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Glucose, Insulin and Non Esterified Fatty Acid Responses to Ladies Finger and Pointed Gourd in Type 2 Diabetes Mellitus

[1]F. Rahman, [1]K. Fatema, [2]A.T.M.A. Rahim and [1]L. Ali

[1]Biomedical Research Group, Bangladesh Institute of Research and Rehabilitation on Diabetes, Endocrine and Metabolic Disorders, Dhaka-1000, Bangladesh

[2]Institute of Nutrition and Food Science, Bangladesh

Corresponding Author: Kaniz Fatema, Department of Epidemiology and Bio-statistics, Bangladesh Institute of Health Sciences, 125/1, Darus Salam, Mirpur, Dhaka-1216, Tel: 880-2-8055352, +8801716502930 Fax: 880-2-8611138

ABSTRACT

Glycemic Index (GI) and Insulin (as measured by C-peptide) responses of Ladies Finger (*Abelmoschus esculentus*) and Pointed Gourd (*Trichosanthes dioica*) from Bangladeshi origin were investigated to help in creating a better food exchange table for diabetic patients. Based on serving size in the Bangladeshi society, the GL of those two food items have also been calculated. Ten diabetic subjects, under a cross-over design, consumed equi-carbohydrate amount (25 g of total carbohydrate) of the vegetables and white bread (WB, as reference food), with a run in period of seven days between the consecutive items. The serum levels of glucose were estimated at 0, 0', 15, 30, 45, 60, 90, 120, 150 and 180 min, respectively, NEFA and c-peptide levels were at 0 and 180 min only. GI and GL were calculated by standard formula. Both Ladies Finger (LF) and Pointed Gourd (PG) showed significantly lower serum glucose value than that of WB. The GI of LF and PG were 56±20 and 76±24, respectively. Both LF and PG showed significantly lower serum c-peptide and serum NEFA response as compared to WB at 180 min ($p<0.01$). The GL of LF and PG were 5 and 6, respectively. In contrast to the general belief that vegetables are rich in dietary fiber and thus necessarily have low glycemic index, the present data shows relatively medium and high glycemic index of LF and PG, respectively. Presence of bioactive natural agents in these vegetables, resulting in the suppression of insulin secretion/action or having stimulatory effect on insulin antagonists, need to be investigated.

Key words: Glycemic index, glycemic load, vegetable, ladies finger, pointed gourd, diet, mixed meal, type 2 diabetes mellitus

INTRODUCTION

The component of the diet that has the greatest influence on blood glucose is carbohydrate. Both the quantity and the type or source of carbohydrate found in foods influence postprandial glucose level. Furthermore, high carbohydrate diets raise plasma insulin to the greatest extent in persons with insulin resistance (Jeppesen *et al*., 1997) which itself is associated with increased risk of obesity, cardiovascular disease, diabetes and hypertension (Reaven, 1995). Thus reducing postprandial glucose and insulin may be beneficial in the management of insulin resistance.

Plasma glucose and insulin responses can be manipulated by altering the amount or source of dietary carbohydrate (Wolever and Bolognesi, 1996). Different carbohydrate foods are digested *in vitro* at different rates (Jenkins *et al*., 1980) and in turn, are directly related to the glucose and insulin responses they elicit (Wolever *et al*., 1988a; Brand-Miller *et al*., 2007; FAO/WHO, 1998). The glycemic responses of foods are classified by using the Glycemic Index (GI) which is a useful indicator for the biological effects of carbohydrates and it can be converted to a practical tool called Glycemic Load (GL) which can be used for routine dietary advice.

Since insulin is known to be atherogenic, a low GI at the expense of hyperinsulinemia may not be useful. Thus a ranking of food based on their insulin secretary capacity along with the glycemic response is necessary. Increased circulating concentrations of Non-Esterified Fatty Acids (NEFA) have been implicated in the pathogenesis of type 2 diabetes (Caroline and Nattrass, 2000). Recently, attention has focused upon a possible role for NEFA in the β-cell and the likely consequences of such a role for the pathogenesis of type 2 diabetes (T2DM).

The GI has been recommended to help guide 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 for 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.

