International Journal of Food Sciences and Nutrition,
November 2012; 63(7): 767–771

informa
healthcare

Effect of broccoli sprouts on insulin resistance in type 2 diabetic patients: a randomized double-blind clinical trial

ZAHRA BAHADORAN[1], MARYAM TOHIDI[2], PANTEA NAZERI[1], MAHYA MEHRAN[1], FEREIDOUN AZIZI[3], & PARVIN MIRMIRAN[4]

[1]*Obesity Research Center, Research Institute for Endocrine Sciences, Shahid Beheshti University of Medical Sciences, Tehran, Iran,* [2]*Prevention of Metabolic Disorders Research Center, Research Institute for Endocrine Sciences, Shahid Beheshti University of Medical Sciences, Tehran, Iran,* [3]*Endocrine Research Center, Research Institute for Endocrine Sciences, Shahid Beheshti University of Medical Sciences, Tehran, Iran, and* [4]*Department of Clinical Nutrition and Diet Therapy, Faculty of Nutrition Sciences and Food Technology, National Nutrition and Food Technology Research Institute, Shahid Beheshti University of Medical Sciences, Tehran, Iran*

Abstract
Use of antioxidant components is a new approach for improvement of insulin resistance (IR) as a main feature of type 2 diabetes and its complications. The aim of this study was to investigate the effects of broccoli sprouts powder (BSP) containing high concentration of sulphoraphane on IR in type 2 diabetic patients. Eighty-one patients were randomly assigned to receive 10 g/d BSP (A, $n = 27$), 5 g/d BSP (B, $n = 29$) and placebo (C, $n = 25$) for 4 weeks. Fasting serum glucose and insulin concentration, glucose to insulin ratio and homoeostasis model assessment of IR (HOMA-IR) index were measured at baseline and again 4 weeks after treatment. Seventy-two patients completed the study and 63 were included in the analysis. After 4 weeks, consumption of 10 g/d BSP resulted in a significant decrease in serum insulin concentration and HOMA-IR ($p = 0.05$ for treatment effect). Therefore, broccoli sprouts may improve IR in type 2 diabetic patients.

Keywords: *type 2 diabetes, insulin resistance, broccoli sprouts, sulphoraphane*

Abbreviations: *BSP, broccoli sprouts powder; FBS, fasting blood glucose; HOMA-IR, homoeostasis model assessment of insulin resistance; IR, insulin resistance; SPN, sulphoraphane*

Introduction

Insulin resistance (IR) usually refers to a defect in the ability of insulin to stimulate glucose uptake. IR in addition to being a characteristic feature of type 2 diabetes is also a risk factor for metabolic and cardiovascular complications (Lebovitz 2001). *In vitro* and *in vivo* models reveal that increased oxidative stress and accumulation of lipid peroxidation metabolites in muscle, liver, adipocytes and pancreatic beta cell contribute to IR, beta cell dysfunction and development of type 2 diabetes (Hoehn et al. 2009; Park et al. 2009; Henriksen et al. 2011). The molecular mechanisms whereby oxidative stress causes IR are not clear, but it seems that activated stress-sensitive signalling pathways, such as nuclear factor kappa B, extracellular signal-related kinase and others, in the absence of appropriate antioxidant defence system in diabetes mediate IR (Evans et al. 2003). Thus, strengthening the antioxidant system may be very important in preventing the activation of these pathways (Evans 2007). The major pharmacological approaches to increase insulin sensitivity are biguanides and thiazolidinediones but a potentially new approach is the use of antioxidant components (Laakso 2001). Studies confirm the beneficial effects of bioactive food components with antioxidant activity including α-lipoic acid, antioxidant vitamins and

Correspondence: Parvin Mirmiran, Department of Clinical Nutrition and Diet Therapy, Faculty of Nutrition Sciences and Food Technology, National Nutrition and Food Technology Research Institute, Shahid Beheshti University of Medical Sciences, P.O. Box: 19395-4763, Tehran, Iran. Tel: + 98 (21) 224 32 500. Fax: + 98 (21) 224 16 264, 224 02 463. E-mail: mirmiran@endocrine.ac.ir

ISSN 0963-7486 print/ISSN 1465-3478 online
DOI: 10.3109/09637486.2012.665043

flavonols, in increasing insulin sensitivity in type 2 diabetes (Evans 2007).

