Translational Article

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Pilot Study of Resveratrol in Older Adults With Impaired Glucose Tolerance

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Background. Resveratrol, a plant-derived polyphenol, has shown promising effects on insulin sensitivity and glucose tolerance in animal models and is also reported to have cardioprotective properties, but human studies are limited. In a pilot study, we tested the hypothesis that resveratrol improves glucose metabolism and vascular function in older adults with impaired glucose tolerance (IGT).

Methods. Ten subjects aged 72 ± 3 years (M ± SD) with IGT were enrolled in a 4-week open-label study of resveratrol (daily dose 1, 1.5, or 2 g). Following a standard mixed meal (110 g carbohydrate, 20 g protein, 20 g fat), we measured 3-hour glucose and insulin area under the curve (AUC), insulin sensitivity (Matsuda index), and secretion (corrected insulin response at 30 minutes). Endothelial function was assessed by reactive hyperemia peripheral arterial tonometry (reactive hyperemia index) before and 90 minutes postmeal. Results did not differ by dose, so data were combined for analysis.

Results. At baseline, body mass index was 29 ± 5 kg/m2; fasting plasma glucose 110 ± 13 mg/dL, and 2-hour glucose 183 ± 33 mg/dL. After 4 weeks of resveratrol, fasting plasma glucose was unchanged, but peak postmeal (185 ± 10 vs 166 ± 9 mg/dL, p = .003) and 3-hour glucose AUC (469 ± 23 vs 428 ± 19, p = .001) declined. Matsuda index improved (3.1 ± 0.5 vs 3.8 ± 0.5, p = .03), and corrected insulin response at 30 minutes was unchanged (0.6 ± 0.1 vs 0.5 ± 0.5, p = .49). There was a trend toward improved postmeal reactive hyperemia index (baseline vs resveratrol postmeal delta = 0.4 ± 0.2 vs 0.2 ± 0.3, p = .06). Weight, blood pressure, and lipids were unchanged.

Conclusions. At doses between 1 and 2 g/day, resveratrol improves insulin sensitivity and postmeal plasma glucose in subjects with IGT. These preliminary findings support the conduct of larger studies to further investigate the effects of resveratrol on metabolism and vascular function.

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RESVERATROL (3,5,4′-trihydroxystilbene) is a plant-derived polyphenolic compound mainly known for its antioxidant and phytoestrogenic properties. Interest in this compound has increased in recent years, first from its identification as a chemopreventive agent for skin cancer and subsequently from reports that it activates sirtuins and extends the life span of lower organisms, including rodents (1). Resveratrol has demonstrated promising effects on insulin secretion, insulin sensitivity, and glucose tolerance in a variety of animal models (2,3). Notably, resveratrol prevented the negative metabolic effects of excess calorie intake, improving glucose tolerance, lowering insulin levels, and significantly increasing survival of middle-aged mice (4). Resveratrol has also been shown to increase mitochondrial biogenesis and appears to mimic the beneficial effects of caloric restriction on glucose metabolism (5–7).

Resveratrol has also been proposed to have cardioprotective effects. Resveratrol possesses weak activity as a phytoestrogen (8), antioxidant properties (9), and has been shown to both enhance synthesis and decrease inactivation of the vasorelaxant nitric oxide (10). Resveratrol may also promote vascular relaxation by inhibiting synthesis of the potent vasoconstrictor thromboxane A2 and by other nitric oxide-independent mechanisms (11).

However, despite the many health claims made on its behalf and its widespread use as a nutritional supplement, formal studies of resveratrol in humans are very limited and no studies of its metabolic effects have been reported. Further,
questions about resveratrol bioavailability, dosing range, and safety also need to be addressed (12–14). We therefore conducted a pilot study of resveratrol treatment as an initial step in assessing its potential to improve glucose tolerance, insulin sensitivity, and vascular function. For this initial investigation, we studied the effects of resveratrol in subjects with impaired glucose tolerance (IGT) who have definite but not-yet-severe metabolic dysregulation, which may be most amenable to intervention. We chose to focus on older adults for two important reasons. First, IGT is in large part an age-related phenomenon, affecting up to 30% of older adults (15) and constitutes a major risk factor for the development of both diabetes and cardiovascular disease (16). In addition, although lifestyle modification was exceptionally effective in preventing progression from IGT to diabetes in older participants (age 60–85) in the Diabetes Prevention Program, metformin was not (17), highlighting the need for alternate pharmacologic approaches for older adults with IGT.