Apart from their nutrient content, fruits, vegetables and cereal products are now regarded as rich sources of Dietary Fiber (DF). A number of studies (Huq *et al*., 2001) have determined the content and composition of DF in a number of local fruits, vegetables and cereals that are mainly available in South East Asian region. Some of these foodstuffs were found rich in DF and can be used frequently in the diet of T2DM patients provided their biological responses are known. The objective of the present study was to determine the proximate nutrient composition of two DF rich vegetables (LF and PG) and estimate their glucose, insulin and NEFA responses in T2DM subjects.

MATERIALS AND METHODS

The study was conducted at the Department of Biomedical Research Group, Research Division, Bangladesh Institute of Research and Rehabilitation in Diabetes, Endocrine and Metabolic Disorders during January 2008 to December 2008.

Nutritional analysis of test meals: Two fiber rich vegetables - Ladies finger and Pointed Gourd were selected for the study of glucose and insulin responses in T2DM subjects. A number of factors influence the glycemic responses of carbohydrate foods. It is therefore desirable to minimize these factors, cooking effect being one of them, in designing any study that intends to determine GI of foods. For that we decided to analyze the proximate nutrients of cooked samples along with of fresh ones. The proximate composition of the test vegetables was comparable with the published data in the Food Composition Tables shown in the tables (Table 1).

Table 1: Nutrient composition of the test meals (g per 100 g)

Meals	Moisture	Protein	Fat	CHO	DF	Ash	Energy
Bread							
Analyzed	2.36	15.23	2.38	64.39	-	2.30	39.90a
Ladies Finger							
Analyzed							
Raw	90.80	0.60	0.12	7.51	2.86	0.97	33.52
Cook	85.40	1.77	0.40	11.49	5.11	0.94	56.64
Reported							
Raw	88.30	1.80	0.10	8.70	3.24	1.10	42.9 b
	88.0	2.20	0.20	7.70	-	-	- c
Pointed Gourd							
Analyzed							
Raw	92.9	0.72	0.20	5.21	2.46	0.97	20.60
Cook	91.6	1.67	0.50	5.50	2.2	0.73	28.78
Reported							
Raw	92.00	2.00	0.30	2.2	3.3	0.50	20.0b
	92.30	2.00	0.30	1.90	-	-	- c

[a]Values were taken from Animal Nutrition Lab, Department of Livestock Services, Farmgate, Dhaka, Bangladesh 2002, [b]Values were taken from Deshio Khadrodrobbyer Pushtiman (1995). [c]Values were taken from Food and Nutrition-Dr. M. Swaminathan

Sample collection: Food samples were purchased from local market of Dhaka city. They were selected at randomly from the stock lot of the seller. Food samples were collected during their pick seasons.

Sample preparation: Edible Portion (EP) of half of the collected food samples were chopped into small pieces. The other half of the samples was then boiled with water for 15 min. Water was added in such a way that there was no water remained at the end of cooking. The boiled samples were taken into a frying pan and fried with margarine and salt for few minutes. Portions of both raw and cooked samples were taken out for moisture and ash analyses.

Analysis of proximate composition: Moisture content was estimated according to the method of Pearson (Kirk and Sawyer, 1991). Estimation of Ash was done using the AOAC (1990) method. The protein content was determined by some micro Kjeldahl method of AOAC (1990). The Soxhlet method was used (AOAC., 1990) for estimation of fat. The nitrogen free extracts (NFE) were considered as total carbohydrate and were calculated by this equation: Carbohydrate (NFE g%) =100 - (moisture + protein+ lipid + DF + ash) g/100 g. Energy value of the samples were estimated and expressed in kilo calorie by multiplying the percentage of protein, lipid and carbohydrate by the Atwater-Bryant factors 4, 9 and 4, respectively.

Dietary fiber analysis: For the estimation of DF, the Neutral Detergent Fiber (NDF) method of Van-Soest and Wine (1967) was adopted.

Biological responses of the test meals (determination of glycemic, insulin and NEFA responses)

Subjects: The subjects were selected among outpatients department of Bangladesh Institute of Research and Rehabilitation on Diabetes, Endocrine and Metabolic Disorders (BIRDEM). A total number of ten diabetic subjects were included in the study. Diabetes was diagnosed and classified

by the WHO criteria. Patients with acute or chronic complication of diabetes mellitus and those using insulin oral contraceptives or steroid were excluded. Pregnancy was also an exclusion criterion. All participants gave their written consent after being fully informed about the nature of study.

