Full Length Research Paper

Glycemic and insulinemic responses to pumpkin and unripe papaya in type 2 diabetic subjects

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Glycemic and Insulinemic responses to pumpkin and unripe papaya by estimating their glycemic index (GI) and insulinemic index (measured by c-peptide) from Bangladeshi origin were investigated in T2DM patients. Based on serving size in the Bangladeshi society, the GL of those two food items were also calculated. Ten T2DM subjects, under a cross-over design, consumed equi-carbohydrate amount (25g of total carbohydrate) of the vegetables and WB (white bread, as reference food), with a run in period of 7 days between the consecutive items. Serum levels of glucose were estimated at 0, 15, 30, 45, 60, 90, 120, 150 and 180 min, respectively, and c-peptide levels were at 0 and 180 min only. GI and GL were calculated by standard formulas. Unripe papaya showed significant lower serum glucose response compared to that of bread and pumpkin. The GI of pumpkin and unripe papaya were 74 ± 42 and 23 ± 7, respectively. The substantially lowered glycemic response and GI values in pumpkin and unripe papaya were not paralleled by an increased insulin response. The GL of Pumpkin and unripe papaya were 6.4 and 1.5, respectively. As judged against the mean values of the international table, pumpkin of Bangladeshi origin is a high GI and unripe papaya is a very low GI food. However, from the dietary practices in Bangladeshi society, pumpkin and papaya may be used as a low and very low GL food respectively.

Key words: Diet, vegetable, unripe papaya, pumpkin, Type 2 diabetes mellitus, glycemic index, glycemic load.

INTRODUCTION

Current epidemiological and physiological research (Colombani, 2004; Dickinson et al., 2005; Frost and Dornhorst, 2000; La Vecchia, 2004) and subsequent dietary recommendations (U.S. Department of Health and Human Services, 2005) are highlighting the importance of improving the overall quality of carbohydrate in the diet. Blood glucose response to the ingestion of carbohydrate-containing foods has been shown to vary dramatically, depending on factors including the molecular structure of the carbohydrate, fiber content and degree of processing (Ludwig, 2002). The blood glucose response to a carbohydrate-containing food is indicated by its glycemic index (GI), (Jenkins et al., 1981) defined as the incremental area under the glucose response curve carbohydrate relative to that produced by a portion of a control food (either glucose or white bread) containing the following consumption of a food portion containing 50 g of same amount of carbohydrate.

GI reflects the effect of carbohydrates in individual foods on the postprandial glycemic response, whereas glycemic load (GL) includes both the GI and total carbohydrate intake; thus, it approximates the total glycemic effect of the diet, which gives an adequate assessment of the total diet (Foster-Powell et al., 2002). Dietary GI and GL can affect carbohydrate metabolism *in vivo*: High GI and GL have been associated with hyperinsulinemia, impaired glucose tolerance and higher circulating insulin-like growth factor (IGF) concentrations (Brand-Miller et al., 2005; Jenkins et al., 1981; Jenkins et al., 1994; Liu et al., 2001; Salmeron et al., 1997). Low-GI

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food has been shown to have reduced postprandial blood glucose and insulin responses and improved the overall blood glucose and lipid concentrations in normal subjects (Jenkins et al., 1987) and patients with diabetes mellitus (Collier et al., 1988).

The GI has been recommended to help in guiding food choices (American Diabetes Association, 1979) because it has been reported that a high GI diet may have adverse health consequences by increasing the risk for chronic disease (Mann, 1980). Evidence suggests that high GI/GL diets may increase the risk for cardiovascular disease (Wei et al., 2000) and T2DM (Wannamethee et al., 2002; Jenkins et al., 1981; Ludwig, 2002; Willett et al., 2002). A high GI diet may increase the risk of chronic disease through the stimulation of hyperglycemia and hyperinsulinemia. In contrast, a low GI diet has been reported to have health benefits (Mann, 1980; Jenkins et al., 1981; Wolever et al., 1994). Epidemiological data indicate that a low GI diet has a protective role against development of T2DM (Wannamethee et al., 2002; Jenkins et al., 1981) coronary heart disease and the metabolic syndrome.

