Glycemic, NEFA and Insulinemic Responeses to Molasses Based and Sucrose Based Cakes (PITHAS) in Type 2 Diabetic Subjects

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Abstract

Glycemic index (GI), C-peptide index (CI) and NEFA index (NI) are important and useful measurement of serum glucose, insulin and NEFA response to a dietary component. This study was undertaken to detemine the GI, CI and NI of two traditional pithas; one baked, locally known as Vapa pitha (VP), and another fried, locally known as Poa pitha (PP). VP was prepared using rice flour, molasses and coconut while PP was prepared with rice flour, sugar, and oil. Eighteen subjects, 9 male and 9 female (mean age HbA_1c 40±6.60 years and mean plasma HbA_1c of 7.68±1.17 mmol/dl) had participated in the study, under a crossover design. They consumed equicarbohydrate of the test meal and bread, with a run–in period of 7 days between the consecutive items.

Both of the pithas showed significantly higher blood glucose levels compared to bread. The area under the glucose response curve (AUGC) of bread, VP and PP were 1625, 1755, and 1922 mmol/l/120 min respectively. Compared to PP, VP showed significantly lower AUGC. The trend of higher glycemic response of VP and PP was reflected in their inlsulinamic responses as revealed from postprandial serum levels of C-peptide. But mean area under C-peptide curve, AUCC of VP (513) was found lower than that of bread (568) and PP (688). Except NEFA index, both GI and Insulinemic index (equivalent to C-peptide index) were found higher in PP compared to VP. The results thus suggests that VP is a better choice for diabetic patients.

Key words: Glycemic index, C-peptide, NEFA, Country cakes (Pithas)

Introduction

Diabetes mellitus is a hereditary metabolic disorder and a universal health problem. However, it is closely linked to diet and nutrition both in respects to its causation and management[1]. To control this disease especially non-insulin dependent diabetes mellitus (NIDDM or Type 2) and its complications, proper dietary regulation is very essential.

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Generally regular dietary menu for diabetic is prepared by balancing carbohydrate based foods. Recent suggestion is that dietary management must be planned for both diabetic and normal healthy people considering the required daily 55-60% of total energy from carbohydrates. Different starchy foods with equivalent amount of carbohydrate have different glycemic response[2]. To assess and measure the digestion and absorption rate of carbohydrate the concept of Glycemic Index (GI) was established[3]. GI reflects the rate at which starchy foods are digested and glucose becomes available in the blood. Increased circulating concentrations of non-esterified fatty acids (NEFA), a pathogenesis factor for Type 2 diabetes[4], also increase hepatic glucose output due to promotion of gluconeogenesis[5]. A reduction in circulating NEFA leads to an increase in insulin mediated glucose uptake[6]. So in addition to GI, it is also essential to know the amount of NEFA response to food in the management of diabetic and cardiovascular diseases.

An interchangeable food mapping is required for perfect diet plan of different types of patients with diabetes. Regular diets have to be planned by incorporating the traditional distinctive culture of the country. Unfortunately diabetic patients in Bangladesh are usually restricted to take traditional pithas. But pithas are home made, easily processed and nutritious food. Most of the pithas are made of rice flour and the calorie value in rice flour and wheat are the same[8]. So they can also be used in breakfast as dessert and snacks by interchanging wheat based bread or Chapatti for type 2 diabetes patients.

The ranking of the pithas, in this context, on the basis of glycemic and NEFA indices (GI & NI) will help to suggest the required amount of these foods for diabetic patients. The purpose of the present study was to compare GI and NI of one parboiled pitha (Vapa Pitha) and one fried pitha (Poa Pitha).

