Novel Soy Germ Pasta Improves Endothelial Function, Blood Pressure, and Oxidative Stress in Patients With Type 2 Diabetes

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OBJECTIVE—To compare the effects of a novel soy germ–enriched pasta, containing isoflavone aglycons, with conventional pasta on endothelial function and cardiovascular risk markers in patients with type 2 diabetes (T2D).

RESEARCH DESIGN AND METHODS—This randomized controlled double-blind crossover study compared one serving/day of soy germ pasta and conventional pasta for 8 weeks for effects on brachial artery flow-mediated vasodilation, blood pressure, plasma lipids, oxidized LDL cholesterol, 8-iso-PGF2α, total antioxidant capacity (TAC), glutathione (GSH), and homocysteine.

RESULTS—Isoflavone-enriched pasta significantly improved arterial stiffness ($P = 0.005$) and reduced systolic ($P = 0.026$) and diastolic ($P = 0.017$) blood pressures. Plasma TAC increased ($P = 0.0002$), oxidized LDL cholesterol decreased ($P = 0.009$), 8-iso-PGF2α decreased ($P = 0.001$), GSH levels increased ($P = 0.0003$), and homocysteine decreased ($P = 0.009$) consistent with a reduction in oxidative stress. No significant changes were observed with conventional pasta.

CONCLUSIONS—Pasta enriched with biologically active isoflavone aglycons improved endothelial function and had beneficial effects on cardiovascular risk markers in patients with T2D.

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The risk of cardiovascular disease and stroke is 3–4 times greater in patients with type 2 diabetes (T2D) than in the general population (1). It results from loss of normal endothelial function and is associated with increased oxidative stress, production of reactive oxygen species (2), and a decrease in antioxidative defense systems (3). Hypertension, dyslipidemia, and hyperhomocysteinemia also contribute to overall morbidity and mortality. In a recent study of patients with hypercholesterolemia, a soy germ–enriched pasta containing isoflavones, in mainly the biologically active aglycon form, significantly improved serum lipids and other markers of cardiovascular risk, including arterial reactivity, beyond that of an American Heart Association Step II diet alone (4). Our objective was to determine whether similar effects from this novel food could be attained in patients with T2D.

RESEARCH DESIGN AND METHODS

—This 20-week study enrolled 26 adults with T2D (13 men/13 women, aged 62 ± 7 years) in a randomized double-blind placebo-controlled crossover design. None had major complications of diabetes and all were on an American Diabetes Association diet. Prior use of estrogen therapy, oral corticosteroids, antioxidant supplements, soy foods or isoflavone supplements, a BMI >35, and smoking were exclusion criteria. The study was conducted with informed consent according to ethical guidelines for research on humans and was approved by the human ethics committee of the University of Perugia School of Medicine.

Two types of dried pasta were used: a soy germ–enriched pasta (Aliveris s.r.l., Perugia, Italy) containing 31–33 mg of total isoflavones per serving predominantly in aglycon form (Pasta+) and conventional pasta (Pasta−), with both packaged identically (4).

Patients were randomized to two groups. One group consumed one serving/day of soy germ–enriched pasta (80 g dry pasta) for 8 weeks followed by conventional pasta for 8 weeks, with a 4-week washout between. The other group consumed these pasta types in reverse sequence. Overnight fasting blood samples were collected at baseline, after washout, and at the end of the diet periods for serum/plasma biochemistries measured by standard methods. Blood pressure was measured and endothelial function assessed from changes in brachial artery flow-mediated vasodilation using ultrasound imaging of artery diameter during reactive hyperemia (5). Methods for data analysis are detailed in the Supplementary Appendix.

RESULTS

—Of the 26 patients enrolled, 6 were withdrawn (4 whose drug therapies were altered, 1 who took antioxidants, and

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1 who was noncompliant to the diets). The mean BMI of the remaining subjects (10 women, 10 men) was 28.3 ± 3.5 kg/m^2, fasting glucose was 122 ± 33 mg/dL, and HbA_{1c} was 6.8 ± 0.65%. After randomization, there were no significant differences between the groups (Pasta+/Pasta− vs. Pasta−/Pasta+) in these measures.