Since insulin is known to be atherogenic, a low GI at the expense of hyperinsulinaemia may not be useful. Thus a ranking of food based on their insulin secretary capacity along with the glycemic response is necessary. Due to the problem in differentiating endogenous and exogenous insulin, measurement of C-peptide is preferred as a measure of serum insulin.

This research work is conducted with pumpkin and unripe papaya, which are very much commonly consumed and popular vegetable in Bangladesh. The nutritional value of these vegetables makes it an excellent choice for both weight control and general health. Pumpkin contains 90% of water and a rich source of vitamin A. Papaya is a well source of enzyme that includes nutrients (rich in Ca, P and vit C) and antioxidants.

The present study was designed to explore these responses for pumpkin and unripe papaya consumed as vegetable (with only boiled preparation) in Bangladeshi type 2 diabetes mellitus subjects.

SUBJECTS AND METHODS

Subjects

Subjects were selected from out patients department (OPD) of Bangladesh Institute of Research and Rehabilitation on Diabetes, Endocrine and Metabolic Disorders (BIRDEM). A total number of ten T2DM subjects (6 males and 4 female) took part in the study. Diabetes was diagnosed and classified by the WHO criteria. The mean (±SD) of plasma HbA$_1$c level was < 8%. Exclusion criteria of the study subject were those suffering from acute and chronic complications of DM using oral contraceptives, steroids, diuretics and insulin, at the pregnant stage and those suffering from any other illness revealed on clinical examinations. Subjects were requested to maintain their usual daily food intake and activity throughout the study period. The purpose and protocol of the study

were explained to the subjects and written consent was obtained.

Tested foods and its preparation

The study included 2 test meals (pumpkin and unripe papaya) and white bread (WB) as reference food. Both test foods and reference food consisted of 25 g available carbohydrates. To get 25 g available carbohydrate weight of white bread, pumpkin and unripe papaya were 39, 258 and 438 g, respectively.

For test food, fresh pumpkin and papaya were purchased from the local Dhaka city market in bulk quantities sufficient to conduct all tests, Pumpkin and unripe papaya were first washed, weighed (258 and 438 g, respectively) and then steamed with water for 5 min. The steamed samples were then taken into a plate. The test meals were served at room temperature. The nutrient composition of the test meals is shown In Table 2.

As reference food freshly baked bread was sliced and portioned to the calculated weight (39 g). Each portion was bagged individually and stored frozen. On the days of trial, white bread portions were removed from the freezer 45 min before serving and allowed to thaw at room temperature.

Experimental procedure

On the first day after selection and taking of consent detailed socio-demographic data, family history of the patients and medical history were taken and physical and clinical examinations were done on the first day of visit using a pre-tested questionnaire. Anthropometric measurements included height, weight; waist circumference and hip circumference were taken. Thereafter, subjects were required to go through the study protocol on four separate occasions (one trial for test food and two repeated trial for the reference food) in the morning after a 10 to12 h overnight fasting and advised not to take any kind of medicine or smoke on the previous day except the prescribed one. The test of the reference food was repeated once in order to obtain at least two values in each subject, thus the precision was improved (Brouns et al., 2005). Test and reference meals were given to patients under a cross-over design with a wash out period of 7 days to avoid the 'second meal effect' (Wolever et al., 1988). Patients were advised to rely on recommended standard carbohydrate diet and also instructed not to eat legumes in the meal preceding the fast. An intravenous cannula was inserted into a superficial vein in the forearm on the day of experiment, drawing the fasting (0 h) blood sample of the patient, subjects were requested to consume the test food with 250 ml plain water (during the protocol of the test potato) or the glucose in 250 ml water (during the protocol of the reference food) in random order at a comfortable place within 10 min. Further blood samples were drawn at 15, 30, 45, 60, 90, 120, 150 and 180 min after the initial intake of sample. Patients took their prescribed medicine at the beginning of the meal. All the information and data obtained were recorded in a predesigned Case Record Form.