Broccoli and broccoli sprouts are rich sources of bioactive components including isothiocyanates, glucosinolates, flavonoids, phenols, carotenoids, antioxidant vitamins and selenium (Nestle 1997). Important of these components is sulphoraphane (SPN; 1-isothiocyanate-4-methylsulfinylbutane), a potent inducer of antioxidant enzymes, which is found in cruciferous vegetables especially in young broccoli sprouts (Fahey and Talalay 1999; Verkerk et al. 2009). The beneficial properties of broccoli sprouts are mainly attributed to high concentration of SPN; studies demonstrate that SPN reduces oxidative stress through the activation of antioxidant response pathways (Wu et al. 2004; Xue et al. 2008; Riedl et al. 2009). Previously, we reported that broccoli sprouts supplementation with standard dose of SPN, in type 2 diabetic patients, significantly decreased lipid peroxidation and increased total antioxidant capacity (Bahadoran et al. 2011). To date, no data are available on the effects of rich sources of SPN on IR. We hence hypothesized that broccoli sprouts may improve IR in type 2 diabetic patients.

Materials and methods

Subjects and study design

This was a parallel, randomized, double-blind and placebo controlled study. Type 2 diabetic patients, aged 18–60 years with a clinical diagnosis of type 2 diabetes for at least 1 year, were recruited from the Iran Diabetes Society. Patients were excluded from the trial if they had severe impairment of cardiac, hepatic or renal function, gestation or lactation and if they used insulin injection or consumed oestrogen, vitamin K-antagonists or antioxidant supplements. Finally, 81 patients were randomly assigned to three groups, A (broccoli sprouts powder (BSP) 10 g/d, $n = 27$), B (BSP 5 g/d, $n = 29$) and C (placebo, $n = 25$), using a computer-generated random number table. Randomization was performed with the use of sealed envelopes. The investigator and participants were blinded to group allocation. In order to assess dietary changes during the study period, 3-day dietary recalls, including 2 week days and 1 weekend day, were collected at baseline and again after 4 weeks from subjects. Since the Iranian food composition table (FCT), with limited data on nutrient content of raw foods and beverages, is incomplete, the US Department of Agriculture (USDA) FCT was used to calculate energy and nutrient intakes. However, the Iranian FCT was used for some national foods that are not listed in the USDA FCT (Azar and Sarkisian 1980). An Excel program was designed to analyse the nutrients of each food item. In Excel sheet, all nutrients of each food item were formulated based on 1 g of it. Weight was measured and body mass index was calculated at baseline and 4 weeks later. Ethics approval for the trial was obtained from the ethics committee of the Research Institute for Endocrine Sciences, Shahid Beheshti University of Medical Sciences. The written consent forms were obtained from the patients at the beginning of the study. This clinical trial has been registered in the Iranian Registry of Clinical Trials at http://www.irct.ir with the following identification: IRCT138901181640N2. The results are reported according to the Consolidated Standards of Reporting Trials guidelines.

Intervention

BSP standardized for SPN (BroccoPhane®) was purchased from the Cyvex Nutrition Company (Irvine, CA, USA) The SPN content of BSP was determined ~22.5 μmol/g by manufacturer using the HPLC method. At baseline, subjects received 28 packets containing either 5 or 10 g of BSP in BSP groups, whereas controls were given 5 g cornstarch powder coloured with chlorophyll. Packets were identically packaged to be indistinguishable. Patients were prescribed one packet daily to be consumed for 4 weeks, preferably with a beverage after meals to reduce gastrointestinal complications; they were also asked to maintain their regular diet and lifestyle during the study period and were contacted every week to evaluate compliance and to enquire regarding any possible side effects. Patients were excluded from the analysis if they consumed $<80\%$ of the packets, changed their medication or reported severe side effects.

