Pinitol Supplementation Does Not Affect Insulin-Mediated Glucose Metabolism and Muscle Insulin Receptor Content and Phosphorylation in Older Humans1,2

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ABSTRACT This study assessed the effect of oral pinitol supplementation on oral and intravenous glucose tolerances and on skeletal muscle insulin receptor content and phosphorylation in older people. Fifteen people (6 men, 9 women; age 66 ± 8 y; BMI 27.9 ± 3.3 kg/m2; hemoglobin A1c 5.39 ± 0.46%, mean ± SD) completed a 7-wk protocol. Subjects were randomly assigned to groups that during wk 2–7 consumed twice daily either a non-nutritive beverage (Placebo group, n = 8) or the same beverage with 1000 mg pinitol dissolved into it (Pinitol group, n = 7, total dose = 2000 mg pinitol/d). Testing was done at wk 1 and wk 7. In the Pinitol group with supplementation, 24-h urinary pinitol excretion increased 17-fold. The fasting concentrations of glucose, insulin, and C-peptide, and the 180-min area under the curve for these compounds, in response to oral (75 g) and intravenous (300 mg/kg) glucose tolerance challenges, were unchanged from wk 1 to wk 7 and were not influenced by pinitol. Also, pinitol did not affect indices of hepatic and whole-body insulin sensitivity from the oral glucose tolerance test and indices of insulin sensitivity, acute insulin response to glucose, and glucose effectiveness from the intravenous glucose tolerance test, estimated using minimal modeling. Pinitol did not differentially affect total insulin receptor content and insulin receptor phosphotyrosine 1158 and 1162/1163 activation in vastus lateralis samples taken during an oral-glucose–induced hyperglycemic and hyperinsulinemic state. These data suggest that pinitol supplementation does not influence whole-body insulin-mediated glucose metabolism and muscle insulin receptor content and phosphorylation in nondiabetic, older people. J. Nutr. 134: 2998–3003, 2004.

KEY WORDS: • d-chiro-inositol • C-peptide • oral glucose tolerance test • intravenous glucose tolerance test

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under the curve (AUC) for C-peptide during a 75-g oral glucose tolerance test (OGTT) in older, nondiabetic people. These data may suggest an association between insulin secretion and the urinary excretion of these compounds. Davis et al. (10) reported that oral supplementation of obese patients who had either type 2 diabetes mellitus or impaired glucose tolerance with 20 mg pinitol · kg⁻¹ · d⁻¹ for 4 wk increased plasma pinitol concentration 48-fold and increased plasma D-chiro-inositol concentration 14-fold but did not influence glucose production, insulin-mediated glucose disposal, or rates of appearance of free fatty acids and glycerol in plasma. The authors suggested that these obese, diabetic, and impaired glucose patients might have been resistant to the effects of pinitol because of inherent abnormalities in the insulin-signaling pathway and that pinitol supplementation might be more effective in people who do not have an underlying defect of insulin action.

The primary purpose of the present study was to assess in older humans who do not have diabetes the effect of oral pinitol supplementation on whole-body glucose uptake and insulin sensitivity using oral and intravenous glucose tolerance tests. The second purpose was to evaluate the effect of pinitol on insulin action in skeletal muscle. Because of a lack of previous research examining pinitol supplementation on insulin signaling, we started this evaluation at the beginning of the insulin-signaling pathway with measurements of skeletal muscle total insulin receptor content and phosphorylated insulin receptor activation in a state of glucose-stimulated hyperglycemia and hyperinsulinemia. A double-blinded, placebo-controlled, repeated measures study design was used. We hypothesized that pinitol supplementation would improve responses to oral and intravenous glucose tolerance challenges, would increase the total insulin receptor content, and would increase insulin receptor phosphorylation on tyrosines 1158, 1162, and 1163. Older persons who were screened to not have type 1 or type 2 diabetes mellitus were chosen for study because they may be at increased risk for impaired insulin sensitivity, yet possibly may be more responsive to pinitol-related improved glucose uptake than young, healthy, insulin-sensitive men and women.