Blood sample was allowed to centrifuge at 3000 rpm for 15 min. The plasma separated was allocated in the labeled eppendrof tubes and preserved at −70°C until biochemical analysis. C-peptide-Glucose ratio was calculated with calculating values of glucose and C-peptide in study participants at zero and 180 min. These ratio evaluated C-peptide (equivalent to insulin) status of the patients in response to their glucose responses after ingestion of test foods.

Laboratory method

Serum glucose was estimated by glucose-oxidase (GOD-PAD) method using reagents from SERA PAK, USA (Trinder, 1969). Insulin (measured by c-peptide as a marker of insulin) was

Table 1. Clinical and socioeconomic characteristics of study subjects (n = 10).

Parameter	Values
Age (years)	37 ± 5
Body Mass Index (BMI)	24 ± 2
Male: Female	3:2
Waist-Hip ratio (WHR)	0.93 ± 0.04
Annual income (USD)	1657 - 5143
HbA_1c (%)	6.6 ± 1.1

Values are expressed as Mean ± SD except range and ratio.

determined by ELISA method using kits from DRG Diagnostics (Germany) and glycosylated hemoglobin (HbA1c) was measured by high-performance liquid chromatography (HPLC) method

Ethical consideration

The protocol was approved by the Ethical Review Committee of the Diabetic Association of Bangladesh (BADAS).

Statistical analysis

All analysis were done using the statistical package for social science (SPSS) software for Windows. The incremental areas under the curve (iAUC) was calculated by the standardized criteria (Wolever et al., 1991), ignoring any area below the baseline. The average iAUC for the two white bread tests was used as the reference value and each subject's individual GI for each food was calculated. To compare THE difference between Means, ANOVA (bonferroni test) was performed where appropriate. All parametric variables were expressed as M ± SD and non-parametric data were expressed in percentage value. P < 0.05 was considered as the statistically significant.

RESULTS

Characteristics of the subjects

Table 1 shows the characteristics of the participants. Participants were made of ten type 2 diabetes subjects (male 6, female 4; age 37 ± 5years, mean±SD). The mean BMI of the study subjects were mean ± SD, 24 ± 2, while the mean waist-hip ratio was found to be 0.93 ± 0.04 and the average HbA_1c was 6.6 ± 1.1 for the study subjects (Table 1).

Glycemic response to the food items

The nutrient composition and the cooking properties of the test foods are shown in Table 2.

Table 3 explained the result of unripe papaya that demonstrated significantly, lower serum glucose response compared to that of bread and pumpkin (increment area under the curve; mean± SD 119 ± 27 in bread and 91 ± 64 in pumpkin vs unripe papaya 29 ± 14;

p < 0.001 and 0.05, respectively). The significant lower glycemic response (p < 0.05) between pumpkin and unripe papaya was reflected in their GI values (mean ± SD; pumpkin 74 ± 42 and unripe papaya 23 ± 7). The GL of pumpkin and unripe papaya were 6.4 and 1.5, respectively (Table 3).

C-peptide response of the food items

Table 4 showed the basal values of serum insulin in all the 3 groups that were matched; the substantially lowered glycemic response and GI values in pumpkin and unripe papaya were not paralleled by an increased insulin response (180 min; mean ± SD: 1.31 ± 0.51, 1.36 ± 0.67 and 1.51 ± 0.72 in case of bread, pumpkin and unripe papaya, respectively). This was also supported by the 180 min C-peptide: glucose ratio (0.23±0.11, 0.22 ± 0.12 and 0.26 ± 0.13 in case of bread, pumpkin and unripe papaya, respectively). Homa%B and Homa%S were calculated and no significant difference has observed among the groups.

DISCUSSION

There are very few vegetables, which are not very seasonal rather available throughout the year. Pumpkin and unripe papaya is one of them and is rich in vitamins and other nutritional contents, comparatively lower price and cooked in different forms, which may indeed, serve as a substitute diet of various health conditions (that is, constipation, overweight etc). The GI is relevant both in preventing and managing diabetes mellitus. This study showed that the blood glucose response produced after consuming unripe papaya was significantly lower when compared with white bread (reference food) and also with pumpkin. As a result, the GI of unripe papaya and pumpkin also reflect the same trend (Table 3). Unripe papaya showed a very low GI, but the pumpkin has just crossed the moderate level of GI. The GI of pumpkin has been studied in South Africa (Walker and Walker, 1984) and unripe papaya in Australia and Philippines showed high GI on population study compared with the

Table 2. Nutrient compositions of the test meals (g per 100 g).

