

Soya protein does not affect glycaemic control in adults with type 2 diabetes

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Evidence from observational, animal and human studies supports a role for soya protein and its isoflavones in the improvement of glycaemic control in type 2 diabetes. The objective of the present study was to determine the effect of isoflavone-rich soya protein on markers of glycaemic control in adults with type 2 diabetes. Using a randomised, crossover, double-blind, placebo-controlled design, adults with diet-controlled type 2 diabetes (n 29) consumed soya protein isolate (SPI) and milk protein isolate (MPI) for 57 d each separated by a 4-week washout. Blood was collected on days 1 and 57 of each treatment period for analysis of fasting HbA1C, and fasting and postprandial glucose, insulin and calculated indices of insulin sensitivity and resistance. Urine samples of 24 h were collected at the end of each treatment period for analysis of isoflavones. Urinary isoflavone excretion was significantly greater following consumption of SPI compared with MPI, and 20.7% of the subjects (n 6) were classified as equal excretors. SPI consumption did not significantly affect fasting or postprandial glucose or insulin, fasting HbA1C, or indices of insulin sensitivity and resistance. These data do not support a role for soya protein in the improvement of glycaemic control in adults with diet-controlled type 2 diabetes and contribute to a limited literature of human studies on the effects of soya protein on the management of type 2 diabetes.

Soya protein: Isoflavones: Type 2 diabetes: Glycaemic control

Type 2 diabetes is consistently recognised as a global epidemic^(1–4) with the estimation that by 2025, the number of adult cases will have reached 300 million worldwide⁽⁵⁾. Diabetes complications lead to morbidity and mortality^(6,7), and not only impact quality of life, but also pose a significant economic burden^(6,8). Thus, strategies to improve diabetes management and reduce complications risk are worth investigation.

Appropriate management of type 2 diabetes can reduce complications through reduction in associated risk factors⁽⁹⁾. Reduction in hyperglycaemia is of particular importance such that a 1% reduction in HbA1C is associated with a 21% reduced risk of diabetic complications⁽¹⁰⁾. Lifestyle is a well-recognised component of diabetes management, and improvements in glycaemic control have been demonstrated in adults with type 2 diabetes with a combination of pharmaceuticals and lifestyle changes⁽¹¹⁾ and with lifestyle changes alone⁽¹²⁾. Diet is a modifiable lifestyle factor that is a well-accepted component of diabetes management⁽⁹⁾. Numerous dietary components have been studied in relation to glycaemic control in adults with type 2 diabetes including carbohydrates⁽¹³⁾, dietary fibre^(14,15), micronutrients⁽¹⁶⁾ and

fatty acids^(17,18). A less traditional dietary component of interest to diabetes management is soya and its constituent protein and isoflavones.

Several lines of evidence support a role for soya in the improvement of glycaemic control. Cross-sectional studies document that the prevalence of type 2 diabetes among Japanese living in Tokyo (where soya consumption is high) is four times less than that of second-generation Japanese Americans living in the United States (where soya consumption is low)^(19,20). More focused studies report that consumption of soya isoflavones⁽²¹⁾, soyabeans⁽²²⁾ and soya foods⁽²³⁾ is associated with reduced fasting and 2-h post-glucose challenge serum insulin in postmenopausal women⁽²¹⁾, reduced incidence of type 2 diabetes in middle-aged Chinese women⁽²²⁾ and reduced glycosuria in postmenopausal women⁽²³⁾.

Animal studies in healthy and diabetic rat, mice and monkey models also provide support for a role of soya in diabetes management. In healthy rats, soya protein significantly reduced fasting glucose, fasting insulin and glucose area under the curve (AUC) following an intravenous glucose tolerance test⁽²⁴⁾, significantly reduced serum insulin⁽²⁵⁾

Abbreviations: AUC, area under the curve; C_{\max} , maximum concentration; HOMA-IR, homeostasis model assessment of insulin resistance; ISI_{Matsuda}, insulin sensitivity index-Matsuda; log, logarithm; QUICKI, quantitative insulin sensitivity check index; MPI, milk protein isolate; SPI, soya protein isolate; T_{\max} , time to C_{\max} .

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and significantly improved insulin secretion⁽²⁶⁾. In healthy monkeys, soya protein significantly increased glucose effectiveness and insulin sensitivity⁽²⁷⁾. In diabetic rats, soya protein significantly reduced HbA1C⁽²⁸⁾, maintained glycaemic control⁽²⁹⁾ and increased GLUT4 expression⁽²⁹⁾, PPAR gamma (PPAR γ)⁽²⁹⁾ and antioxidant enzymes⁽²⁸⁾. Finally, in diabetic mice, soya protein significantly reduced carbohydrate oxidation⁽³⁰⁾, fasting glucose⁽³¹⁾ and fasting insulin⁽³¹⁾; upregulated expression of insulin regulatory genes⁽³¹⁾ and isoflavones significantly reduced fasting glucose and HbA1C and improved glucose tolerance⁽³²⁾.