Laboratory measurements

Fasting venous blood samples were taken after 12–14 h overnight fast, at baseline and again 4 weeks after intervention. Serum was separated and frozen at -70°C, on the day of blood collection for biochemical analysis. Fasting plasma glucose was measured by the enzymatic colorimetric method using a glucose oxidation kit (Pars Azmun Company, Tehran, Iran). Serum insulin concentrations were measured using enzyme-linked immunosorbent assays kit (Mercodia, Uppsala, Sweden). The intra assay coefficients of variation of all assays were $<5\%$. The fasting glucose to insulin ratio was estimated as the insulin sensitivity index (Silfen et al. 2001). IR was estimated using the homoeostasis model assessment of IR (HOMA-IR) index, which is defined as fasting plasma insulin (mU/l) times fasting plasma glucose (mmol/l) divided by 22.5 (Hanley et al. 2002).

Statistical methods

Statistical analysis was carried out with SPSS, version 16.0 (SPSS, Inc., Chicago, IL, USA). The Kolmogorov–Smirnov test was used to test for a

normal distribution. Differences between the three groups at baseline were tested with one-way ANOVA, and paired Student's t test was used to compare baseline and 4-week values in each group. The effects of the BSP were compared by analysis of covariance (ANCOVA) with 4-week values as dependent variables, baseline values as covariates and treatment group as a fixed factor. When the analysis indicated a significant effect of BSP, the groups were compared pairwise by the Bonferroni test. P values <0.05 were considered significant.

Results

Seventy-two patients completed the study. The participants who consumed less than 80% of BSP doses or changed the medications through the study period were excluded from the analysis; finally, the data for 63 patients [10 g/d BSP ($n = 21$), 5 g/d ($n = 22$) and placebo ($n = 20$)] were analysed (Figure 1). Mean age of participants was 53 ± 6.1, 50 ± 6.0 and 52 ± 7.5 years in groups A, B and C, respectively. Mean values for baseline body mass index were 27.8 ± 5.0, 29.2 ± 3.6 and 28.7 ± 4.0 kg/m^2 in these three groups, respectively. No significant differences between the three groups were seen for age, sex, body mass index, consumption of antidiabetic medications or duration of diabetes. Based on self-reports subject, lifestyles were unchanged throughout the study. There was no significant difference between the groups in total energy and nutrient intakes, as estimated by 3-day dietary recalls. The body mass index of participants was not significantly changed throughout the study. No serious adverse events or side effects were reported. Before and after biochemical values and the effects of BSP on serum fasting glucose, insulin concentration, insulin sensitivity and IR indices are presented in Table I. As compared with baseline, after 4 weeks BSP treatment, fasting serum glucose significantly decreased in groups A and B, and serum insulin concentration and the IR index significantly decreased only in group A. Comparison of treatment effects between the three groups with baseline values as covariates revealed that supplementation with 10 g/d BSP resulted in a decrease in serum insulin and IR index as HOMA-IR. After 4 weeks as compared with baseline there was no significant difference in fasting glucose to insulin ratio as insulin sensitivity index. Mean differences in values compared with baseline are presented in Figure 2.

Discussion

Hyperinsulinaemia and IR are major determinants of the markedly increased risk of cardiovascular complications in type 2 diabetic patients (Lee et al. 2010). Treatment of IR in these patients is a potential target for the improvement of glycaemic control and decrease in cardiovascular disease risk factors (Reddy et al. 2010). In this study, 4-week supplementation