Meterials and Methods

Subjects

The study was conducted at the Department of Cell and Molecular Biology, Research Division, Bangladesh Institute of Research and Rehabilitation in Diabetes, Endocrine and Metabolic Disorders (BIRDEM) during a 1 year period. A total number of (18) Type 2 diabetic subjects, equally distributed in male and female, mean age of 40±6.6 years (range 30-55 years), Body Mass Index (BMI) between 19-25 (mean BMI 24.47±2.15), HbA_1c<10% (mean HbA_1c HbAc 7.68±1.17), duration of diabetes 6 months to 5 years were included in the study. Subjects suffering from acute and chronic complications of diabetes mellitus or any other diseases as determined clinically, taking any oral contraceptives, steroids, diuretics and insulin, or with pregnancy were excluded from this study. All patients had normal blood pressure values during the study. Participants were fully informed about the experimental nature of the study. All subjects gave their consents to participate in the study and the study was approved by all ethical considerations of BIRDEM.

Preparation of test meal

The study included 3 test meals: White bread (78g) as a reference food, Poa pitha (100g) and Vapa pitha (63g) as experimental foods; all having 50 g equivalent of available carbohydrates. Each Poa pitha was made by Atap rice (rice from milled dried paddy), flour (50g) with sugar (15g), and small amount of salt and deep-fried in soybean oil. Vapa pitha was made by boiled rice (55g) with coconut (10g), Gur (molasses) (5g) and small amount of salt. Addition of salt to starchy foods has been shown not to influence glycemic responses. Both pithas were prepared by Bangladeshi traditional cooking methods. On the other hand, the freshly baked bread was sliced and portioned to the calculated weight (74g). The meals were served with 250ml water.

Study design

Subjects participated in 3 trials within a period of 4-6 weeks. Test and reference meals were given to patients in a cross over design with a wash out period of 3 days. Patients were advised to rely on recommended standard carbohydrate diet. An intravenous cannula was inserted into a superficial vein in the forearm at the day of experiment, and the fasting (0 hr) blood sample was drawn followed by the serving of meals. They were requested to ingest the meal over a period of minimum 10 and 15 minutes. Patients took their prescribed medicine at the beginning of the meal.

Blood collection protocol

Overnight fasting blood samples (5ml) was drawn zero minute before test meal serving and then subjects were given recommended meals. Five milliliter of blood was then drawn at 30, 60, 120 and 180 minutes. Blood samples were collected into heparinized glass tubes followed by centrifugation at 3000 rpm for 15 minutes. The plasma separated was allocated in the labeled eppendorfs and preserved at–70^{0}C until biochemical analysis.

Laboratory analysis

Serum glucose was estimated by glucose oxidase (GOD-PAD) method[7] using regents from SERA PAK, USA. Optical densities obtained were used to calculate values of unknown samples using a software (Kinetic Calculation). A variant hemoglobin testing system (Bio-Rad model) using a modified HPLC method was used to estimate the percentage of HbA_1c. On the other hand, 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. All results were calculated by extrapolating the standard curve.

Serum C-peptide was determined by ELISA method using kits from DRG diagnostics, (Germany). C-peptide ELISA Kit is based on the competition principle and microplate separation. An unknown amount of C-peptide present in the unknown sample and fixed amount of C-peptide conjugate compete for the binding sites of a polyclonal C-

peptide antiserum coated onto the wells. In a second step, the enzyme complex was washed off. Having added the substrate solution, the concentration of C-peptide in the samples was inversely proportional to the optical density measured.

Determination of glycemic and ilnsulinemic indices

The glycemic index (GI) quantifies the glucose change after eating a certain food compared to the change after eating a similar amount of reference carbohydrate. Practically, it is calculated as the incremental area under the postprandial blood glucose response curve of a test food expressed as a percent of the response to the same amount of carbohydrate from a standard food taken by the same subject[8]. Other indices i.e., C-peptide-Glucose ratio, NEFA-Glucose ratio, and C-peptide-NEFA ratio were calculated with circulating values of glucose, C-peptide, and NEFA in study participants at zero and 180 minutes. These ratios evaluated NEFA and C-peptide (equivalent to Insulin) status of the patients in response to their glucose responses after ingestion of test foods.

Statistical analysis

AUC of glucose, NEFA, and C-peptide responses were calculated by the trapezoid rule using software developed by BIRDEM.