**Methods**

Adults aged 65 and older were screened with a 75-g oral glucose tolerance test and those with fasting plasma glucose <126 mg/dL and 2-hour glucose ≥140 mg/dL were eligible to enroll. Subjects were excluded if they had a recent cardiovascular event, evidence of significant liver or renal disease; any active cancer; or prior history of estrogen-dependent neoplasm. Because of the possibility of CYP450-related drug interactions (18), treatment with the following drugs was exclusionary: antiepileptics, mefloquine, cyclosporine, tacrolimus, HIV protease inhibitors, or high-dose statin therapy (>20 mg atorvastatin or rosuvastatin; >40 mg simvastatin, pravastatin, or lovastatin). Individuals taking resveratrol or antioxidant vitamins (other than a standard multivitamin preparation) within the prior 3 months were also excluded. The study protocol was approved by the Albert Einstein College of Medicine Institutional Review Board, and all participants provided written informed consent.

Resveratrol capsules were obtained from Biotiva, LLC, and independent verification of the resveratrol content of the capsules used in this study was performed in the laboratory of Rong-Fong Shen, PhD, Proteomics and Analytical Biochemistry Unit, National Institute on Aging at the National Institutes of Health. Subjects were randomly assigned to take open-label resveratrol for 4 weeks in one of the three doses: 1, 1.5, and 2 g/day, taken in divided doses. Subjects were instructed to maintain their usual dietary and physical activity patterns during their participation in the study.

**Standard Meal Test**

Subjects were studied following an overnight fast and after a test meal containing 110 g carbohydrate, 20 g protein, and 20 g fat. The meal consisted of standard breakfast foods: cereal, bread, juice, and milk. Blood sampling was performed fasting and 30, 60, 120, and 180 minutes following the meal through an indwelling intravenous catheter. Subjects were instructed to consume a standard meal and snack at home on the night prior to the standard meal test, in order to minimize metabolic variability between tests. The assigned resveratrol dose was administered with the standard meal during the test conducted at the conclusion of the 4-week treatment period. Insulin sensitivity was estimated using homeostasis model assessment (HOMA-IR): insulin$_{fasting}$ (mU/mL) × glucose$_{fasting}$ (mmol/L)/22.5 (19) and also from insulin and glucose levels obtained following the standard meal challenge using the Matsuda index: 10,000/√[(fasting plasma glucose × fasting plasma insulin) (mean glucose × mean insulin)] (20,21). Insulin secretion was estimated using the corrected insulin response at 30 minutes: insulin$_{30min}$ (μU/mL)/glucose$_{30min}$ (mg/dL) × (glucose$_{30min}$ [mg/dL] − 70) (19). β-Cell function was assessed using the oral disposition index (DI0) calculated using the formula: (ΔI0−30/ΔG0−30)/(1/I0) (22). Insulin and glucose area under the curve (AUC) were calculated using the trapezoidal method. Percent body fat was measured using bioimpedance analysis (RJL Systems, Clinton Township, MI).

Endothelial function testing was performed fasting and 90 minutes following the standard meal, using reactive hyperemia peripheral arterial tonometry (RH-PAT) (23,24). RH-PAT employs a finger plethysmographic device to detect pulsatile arterial volume changes, which are sensed by a pressure transducer and transferred to a computer for analysis (EndoPAT; Itamar Medical). Studies are performed with the patient at rest, in a comfortable thermoneural environment, and any antihypertensive medications were held until after completion of the 90-minute postmeal test. Fingertip probes are placed on the index finger of both hands, and 5 minutes of baseline recording is obtained. Blood flow is then occluded in one arm for 5 minutes, using a standard blood pressure cuff. Recording continues in both fingers during occlusion and for 5 minutes after release of the cuff. The reactive hyperemia index is calculated as the ratio of the average pulse amplitude in the posthyperemic phase divided by the average baseline amplitude, with normalization to the signal in the control arm to compensate for any systemic changes. Test–retest repeatability testing in our laboratory among healthy controls resulted in a coefficient of variability of 15.2% for tests performed 2 hours apart and 16.4% for tests performed 1–4 weeks apart.

Assays were performed in the core laboratories of the Einstein Institute for Clinical and Translational Research: chemistry profiles, complete blood count, urinalysis, glucose, lipoproteins, insulin (radioimmunoassay), high sensitivity c-reactive protein (latex-enhanced turbidimetric assay), and adiponectin (radioimmunoassay; Linco).

**Statistical Analysis**

Data are presented as mean (±SD) for baseline values and mean (±SEM) for baseline versus resveratrol comparisons. Baseline and posttreatment variables (peak and AUC glucose,
insulin, Matsuda index, etc.) were compared using a paired t-test. Since there were no apparent differences among the three resveratrol doses studied, the groups were combined for analysis. For RH-PAT index, the pre- and postmeal delta was also analyzed (baseline vs resveratrol) using paired t-test. Data analyses were performed using GraphPad Instat, v3.