Name of test food	Moisture (mg)	Protein (mg)	Fat (mg)	CHO (mg)	Minerals (mg)	DF (mg)	Energy (Kcal)	Vit A (µgm)	Ca (mg)	Fe (mg)	P (mg)	Vit C (mg)
Pumpkin	88.3	0.5	0.1	9.7	0.6	0.8	42	1550	10	1.1	10	2 [a]
Papaya	92.0	0.7	0.2	5.7	0.5	0.9	27	0	28	0.9	40	12 [a]
Bread	2.36	15.23	2.38	64.3	2.30	0.78	39.90 [b]					

Sources: [a]Nutritional value by C. Gopalan, B.V. RAMA SASTRI and Balasubra manian (page 50-51). [b]Values were taken from "Animal Nutrition Lab, Department of Livestock Services", Farmgate, Dhaka, Bangladesh 2002.

Table 3. Glycemic response of the study subjects (n=10) at different time intervals after ingestion of test meals.

Test foods	Serum glucose (mmol/l)									iAUC (mmol/l)	GI	GL
	0 min	15 min	30 min	45 min	60 min	90 min	120 min	150 min	180 min			
Bread	7.2 ± 1.3	7.2 ± 1.2	8.1 ± 1.6	8.8 ± 1.7	9.0.± 1.6	8.3 ± 1.5	7.2 ± 1.5	6.5 ±1.6	5.9 ± 1.3	119 ± 27		
	100	101 ± 2.9	112 ± 4.6	121 ± 5.3	125 ± 3.6	115 ± 9	99.7 ± 12.2	90 ± 14.6	82 ± 11.2			
Pumpkin	7.5± 1.5	7.5 ± 1.3	8.4 ± 1.3	8.7± 1.3	8.5 ± 1.4	7.5± 1.7	7.0 ± 1.6	6.5 ± 1.5	6.3± 1.4	91 ± 64	74 ± 42	6.4
	100	100.3 ± 6	113 ± 12	117 ± 10.3	114 ± 13	101 ± 16	92.3 ± 13.7	87 ± 12.2	84.6 ± 10.3			
Unripe papaya	7.7 ± 0.9	7.4 ± 0.8	8.0 ± 0.8	8.0 ± 1.0	7.6±1.2	7.0 ± 1.3	6.6 ± 1.4	6.3 ± 1.2	6.0 ± 1.3	29 ± 14a***b*	23 ± 7b*	1.5
	100	96 ± 2.0	102 ± 5.3	104 ± 8.0	99 ± 10	93 ±12	85 ± 14	82 ± 12	76 ± 12			

*p < 0.05 and **p < 0.01 ***p < 0.001 were taken as the level of significance as compared to reference food in Mann-Whitney 'U' test. a. Bread; b.Pumpkin; c. Unripe Papaya. To calculate GL, serving size was 90g/serve (pumpkin) and 120 g/serve (unripe papaya).

reference food white bread, respectively (Guevarra and Panlasigui, 2000; Brand-Miller et al., 1995).

For a particular food to be taken by diabetic patients, it is also important to consider how rapidly the glucose level rise and fall. Though the result of three test meals have shown that at post-prandial stage after three hours, all the foods were stayed at same serum glucose levels, but looking at the dynamics of blood glucose changes seems that the foods exhibit different timing of blood glucose response. For example, the peak rise of pumpkin and unripe papaya was at 45 min and sharp fall at 180 min and for bread, the peak rise of blood glucose was at 60 min. The glucose response dynamics and the GI depend largely on the rate of digestion and the rapidity of absorption of carbohydrates. Considering this, it can be suggested that unripe papaya might be better choice than pumpkin. Insulin is the central hormone in maintaining blood glucose homeo-stasis and it has a life saving role. Since c-peptide is secreted in equimolar concentration with insulin and since it has a much higher half-life than insulin, it was used as a marker for the insulin response of the subjects. Higher level of insulin in blood (hyperinsulinemia) has been shown to be associated with increased atherosclerosis leading to cardiovascular disorders (Kaplan et al., 1996). In this context, effect of pumpkin and unripe papaya on serum insulin has important implica-tions. In this study, both pumpkin and unripe papaya produced a negative value of the absolute increment of c-peptide (as a measure of insulin). Compared with the reference food, the absolute increment of c-peptide showed a decreasing

Table 4. C-peptide status of the study subjects (n = 10) after feeding different test meals.