Plasma isoflavones were undetectable at baseline and when consuming Pasta−. When consuming Pasta+, serum daidzein, glycitein, and genistein concentrations ranged 12–255 nmol/L, 11–128 nmol/L, and 15–167 nmol/L, respectively, consistent with previously reported data (4).

Brachial artery flow-mediated vasodilation increased ~5% after consuming Pasta+ and decreased with Pasta− (Fig. 1 and Supplementary Appendix); this difference was highly significant ($P = 0.0005$). Consistent with improved endothelial function was a significant reduction in systolic ($P = 0.026$) and diastolic ($P = 0.017$) blood pressures. Pasta+ lowered systolic blood pressure from 133 ± 1 at baseline to 126 ± 12 mmHg, and diastolic blood pressure from 79 ± 9 at baseline to 73 ± 10 mmHg. Pasta− had no effect on blood pressure.

The effect of the two pastas on serum total cholesterol was in the opposite direction, with the difference being significant ($P = 0.025$). Favorable, albeit not significant, differential trends were observed with Pasta+ on serum LDL cholesterol, HDL cholesterol, and triglyceride levels.

Several markers of oxidative stress improved after consuming Pasta+ (Fig. 1 and Supplementary Appendix). Plasma total antioxidant capacity (TAC) increased significantly ($P = 0.0002$) in comparison with Pasta−. Plasma 8-iso-PGF2α concentration was reduced ($P = 0.001$) and glutathione (GSH) increased ($P = 0.0003$). Consistent with these changes was a reduction in lipid peroxidation, as observed by a decrease in plasma oxidized LDL cholesterol ($P = 0.009$). Serum homocysteine concentration was lower ($P = 0.017$) after Pasta+ compared with Pasta−, but there was no effect of either pasta on serum cysteine or interleukin-6 levels. At baseline, all patients were in good glycemic control and, consequently, neither pasta influenced plasma insulin, glucose, or HbA_{1c} levels.

CONCLUSIONS—This novel soy germ pasta was developed with the objective of delivering biologically active isoflavone aglycons (4) at levels typically