MATERIALS AND METHODS

Subjects. Sixteen subjects (6 men and 10 women), age 50 y or above, were recruited from the Little Rock and central Arkansas region to participate in this study. Exclusion criteria included the following: 1) current acute or chronic disease, or drug use known to alter carbohydrate metabolism, 2) a previous or current diagnosis of type 1 or type 2 diabetes mellitus, 3) performance of >40 min of physical exercise 4 or more d/wk or the initiation of a structured exercise program in the past 3 mo, 4) body weight instability >2 kg in the past 3 mo, and 5) impaired renal function (serum creatinine concentration >150 μmol/L). All women were postmenopausal for at least 2 y. The subjects' habitual dietary practices were not used as a screening criterion. Before admittance into the study, each subject successfully completed an evaluation that included a physical examination, a resting electrocardiogram, a 75-g OGTT, and routine blood screening criterion. Before admittance into the study, each subject received monetary compensation for participating.

Experimental design. Each subject completed a 7-wk protocol. Week 1 was used to complete baseline testing and evaluations (Baseline). Weeks 2–7 comprised the period of supplementation. Testing and evaluations were repeated at wk 7 (Post). At wk 1 and wk 7, each subject resided at a general clinical research center on the night immediately before the intravenous glucose tolerance test (IVGTT) procedure.

Dietary control. For 3 d during study wk 1 and 7, each subject was provided with controlled, macronutrient-defined meals, which they were required to completely consume. Each subject's total energy intake was estimated using the gender-specific Harris-Benedict equation (11), plus a 75% allowance for the energy expenditure of habitual daily physical activity (12,13). The total energy intake of each menu consisted of 15% protein, 35% fat, and 50% carbohydrate.

Supplementation. During wk 1, each subject consumed, twice daily, a placebo drink consisting of 30 mL of a saccharin-sweetened, cherry-flavored beverage. During wk 2–7, the subjects in the Placebo group continued to consume the same beverage twice daily, whereas the subjects in the Pinitol group consumed, twice daily, the same beverage with 1000 mg pinitol (INZITOL, CAS #10284–63-6, 3-O-methyl-2-k-glucofuranosyl-α-D-glucose, 96% purity based on high performance liquid chromatography analysis, Hema- netics) dissolved into each beverage (total pinitol intake 2000 mg/d). Presudy blinded taste tests established that the sweetness and the flavor of the beverage completely masked any taste of the pinitol. Dietary technicians who were otherwise not involved in the study made up the placebo and pinitol beverages. The subjects and the investigators were unaware of the group assignments of the subjects.

OGTT. At wk 1 and 7, OGTTs were performed. Each subject came to the laboratory in the morning after an overnight 12-h fast. While the subject was in a postabsorptive state, a catheter was inserted into an antecubital vein, and blood was drawn. The subject then consumed either the placebo or the pinitol beverage (which was void of energy), and a 75-g glucose solution was administered. The oral glucose solution was administered intravenously at the same time but using a 50% (wt:v) glucose solution over a 20-s period. For each subject, OGTT procedures, all blood samples were collected into heparinized tubes. The tubes were centrifuged, and aliquots of plasma were either stored unfrozen at 4°C and subsequently analyzed for insulin and C-peptide concentrations, using double-antibody radioimmunoassays, or stored frozen at –20°C until analyzed.

IVGTT. At wk 1 and 7, an IVGTT was performed the day after the OGTT. The subjects resided overnight at the General Clinical Research Center and remained fasting for 12 h before starting the procedure. A catheter was inserted into an antecubital vein, and 3 fasting blood samples were drawn. Each subject then consumed either the placebo or the pinitol beverage and was administered 300 mg glucose/kg by intravenous injection of precisely portioned quantities of a 50% (wt:w) glucose solution over a 60-s period. For each subject, the amount of glucose solution given at wk 1 and 7 was exactly the same. Blood samples (2 mL) were taken at 2, 3, 4, 5, 6, 8, 10, 12, 14, 16, 19, 22, 25, 30, 40, 50, 60, 70, 90, 100, 120, 140, 160, and 180 min. 24-h urine collections. Timed 24-h urine collections were made during d 2 of dietary control at wk 1 and 7. The subjects were instructed to collect all voided urine into a disposable 4-L polyethylene container and to keep the container chilled, using a cooler and ice packs or a refrigerator. Each urine-filled container was returned to the laboratory the morning that the collection ended, 10 mL of 1.0 normal acetic acid was mixed into the container as a preservative, and an aliquot was placed in polypropylene tubes and was stored at –20°C until analyzed.