Test foods	Serum C-peptide (ng/ml)		AICP (ng/ml)	HOMA B%		HOMA S%		C-peptide: Glucose	
	00 min	180 min		0 min	180 min	0 min	180 min	0 min	180 min
Bread	0.96 ± 0.4	1.31 ± 0.5	0.35 ± 0.4	105.5 ± 44.2	203.0 ± 126.4	38.0 ± 19.9	30.0 ± 30.0	0.13 ± 0.06	0.23 ± 0.11
Pumpkin	1.35 ± 0.64	1.36 ± 0.67	-0.01 ± 0.05	135.1 ± 99.3	186.6 ± 128.7	29.3 ± 16.8	30.6 ± 17.4	0.19 ± 0.12	0.22 ± 0.12
Unripe papaya	1.59 ± 0.87	1.51 ± 0.72	-0.07 ± 0.8	136.4 ± 66.4	228.1 ± 129.0	26.5 ± 16.1	27.2 ± 14.7	0.21 ± 0.11	0.26 ± 0.13

Results expressed as mean ± SD and median (range); *$p < 0.05$ was taken as the level of significance as compared to reference food in One-Way ANOVA test. a. Bread; b. pumpkin; c. unripe papaya. AICP; absolute incremental changes of C-peptide over basal values. HOMA %B, Insulin secretory capacity by Homeostasis Model Assessment; HOMA %S, Insulin sensitivity by Homeostasis Model Assessment.

tendency in two test meals. Thus, the beneficial GI values of pumpkin and unripe papaya does not seem to be consequence of hyperinsulinemia and a very good dietary component for the diabetic patients.

However, the effect of GI on insulin response may also depend upon insulin sensitivity too.

In Bangladesh, non vegetarian diet is being practiced and the amount of vegetable in daily diet is also very limited. As a result, considering the serving size, both unripe papaya and pumpkin showed very low GL vegetables (1.5 and 6.4, respectively). Moreover, from this study, unripe papaya is regarded as a very low GI and GL type vegetable. So, diabetic patient could take a bit, accessibly, as a mixed meal and could also use it as a substitute diet for various high carbohydrate content food. Moreover, these GI and GL property is not due to their insulin secretion, but may be attributed to their technology of production and cooking process. Higher serving size, however, may turn these varieties into high GL and health providers should make sure that people are aware of this fact.

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REFERENCES

American Diabetes Association (1979). Principles of nutrition,dietary recommendations for individuals with diabetes mellitus. Diabetes, 28(11): 1027-1030.

Brand Miller J, Pang E, Broomhead L (1995). The glycaemic index of foods containing sugars: comparison of foods with naturally occurring v. added sugars. Br. J. Nutr., 73: 613–23.

Brand-Miller JC, Liu V, Petocz P, Baxter RC (2005). The glycemic index of foods influences postprandial insulin-like growth factor-binding protein responses in lean young subjects. Am. J. Clin. Nutr., 82: 350–354.

Brouns F, Bjorck I, Frayn KN, Gibbs AL, Lang V, Slama, Wolever TMS (2005). Glycaemic index methodology. Nutr. Res. Rev., 18: 145-171.

Collier GR, Giudici S, Kalmusky J (1988). Low glycemic index starchy foods improve glucose control and lower serum cholesterol in diabetic children. Diabetes Nutr. Metab., 1: 1–9.

Colombani PC (2004). Glycemic index, load - dynamic dietary guidelines in the context of diseases. Physiol. Behav., 83: 603–610.