Study design: 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 the visit using a pre-tested questionnaire. The body weight in Kilogram was measured by using appropriate scales on bare foot (Detect-Medic, Detect Scales INC and USA). Body Mass Index (BMI) and waist-hip ratio of the subjects were calculated by appropriate formula. 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.*, 1988b). Patients were advised to rely on recommended standard carbohydrate diet and also instructed not to eat legumes in the meal preceding the fast. The subjects were requested to fast overnight (8-10) and suggested not to take medicine. An intravenous cannula was inserted into a superficial vein in the forearm. Fasting blood sample (5 mL) was drawn at 0 min before meal serving. After that the subjects were given recommended meals to eat within the stipulated period of time (10-15 min). Five milliliter of blood was then drawn at 15, 30, 45, 60, 90, 120, 150 and 180 min. Blood sample was allowed to centrifuge at 3000 rpm for 15 min. Separated serum was allocated in the labeled eppendrof tubes and preserved at -70°C until biochemical analysis.

Test foods: The study included 3 test meals: 38 g white bread as a reference food while 217 g Ladies finger and 379 g Pointed gourd as test foods. All the meals were equivalent to 25 g total carbohydrates.

Laboratory method: Serum glucose was estimated by glucose-oxidase (GOD-PAD) method using reagents from SERA PAK, USA (Trinder, 1969), C-peptide was determined by ELISA method using kits from DRG Diagnostics, (Germany) and Plasma NEFA was measured by colorimetric method using kits from Randox, UK, (Cat No FA 115). The measurement has been done using Microplate Reader, adopted in the Research Division, BIRDEM.

Ethical consideration: The protocol was approved by the Ethical Review Committee of the Diabetic Association of Bangladesh.

Statistical analysis: All analysis was 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. To compare difference between Means, ANOVA (bonferroni test), Mann-Whitney U tests were performed where appropriate. All parametric variables were expressed as M±SD and non-parametric data were expressed as percentages. $p<0.05$ was considered as the level of significance.

RESULTS

Proximate composition: The results of the proximate analysis of the test vegetables are presented in Table 1 on the basis of raw and cooked samples. Results showed that Ladies Finger when cooked contained highest carbohydrate content (11.49%) followed by raw Ladies Finger

(7.51%), cooked Pointed Gourd (5.55%) and raw Pointed Gourd (5.21%). Likewise, the content of DF was highest in cooked LF (5.11%) and lowest in cooked PG (2.2%).

The proximate composition of the test vegetables was comparable with the published data in the Food Composition Tables as shown in the same tables (Table 1). It was comparable in terms of moisture content, which was greater than 90% in PG but in cooked LF moisture content was found lower than that of reported values. The protein content was low in raw LF, but it was highest in cooked LF. The fat content of the calculated value (both LF and PG) was very close to the reported values. But in cooked sample it was increased. In raw LF the carbohydrate content was lower than that of reported values but cooked sample contained highest carbohydrate content. The carbohydrate content of PG was higher than the reported value.

Biological responses: The BMI of the study subjects ranged between 20.42 and 28.53 with a mean of 24.14±2.45 while they had a mean waist-hip ratio of 0.90±0.04 (Table 2). The mean of plasma HbA_1C level in the study subjects was 6.57±1.02. Subjects taking LF and PG had significantly lower glucose responses than that of WB ($p = 0.05, 0.01$) at 30, 60 and 90 min. LF and PG statistically differed ($p = 0.05, 0.01$) with each other at 30 and 45 min. The increment Area under the Curve (iAUC) values of LF and PG were significantly differed ($p = 0.001, 0.01$) with WB (iAUC 355±67 in WB, 190±37 in LF and 258±39 in PG) (Table 3). LF also showed significantly lower iAUC value than that of PG ($p = 0.01$). There was also no significant difference regarding their GI values (LF 56 and PG 76). Both LF and PG showed significantly lower serum C-peptide and serum NEFA response as compared to Bread at 180 min ($p<0.01$). Postprandial serum C-peptide values for WB were 3.81±0.80, LF 2.78±1.00; PG 3.04±0.83 (Table 4) and NEFA values were 0.67±0.13, 0.70±0.15 and 0.61±0.17 mmol L^{-1} (Table 5), respectively.