The majority of human intervention studies that have investigated effects of soya in relation to type 2 diabetes have involved subjects who have diabetic complications^(33–37) and/or are taking glycaemic medications^(38–45). Early studies of adults with type 2 diabetes focused on soya fibre and demonstrated significant improvements in acute postprandial glucose⁽⁴⁴⁾ and glucose AUC⁽⁴¹⁾ after 4 weeks. Another study evaluated 6 weeks of a supplement that combined soya protein with soya fibre and found no significant effects on fasting glucose, insulin or HbA1C⁽³⁹⁾. A number of studies have included subjects with diabetic nephropathy and demonstrated beneficial effects of soya protein on renal function^(33–35), but not on fasting glucose or HbA1C following durations of 7⁽³⁵⁾–8⁽³³⁾ weeks, except for a 4-year study that found significantly reduced fasting glucose⁽³⁴⁾. Soya has also been shown to significantly reduce fasting glucose and HbA1C when consumed as part of a meal replacement for 3–12 months⁽³⁸⁾. Related studies of isoflavone supplements have been primarily conducted in postmenopausal women with type 2 diabetes and consistently found no significant effects on fasting glucose^(37,40,45), insulin^(37,45), HbA1C^(40,45) or homeostasis model assessment of insulin resistance (HOMA-IR)⁽⁴⁵⁾, following consumption of isoflavones at doses of 50 mg for 4 weeks⁽⁴⁰⁾, 132 mg for 12 weeks⁽⁴⁵⁾ or 177 mg for 12 weeks⁽³⁷⁾. Most relevant to the present study is a study of thirty-two postmenopausal women with diet-controlled type 2 diabetes in which 12 weeks of soya protein (30 g soya protein; 132 mg isoflavones) significantly reduced fasting insulin, HbA1C and insulin resistance when compared to cellulose⁽⁴⁶⁾, albeit results could be attributed to the higher quantity (rather than different type) of protein in the soya protein treatment, consistent with evidence of beneficial effects of high-protein diets in subjects with type 2 diabetes^(47–49).

Evidence from epidemiological, animal and some human intervention studies that support a role for soya in diabetes management justifies a more focused human study, particularly in those without diabetic complications who are not taking glycaemic medications. Thus, the purpose of the present study was to compare the effect of soya protein isolate (SPI) with milk protein isolate (MPI) on markers of fasting and postprandial glycaemic control in adults with diet-controlled type 2 diabetes.

Methods

Subjects

Subjects were adult (> 19 years old) males or postmenopausal females (no menstrual bleeding for at least 12 months or > 50 years old with a hysterectomy) who were diagnosed with type

2 diabetes (fasting plasma glucose ≥ 7.0 mmol/l⁽⁹⁾) with stable glycaemic control (HbA1C $\leq 8\%$), managing their diabetes with a stable diet (not currently undergoing dietary change) and medication stable (no changes in medications used) for at least 3 months. Subject exclusionary criteria included premenopausal women; BMI > 35 kg/m²; use of oral hypoglycaemic agents, insulin therapy or lipid-lowering medications; occurrence of diabetic complications; use of hormone replacement therapy; alcohol consumption > 2 drinks/d or > 7 drinks/week; antibiotic use within 3 months; allergy to soybeans or milk; soya consumption of > 3 servings/week; vegans.

Potential subjects were recruited using recruitment posters, emails, newspaper advertisements and support from local Diabetes Education Centres. Potential subjects were screened with a telephone/email eligibility questionnaire followed by an in-person meeting that included an in-depth eligibility questionnaire and discussion of the study details. Eligible and interested subjects received a study welcome letter and attended an orientation where they received a study handbook and were instructed on all study details. A total of thirty-four subjects started the study.

Study design

The study employed a randomised, crossover, double-blind, placebo-controlled design that consisted of two 57-d treatment periods separated by a 28-d washout period. Subjects visited the Human Nutraceutical Research Unit of the University of Guelph on days 1, 15, 29, 43 and 57 of each treatment period. The present study was conducted according to the guidelines laid down in the Declaration of Helsinki, and all procedures involving human subjects were approved by the University of Guelph Research Ethics Board (REB #DE009). Written informed consent was obtained from all subjects.

Sample size and treatment randomisation

Sample size was estimated at twenty-two subjects for HbA1C and thirty-four subjects for glucose, using an effect size of 10%, SD estimates of 1.19 for HbA1C and 1.40 for glucose in accordance with the literature^(13,14,39,46,47), a power of 80%, an α of 0.05 and two-sided testing (SPSS Sample Power, version 2.0; Teaneck, NJ, USA). The investigators randomised the participants to treatment order by placing equal numbers of the two possible treatment orders into an envelope and randomly selecting an order group for each participant as they started the study. Seventeen subjects were assigned to consume SPI followed by MPI, and seventeen participants were assigned to consume MPI followed by SPI. Both subjects and investigators were blinded to the treatments.

Study treatments and diet

The study treatments included a SPI and MPI beverage powder (Solae LLC, St Louis, MO, USA) that were provided in two 29 g packets/d. The treatment protein powders were comparable in energy and nutrient contents and contributed a daily total of 837 kJ, 8–9 g carbohydrate, 40 g protein from soya (SPI) or milk (MPI), 88 mg (SPI) or 0 mg (MPI) isoflavones (expressed as aglycone equivalents with an

isoflavone distribution of 65% genistein, 31% daidzein and 4% glycitein), 1 g fat, 0–10 mg cholesterol and 1400–1600 mg Ca. The SPI was made with SUPRO(R)SOY isolated soya protein with isoflavones. The SPI was water washed to preserve isoflavone content. The MPI was made with MPI (TMP 1240; New Zealand Milk Products, Inc., now Fonterra Brands America, Fort Lauderdale, FL, USA), in which casein and whey proteins were isolated together from milk (information provided by Solae LLC).

Subjects supplemented their habitual diets with the treatment protein powders and were provided with multiple examples of ways to consume them but encouraged to reconstitute them with water with the option of adding Nestle™ flavour packets. To avoid excess protein and Ca intakes, subjects were counselled by a registered dietitian to replace certain foods (e.g. milk, cheese, lunch meat) with their treatment protein powders. To create a more consistent exposure to the isoflavones within the SPI, subjects were instructed to consume their two treatment protein powder packets at separate times each day at least 4 h apart.

