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Additional information related to Study Design and Methods

Recruitment of Subjects: The patients enrolled were selected from 20 previously studied and well characterized T2D patients being followed at the Diabetes Clinic of the Department of Internal Medicine of the University of Perugia. These patients were screened by measurement the t1/2 using the [^{13}C]octanoic acid breath test [1, 2]. From this screening 50% (n=10) were found to have a t1/2 of >120 mins defined as the upper limit of normal by the method used [2]. This proportion of patients with was consistent with the reported frequency of gastroparesis in this population [3-7]. At the randomization visit and each subsequent visit, all subjects were advised to maintain their usual diabetes diet and the same level of physical activity throughout the study. The study was conducted according to the ethical guidelines for research on humans and the protocol was approved by the Human Ethics Committee of the University of Perugia School of Medicine, Perugia, Italy. All patients signed a written consent form at time of randomization and the study was initiated prior to requirements for registration of clinical trials.

Description of Pastas: The two types of pasta used in this study were indistinguishable from the packaging and were similar in shape, color and taste. Both were manufactured in spaghetti shaped forms by an Italian pasta manufacturer (Bianconi srl, Bastardo, Umbria, Italy). The soy germ pasta (Aliveris srl, Perugia, Italy) was made of Durum wheat semolina to which 2% of soy germ was blended, while the conventional pasta was made only of Durum wheat semolina. The soy germ-enriched pasta contained 31-33 mg of total isoflavones per serving (80 g) and the soy protein content was negligible at 0.8 g/serving [8]. The energy value/100g of both pastas was similar at 356 Kcal and the macro-composition consisted of 12 g total protein, 73.5 g carbohydrate, and 1.5 g fat. Compliance to the soy germ pasta diets was determined from plasma isoflavone concentrations [9], and for conventional pasta from counting the number of packages of pasta returned.

Study design: In this randomized, double-blind, placebo controlled cross-over design neither the patients nor the researchers were aware of the randomization schedule. The 10 patients were randomly assigned to two groups; 5 patients were assigned to a group that was given a daily serving of soy germ-enriched pasta (80g) for 8 weeks, and the other 5 patients (control group) consumed the same amount of conventional pasta for the same period. Both pastas were consumed either at lunchtime or in the evening on the background of a standard ADA diabetic diet consisting of 55% CHO as energy, 30% of lipid, 15% protein. Compliance to the diets was monitored by a diet questionnaire. After a 4-week washout period during which time neither pasta was consumed, the participants were then given the two pastas in reverse sequence for an additional 8 weeks. At baseline (visit 1) and then at the end of each phase of the trial, 8-wk (visit 2), 12-wk (visit 3) and 20-wk (visit 3), blood (10 mL) was obtained for the measurement of fasting and postprandial glucose and insulin concentrations in response to a standard meal. Blood samples were taken at time 0, 30, 60, 90, and 120 min following the standardized meal which consisted of 125 g of bread, 35 g of cooked ham, 35 g of cheese and an apple. The macronutrient content the meal was total protein 23.1 g, lipids 22.3 g, carbohydrates 83.7 g, and fiber 11.1 g with an energy value of 607 kcal.

Measurement of Gastric Emptying Time: On the second day, gastric emptying time was measured by the [^{13}C]octanoic acid breath test [1, 2] after administration of 91 mg of [^{13}C]octanoic acid in a standardized meal consisting of one scrambled egg, 50 g of prosciutto ham, 10 g of butter, 60 g of white bread, and 100 mL water. The total energy intake was 480 kcal with a macronutrient composition of total protein 19 g (24%), fat 16 g (44%), and carbohydrate 26 g (32%).

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Symptomology assessed using the Gastroparesis Cardinal Symptom Index (GCSI) questionnaire: The severity of symptoms of gastroparesis was assessed in each patient at baseline and after consuming the pastas for 8-weeks using a validated GCSI questionnaire [10] in which each patient assessed on a scale of 0 (none) to 5 (severe), the extent of the following 9 symptoms: nausea, retching, vomiting, stomach fullness, ability to finish a normal-sized meal, feeling of excessive postprandial fullness, loss of appetite, extent of bloating, and signs of a visibly larger stomach. Since these T2D patients were managed with diet alone we did not expect the patients to report severe symptoms despite the delayed t1/2. This was supported by the patient reported GCSI scores.

Analytical measurements: Plasma glucose was measured by using a glucose analyser using an oxidase method (Menarini, Firenze Italy). Serum insulin was assayed using a routine immunoassay. Plasma total and individual isoflavones were measured by GC-MS as described previously [9]. The octanoic acid breath test [1, 2] was performed using a isotope ratio mass spectrometer (Delta S, Finnigan Matt, Bremen, Germany; ABCA Europa Scientific, Crewe, UK).