Table 2: Characteristics of the subjects (n = 10)

Characters	Values
Age (years)	41.85±5.90
Male-Female ratio	1:1
Annual Income (USD)	1000-10289
Body Mass Index (BMI)	24.14±2.45
Waist-Hip Ratio (WHR)	0.09±0.04
HbA_1c (%)	6.57±1.02

Values are expressed as Mean±SD except range and ratio

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

	Serum glucose (mmol L^{-1})					
Test foods	0/0' min	15 min	30 min	45 min	60 min	90 min
White bread	6.5±1.4 (100)	7.7±1.3 (120±18)	8.3±1.0 (130±12)	9.2±1.4 (143±12)	9.9±1.6 (153±15)	9.8±1.9 (151±9.3)
Ladies finger	6.3±1.0 (100)	8.4±1.7 (133±16)	8.2±0.9 (131±13)	7.8±0.8 (124±10)a*	7.4±0.8 (119±15)a**	7.5±1.3 (119±12)a*
Pointed gourd	5.7±0.9 (100)	7.5±0.7 (132±20)	7.1±0.7 (124±20)a* b*	8.7±0.9 (152±13)b**	7.6±0.7 (133±10)a**	7.3±1.6 (127±14)a*

	Serum glucose (mmol L^{-1})					
Test foods	120 min	150 min	180 min	iAUC (mmol L^{-1} at 3 h)	Glycemic Index (GI)	Glycemic Load (GL)
White Bread	8.7±2.1 (133±10)	7.4±1.7 (115±16)	6.4±1.5 (98±7)	355±67		
Ladies Finger	7.4±1.0 (118±12)	7.0±1.1 (112±8)	5.9±0.6 (95±11)	190±37a***	56±20	5
Pointed Gourd	7.6±2.2 (130±27)	6.5±1.2 (112±13)	6.0±0.9 (105±9)	258±39a** b**	76±24	6

Results expressed as Mean±SD; *$p<0.05$ and **$p<0.01$ ***$p<0.001$ were taken as the level of significance. a, White Bread; b, Ladies Finger; c, Pointed Gourd. To calculate GL serving size was 60 g/serve (rice); n, Number of subjects; iAUC, Increment area under the curve

Table 4: Fasting and Postprandial serum C-peptide responses and C-peptide – Glucose ratio of the study subjects (n = 10) after ingestion of test meals

	Serum C-peptide (ng mL^{-1})			C-peptide: Glucose ratio	
Test foods	0 min	180 min	AICP (ng mL^{-1})	0 min	180 min
Bread	2.7±1.0 (100)	3.8±0.80 (148.2±39.2)	1.04±0.68	0.45±0.21	0.63±0.21
Ladies finger	3.0±0.8 (100)	2.7±1.00 (92.8±22)a*	-0.21±0.73a*	0.46±0.17	0.45±0.21
Pointed gourd	2.7±1.1 (100)	3.0±0.8 (118.8±31.6)	0.31±0.82	0.48±0.21	0.51±0.17

Results expressed as Mean±SD; *p<0.05, **p<0.01 was taken as the level of significance. a, Bread; b, Ladies Finger; c, Pointed Gourd. AICP: Absolute incremental changes of C-peptide over basal values

Table 5: Fasting and Postprandial serum NEFA responses and NEFA– Glucose ratio of the study subjects (n = 7) after ingestion of test meals

	Serum NEFA (mmol L^{-1})			NEFA: Glucose ratio	
Test foods	0 min	180 min	AIN	0 min	180 min
Bread	0.33±0.13 (100)	0.67±0.21 (219.9±114)	0.31±0.26	0.05±0.01	0.10±0.02
Ladies finger	0.74±0.10 (100)	0.70±0.15 (95±23)a***	-0.04±0.16a**	0.11±0.02a**	0.11±0.02
Pointed gourd	0.56±0.07 (100)	0.61±0.17 (109±36)a**	0.04±0.19a*	0.10±0.02a**	0.10±0.03