Subjects maintained their habitual lifestyle habits with specific dietary restrictions to minimise background phytoestrogen consumption including the avoidance of all soya products (soya lecithin and soya oil were permitted), flaxseed, beans, legumes and whole grains. Furthermore, since the treatment protein powders were fortified with Ca, subjects were instructed to avoid fluid milk and Ca-fortified beverages. Subjects were also instructed to avoid all natural health products, protein bars, green tea and limit alcohol consumption to <7 drinks/week.

Anthropometric and body measurements

Baseline measurements obtained on study day 1 of treatment period 1 included height, blood pressure and heart rate. Height was measured without shoes, using a stadiometer (SECA Portable Stadiometer 214, Hanover, MD, USA). Sitting blood pressure was measured in duplicate on the left arm after 5 min of rest using an automatic blood pressure monitor (UA-767PC Blood Pressure Monitor; A&D Medical, Milpitas, CA, USA).

Fasted body weight was measured on study days 1, 15, 29, 43 and 57 of each treatment period using a calibrated digital scale (SV 100; Acculab North America, Edgewood, NY, USA) with subjects wearing light clothes without shoes. BMI was calculated as body weight (kg)/height (m²). Waist and hip circumferences were measured on study days 1 and 57 of each treatment period using a metric tape measure. Waist circumference was measured next to bare skin at the midway point between the iliac crest and the lower rib, and hip circumference was measured about the largest part of the buttocks next to light clothing. Body composition was measured on study days 1 and 57 of each treatment period using bioelectrical impedance analysis (BodyStat 1500™; BodyStat Ltd, Tampa, FL, USA). The procedure involved the subject resting in a supine position with two electrodes placed on their right hand (one below the first two knuckles and one on the wrist bone) and two electrodes placed on their right foot (one below the middle toe and one on the front of the ankle). To ensure proper hydration, subjects were instructed to consume at least 500 ml of water in the

12 h preceding their bioelectrical impedance analysis measurement.

Food records

Three-day food records were completed once before the study and on study days 2–4, 26–28 and 54–56 of each treatment period. Subjects were provided with predated sheets that included spaces to provide the time of consumption, amount and description of food and/or beverage. Subjects were trained to complete food records and encouraged to maximise detail, include brand names and submit package labels and recipes. Food records were analysed and averaged across each of the 3 d for intakes of energy, macronutrients, dietary fibre, cholesterol and Ca using ESHA Food Processor (version 9.81; ESHA Research, Salem, OR, USA).

Study diaries

Subjects were provided with a study diary for every day of each treatment period, in which they recorded information such as how and when they consumed their treatment powder, treatment powder tolerance issues or adverse effects, exercise, medications and any other information they deemed important. Study diaries were reviewed by a study coordinator at each study visit.

Urine collection and analysis

Three consecutive 24-h urine collections were completed on study days 54–56 of each treatment period. Urine was collected into opaque 3-litre plastic bottles (VWR International, West Chester, PA, USA) containing 3 g ascorbic acid and kept refrigerated. Subjects were also provided with a 1-litre plastic bottle (Nalgene™), a lunch-size cooler bag and an ice pack for collection of urine in transport with instructions to transfer their urine to the 3-litre bottle. Volume of each 24-h urine collection was recorded, the collection was gently mixed and urine was aliquoted into 15 ml conical tip polypropylene tubes (Sarstedt™) and frozen at –20°C. Samples from every 24-h urine collection were analysed for creatinine using an enzymatic UV method (Randox Laboratories Canada Ltd, Mississauga, Ont., Canada) on a Roche Hitachi 911 auto-analyzer with an inter-assay variability of 2.85%. Samples from every consecutive 3-d urine collection were thawed and proportionally combined to create a pooled sample, which was analysed for isoflavones (genistein and daidzein) and isoflavone metabolites (*O*-desmethylangolensin and equol) using GCMS as previously described⁽⁵⁰⁾. Inter-assay variability was 3.1% for genistein, 4.2% for daidzein, 4.9% for *O*-desmethylangolensin and 4.0% for equol.

Blood collection and analysis

A fasted blood sample and an oral glucose tolerance test were completed on study days 1 and 57 of each treatment period. Subjects were instructed to avoid all food and beverages (except water that was encouraged) for 12 h prior and to avoid alcohol, strenuous activity and over-the-counter medications for 72 h before their blood samples. A catheter

was inserted into the forearm antecubital vein by a qualified technician and kept patent with normal saline to facilitate periodic blood sampling. A baseline blood sample was collected into tubes containing EDTA, sodium heparin or no preservatives. Subjects then consumed 75 g of glucose solution (Trutol[®] 75; Nerl Diagnostics, East Providence, RI, USA), after which blood samples were collected at 15, 30, 60, 90, 120, 150 and 180 min into tubes containing sodium heparin or no preservatives. Blood containing EDTA (from the baseline fasting samples) was frozen at -20°C until analysis for HbA1C using HPLC at Guelph General Hospital in Guelph, Ont., Canada. Inter-assay variation for HbA1C was 4.02%. Blood containing sodium heparin was kept on ice until centrifugation at 1500 g at 5°C for 15 min, after which plasma was aliquoted into cryovials and stored at -80°C until analysis for glucose in duplicate using a YSI Model 2300 STAT PLUS Glucose Analyzer (YSI, Inc., Yellow Springs, OH, USA). Intra- and inter-assay variations for glucose were 0.91 and 2.07%, respectively. Blood containing no preservatives was left at room temperature for 30 min before centrifugation at 1500 g at 5°C for 15 min, after which serum was aliquoted into cryovials and stored at -80°C until analysis in duplicate for insulin using a single antibody-coated tube RIA with ^{125}I -labelled hormone (Coat-a-Count; Diagnostic Products Corporation, Los Angeles, CA, USA). Intra- and inter-assay variations for insulin were 2.28 and 13.8%, respectively. To reduce effects of inter-assay variability in HbA1C, glucose and insulin, all samples from the same subject were analysed in the same batch.