Data analysis: This was a preliminary study and no sample-size calculations were performed when the study was planned. Group data are expressed as mean ± SEM, 95% confidence intervals (CI) or, in the case of GCSI data, medians and (range). Plasma glucose and insulin responses to standard meal were summarized as the mean value of the measures obtained from each patient after the standard meal. Data from this crossover study were analyzed according to Hills and Armitage [11]. Patient response to dietary intervention was the difference between the values obtained at the end and at the beginning of each diet intervention period. Lack of treatment by period interactions (sequence effects) was preliminarily addressed by testing equality of the mean response during both periods between the two groups defined by sequence of diets (i.e., between the group who received soy-enriched pasta in the first period followed by the conventional pasta in the second period, and the group fed the two pasta types in the reverse order). A significance level of p=0.10 was conservatively used for that test. Mann-Whitney tests were used to assess significance of the interaction, period, and treatment effects.
As baseline samples were not collected under controlled conditions, to assess treatment effects we also performed an alternative analysis to the classical crossover study limited to a pairwise comparison of data from the end of each study period. To this purpose, the Wilcoxon signed-rank test for paired data was used.
Differences in proportions were tested using Fisher's exact test or chi-square statistics. Statistical analyses were two-sided and performed using Stata Statistical Software (Version 11.2, Stata Corporation, College Station, TX).

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Supplementary Table 1. Mean (±SEM) plasma glucose and insulin responses in 10 patients with documented diabetic gastroparesis at baseline and after 8 weeks dietary inclusion of a soy germ pasta containing isoflavones aglycons, or conventional pasta lacking isoflavones.

	Baseline	At 8-weeks	Change	P value vs baseline
Glucose (mg/dL):				
Soy germ pasta	**187 ± 9**	**191 ± 8**	**4 ± 5**	**0.75**
Conventional pasta	**194 ± 11**	**199 ± 11**	**5 ± 4**	**0.75**
			p>0.9*	
Insulin (mg/dL):				
Soy germ pasta	**76 ± 16**	**73 ± 13**	**-5 ± 5**	**0.75**
Conventional pasta	**73 ± 11**	**67 ± 12**	**-5 ± 10**	**>0.9**
			p.0.9*	

Analyses are based on the mean values of plasma glucose or insulin levels obtained after a standardized meal

***** p values for the difference between changes from baseline observed in the two pasta treatments according to (15). Significance values for the analysis limited to pairwise comparison of data from the end of each study period were p=0.2859 for glucose and p=0.3199 for insulin.

References

[1] Ghoos YF, Maes BD, Geypens BJ, Mys G, Hiele MI, Rutgeerts PJ, et al. Measurement of gastric emptying rate of solids by means of a carbon-labeled octanoic acid breath test. Gastroenterology. 1993 Jun;104(6):1640-7.
[2]. Perri F, Ghoos YF, Maes BD, Geypens BJ, Ectors N, Geboes K, et al. Gastric emptying and Helicobacter pylori infection in duodenal ulcer disease. Dig Dis Sci. 1996 Mar;41(3):462-8.
[3]. Horowitz M, Harding PE, Maddox AF, Wishart JM, Akkermans LM, Chatterton BE, et al. Gastric and oesophageal emptying in patients with type 2 (non-insulin-dependent) diabetes mellitus. Diabetologia. 1989 Mar;32(3):151-9.
[4]. Cotroneo P, Grattagliano A, Rapaccini GL, Manto A, Mancini L, Magnani P, et al. Gastric emptying rate and hormonal response in type II diabetics. Diabetes Res. 1991 Jun;17(2):99-104.
[5]. Rabine JC, Barnett JL. Management of the patient with gastroparesis. J Clin Gastroenterol. 2001 Jan;32(1):11-8.
[6]. Horowitz M, O'Donovan D, Jones KL, Feinle C, Rayner CK, Samsom M. Gastric emptying in diabetes: clinical significance and treatment. Diabet Med. 2002 Mar;19(3):177-94.
[7]. Frank L, Kleinman L, Ganoczy D, McQuaid K, Sloan S, Eggleston A, et al. Upper gastrointestinal symptoms in North America: prevalence and relationship to healthcare utilization and quality of life. Dig Dis Sci. 2000 Apr;45(4):809-18.
[8]. Clerici C, Setchell KDR, Battezzati PM, Pirro M, Giuliano V, Asciutti S, et al. Pasta naturally enriched with isoflavone aglycons from soy germ reduces serum lipids and improves markers of cardiovascular risk. J Nutr. 2007 Oct;137(10):2270-8.
[9]. Setchell KDR, Cole SJ. Method of defining equol-producer status and its frequency among vegetarians. J Nutr. 2006 Aug;136(8):2188-93.
[10]. Revicki DA, Rentz AM, Dubois D, Kahrilas P, Stanghellini V, Talley NJ, et al. Development and validation of a patient-assessed gastroparesis symptom severity measure: the Gastroparesis Cardinal Symptom Index. Aliment Pharmacol Ther. 2003 Jul 1;18(1):141-50.
[11]. Hills M, Armitage P. The two-period cross-over clinical trial. Br J Clin Pharmacol. 1979 Jul;8(1):7-20.