Results expressed as Mean±SD; *p<0.05, **p<0.01 was taken as the level of significance. a, Bread; b, Ladies Finger; c, Pointed Gourd. AIN: Absolute incremental changes of NEFA over basal values

DISCUSSION

Diet is considered to be the cornerstone in the management of diabetes mellitus. To create a better diet plan for T2DM the present study showed that the blood glucose response after consuming ladies finger was significantly lower when compared with bread ($p<0.01$) and also with pointed gourd. This lower glycemic response was also reflected in the GI value. The glycemic index of ladies finger had just crossed the lower limit of GI value and pointed gourd showed a higher GI value. A number of factors such as the presence of fibre, cooking procedure, processing, nature of starch etc contributed to the response of glucose level. It is also evident that vegetables are low in carbohydrate and rich in dietary fibre, so their glycemic index will be low which are generally advantageous for diabetic patients.

High fiber was believed to be able to reduce the blood glucose response and hence lower the GI value. Here we found that the dietary fiber of ladies finger (both analyzed and reported) is higher than pointed gourd and it was reflected in the GI value of ladies finger also.

Allowing for a particular food for 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 staying at same serum glucose levels. But looking at the dynamics of blood glucose changes it seems that the foods exhibit different timing of blood glucose response. For example in LF, there was a peak rise to 133% at 15 min and a sharp fall at 180 min (95%). For bread the peak rise of blood glucose was 153% at 60 min. The rise of blood glucose level to a peak in PG was at 45 min (152%) with inconsistencies in rise and fall of the response curve. The glucose response dynamics and hence the GI depends largely on the rate of digestion and the rapidity of absorption of carbohydrates. The sharp rise of Glucose Response Curve (GRC) of LF to its peak within 15 min, therefore, might be due to rapid digestion and absorption of its glycemic carbohydrates. However the fall of the GRC at 180 min after maintaining approximately constant plateau through out the experimental period is interesting but difficult to

explain (Table 3). On the other hand, timing of glucose responses to PG has been found different than that of LF; its GRC has shown a relatively late rise and early fall than that of LF (Table 3). A slower digestion and absorption rate of carbohydrate alone, however, may not explain this glucose response phenomenon. To prescribe a particular food for diabetic patients it is important to consider how rapidly the glucose level rise and fall. For this it can be said that PG is a better choice than LF. This factor must be considered when these vegetables are added in the diabetic patient's diet.

Based on the serving size in the Bangladeshi society, these two vegetables can be considered as a very low GL food. And though the GI of these vegetables are medium and high respectively but their GL is low; so if we should give special attention to the serving size of these vegetables, it would be beneficial for us.

Though LF and PG raises blood glucose level sharply which excite β-cell to release insulin, it shows that in 180 min LG and PG release less c-peptide compared to white bread (Table 4). In LF, fasting c-peptide level reduced from 3 to 2.78 ng mL^{-1} after 180 min of ingestion (Table 4) which could play a beneficial role in cardiovascular disease.

Like insulinemia, NEFA was also reduced in LF at 180 min. The literature suggests that reducing postprandial NEFA levels optimizes insulin stimulated glucose uptake in muscle, thereby increasing insulin sensitivity. From this viewpoint of NEFA, it seems LF has beneficial effect on dyslipidemia (Table 5).

It may be concluded that LF and PG could be categorized as having medium and high GI and low GL values without any adverse insulin responses. In this study the subjects took only cooked LF or PG. But in our culture we take food as a mixed meal that alters the glycemic index of a food. When a particular food is eaten along with other foods, the blood glucose response and glycemic index will vary, depending on the proportion of carbohydrate, protein and fat in the mixed meal.

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REFERENCES

AOAC, 1990. Official Methods of Analysis of the AOAC. 15th Edn., Association of Official Analytical Chemists, Arlington VA.

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

Brand-Miller, J.C., K. Fatema, C. Middlemiss, M. Bare, V. Liu, F. Atkinson and P. Petocz, 2007. Effect of alcoholic beverage on postprandial glycemia and insulinemia in lean, young, healthy adults. Am. J. Clin. Nutr., 85: 1545-1551.