Data and statistical analyses

AUC for glucose and insulin was calculated from the oral glucose tolerance test data using GraphPad Prism (GraphPad Software, Inc., San Diego, CA, USA). Surrogate indices of insulin sensitivity and resistance were calculated including the logarithm (log) of HOMA-IR⁽⁵¹⁾, quantitative insulin sensitivity check index (QUICKI)⁽⁵²⁾ and insulin sensitivity index-Matsuda (ISI_{Matsuda})⁽⁵¹⁾. Maximum concentrations (C_{\max}) and time to C_{\max} (T_{\max}) were identified from the oral glucose tolerance test data for glucose and insulin. Insulin secretion was calculated using the method of Phillips *et al.*⁽⁵³⁾.

All statistical analyses were performed using the Statistical Analysis System (version 9.1; Cary, NC, USA) with $P < 0.05$ considered statistically significant. Data were examined for normality using stem leaf diagrams, box plots and residual plots, and it was determined that urinary isoflavones, fasting insulin, insulin AUC, insulin C_{\max} , insulin T_{\max} , log HOMA, QUICKI, ISI_{Matsuda} and insulin secretion required log transformation.

The effect of study treatment on anthropometric data at study day 57 was determined using repeated-measures analysis of covariance including study day 1 values as a covariate and controlling for subject, treatment order and treatment, followed by the Tukey's test for multiple comparisons.

The effect of study treatment on energy and nutrient intake data was determined using repeated-measures ANOVA, controlling for subject, treatment order and treatment, followed by the Tukey's test for multiple comparisons.

The effect of study treatment on markers of glycaemic control values at study day 57 was determined using repeated-measures analysis of covariance including study day 1 values as a covariate and controlling for subject, treatment order and treatment, followed by the Tukey's test for multiple comparisons. To ensure the washout period was adequate, repeated-measures ANOVA was performed on study day 1 values for markers of glycaemic control, controlling for subject, treatment order and treatment. Markers of glycaemic control were further analysed to account for variation in urinary equol by inclusion of equol excretor status as a covariate and the interaction between equol excretor status and treatment in the statistical model.

Results

Subject withdrawals and exclusions

During the study, four subjects withdrew due to dislike of the study treatment powders (n 1), cancer diagnosis (n 1) and personal issues (n 2). After the study, data from one subject were excluded due to a baseline HbA1C $> 8\%$. A total of twenty-nine subjects (sixteen males and thirteen females) were included in the statistical analysis, out of which fourteen subjects consumed SPI followed by MPI and fifteen subjects consumed MPI followed by SPI. All subjects consumed the SPI and MPI treatments every day during each treatment period with no reports of tolerance issues or adverse effects.

Subject characteristics

Subject characteristics at baseline are presented in Table 1. Subjects had good glycaemic control (baseline fasting plasma glucose of 6.76 mmol/l and HbA1C of 5.89%), were not taking any medications to modulate glycaemic control or lipids and were taking an average of 1.80 medications with the top three types being ace inhibitors (n 8), aspirin (n 6) and β -blockers (n 5). Subject characteristics during the study are presented in Table 2 and show that there were no significant differences in body weight, BMI, body fat, waist or hip circumferences or waist:hip ratio between the SPI and MPI treatments.

Table 1. Baseline subject characteristics (Mean values and standard deviations, n 29)

	Mean	SD
Age (years)	60.1	9.64
Body weight (kg)	83.4	10.9
BMI (kg/m ²)	29.6	4.07
Body fat (%)	38.5	10.1
Waist circumference (cm)	99.0	7.48
Hip circumference (cm)	109.2	8.05
Waist:hip ratio	0.92	0.07
Blood pressure (mmHg)		
Systolic	134.7	16.1
Diastolic	83.7	8.51
Heart rate (beats/min)	65.9	8.49
Time since diagnosis (months)	40.8	57.3
Number of medications (n)	1.80	0.95
Fasting plasma glucose (mmol/l)	6.76	1.30
HbA1C (%)	5.89	0.62

Table 2. Subject characteristics during the study
(Mean values and standard deviations, *n* 29)

	MPI				SPI			
	Day 1		Day 57		Day 1		Day 57	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Body weight (kg)	83.4	10.9	83.2	10.4	83.1	10.5	83.1	10.2
BMI (kg/m ²)	29.5	3.77	29.4	3.75	29.3	3.59	29.2	3.62
Body fat (%)	38.0	10.8	37.5	12.4	37.3	9.31	36.2	10.6
Waist circumference (cm)	98.8	7.77	99.2	7.23	98.6	7.72	99.1	8.03
Hip circumference (cm)	109.7	8.29	108.2	8.16	108.0	8.02	107.7	7.56
Waist:hip ratio	0.91	0.07	0.92	0.06	0.91	0.06	0.92	0.05

MPI, milk protein isolate; SPI, soya protein isolate.

Energy and nutrient intakes

Protein ($P<0.0001$) and Ca ($P<0.0001$) intakes were significantly higher during the study compared with before the study (Table 3). During the study, with the exception of SFA, which was significantly lower during the SPI compared with the MPI treatment ($P=0.04$), there were no significant differences between the SPI and MPI treatments in intakes of energy, macronutrients, dietary fibre, cholesterol or Ca (Table 3).