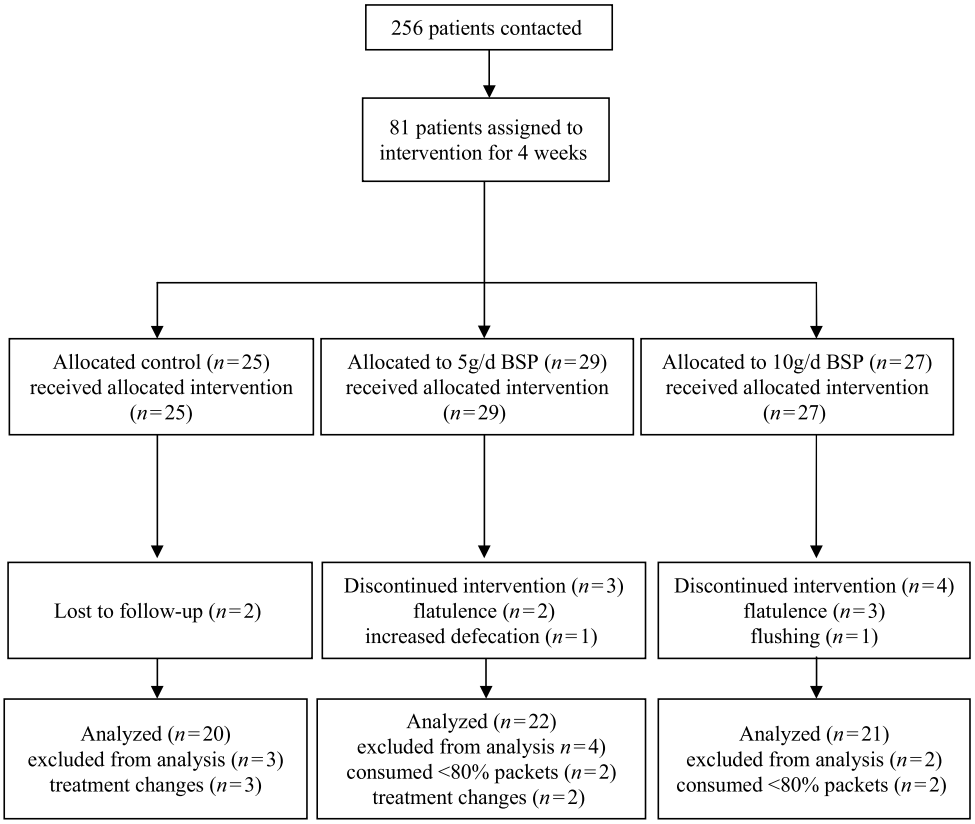


Figure 1. Flow chart of the study. BSP, Broccoli sprouts powder.

Table I. Fasting serum glucose and insulin concentration, insulin sensitivity and IR indices at baseline and after 4 weeks in BSP and placebo groups*.

	10 g/d BSP ($n = 21$)				5 g/d BSP ($n = 22$)				Placebo ($n = 20$)				
	Baseline		4 Weeks		Baseline		4 Weeks		Baseline		4 Weeks		
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	*P* for treatment effect †
Fasting glucose (mmol/l)	9.38	3.63	7.48‡	3.83	8.13	2.88	7.03‡	2.88	8.10	2.75	7.42	3.13	NS
Insulin (mU/l)	5.20	4.11	4.35‡,¶	3.11	7.60	4.61	7.31	5.03	6.50	3.90	7.81	6.99	<0.05
Fasting glucose to insulin ratio	2.71	3.55	2.32	3.19	1.43	0.94	1.31	1.08	2.0.1	1.95	1.93	1.88	NS
HOMA-IR	2.21	2.04	1.55‡,¶	1.32	2.81	2.03	2.44	2.11	2.22	1.42	2.58	3.24	<0.01

Note: BSP, broccoli sprouts powder; HOMA-IR, homeostasis model for IR; * Mean values and standard deviations (there were no significant differences between three groups at baseline); † ANCOVA with 4-week values as dependent variables, baseline values as covariates and treatment group as fixed factor; ‡ Significantly different from baseline (paired *t* test; $p < 0.05$); ¶ Significantly different from controls (Bonferroni pairwise comparisons in general linear model; $p < 0.05$).

with 10 g/d BSP decreased fasting serum insulin and IR index. There were no significant changes in lifestyle, dietary intake or medication of subjects throughout the study duration; hence any changes in variables were probably a result of the BSP treatment.