Caroline, M.A. and M. Nattrass, 2000. Fatty acids and insulin secretion. Diabetes Obes. Metab., 2: 213-221.

FAO/WHO, 1998. Carbohydrate in human nutrition. Report of Joint FAO/WHO Expert Consultation Paper No. 66. FAO Food and Nutrition, Rome.

Huq, F., K. Fatema and A.T.M.A. Rahim, 2001. Content and composition of dietary fiber in some selected bangladeshi vegetables. Diab. Endocr. J., 29: 37-41.

Jenkins, D.J., T.M. Wolever, R.H. Taylor, H. Barker and H. Fielden *et al.*, 1981. Glycaemic index of foods: A physiological basis for carbohydrate exchange. Am. J. Clin. Nutr., 24: 362-364.

Jenkins, D.J., T.M. Wolever, R.H. Taylor, H. Ghafari, A.L. Jenkins, H. Barker and M.J. Jenkins, 1980. Rate of digestion of foods and postprandial glycaemia in normal and diabetic subjects. Br. Med. J., 281: 14-17.

Jeppesen, J., P. Schaaf, C. Jones, M.Y. Zhou, Y.D. Chen and G.M. Reaven, 1997. Effects of low-fat, high carbohydrate diets on risk factors for ischemic heart disease in postmenopausal women. Am. J. Clin. Nutr., 65: 1027-1033.

Kirk, R.S. and R. Sawyer, 1991. Pearson's Composition and Analysis of Foods. 9th Edn., Longman Scientific and Technical, England, pp: 607-617.

Ludwig, D.S., 2002. The glycemic index: Physiological mechanisms relating to obesity, diabetes and cardiovascular disease. JAMA, 287: 2414-2423.

Mann, J.I., 1980. Diet and diabetes. Diabetologia, 18: 89-95.

Reaven, G.M., 1995. Pathophysiology of insulin resistance in human disease. Physiol. Rev., 75: 473-486.

Trinder, P., 1969. Determination of glucose in blood using glucose oxidase with an alternative oxygen receptor. Ann. Clin. Biochem., 6: 24-27.

Van Soest, P.J. and H. Wine, 1967. Use of detergents in the analysis of fibrous feeds. IV. Determination of plant cell-wall constituents. J. Assoc. Official Anal. Chem., So: 50-55.

Wannamethee, S.G., A.G. Shaper, I.J. Perry and K.G. Alberti, 2002. Alcohol consumption and the incidence of type II diabetes. J. Epidemiol. Community Health, 56: 542-548.

Wei, M., L.W. Gibbons, T.L. Mitchell, J.B. Kampert and S.N. Blair, 2000. Alcohol intake and incidence of type 2 diabetes in men. Diabetes Care, 23: 18-22.

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

Wolever, T.M.S., D.J.A. Jenkins, G.R. Collier, R. Lee, G.S. Wong and R.G. Josse, 1988a. Metabolic response to test meals containing different carbohydrate foods: 1 Relationship between rate of digestion and plasma insulin response. Nutr. Res., 8: 573-581.

Wolever, T.M.S., D.J.A. Jenkins, A.M. Ocana, V.A. Rao and G.R. Collier, 1988b. Second-meal effect: Low-glycemic-index foods eaten at dinner improve subsequent breakfast glycemic response. Am. J. Clin. Nutr., 48: 1041-1047.

Wolever, T.M., D.J. Jenkins, A.L. Jenkins and R.G. Josse, 1991. The glycemic index: Methodology and clinical implications. Am. J. Clin. Nutr., 54: 846-854.

Wolever, T.M., P.N. Nguyen, J.L. Chaisson, J.A. Hunt and R.G. Josse *et al.*, 1994. Determinants of diet glycemic index calculated retrospectively from diet records of 342 individuals with non-insulin dependent diabetes mellitus. Am. J. Clin. Nutr., 59: 1265-1269.

Wolever, T.M.S. and C. Bolognesi, 1996. Source and amount of carbohydrate affect postprandial glucose and insulin in normal subjects. J. Nutr., 126: 2798-2806.