RESULTS

Ten subjects (seven female) with a mean age of 72 ± 3 years were enrolled. The subjects were overweight to obese and moderately insulin resistant, with a mean body mass index of 29 ± 5 kg/m² and HOMA-IR 5.1 ± 1.8. Mean fasting and 2-hour plasma glucose were 110 ± 13 mg/dL and 183 ± 33 mg/dL, respectively. Four subjects were being treated with antihypertensive medications and three with statins; doses of these medications remained unchanged during the study.

After 4 weeks of resveratrol, fasting glucose was unchanged, but peak postmeal glucose (185 ± 10 vs 166 ± 9 mg/dL, p = .003) and 3-hour glucose AUC (469 ± 23 vs 428 ± 19, p = .001) declined significantly (Figure 1a). Furthermore, postmeal insulin AUC fell by 18 ± 25% (p = .05; Figure 1b), and insulin sensitivity (using the Matsuda index) improved following treatment with resveratrol (3.1 ± 0.5 vs 3.8 ± 0.5, p = .03; Figure 2). Changes in HOMA-IR were not significant (3.6 ± 0.5 vs 3.3 ± 0.3, p = .13). Insulin secretion, as measured by corrected insulin response at 30 minutes, did not change significantly (0.60 ± 0.1 vs 0.50 ± 0.1, p = .49). Disposition index, a measure of β-cell function adjusted for insulin sensitivity, was 1.43 at baseline and 1.60 after resveratrol (p = .52). Weight, percent body fat, blood pressure, and fasting lipid profile were unchanged (Table 1). We also found no changes in the levels of high sensitivity c-reactive protein and adiponectin.

Endothelial function was assessed by RH-PAT (pre- and postmeal) during the standard meal test at baseline and after 4 weeks of resveratrol (Figure 3). Fasting RH-PAT index was essentially unchanged (2.3 ± 0.16 vs 2.2 ± 0.15, p = .8), but there was a trend toward improved postmeal endothelial function (higher RH-PAT score) following resveratrol.

Table 1. Metabolic and Safety Variables at Baseline and After 4 Weeks Treatment With Resveratrol

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>Resveratrol</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weight (kg)</td>
<td>74.0 (4.2)</td>
<td>73.6 (4.1)</td>
<td>.95</td>
</tr>
<tr>
<td>Body fat (%)</td>
<td>34.7 (4.8)</td>
<td>35.2 (4.8)</td>
<td>.94</td>
</tr>
<tr>
<td>Cholesterol (mg/dL)</td>
<td>176 (10)</td>
<td>184 (12)</td>
<td>.62</td>
</tr>
<tr>
<td>HDL cholesterol (mg/dL)</td>
<td>51 (4)</td>
<td>50 (4)</td>
<td>.86</td>
</tr>
<tr>
<td>Triglycerides (mg/dL)</td>
<td>103 (13)</td>
<td>113 (13)</td>
<td>.59</td>
</tr>
<tr>
<td>LDL cholesterol (mg/dL)</td>
<td>104 (9)</td>
<td>111 (11)</td>
<td>.63</td>
</tr>
<tr>
<td>hs-CRP (mg/mL)</td>
<td>2.3 (0.6)</td>
<td>2.8 (0.8)</td>
<td>.62</td>
</tr>
<tr>
<td>Adiponectin (µg/mL)</td>
<td>8.5 (1.0)</td>
<td>8.3 (1.0)</td>
<td>.89</td>
</tr>
<tr>
<td>Fasting glucose (mg/dL)</td>
<td>103 (4)</td>
<td>103 (4)</td>
<td>.99</td>
</tr>
<tr>
<td>Fasting insulin (µU/mL)</td>
<td>14.1 (1.8)</td>
<td>12.4 (1.0)</td>
<td>.42</td>
</tr>
<tr>
<td>CIRγ0t</td>
<td>0.6 (0.1)</td>
<td>0.5 (0.1)</td>
<td>.49</td>
</tr>
<tr>
<td>Serum creatinine (mg/dL)</td>
<td>0.8 (0.05)</td>
<td>0.8 (0.04)</td>
<td>.99</td>
</tr>
<tr>
<td>AST (U/L)</td>
<td>18.3 (1.1)</td>
<td>19.1 (1.0)</td>
<td>.60</td>
</tr>
<tr>
<td>ALT (U/L)</td>
<td>15.2 (1.1)</td>
<td>16.9 (1.5)</td>
<td>.37</td>
</tr>
<tr>
<td>BP systolic (mmHg)</td>
<td>129 (5)</td>
<td>126 (5)</td>
<td>.68</td>
</tr>
<tr>
<td>BP diastolic (mmHg)</td>
<td>72 (2)</td>
<td>70 (3)</td>
<td>.59</td>
</tr>
</tbody>
</table>