Urinary isoflavone excretion

Urinary excretion of genistein, daidzein and *O*-desmethylan-golensin were significantly higher following consumption of the SPI compared to the MPI ($P<0.0001$; Table 4). Further analysis of urinary equol data within the SPI treatment showed that six subjects (20.7% of subjects) could be classified as equol excretors (equol excretion >1000 nmol/l⁽⁵⁴⁾) and twenty-three subjects could be classified as equol non-excretors. Among the equol excretors, urinary equol excretion was significantly higher following consumption of the SPI compared to the MPI ($P<0.0001$; Table 4).

Markers of glycaemic control

There were no significant differences in day 1 concentrations of fasting plasma glucose, HbA1C, glucose C_{max} , glucose T_{max} (Table 5), postprandial glucose (Fig. 1(a)), glucose AUC (Fig. 1(a)), fasting serum insulin, insulin C_{max} , insulin T_{max} , log HOMA-IR, QUICKI, ISI_{Matsuda}, insulin secretion (Table 5), postprandial insulin (Fig. 2(a)) or insulin AUC (Fig. 2(a)), providing evidence that the washout period between treatments was sufficient.

Plasma glucose parameters, including fasting glucose, HbA1C, glucose C_{max} , glucose T_{max} (Table 5), postprandial glucose (Fig. 1(b)) and glucose AUC (Fig. 1(b)), were not significantly affected by consumption of SPI compared with MPI. Similarly, serum insulin parameters, including fasting insulin, insulin C_{max} , insulin T_{max} , log HOMA-IR, QUICKI, ISI_{Matsuda}, insulin secretion (Table 5), postprandial insulin (Fig. 2(b)) and insulin AUC (Fig. 2(b)), were not significantly affected by consumption of SPI compared with MPI. Inclusion of equol excretor status as a covariate in the statistical model did not change any of the results, and there was no significant interaction between equol excretor status and treatment for any of the markers of glycaemic control.

Table 3. Dietary intake during the study
(Mean values and standard deviations, *n* 29)

	Pre-study*		MPI†		SPI†	
	Mean	SD	Mean	SD	Mean	SD
Energy (kJ)	8322	2457	8950	2597	8598	2149
Protein (g)	87.3 ^a	22.6	120.1 ^b	28.4	118.6 ^b	20.0
Carbohydrates (g)	239.7	73.1	237.4	81.4	230.2	67.2
Total fat (g)	75.6	33.5	78.8	30.9	73.4	26.9
SFA	22.0 ^{a,b}	10.5	24.4 ^a	11.7	22.2 ^b	8.43
MUFA	22.3	14.3	21.2	10.3	20.7	11.0
PUFA	10.7	5.46	10.3	4.31	10.1	5.18
Dietary fibre (g)	24.6	8.50	24.0	9.68	22.8	7.71
Cholesterol (g)	253.0	132.4	276.1	148.3	261.1	97.6
Ca (mg)	920.7 ^a	442.3	1959 ^b	456.0	2002 ^b	462.6

MPI, milk protein isolate; SPI, soya protein isolate.

^{a,b} Mean values within a row with unlike superscript letters were significantly different ($P<0.05$).

* Values are based on the results of one 3-d food record completed before the study.

† Values are based on the average results of three 3-d food records completed on days 2–4, 26–28 and 54–56 and include contributions from the study treatment powders.

Table 4. Urinary isoflavone excretion after consumption of MPI and SPI
(Geometric mean values and 95% confidence intervals, *n* 29)*

	MPI		SPI	
	Geometric mean	95% CI	Geometric mean	95% CI
Genistein (nmol/24 h)	90.9 ^a	44.4, 186.2	11 849 ^b	9855, 14 247
Daidzein (nmol/24 h)	134.3 ^a	65.6, 275.0	13 767 ^b	12 175, 15 567
ODMA (nmol/24 h)	31.8 ^a	17.9, 56.5	7708 ^b	3842, 15 465
Equol (nmol/24 h)†	21.8 ^a	17.4, 27.3	12 965 ^b	10 140, 16 577

MPI, milk protein isolate; SPI, soya protein isolate; ODMA, *O*-desmethylangolensin.^{a,b} Mean values within a row with unlike superscript letters were significantly different ($P < 0.0001$).

* Data were log-transformed before statistical analysis and presented as geometric mean (95% CI).

† Data are presented for equol excretors only (*n* 6).

Discussion

The purpose of the present study was to determine the effect of soya protein on markers of glycaemic control in adults with diet-controlled type 2 diabetes. The present study is unique as it included subjects with diet-controlled type 2 diabetes who were not on glycaemic medications, a treatment and control group with equal amounts of protein and an evaluation of fasting and postprandial glycaemic response. Subjects had been diagnosed with type 2 diabetes for an average duration of 40.8 months, and their average baseline fasting plasma glucose of 6.76 mmol/l and HbA1C of 5.89% indicated good diabetes management⁽⁹⁾ and made them candidate to explore the potential for soya to prevent risk of diabetes complications.

Anthropometric measurements did not significantly change during the present study. Since weight loss in general, and abdominal fat loss in particular, can improve glycaemic control in type 2 diabetes⁽⁵⁵⁾, the lack of significant changes in these measures reduced any potential confounding effects on the study's primary glycaemic control endpoints. Analysis of 3-d food records revealed that protein and Ca intakes were significantly higher during the study compared with

before the study, which was expected due to the 40 g protein and 1400–1600 mg Ca content of the daily study treatment powder. Since increased protein intake can improve glycaemic control in type 2 diabetes^(47–49), the lack of significant difference in protein intake between the SPI and MPI treatments allowed for a focus on protein type rather than amount. Ca intakes were also not significantly different between treatments minimising any potential confounding effects of Ca on glycaemic control⁽⁵⁶⁾.

