6</td>
<td>155.0</td>
<td>232.5</td>
</tr>
<tr>
<td>Glycaemic load</td>
<td>15.7</td>
<td>25.1</td>
<td>37.7</td>
</tr>
</tbody>
</table>

**Table 2**

Anthropometric and clinical parameters in the study population at baseline according to the dose of Fruit Up® containing 2.5, 4.0 and 6.0 g of pinitol.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Low dose (2.5 g)</th>
<th>Intermediate dose (4.0 g)</th>
<th>High dose (6.0 g)</th>
<th>p-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>n (men/women)</td>
<td>10/4 (6/6)</td>
<td>10/3 (7/7)</td>
<td>10/4 (6/6)</td>
<td></td>
</tr>
<tr>
<td>Age (years)</td>
<td>31.0 ± 9.9</td>
<td>33.2 ± 4.7</td>
<td>33.1 ± 6.2</td>
<td>0.760</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>24.1 ± 2.8</td>
<td>23.8 ± 3.7</td>
<td>23.4 ± 3.1</td>
<td>0.870</td>
</tr>
<tr>
<td>Waist (cm)</td>
<td>83.2 ± 9.7</td>
<td>82.3 ± 10.0</td>
<td>83.9 ± 9.8</td>
<td>0.955</td>
</tr>
<tr>
<td>Systolic BP (mmHg)</td>
<td>118 ± 12</td>
<td>117 ± 11</td>
<td>115 ± 10</td>
<td>0.908</td>
</tr>
<tr>
<td>Diastolic BP (mmHg)</td>
<td>71 ± 9</td>
<td>73 ± 12</td>
<td>71 ± 8</td>
<td>0.861</td>
</tr>
<tr>
<td>Glucose (mg/dl)</td>
<td>89.5 ± 7.2</td>
<td>86.1 ± 7.8</td>
<td>90.2 ± 5.6</td>
<td>0.360</td>
</tr>
<tr>
<td>Insulin (μU/ml)</td>
<td>5.55 ± 2.42</td>
<td>4.99 ± 2.76</td>
<td>4.26 ± 2.18</td>
<td>0.508</td>
</tr>
</tbody>
</table>

Data are expressed as mean ± standard deviation. HOMA-IR index was estimated as fasting insulin (μU/ml) × fasting glucose (mg/dl)/405.
load when compared with a placebo. These data are in accordance with findings in animal models in which a single dose of pinitol reduced the plasma response to an oral glucose challenge in non-diabetic and streptozotocin diabetic mice (Bates et al., 2000; Narayanan, Joshi, Mudjumdar, & Dhekne, 1987). Only two previous studies have evaluated the effect of oral pinitol supplementation at several doses on the glycaemia response to an oral carbohydrate challenge in humans. Kang et al. (2006) showed that a dose of 1.2 g of pinitol one hour prior to a meal tolerance test reduced glucose AUC but not insulin AUC in diabetic patients. In contrast, Stull et al. (2009) reported that acute oral administration of 1.0 g of pinitol 1 h prior to an oral glucose tolerance test did not influence a fasting- and oral glucose-induced rise in plasma glucose and insulin concentrations in older non-diabetic subjects. The fact that our study population was younger and the pinitol consumption higher than that of the aforementioned studies could explain these differences. With regard to chronic pinitol supplementation studies in humans, the literature is ambiguous. Some studies have observed a glucose-lowering effect of pinitol (Kim et al., 2005, 2007, 2012), while others have not found a differential response (Campbell et al., 2004; Choi et al., 2011; Davis et al., 2000).

In line with previous studies (Davis et al., 2000; Stull et al., 2009), we observed that intake of 2.5, 4.0 and 6.0 g of pinitol led to increasing pinitol plasma levels, while pinitol plasma levels were undetectable when a placebo beverage was consumed. Our data show that pinitol supplementation at different doses increases pinitol concentration in the bloodstream over a 240-min test period. Moreover, they demonstrate that pinitol absorption is not proportional to the dose administered. In pharmacokinetic terms, absorption is not first-order kinetic; thus, a saturable absorption would be expected as a result of a probable competition between pinitol and the other products contained in the pinitol-enriched beverage, with a marked effect being produced as the dose increases. Our results also show that glucose levels are almost normal in the first hour after consumption of pinitol, though...
Maximum plasma concentration, area under the curve of pinitol determined in between variables was determined using Spearman’s correlation coefficient.

The precise mechanism by which pinitol may influence glucose metabolism is not well defined. The classic actions of insulin involve increased glucose uptake from the bloodstream and its metabolism in peripheral tissues. However, non-oxidative and oxidative glucose disposal through activation of glycogen synthase and mitochondrial pyruvate dehydrogenase are not completely explained by current models. A recent review (Larner et al., 2010) has proposed a model in which an alternative pathway of insulin coupled to G protein activates phospholipase D. The action of phospholipase D could lead to the release of insulin’s second messenger from an IPG lipid precursor in the inner and/or outer leaflets of the plasma membrane, or may be released inside the cytoplasm or outside the cell as a secondary messenger, and may move back into the cell of origin or into neighbouring cells via an ATP-dependent inositol glycan transporter (Coady, Wallendorff, Gagnon, & Lapointe, 2002). Inside the cell, IPG activates glycogen synthase by acting as insulin’s second messenger. Thus, by acting as insulin’s second messenger, pinitol could increase insulin sensitivity. In fact, we can report a negative correlation between glucose and insulin secretion levels in subjects receiving the placebo beverage, thus suggesting that glucose levels drop as insulin secretion rises. In contrast, this trend was not observed in volunteers consuming the pinitol-enriched product, in whom plasma insulin and glucose concentrations were lower, which points to higher insulin sensitivity in these subjects.

It has been suggested that pinitol is converted to d-chiro-inositol. Davis et al. (2000) reported that the plasma concentrations of pinitol and d-chiro-inositol increased 48-fold and 14-fold, respectively, in fasting samples taken after 4 weeks of pinitol supplementation (20 mg/kg). They suggested that the 14-fold increase in plasma d-chiro-inositol concentration was consistent with an in vivo conversion of pinitol to d-chiro-inositol. However, in another study of urinary excretion of pinitol and d-chiro-inositol, the lack of an alteration in urinary d-chiro-inositol excretion did not support the existence of in vivo conversion (Campbell et al., 2004), although the authors failed to detect differences in plasma pinitol concentration after 6 weeks of supplementation with 2.0 g of pinitol.

As far as we know, ours is the only study to evaluate the efficacy of pinitol in young normal fasting glucose subjects. Moreover, we have extended the scope of the study in order to assess the optimal dose of pinitol for producing an improvement in the glucose metabolism in a normoglycaemia state. A strength of this study is that we have employed a randomised, placebo-controlled, and cross-over study design in which the diet was controlled by an experienced dietician prior to initiation of the trial. Moreover, the protocol measurements were performed in a clinical research...
setting. On the other hand, we should point out that our study involved a relatively small, though homogenous, sample of healthy subjects. We cannot say if the metabolic changes we observed are maintained over longer periods of time in these or other individuals with impairment of glucose metabolism.

4. Conclusion

In summary, our findings demonstrate the positive effect of oral pinitol supplementation on glucose and insulin levels in normal fasting glucose subjects. This dietary intervention would appear to be an effective first-step strategy for treating hyperglycaemia in subjects. This dietary intervention would appear to be an effective first-step strategy for treating hyperglycaemia and related insulin resistance states, although future research is warranted to evaluate whether chronic doses of pinitol are effective in subjects with altered glucose metabolism.

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References