Analytical methods and calculations. For the OGTT and IVGTT procedures, all blood samples were collected into heparinized tubes. The tubes were centrifuged, and aliquots of plasma were either analyzed for glucose concentration, using the oxidase method standard for a Beckman glucose analyzer (model 6517, Beckman Instruments), or stored frozen at –20°C and subsequently analyzed for insulin and C-peptide concentrations, using double-antibody radioimmunoassays as described by Engdahl et al. (15). For both
the OGTT and IVGTT, the integrated AUC for glucose, insulin, and C-peptide were calculated using the trapezoidal method (16). For the OGTT, estimates of hepatic and whole body (hepatic plus peripheral) insulin sensitivities were calculated using the formulas described by Matsuda and DeFronzo (17). For the IVGTT, estimates of insulin sensitivity, acute insulin response to glucose, and glucose effectiveness were calculated using the MINMOD Millennium computer program (version 5.0, 2001) (18). The fasting-state plasma samples collected as part of the OGTTs and aliquots from the 24-h urine program were processed for analyses of inositols by a modification of a previous method (2,10). The purified inositol fractions were derivatized by heating with 100 μL of 10% pentfluoropropionylimidazole in acetonitrile for 2 h at 65°C. These samples were then analyzed in a chromatograph on a 25-m 0.25-mm i.d. Chiral-Val column (Alltech) and were analyzed in the negative-ion mode with a mass spectrometer (Agilent Technologies model 5973) for myo-inositol, d-chiro-inositol, and pinitol. The muscle samples were analyzed for total insulin receptor (β-subunit) content, insulin receptor phosphorytrosine pY1158 activation, and insulin receptor phosphotyrosine pYpY1162/1163 activation, using ELISA kits from Biosource International. These analyses determined if pinitol supplementation altered total insulin receptor content (independent of its phosphorylation status) and insulin-stimulated receptor phosphorylation on tyrosines 1158, 1162, and 1163, which are known to initiate insulin receptor autophosphorylation and subsequent insulin signaling.

**Body composition.** Body weight was measured twice each wk to the nearest 0.1 kg (model 15S scale, Ohaus). All weights were taken in the morning after a 12-h overnight fast and soon after the subject had voided. Nude body weight was calculated as total body weight minus clothing weight. At wk 1, body weight without shoes was measured to the nearest 0.1 cm using a stadiometer (Country Tech, model 67031). At wk 1 and 7, whole body density was determined using a whole body plethysmograph (BOD POD, Life Measurement) (19). All body-density measurements were done in the morning after a 12-h overnight fast, soon after the subject had voided, and with the subject wearing only a tight-fitting bathing suit and a swimming cap. Percentage of body fat and fat-free mass were estimated from body density using the two-compartment model equation of Siri (20).

**Statistical analyses.** Values are reported as means ± SD. For each of the dependent variables, the difference between the Placebo group and the Pinitol group at wk 1 (baseline) was determined using one-way ANOVA. The main effects of group (Placebo vs. Pinitol) and time (wk 1 vs. wk 7) and the group-by-time interaction for each of the dependent variables were determined using two-way, repeated measures ANOVA. Post-hoc comparisons among the 4 mean values for a given variable were done using Student’s t test. Statistical significance was assigned if P ≤ 0.05. All data processing and calculations were performed using JMP Statistical Discovery Software (SAS Institute).

### RESULTS

**Characteristics, body composition, and plasma and urine inositols.** At baseline, there were no significant differences in mean age, height, weight, percentage of body fat, and fat-free mass between the Placebo and Pinitol groups (Table 1). Over time, from wk 1 to wk 7, there were no changes in weight, percentage of body fat, and fat-free mass, and there were no differences in responses over time for these dependent variables (i.e., there were no significant group-by-time interactions).

The plasma concentrations and 24-h urinary excretions of myo-inositol, d-chiro-inositol, and pinitol were not different between the Placebo and Pinitol groups at baseline, and were not changed at wk 7, except for urinary pinitol excretion in the Pinitol group (17-fold increase; group-by-time interaction, P < 0.0001) (Table 1).