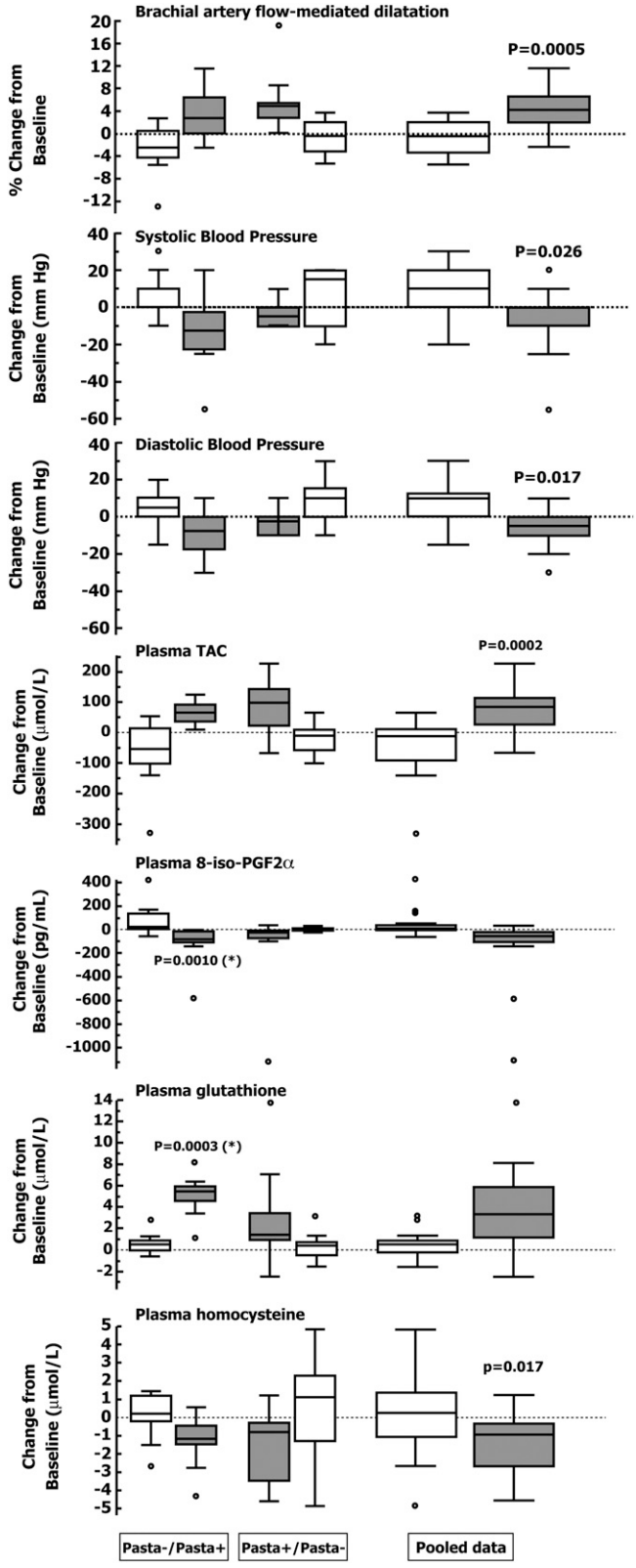

Figure 1—*Box plots showing changes from baseline in brachial artery flow-mediated dilatation, systolic and diastolic blood pressures, TAC in plasma, plasma 8-iso-PGF2α concentrations, plasma GSH concentration, and serum homocysteine concentration measured in adult T2D patients* (n = *20) at baseline and after consuming pasta enriched with isoflavones from soy germ (Pasta+, gray-shaded box and circles) or conventional pasta (Pasta−, control, white box and circles) for 8 weeks. Effects are also shown separately during each sequence of administration of the two pasta types (Pasta−/Pasta+,* n = *11, and Pasta+/Pasta−,* n = *9). The line in the middle of*

found in soy foods in the Asian diet (6) and not to satisfy the 1999-approved Federal Drug Administration cardiovascular health claim for soy protein (7). A high dietary intake of isoflavone aglycons may explain why soy foods appear beneficial to Asians (8) and why pure aglycon supplements, such as genistein (9) or *S*-(-)equol (10), are proving efficacious. Because Italians consume pasta almost daily, this facilitated dietary compliance, which was confirmed by the expected increase in plasma isoflavone concentrations (4) or from returned package counts.

Although no significant changes were observed with either pasta on serum total and LDL cholesterol, which were relatively normal at baseline, serum triglycerides showed a decreasing trend that was greater with Pasta+ than Pasta−. Despite a lack of effect on serum lipids, impressive effects on other important surrogate markers of cardiovascular risk were observed. Endothelial dysfunction, an early marker of cardiovascular disease, has prognostic value in assessing cardiovascular risk (11) and was significantly improved by Pasta+. This finding corroborates previous data in hypercholesterolemic patients (4) and is consistent with the vasodilatory effects of isoflavone aglycons (9). With Pasta+, highly significant 8 and 7% reductions, respectively, in systolic and diastolic blood pressures occurred, and considering the small sample-size, the magnitude of the effect on endothelial function and blood pressure is impressive and clinically relevant.

Because isoflavones have antioxidant activity (12), the finding of highly significant beneficial effects of Pasta+ on several markers of oxidative stress, and on serum GSH, a key constituent of the antioxidant defense system, was not unexpected but important given that increased oxidative stress is seen in T2D (13). Furthermore, hyperhomocysteinemia is an independent risk factor for cardiovascular disease in diabetes (14), and Pasta+ lowered serum levels of this atherogenic and prothrombotic amino acid.

In summary, this isoflavone-enriched soy germ pasta, unlike conventional pasta, significantly improved multiple markers of cardiovascular risk in patients with T2D. Dietary inclusion of this novel pasta may provide benefits to diabetic patients beyond those achieved by an American Diabetes Association diet alone.