Notes: Data obtained during standard meal challenge; data are mean (SEM); p values calculated from paired t-tests. ALT= alanine aminotransferase; AST= aspartate aminotransferase; CIRγ0t= corrected insulin response at 30 minutes; HDL= high density lipoprotein; hs-CRP= high sensitivity c-reactive protein; LDL= low density lipoprotein.
be better tolerated in older individuals (26). The use of meal challenge data to assess insulin sensitivity, rather than “gold standard” techniques, such as the euglycemic clamp, is an acknowledged limitation of our study. However, meal challenge protocols to measure insulin sensitivity have been widely used (25,26) and application of the Matsuda algorithm in this context has been validated (in nondiabetic and diabetic subjects), showing reasonable correlation \( r = .55 \) to \( .70 \) with insulin sensitivity obtained during a frequently sampled intravenous glucose tolerance test (22,27). Future studies will be needed to confirm our finding of improved insulin sensitivity with resveratrol and to define the relative contributions of hepatic and peripheral insulin action. We were unable to detect significant changes in insulin secretion using calculated measures based on 30-minute glucose and insulin (corrected insulin response at 30 minutes) or using the oral disposition index, but tests with better sensitivity (c-peptide modeling or hyperglycemic clamp techniques) may be more informative.

Although in vivo evidence of resveratrol’s metabolic effects has been quite consistent, the relevant mechanisms are likely complex and remain incompletely understood. Resveratrol has been reported to be an activator of the mammalian sirtuin, Sirt1, which is involved in processes such as cell survival and glucose homeostasis (28,29). However, resveratrol’s activation of Sirt1 has been questioned and there is evidence that alternate pathways, including activation of AMP kinase, could be responsible for some of resveratrol’s metabolic effects (4,30).

Concerns have been raised about resveratrol’s bioavailability in vivo, and the appropriate doses for use in human studies are not known (31). Animal studies have employed doses from 5 to 400 mg/kg/day, and human clinical trials (mostly single-dose or short-term studies) have also included a wide range of doses, from 5 to 5,000 mg/day. We chose to explore the dose range of 1–2 g/day, balancing the strategy of testing higher doses for “proof of concept” with concern about possible drug toxicity since little human safety data are currently available for resveratrol (32). In our study, meal tolerance improved with these doses, but it did not return to levels seen in similar aged subjects with normal glucose tolerance studied using the same standard meal protocol (33). Further, we were unable to detect differences within the narrow dose range studied and expect that a wider range of resveratrol doses will be necessary to reveal evidence of a dose–response.

The vascular effects of resveratrol on isolated tissues or animal models are well described and include decreased platelet aggregation via inhibition of cyclooxygenase-1 and weak estrogen receptor agonist and antioxidant properties (34–36). Human data are limited, but a study of red grape polyphenols (containing trace amounts of resveratrol) showed improvement in flow-mediated vasodilation (37), and others have reported reduction in oxidative stress and increased expression of antioxidant genes following a single dose of resveratrol (38). We hypothesized that endothelial function
would be enhanced in our subjects following treatment with resveratrol, both directly through effects on nitric oxide and also potentially as a consequence of improved glucose tolerance. In a previous study, we demonstrated reduced postmeal RH-PAT index in IGT, the timing of which coincided with peak postmeal glucose levels (32). These dual effects of resveratrol on endothelial function (directly and via improvement in glucose tolerance) may be synergistic. However, with the current study design, we are unable to determine whether any observed vascular effects may be direct or mediated by improvements in glucose metabolism, and further studies are needed to explore this. Further, our results are of borderline statistical significance and require confirmation.

Results of this pilot study should be interpreted with caution given the open-label uncontrolled study design. Although weight and reported physical activity levels did not change during the 4-week treatment period, it is possible that subtle changes in diet and/or exercise could have influenced these results. Furthermore, with the small number of subjects, we were likely underpowered to detect differences in some study outcomes, especially RH-PAT. Finally, although resveratrol was well tolerated and appeared to be safe in this cohort, much larger and longer-duration studies will be needed to appropriately assess safety concerns.

In conclusion, this pilot study provides the first evidence in humans that resveratrol may possess clinically relevant effects on glucose metabolism and vascular function. Future studies should include formal randomized placebo-controlled trials and efforts to explore the potential mechanisms for resveratrol’s cardiometabolic effects.

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SUPPLEMENTARY MATERIAL
Supplementary Table 1 can be found at: http://biomedgerontology.oxfordjournals.org.

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