**OGTT and IVGTT.** At baseline, there were no differences between the Placebo and Pinitol groups for any of the OGTT and IVGTT parameters (Table 2). With supplementation, there were no significant time-dependent or group-by-time-dependent changes in any of these parameters, with the exception of a group-by-time dependent change in the fasting C-peptide concentration (P = 0.048) and the decrease over time in the whole body insulin sensitivity index (P = 0.008) with the OGTT. The group-by-time interaction for fasting C-peptide indicated that this variable increased over time in the Pinitol group but not in the Placebo group. Post-hoc

### TABLE 1

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Placebo Baseline</th>
<th>Placebo Post</th>
<th>Pinitol Baseline</th>
<th>Pinitol Post</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y</td>
<td>65 ± 6</td>
<td>66 ± 11</td>
<td>71 ± 21</td>
<td>77 ± 6.2</td>
</tr>
<tr>
<td>Height, m</td>
<td>1.73 ± 0.13</td>
<td>1.67 ± 0.07</td>
<td>1.75 ± 0.62</td>
<td>1.78 ± 6.2</td>
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<tr>
<td>Weight, kg</td>
<td>84.2 ± 12.7</td>
<td>84.6 ± 13.1</td>
<td>40.9 ± 7.9</td>
<td>37.5 ± 12.0</td>
</tr>
<tr>
<td>Body fat, %</td>
<td>39.9 ± 8.8</td>
<td>40.9 ± 7.9</td>
<td>47.9 ± 6.8</td>
<td>47.9 ± 6.5</td>
</tr>
<tr>
<td>Fat-free mass, kg</td>
<td>50.9 ± 13.6</td>
<td>50.2 ± 12.0</td>
<td>47.9 ± 6.8</td>
<td>47.9 ± 6.5</td>
</tr>
<tr>
<td>Plasma myo-inositol, μmol/L</td>
<td>34 ± 6</td>
<td>36 ± 7</td>
<td>33 ± 6</td>
<td>36 ± 4</td>
</tr>
<tr>
<td>D-chiro-inositol, μmol/L</td>
<td>0.90 ± 0.24</td>
<td>1.16 ± 0.51</td>
<td>1.77 ± 1.63</td>
<td>0.51 ± 0.32</td>
</tr>
<tr>
<td>Pinitol, μmol/L</td>
<td>0.34 ± 0.41</td>
<td>1.19 ± 1.94</td>
<td>0.31 ± 0.29</td>
<td>1.07 ± 0.93</td>
</tr>
<tr>
<td>Urine myo-inositol, μmol/d</td>
<td>167 ± 99</td>
<td>197 ± 135</td>
<td>146 ± 71</td>
<td>213 ± 151</td>
</tr>
<tr>
<td>D-chiro-inositol, μmol/d</td>
<td>50 ± 35</td>
<td>47 ± 32</td>
<td>40 ± 22</td>
<td>42 ± 23</td>
</tr>
<tr>
<td>Pinitol, μmol/d</td>
<td>7.4 ± 9.9a</td>
<td>1.4 ± 1.2a</td>
<td>4.4 ± 8.6a</td>
<td>77.5 ± 31.8b</td>
</tr>
</tbody>
</table>

1 Values are mean ± SD (median); n = 8 Placebo group (5 women, 3 men); n = 7 Pinitol group (4 women, 3 men).
2 Group-by-time interaction, P < 0.0001. Values without a common superscript letter differ, P < 0.05.
analysis using Student's t test established that wk 7 fasting C-peptide was different between the Placebo and Pinitol groups. This finding was not supported by the fasting C-peptide results from the IVGTT. The decrease over time in C-peptide was different between the Placebo and Pinitol groups at baseline (Table 2). Over time, from wk 1 to wk 7, the group-by-time interactions.

**Muscle insulin receptors.** At min 60 of the OGTT, just before the vastus lateralis samples were obtained, plasma glucose and insulin concentrations were ~180 and ~600%, respectively, of the pre-OGTT concentrations. The vastus lateralis total insulin receptor content and the amount of insulin receptor tyrosine phosphorylated at tyrosines 1158 and 1162/1163 were not different between the Placebo and Pinitol groups at baseline (Table 2). Over time, from wk 1 to wk 7, the activation of these receptors was not changed (i.e., no main effect of time) or influenced by pinitol (i.e., no significant group-by-time interactions).