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C.C. and K.D.R.S. were principal investigators responsible for the conception and design of the study and prepared the manuscript with support from the coauthors. E.N. was responsible for recruitment, enrollment, clinical care, and follow-up of patients and performed the clinical and biochemical tests, data collection, and processing. P.M.B. performed all the statistical analysis of data. S.A., D.C., and F.G. performed the clinical and biochemical tests, data collection, and processing. N.C. was responsible for recruitment, enrollment, clinical care, and follow-up of patients. V.G. and S.G. performed the clinical and biochemical tests, data collection, and processing. G.P. and G.D.M. were responsible for recruitment, enrollment, clinical care, and follow-up of patients.

References

1. Beckman JA, Creager MA, Libby P. Diabetes and atherosclerosis: epidemiology, pathophysiology, and management. JAMA 2002;287:2570–2581
2. Guerci B, Böhme P, Kearney-Schwartz A, Zannad F, Drouin P. Endothelial dysfunction and type 2 diabetes. Part 2: altered endothelial function and the effects of treatments in type 2 diabetes mellitus. Diabetes Metab 2001;27:436–447
3. Hill MF. Emerging role for antioxidant therapy in protection against diabetic cardiac complications: experimental and clinical evidence for utilization of classic and new antioxidants. Curr Cardiol Rev 2008;4:259–268
4. Clerici C, Setchell KDR, Battezzati PM, et al. Pasta naturally enriched with isoflavone aglycons from soy germ reduces serum lipids and improves markers of cardiovascular risk. J Nutr 2007;137:2270–2278
5. Celermajer DS, Sorensen KE, Gooch VM, et al. Non-invasive detection of endothelial dysfunction in children and adults at risk of atherosclerosis. Lancet 1992;340:1111–1115
6. Messina M, Nagata C, Wu AH. Estimated Asian adult soy protein and isoflavone intakes. Nutr Cancer 2006;55:1–12
7. Food and Drug Administration. Food labeling, health claims, soy protein, and coronary heart disease. Fed Regist 1999;57:699–733
8. Setchell KDR, Clerici C. Equol: history, chemistry, and formation. J Nutr 2010;140:1355S–1362S
9. Squadrito F, Altavilla D, Crisafulli A, et al. Effect of genistein on endothelial function in postmenopausal women: a randomized, double-blind, controlled study. Am J Med 2003;114:470–476
10. Ishiwata N, Melby MK, Mizuno S, Watanabe S. New equol supplement for relieving menopausal symptoms: randomized, placebo-controlled trial of Japanese women. Menopause 2009;16:141–148
11. Vita JA, Keaney JF Jr. Endothelial function: a barometer for cardiovascular risk? Circulation 2002;106:640–642
12. Ruiz-Larrea MB, Mohan AR, Paganga G, Miller NJ, Bolwell GP, Rice-Evans CA. Antioxidant activity of phytoestrogenic isoflavones. Free Radic Res 1997;26:63–70
13. Gopaul NK, Anggård EE, Mallet AI, Betteridge DJ, Wolff SP, Nourooz-Zadeh J. Plasma 8-epi-PGF2 alpha levels are elevated in individuals with non-insulin dependent diabetes mellitus. FEBS Lett 1995;368:225–229
14. Hermansen K, Søndergaard M, Høie L, Carstensen M, Brock B. Beneficial effects of a soy-based dietary supplement on lipid levels and cardiovascular risk markers in type 2 diabetic subjects. Diabetes Care 2001;24:228–233

the box represents the median or 50th percentile of the data, and the box extends from the 25th to the 75th percentile, an interval referred to as the interquartile range. The lines emerging from the box extend to the upper and lower adjacent values (i.e., the largest data point less than or equal to the 75th percentile plus 1.5 times the interquartile range, and the smallest data point greater than or equal to the 25th percentile minus 1.5 times the interquartile range, respectively). If the examined data come from a normal distribution, one would expect the interval between the adjacent values to include 99.3% of the data. Observed points more extreme than the upper or lower adjacent values, if any, are plotted individually. Significance levels ($P < 0.20$) *for the comparison of the effects of the two pasta types are reported.*