All analysis was done using the Statistical Package for Social Science (SPSS) software for Windows. All parametric variables were expressed as mean ±SD and non-parametric data were expressed as median (range). To compare difference between means ANOVA (Bonferroni test), Mann-Whitney 'U' tests and Paired t-test were performed where appropriate. The level of significance in all cases was considered at $P<0.05$.

Results

Glycemic, C-Peptide, and NEFA status of the study subjects

The mean plasma HbA_1c level of the study subjects was 7.68±1.17 mmol/l indicating their overall diabetes status during the last three months. Glycemic status of the subjects after feeding different test meals is featured in Figure 1. The values of fasting glucose levels in three trials did not show any statistical difference. Postprandial glucose values were measured at 30, 60, 120 and 180 minutes. Mean area under the glucose curve (AUGC) of PP (1922) and VP (1755) did not show any statistical difference compared to bread (1625) (Figure 2).

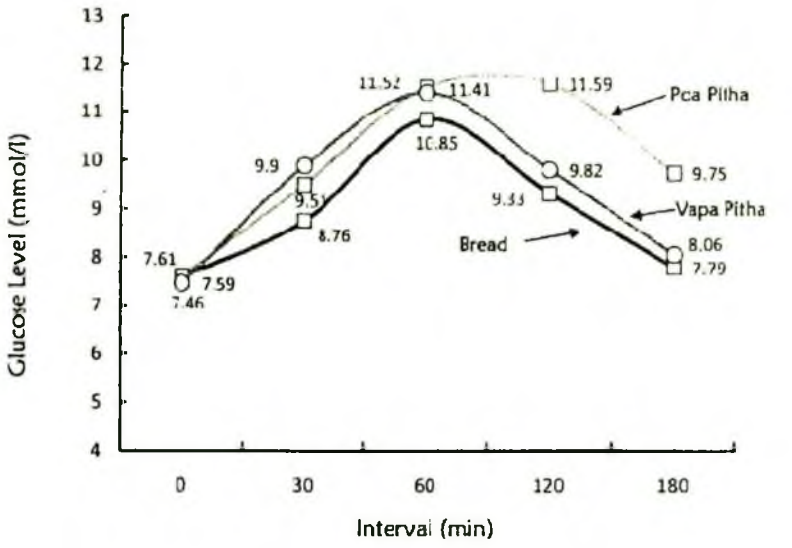

Figure 1 : Comparison of glucose response curves of different test meals.

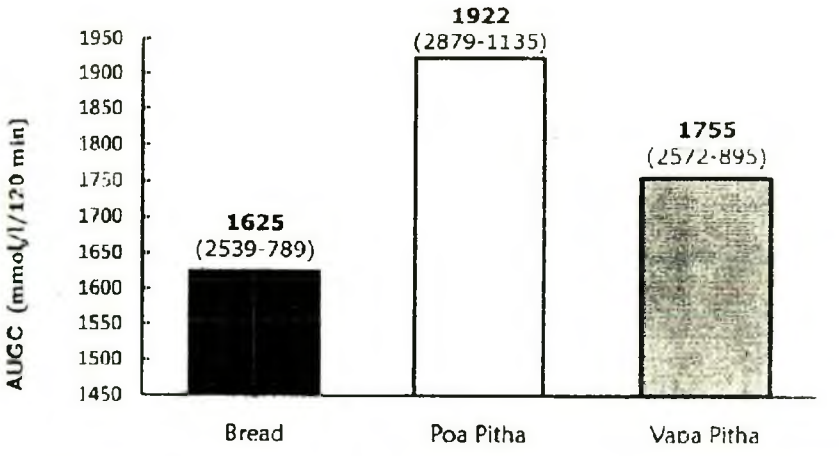

Figure 2 : Comparison of mean area under the glucose response curve (AUGC) of different test foods (values in parentheses are ranges).

Figure 3 showed fasting (0 min) and postprandial C-peptide levels (at 30, 60, 120 and 180 min). The fasting levels of the test foods were insignificantly different. AUC of C-peptide (AUCP) was then calculated. AUCP of PP (688) and VP (513) showed no differences among them but PP showed significant differences compared to bread (568) and VP (Figure 4).