Urinary isoflavone excretion was significantly higher following consumption of the SPI compared with the MPI, providing evidence that subjects consumed the SPI during the SPI treatment period and avoided external sources of phyto-oestrogens during the MPI treatment period. Urinary equol data revealed that six out of the twenty-nine subjects (20.7%) could be classified as equol excretors, which is lower than the 28–50% prevalence rates observed in studies of adults without diabetes^(54,57–60). Prevalence of equol excretor status has not been identified in previous studies of adults with type 2 diabetes; however, a study of adults with type 1 diabetes reported that increasing concentration of serum equol was associated with increasing severity of renal

Table 5. Plasma glucose and serum insulin parameters before and after consumption of MPI and SPI(Mean values and 95% confidence intervals, *n* 29)

	MPI				SPI			
	Day 1		Day 57		Day 1		Day 57	
	Mean	95% CI	Mean	95% CI	Mean	95% CI	Mean	95% CI
Fasting plasma glucose (mmol/l)	6.92	6.41, 7.43	6.89	6.46, 7.32	6.73	6.24, 7.22	7.01	6.50, 7.52
HbA1C (%)	5.94	5.69, 6.19	5.97	5.74, 6.20	5.92	5.67, 6.17	5.91	5.66, 6.16
Postprandial plasma glucose								
Plasma glucose C_{max} (mmol/l)	14.2	13.0, 15.5	14.3	13.2, 15.3	14.0	12.8, 15.3	14.1	12.9, 15.3
Plasma glucose T_{max} (min)	73.5	65.1, 81.9	82.8	71.5, 94.0	73.5	63.1, 83.8	80.7	72.6, 88.8
Fasting serum insulin (pmol/l)*	66.0	54.9, 79.4	69.4	56.6, 85.2	61.6	50.2, 75.6	61.6	50.2, 75.6
Postprandial serum insulin								
Serum insulin C_{max} (pmol/l)*	554.5	433.7, 708.9	501.7	376.6, 668.3	522.2	400.1, 681.5	477.2	373.3, 610.2
Serum insulin T_{max} (min)*	86.5	74.9, 99.8	91.8	81.2, 103.8	94.6	82.0, 109.2	102.5	92.5, 113.6
Log (HOMA)*	2.94	2.40, 3.61	3.10	2.47, 3.88	2.64	2.11, 3.30	2.77	2.26, 3.40
QUICKI*	0.24	0.23, 0.26	0.24	0.23, 0.25	0.25	0.23, 0.26	0.24	0.23, 0.26
ISI _{Matsuda} *	3.53	2.87, 4.33	3.53	2.87, 4.33	3.78	2.96, 4.83	3.82	3.05, 4.78
Insulin secretion*	5.53	3.67, 8.33	5.26	3.87, 7.15	5.26	3.79, 7.30	4.71	3.19, 6.95

MPI, milk protein isolate; SPI, soya protein isolate; C_{max} , maximum concentration; T_{max} , time to C_{max} ; HOMA, homeostasis model assessment; QUICKI, quantitative insulin sensitivity check index; ISI_{Matsuda}, insulin sensitivity index-Matsuda.

* Data were log-transformed before statistical analysis and presented as geometric mean (95% CI).

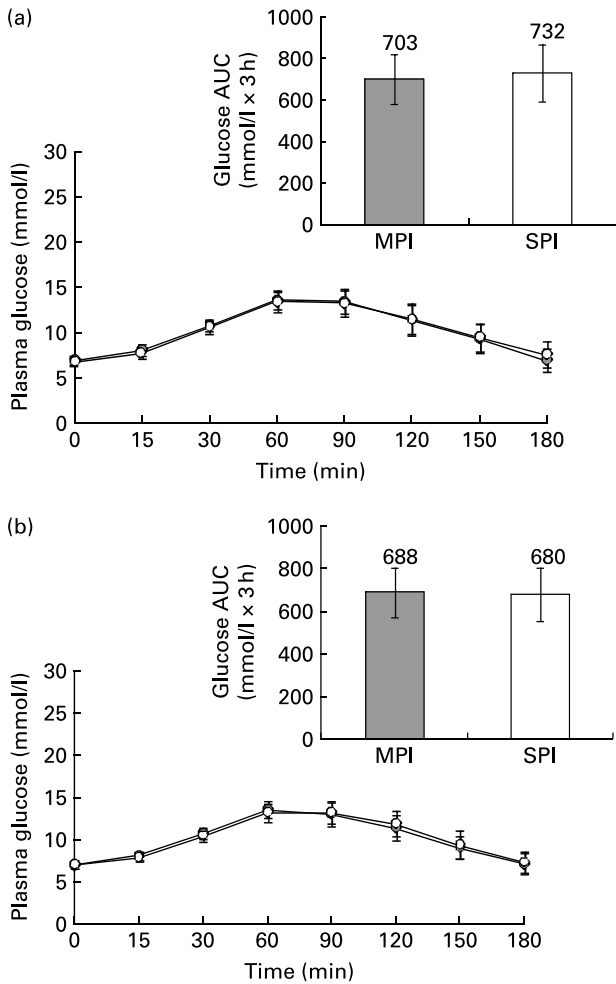


Fig. 1. Postprandial plasma glucose. Data are means and 95% CI (*n* 29) presented at each time point in the line graphs and summarised across time points as area under the curve (AUC) in the bar graphs. (a) Data from day 1 and (b) data from day 57. MPI, milk protein isolate (●—); SPI, soya protein isolate (○—).