**DISCUSSION**

The results of this study suggest that 6 wk of oral pinitol supplementation by older, nondiabetic people does not influence oral or intravenous glucose tolerances, the insulin and C-peptide responses to these challenges, or estimates of hepatic and whole body insulin sensitivity. These data support the findings from the only other pinitol supplementation study in humans (10). Davis et al. (10), reported that supplementation of obese people (mean BMI, 37 ± 5 kg/m²) who had type 2 diabetes (82% of subjects) or impaired glucose tolerance (18% of subjects) with 20 mg pinitol · kg⁻¹ · d⁻¹ for 4 wk did not influence basal or insulin-stimulated glucose kinetics (assessed before and during the final 30 min of a 4-h period of hyperinsulinemia and euglycemia).

Whereas Davis et al. (10) did not report the mean body weight of their subjects, it may be reasonable to estimate it at about 106 kg, based on the mean BMI of 36.6 kg/m² and an estimated height of 1.7 m (the mean height of the subjects in the present study). Assuming this weight, the subjects in the Davis et al. (10) study likely consumed 2100 mg pinitol/d, a dose just higher than the 2000 mg pinitol/d consumed by the people in the present study. Davis et al. (10) reported that the plasma concentrations of pinitol and d-chiro-inositol increased 48-fold and 14-fold, respectively, in fasting samples taken after 4 wk of pinitol supplementation and with the final dose of pinitol consumed "just before" the blood collection. These authors 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. The urinary excretions of pinitol and d-chiro-inositol were not reported.

In the present study, the 17-fold increase in the 24-h urinary excretion of pinitol in the Pinitol group is consistent with compliance by these participants with the supplementation. The median 84 μmol/d urinary excretion is ~0.8% of the amount of pinitol consumed daily in the supplement. The lack of change in urinary d-chiro-inositol excretion does not support the suggestion of in vivo conversion of pinitol to d-chiro-inositol, although additional acute dosing studies are warranted to clarify this issue. The lack of change from wk 1 to wk 7 in plasma pinitol concentration in the Pinitol group suggests that orally ingested pinitol is cleared from the bloodstream before 12 h. The dramatic difference in plasma pinitol results between this study (no change) and Davis et al. (10) (48-fold increases).
increase) is likely because of the timing of the blood draw in relation to the most recent pinitol dosing (~12 h and just before, respectively).

In contrast with the results of the present study and those of Davis et al. (10) with pinitol supplementation, Nestler et al. (21) reported that oral supplementation of D-chiro-inositol (1200 mg/d vs. placebo control) for 6 to 8 wk decreased plasma insulin AUC during an OGTT in women with polycystic ovary syndrome. It is unknown whether the decreased insulin AUC was because of decreased insulin secretion or increased insulin clearance. Nestler et al. (21) did not report plasma D-chiro-inositol concentrations.

Animal research suggests that pinitol supplementation positively influences glucose metabolism. Narayanan et al. (7) reported that the oral dosing of pinitol, extracted from the leaves of Bougainvillea spectabilis and dissolved into water, lowered fasting plasma glucose concentrations in normal albino mice (30, 60, and 120 min after a single 10 mg pinitol/kg body weight dose) and alloxan-treated diabetic albino mice (120 and 240 min after 5, 10 mg pinitol/kg doses over a 72-h time period), and reduced the plasma glucose response to an oral glucose challenge in normal albino mice (after a single 10 mg pinitol/kg dose). Bates et al. (8) reported that oral dosing of pinitol (5, 10, and 100 mg D-pinitol/kg) to STZ-induced diabetic mice decreased plasma glucose progressively over a 6-h time period, with the greatest decrease (20%) observed with the 100 mg/kg dose. Plasma insulin concentration was not changed by pinitol in these mice. A similar decline (21%) in plasma glucose occurred when 100 mg pinitol/kg was administered by intraperitoneal (i.p.) injection, and lower plasma glucose was chronically maintained in STZ-diabetic mice that received 100 mg pinitol/kg i.p. injections twice daily for 11 d. D-pinitol also acutely increased basal but not insulin-stimulated 2-deoxyglucose uptake into cultured L6 muscle cells. In contrast, plasma glucose and insulin concentrations were not influenced by acute oral or i.p. doses of pinitol in normal and obese diabetic (ob/ob) mice, and pinitol did not influence the rate of glucose disappearance from the bloodstream during the 40 min after the i.p. administration of insulin in STZ-treated mice (8). Collectively, these studies in mice (7,8) suggest that pinitol influences plasma glucose most consistently in induced diabetic states, with more variable results obtained in basal and naturally occurring hyperglycemic states. The impact of acute pinitol supplementation on plasma glucose in humans is undocumented and requires investigation. The humans in the present study consumed a somewhat higher dose of pinitol (13 mg pinitol·kg⁻¹·d⁻¹; 26 mg pinitol·kg⁻¹·d⁻¹) than Narayanan et al. used (10 mg pinitol·kg⁻¹·d⁻¹) and less than Bates et al. used (100 mg pinitol·kg⁻¹·d⁻¹) in their mice studies.