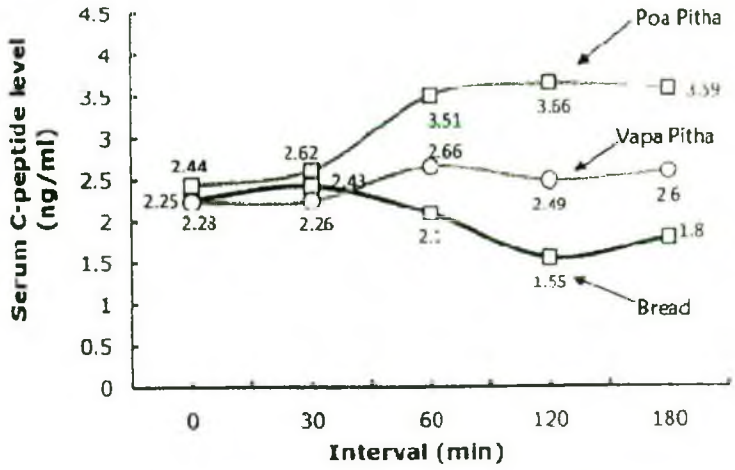

Figure 3 : Comparison of serum C-peptide levels of the study subjects after feeding different test meals.

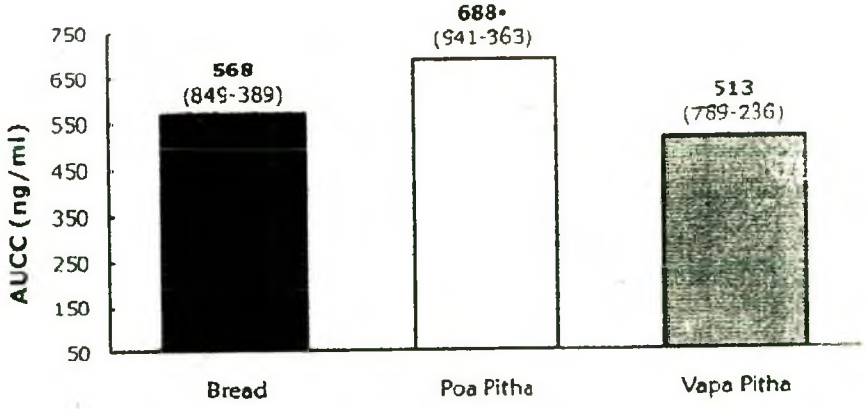

Figure 4 : Comparison of mean area under the C-peptide curve (AUCC) of different test foods; range is given in parantheses; *AUCC of Poa Pitha showed significant different with Bread and Vapa Pitha.

Circulating NEFA values at fasting and at the end of experiment was estimated and presented in Table 1. Absolute change of NEFA levels were determined and found to be in decreased mode. Absolute increment of NEFA of PP was found higher than VP.

Table 1 : Fasting and Postprandial serum NEFA levels of the study subjects after feeding the different test meals[1].

Test foods	Serum NEFA (mmol/l)		ADN of NEFA
	0 min	180 min	
Bread	0.617	0.322	-0.29±0.11
PP	0.564	0.334	-0.23±0.13
VP	0.684	0.379	-0.31±0.17

[1]Result expressed as Mean±SD; ANOVA (Bonferroni) test performed to test the difference between means; n, Number of subjects; ADN, Absolute decrease of NEFA, Non-esterified fatty acid.

The ratio of glucose, C-peptide, and NEFA

Fasting and postprandial ratios of Glucose of C-peptide were calculated to find correlation between glycemic and insulinemic responses of the test meals (Figure 5).