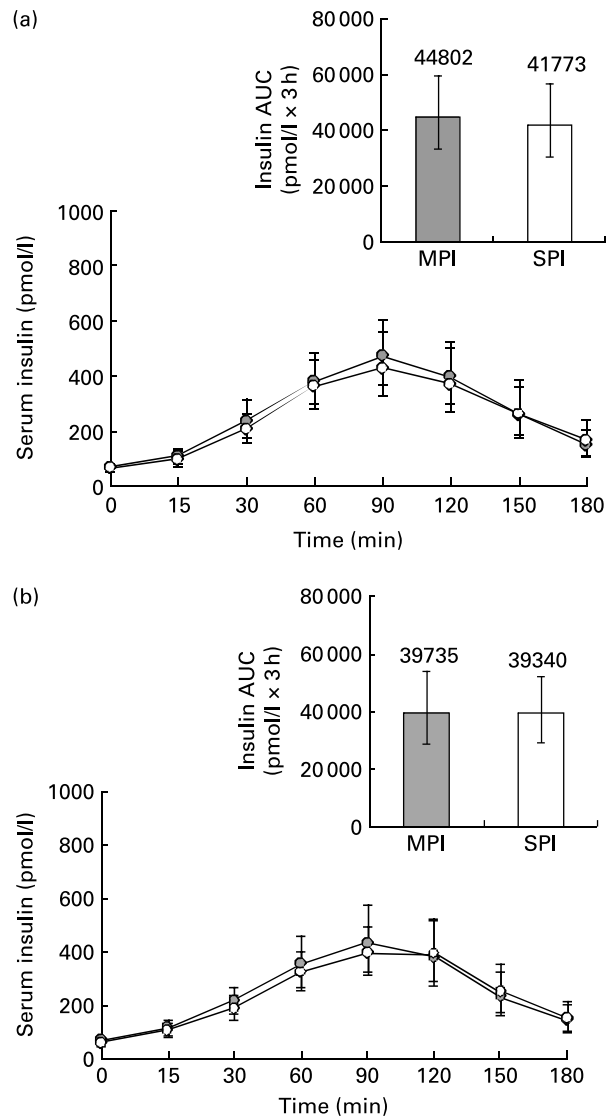


Fig. 2. Postprandial serum insulin. Data were log-transformed before statistical analysis and are geometric means and 95% CI (*n* 29) presented at each time point in the line graphs and summarised across time points as area under the curve (AUC) in the bar graphs. (a) Data from day 1 and (b) data from day 57. MPI, milk protein isolate (●—); SPI, soya protein isolate (○—).

disease⁽⁶¹⁾. The equal excretion data of the present study warrant further exploration of equol production in type 2 diabetes.

Fasting or postprandial glucose was not significantly affected by 8 weeks of SPI (40 g soya protein and 80 mg isoflavones) relative to MPI (40 g protein) in the present study. This is consistent with previous studies of adults with type 2 diabetes, which have found no significant effect on fasting glucose with consumption of a soya protein and fibre supplement (50 g soya protein, 165 mg isoflavones and 20 g soya fibre) for 6 weeks⁽³⁹⁾, soya foods (0.5 g soya protein/kg body weight/d, mg isoflavones not reported) for 8 weeks⁽³⁶⁾, soya protein (30 g SPI and 132 mg isoflavones for 12 weeks⁽⁴⁶⁾, 35% of daily protein as soya, mg isoflavones not reported for 7 weeks⁽³⁵⁾ or 0.5 g SPI/kg body weight/d, mg isoflavones not reported for 8 weeks⁽³³⁾ or isoflavone supplements (50 mg for 4 weeks⁽⁴⁰⁾, 132 mg for 12 weeks⁽⁴⁵⁾ or 177 mg for 12 weeks⁽³⁷⁾). In contrast, other studies of adults with type 2 diabetes have found significant reductions in fasting glucose following consumption of soya protein meal (12 g soya protein and 50 mg isoflavones) for

3 months⁽⁴³⁾, a soya protein supplement (5.22 g soya protein, mg isoflavones not reported) for 12 weeks⁽⁴²⁾, soya protein (14–17 g soya protein and 36–48 mg isoflavones) for 4 years⁽³⁴⁾ and a soya-based meal replacement (amounts of soya protein and isoflavones not reported) for 3 and 6, but not 12 months⁽³⁸⁾.

HbA1C was also not significantly affected by 8 weeks of SPI relative to MPI in the present study. This is consistent with previous studies of adults with type 2 diabetes, which have also found no significant change in HbA1C following consumption of a soya and fibre supplement (50 g soya protein, 165 mg isoflavones and 20 g soya fibre) for 6 weeks⁽³⁹⁾, diets containing soya protein for 7–8 weeks^(33,35,36) or an isoflavone supplement (50 mg for 4 weeks⁽⁴⁰⁾ or 132 mg for 12 weeks⁽⁴⁵⁾). However, other studies of adults with type 2 diabetes have reported significant reductions in HbA1C

following consumption of SPI (30 g soya protein and 132 mg isoflavones) for 12 weeks⁽⁴⁶⁾, a soya-based meal replacement for 12 months⁽³⁸⁾ and soya protein meal (12 g soya protein and 50 mg isoflavones) for 3 months⁽⁴³⁾. Since HbA1C most accurately reflects glycaemic control in the previous 2–3 months⁽⁶²⁾, it is possible that the 8-week treatment period of the present study was insufficient to reveal an effect. Although dietary interventions in adults with type 2 diabetes have demonstrated significant reductions in HbA1C after 4 weeks^(47,63), the only soya interventions to do so have been of 3^(43,46) and 12⁽³⁸⁾ month durations.