Dickinson S, Brand-Miller J (2005). Glycemic index, postprandial glycemia and cardiovascular disease. Curr. Opin. Lipidol., 16: 69–75.

Foster-Powell K, Holt SH, Brand-Miller JC (2002), International table of glycemic index and glycemic load values. Am. J. Clin. Nutr., 76: 5–56.

Frost G, Dornhorst A (2000). The relevance of the glycemic index to our understanding of dietary carbohydrates. Diabet Med., 17: 336–345.

Guevarra MTB, Panlasigui LN (2000). Blood glucose responses of diabetes mellitus type II patients to some local fruits. Asian Pacific J. Clin. Nutr., 9: 303-308.

Jenkins DJ, Jenkins AL, Wolever TM (1994). Low glycemic inde: lente carbohydrates and physiological effects of altered food frequency. Am. J. Clin. Nutr., 59(suppl): 706S–709S.

Jenkins DJ, Wolever TM, Taylor RH (1981). Glycemic index of foods: a physiological basis for carbohydrate exchange. Am. J. Clin. Nutr., 34: 362–366.

Jenkins DJA, Cuff D, Wolever TMS (1987). Digestibility of carbohydrate foods in an ileostomate: relationship to dietary fiber, in vitro digestibility and glycemic response. Am. J. Gastroenterol., 82: 709–717.

La Vecchia C (2004). Mediterranean diet and cancer. Public Health Nutr., 7: 965–968.

Liu S, Manson JE, Stampfer MJ (2001). Dietary glycemic load assessed by food-frequency questionnaire in relation to plasma high densitylipoprotein cholesterol and fasting plasma triacylglycerols in postmenopausal women. Am. J. Clin. Nutr., 73: 560–566.

Ludwig DS (2002). The glycemic index: physiological mechanisms relating to obesity, diabetes and cardiovascular disease. JAMA, 287: 2414–2423

Mann JI (1980). Diet and diabetes. Diabetologia, 18: 89-95.

Salmeron J, Manson JE, Stampfer MJ, Colditz GA, Wing AL, Willett WC (1997). Dietary fiber, glycemic load, and risk of non-insulin dependent diabetes mellitus in women. JAMA, 277: 472–477.

Trinder P (1969). Determination of glucose in blood using glucose oxidase with an alternative oxygen acceptor. Ann. Clin. Biochem., 6: 24–27.

Trinder P (1969). Determination of glucose in blood using glucose oxidase with an alternative oxygen acceptor. Ann. Clin. Biochem., 6: 24–27.

U.S. Department of Health and Human Services and U.S. Department of Agriculture. Dietary Guidelines for Americans (2005). Washington, DC: U.S. Government Printing Office.

Walker ARP, Walker BF (1984). Glycaemic index of South African foods determined in rural blacks—a population at low risk of diabetes. Hum. Nutr. Clin. Nutr., 38C: 215–222.

Wannamethee SG, Shaper AG, Perry IJ, Alberti KG (2002). Alcohol consumption and the incidence of type II diabetes. J. Epidemiol. Community Health, 56: 542–548.

Wei M, Gibbons LW, Mitchell TL, Kampert JB, Blair SN (2000). Alcohol intake and incidence of type 2 diabetes in men. Diabetes Care, 23: 18–22.

Willett W, Manson J, Liu, S (2002). Glycemic index, glycemic load, and risk of type 2 diabetes. Am. J. Clin. Nutr., 76(1): 274S–280S.

Wolever TMS, Jenkins DJA, Ocana AM, Rao VA, Collier GR (1988). Second-meal effect: low-glycemic-index foods eaten at dinner improve subsequent breakfast glycemic response. Am. J. Clin. Nutr., 48: 1041 - 1047.

Wolever TM, Jenkins DJ, Jenkins AL, Josse RG (1991). The glycemic index: Methodology and clinical implications. Am. J. Clin. Nutr., 54: 846-854.

Wolever TMS, Katzman-Relle L, Jenkins AL, Vuksan V, Josse RG, Jenkins DJA (1994). Glycaemic index of 102 complex carbohydrate foods in patients with diabetes. Nutr. Res., 14: 651-659.