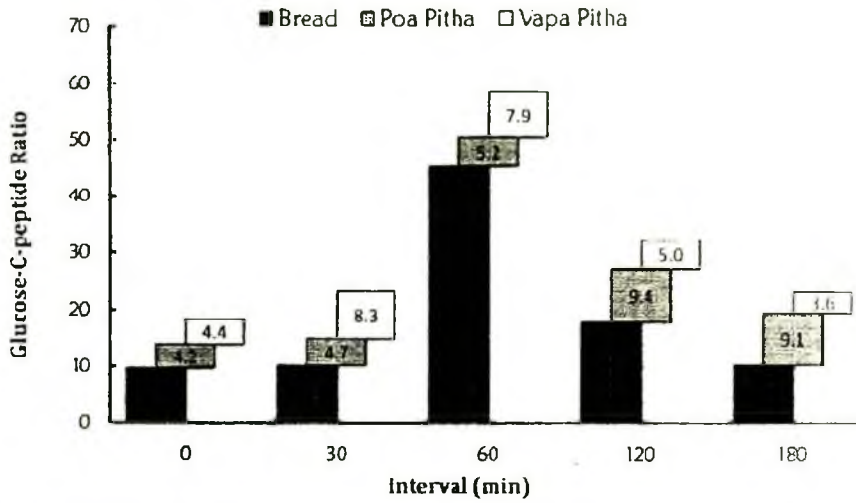

Figure 5 : Fasting and postprandial ratios of Glucose-C-peptide ratio

On the other hand, Glucose-NEFA ratio was calculated to evaluate the effect of fatty acids on peripheral glucose utilization and C-peptide-NEFA ratio was calculated to find NEFA stimulation on insulin secretion. These results are presented in Table 2.

Table 2 : Fasting and postprandial ratio among glucose, C-peptide and NEFA

Test foods	Glucose : NEFA		C-peptide : NEFA	
	0 min	180 min	0 min	180 min
Bread	12.98	25.01	0.84	0.50
PP	14.18	35.05	0.40	0.30
VP	11.46	54.97	0.41	0.19

Glycemic, C-Peptide and NEFA Indices

The glycemic Index (GI) refers to the blood-glucose rising potential of carbohydrates. All three indices are presented in Table3.

Table 3 : Glycemic Index (GI), NEFA Index (NI), and C-peptide Index (CI) for the two test foods of the study subject (n=18)

Test foods	Glycemic Index	C-Peptide Index	NEFA Index
PP	**125** (182-67)	**144** (177-57)	**93** (911-8)
VP	**116** (180-47)	**81** (165-42)	**98** (367-3)
Significance	P<0.059	P<0.02	P<0.41

Result expressed as mean and values given in parentheses are range. Paired students''t' test was performed to calculate difference between means. Mann-Whitney Wilcoxon Rank Sum Test was done to calculate the index. n, Number of subjects.

Discussion

Dietary recommendations play a central role in the management of diabetes mellitus and its complications. Diet may be scientifically excellent but it is useless if the patient does not take it. Starchy foods with a low postprandial glycemic response are generally advantageous for diabetic patients. In our country traditional cakes (pithas) are one of the most popular foods that can be excellent part of the diet of diabetic patients for their high nutritional value.

In the present study PP and VP were investigated to find out their glycemic characteristics in order to find their suitability as component of the diet of the diabetics. The findings suggests that equicarbohydrate amount of VP produces lower glycemic responses as compared to PP but both PP and VP produces higher glycemic responses as compared to bread. This notion needs to be kept in mind when providing dietary advice to diabetic patients and calculating their calorie requirements. However, the results clearly demonstrated that VP is better than PP from the point of glycemic responses and AUGC. Since PP is sugar based and VP is molasses based food, it can be predicted from the findings that molasses increases blood glucose levels slowly than sugar. Molasses has other advantages too, for example it is Ca-rich food. Coconut in VP is also nutritious. On the other hand, VP is also a better choice than PP from the standpoint of NEFA responses. In the study NEFA index of PP and VP did not differ significantly indicating their indifferent roles in insulin secretion. Again, from the view of postprandial NEFA responses, it can also be concluded that VP is a better choice in a diabetic diet than PP.

As Glycemic and NEFA indexes are more useful markers in ranking the carbohydrate containing food compared to the absolute blood levels of those parameters, it is now necessary to study the other common varieties of traditional pithas used in our country.

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