Fasting or postprandial insulin was also not significantly affected by 8 weeks of SPI relative to MPI in the present study. This is consistent with previous studies of adults with type 2 diabetes, which have also found no significant change in fasting insulin following consumption of an acute meal with 10 g soya polysaccharide⁽⁴⁴⁾, a soya and fibre supplement (50 g soya protein, 165 mg isoflavones and 20 g soya fibre) for 6 weeks⁽³⁹⁾, a soya-based meal replacement for 3–12 months⁽³⁸⁾ or isoflavone supplements (132 mg⁽⁴⁵⁾ or 177 mg⁽³⁷⁾) for 12 weeks. In contrast, fasting insulin has been significantly reduced in adults with type 2 diabetes following consumption of SPI (30 g soya protein and 132 mg isoflavones) for 12 weeks⁽⁴⁶⁾. It is relevant that degree of impairment in insulin secretion and/or resistance will vary⁽⁹⁾ and influence interpretation, such that decreased insulin would be favourable in those with insulin resistance and increased insulin would be favourable in those with impaired insulin secretion. Thus, heterogeneity of disease progression among subjects could cause variation that precludes clear demonstration of an effect.

The present study calculated log HOMA-IR as a measure of insulin sensitivity and found that it was not significantly affected by 8 weeks of SPI, consistent with a previous study of postmenopausal women with type 2 diabetes who consumed a 132 mg isoflavone supplement for 12 weeks⁽⁴⁵⁾. In contrast, HOMA-IR was significantly reduced in postmenopausal women with diet-controlled type 2 diabetes who consumed SPI (30 g soya protein and 132 mg isoflavones) for 12 weeks⁽⁴⁶⁾ and in postmenopausal women with metabolic syndrome who consumed soya protein (15 g soya protein and 84 mg isoflavones) or soya nuts (11.3 g soya protein and 102 mg isoflavones) for 8 weeks⁽⁶⁴⁾.

The present study also calculated other indices of glycaemic control (glucose and insulin C_{max} and T_{max} , QUICKI, $ISI_{Matsuda}$ and insulin secretion), none of which were significantly affected by 8 weeks consumption of SPI relative to MPI. These indices have not been included in other soya intervention studies in adults with type 2 diabetes, yet their reflection of the postprandial glycaemic response and correlation with HbA1C⁽⁶⁵⁾ justify their inclusion in future studies.

Overall, soya intervention studies that have examined glycaemic control in adults with type 2 diabetes have yielded inconsistent results. Variation can come from study design, particularly if there is a lack of control group^(37,42,43). Variation can also come from the treatment and control groups themselves such as the combination of soya protein and isoflavones with soya fibre⁽³⁹⁾, the mismatch of protein content between the soya treatment and control⁽⁴⁶⁾ or the possibility that other soybean components (besides protein, isoflavones and fibre) were not accounted for, yet may influence

glycaemic control; for example, pinitol isolated from soyabeans has been shown to significantly reduce postprandial glucose when consumed before a rice meal in adults with type 2 diabetes⁽⁶⁶⁾. Finally, variation can come from differences in baseline concentrations of glucose, insulin and HbA1C. Soya intervention studies of adults with type 2 diabetes, which have found significant improvements in glucose^(34,38,42,43), HbA1C^(38,43,46) or insulin⁽⁴⁶⁾, have had baseline values of glucose of 7.29–9.79 mmol/l^(34,42,43,46), HbA1C of 6.83–9.60 %^(38,43,46) and insulin of 116.0 pmol/l⁽⁴⁶⁾.

There are limitations of the present study, which deserve mention. Subjects were not representative of all those with type 2 diabetes in that they were managing their diabetes without insulin or medications and had good glycaemic control, which contrasts a Canadian study of adults with type 2 diabetes documenting glycaemic medication use at 73 % and average HbA1C of 7.3 %⁽⁶⁷⁾. Furthermore, although the 8-week treatment duration was employed to minimise the likelihood that a subject would need to initiate oral hypoglycaemic medications and to reduce the subject burden of total intervention duration, it may not have been sufficient to detect significant effects. Finally, since the sample size calculation estimated a need for thirty-four subjects to detect changes in plasma glucose, the completion of twenty-nine subjects means that the power was lower than anticipated. Despite these limitations, strengths include the fact that subjects were not taking any medications to manage their diabetes thereby eliminating a possible confounding effect on results, the matching of protein content between the SPI and MPI treatments and the inclusion of postprandial measures of glycaemic control.

In summary, consumption of soya protein for 8 weeks did not significantly affect fasting or postprandial measures of glycaemic control in a sample of twenty-nine adults with diet-controlled diabetes. The present study adds to the literature with its unique qualities of matching for protein content between the SPI and MPI treatments and including postprandial measures of glycaemic control. Information gained from the present study provides valuable insight for future direction of research exploring the effects of soya for the prevention and management of type 2 diabetes.

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provided medical expertise throughout the subject recruitment and data collection and contributed to the manuscript preparation. G. A. D. contributed to the study design, provided statistical expertise throughout the statistical analysis and results interpretation and contributed to the manuscript preparation. J. W. L. performed the urinary isoflavone analyses and contributed to the manuscript preparation. A. M. D. designed the study, secured the funding, supervised the subject recruitment and data collection, supervised the glucose and insulin laboratory analyses, directed the data and statistical analyses, directed the interpretation of results and directed the manuscript preparation.

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