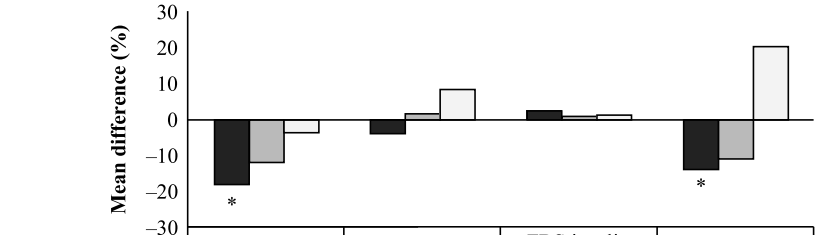
The effect of broccoli sprouts on IR has not yet been investigated and the mechanisms of any possible effects are unknown. It appears that the effect of broccoli sprouts on the improvement of hyperinsulinaemia and IR observed in this study is related to the antioxidant properties of bioactive components mainly SPN. SPN is an activator of transcription factor NF-E2-related factor-2 (Xue et al. 2008), which is a potential regulator of the cellular redox homoeostasis through its capacity to induce the expression of enzymes that detoxify reactive oxygen species and other antioxidant proteins (Li et al. 2009; Tan et al. 2011). *In vitro*, animal and human studies have reported the beneficial biological effects of SPN via induction of important cellular antioxidants and phase 2 enzymes, including superoxide dismutase, catalase, NAD(P)H:quinine oxidoreductase 1, glutathione, glutathione peroxidase, glutathione reductase and glutathione-s-transferase (Wu et al. 2004; Riedl et al. 2009). Enhancement of the endogenous antioxidant network inhibits stress-sensitive signalling pathways and consequently prevents IR and other long-term complications of diabetes (Evans 2007).

However, other bioactive compounds such as flavonoids, phenols, carotenoids, antioxidant vitamins and selenium may contribute to beneficial effects of broccoli sprouts on IR, but low content of these components in the BSP doses in this study does not appear to have independent effects on these results; synergistic effects of these components with high SPN concentration in BSP may be a logical reason for the observed results.

The doses used in this study provided 225 and 112 μmol SPN isothiocyanates daily per 10 and 5 g BSP doses, respectively. There were no serious side effects, but mild gastrointestinal events, including flatulence were reported by some patients.

This study does have a few limitations; study duration was 4 weeks and only one sample was obtained following intervention.

In conclusion, 4 weeks supplementation with BSP rich in SFN had favourable effects on decrease in serum insulin and improvement of IR in type 2 diabetes. Further studies with longer duration and



	FBS	Insulin	FBS:insulin ratio	HOMA-IR
■10g/d BSP (n=21)	–18.2	–4.3	2.3	–14.2
▒5g/d BSP (n=22)	–12	1.4	0.8	–11.3
□Placebo (n=20)	–3.8	8.2	1.1	20.3

Figure 2. Mean differences in FBS, serum insulin concentration, FBS to insulin ratio and HOMA-IR, compared with baseline values in the three groups (*significant difference as compared with control using one-way analysis of variance, $p < 0.05$). BSP, broccoli sprouts powder; FBS, fasting blood glucose; HOMA-IR, homeostasis model assessment of IR assessment.

different doses are needed to confirm these effects and their related mechanisms.

Acknowledgement

The authors express appreciation to the participants of this study and would like to acknowledge Ms N. Shiva for language editing of the manuscript and the assistance given by Mr N. Bahadoran for the purchase of broccoli sprouts powder.

Declarations of interest: This study was funded by the Research Institute of Endocrine Sciences, Shahid Beheshti University of Medical Sciences, Tehran, Iran. The authors report no conflict of interest. The authors alone are responsible for the content and writing of the paper.

References

Azar M, Sarkisian E. 1980. Food composition table of Iran National Nutrition and Food Research Institute. Shahid Beheshti University Press. Tehran: Iran.

Bahadoran Z, Mirmiran P, Hosseinpanah F, Hedayati M, Hosseinpour-Niazi S, Azizi F. 2011. Broccoli sprouts reduce oxidative stress in type 2 diabetes: a randomized double-blind clinical trial. Eur J Clin Nutr 65(8):972–977.

Evans JL. 2007. Antioxidants: do they have a role in the treatment of insulin resistance? Indian J Med Res 125:355–372.