The mechanism by which pinitol might influence plasma glucose is not known. Based on their studies in mice, Bates et al. (8) suggested that pinitol might have a direct effect on glucose transport that was independent of insulin, although the possibility that pinitol might somehow interact with the insulin-signaling pathway was not excluded. Pinitol and D-chiro-inositol are structurally similar to the inositol phosphates that influence insulin signaling via phosphatidylinositol 3-kinase (8). It is of interest to note that the pinitol-induced increase in basal-state glucose uptake into L6 muscle cells was prevented by incubating the cells with a phosphatidylinositol 3-kinase inhibitor (8). It is unknown whether pinitol directly influences glucose metabolism or only after in vivo conversion to D-chiro-inositol (10). D-chiro-inositol was proposed to influence glucose metabolism by accelerating the dephosphorylation of glycogen synthase and pyruvate dehydrogenase, rate-limiting enzymes of nonoxidative and oxidative glucose disposal (22). The influence of pinitol on these enzymes is undocumented in humans.

The urinary D-chiro-inositol excretion of the subjects in this study (40–50 μmol/d) is much higher than reported for younger (age 43 y) borderline obese (BMI 29.5 kg/m²) men and women with normal glucose tolerance (median 2.1 μmol/d) (2), and for postmenopausal women (age 61 y; BMI 28.3 kg/m²) (7.1 μmol/d) (9), but comparable with that of older men (age 62 y; BMI 29.8 kg/m²) (38.9 μmol/d) (9). Urinary D-chiro-inositol excretion is documented to increase with worsening glucose control (2) and to be higher in older persons with greater C-peptide AUC measured during an OGTT, an indirect indicator of both pancreatic β-cell activity and insulin secretion (9,23). Differences in urinary D-chiro-inositol excretion may relate to differences in insulin sensitivity, even among persons with clinically normal glucose control (9). Future research is warranted to evaluate whether urinary D-chiro-inositol excretion might be an early and sensitive indicator of latent insulin resistance.

The strengths of this study include performing the protocol measurements in a clinical research setting, acclimating all of the subjects to a uniform macronutrient-defined diet before testing, and evaluating glucose metabolism by multiple means (i.e., OGTT and IVGTT). Although the OGTT and IVGTT techniques indirectly assess insulin-mediated glucose metabolism, the indices of insulin sensitivity are highly correlated with the accepted standard technique, the glucose clamp (17). The study limitations include the small number of subjects, the use of a parallel study design, and the inability to evaluate potential acute changes in skeletal muscle insulin receptor activation, because muscle samples were not obtained in a basal state.

In summary, the results of this study indicate that the daily oral ingestion of pinitol for 6 wk by nondiabetic older humans does not positively influence insulin-mediated glucose metabolism.

ACKNOWLEDGMENTS

Sincere thanks go to the volunteers who participated in this study. We thank the University of Arkansas for Medical Sciences General Clinical Research Center dietary and nursing staffs for their dedicated support of this research. We also would like to thank Roy Verdery, MD, PhD, for providing medical coverage; Arlene Sullivan, APN, for providing advanced nursing coverage; Nadine Cornell, MS, RD, for providing dietary coverage; and Aaron Roland and Annie Mahon for providing technical assistance to the project. The Humanetics Corporation is thanked for providing the pinitol powder.

LITERATURE CITED