Evans JL, Goldfine ID, Maddux BA, Grodsky GM. 2003. Are oxidative stress-activated signaling pathways mediators of insulin resistance and beta-cell dysfunction? Diabetes 52(1):1–8.

Fahey J, Talalay P. 1999. Antioxidant function of sulforaphane: a potent inducer of phase 2 detoxification enzymes. Food Chem Toxicol 37:973–979.

Hanley AJ, Stern MP, Williams K, Haffner SM. 2002. Homeostasis model assessment of insulin resistance in relation to the incidence of cardiovascular disease. Diabetes Care 25:1177–1184.

Henriksen EJ, Diamond-Stanic MK, Marchionne EM. 2011. Oxidative stress and the etiology of insulin resistance and type 2 diabetes. Free Radic Biol Med 51(5):993–999.

Hoehn KL, Salmon AB, Hohnen-Behrens C, Turner N, Hoy AJ, Maghzal GJ, Stocker R. 2009. Insulin resistance is a cellular antioxidant defense mechanism. Proc Natl Acad Sci USA 106(42):17787–17892.

Laakso M. 2001. Insulin resistance and its impact on the approach to therapy of Type 2 diabetes. Int J Clin Pract 121:8–12.

Lebovitz HE. 2001. Insulin resistance: definition and consequences. Exp Clin Endocrinol Diabetes 109:S135–S148.

Lee SH, Park SA, Ko SH, Yim HW, Ahn YB, Yoon KH. 2010. Insulin resistance and inflammation may have an additional role in the link between cystatin C and cardiovascular disease in type 2 diabetes mellitus patients. Metabolism 59(2):241–246.

Li J, Ichikawa T, Janicki JS, Cui T. 2009. Targeting the Nrf2 pathway against cardiovascular disease. Expert Opin Ther Targets 13: 785–794.

Nestle M. 1997. Broccoli sprouts as inducers of carcinogen-detoxifying enzyme systems: clinical, dietary, and policy implications. Proc Natl Acad Sci USA 94(21):11149–11151.

Park K, Gross M, Lee DH, Holvoet P, Himes JH, Shikany JM, Jacobs DR. 2009. Oxidative stress and insulin resistance: the coronary artery risk development in young adults study. Diabetes Care 32(7):1302–1307.

Reddy KJ, Singh M, Bangit JR, Batsell RR. 2010. The role of insulin resistance in the pathogenesis of atherosclerotic cardiovascular disease: an updated review. J Cardiovasc Med (Hagerstown) 11(9):633–647.

Riedl MA, Saxon A, Diaz-Sanchez D. 2009. Oral sulforaphane increases Phase II antioxidant enzymes in the human upper airway. Clin Immunol 130(3):244–251.

Silfen ME, Mavibo AM, McMahon DJ, Levine LS, Murphy AR, Oberfield SE. 2001. Fasting glucose to insulin ratio is a simple and useful measure of insulin resistance in young girls. J Clin Endocrinol Metab 86:2863–2868.

Tan Y, Ichikawa T, Li J, Si Q, Yang H, Chen X. 2011. Diabetic down-regulation of Nrf2 activity via ERK contributes to oxidative stress-induced insulin resistance in cardiac cells *in vitro* and *in vivo*. Diabetes 60(2):625–633.

Verkerk R, Schreiner M, Krumbein A. 2009. Glucosinolates in brassica vegetable: the influence of the food supply chain on intake, bioavailability and human health. Mol Nutr Food Res 53: S219–S266.

Wu L, Noyan MH, Facci M. 2004. Dietary approach to attenuate oxidative stress, hypertension, and inflammation in the cardio-vascular system. Proc Natl Acad Sci 101(18):7094–7099.

Xue M, Qian Q, Adaikalakoteswari A, Rabbani N, Babaei-Jadidi R, Thornalley PJ. 2008. Activation of NF-E2-related factor-2 reverses biochemical dysfunction of endothelial cells induced by hyperglycemia linked to vascular disease. Diabetes 57: 2809–2817.