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Abstract

Corn is one of the most important cereals for the nutrition of large groups of the Latin American population and arepa is a very popular corn-based food preparation. In order to study the arepa consumption effects on glucose and insulin response when β -glucans were added to this preparation, three different meals (white bread, corn flour arepa and arepa with β -glucans) were served as breakfast to fourteen young subjects in random order after an overnight fast, to determine basal and postprandial glucose and insulin levels. One way ANOVA test was carried out to compare glucose and insulin responses between test meals and between basal and postprandial (glucose and Insulin) levels of each meal. Ingestion of white bread and corn arepa increased postprandial insulin 70.1% and 51.8% respectively, but β -glucan arepa increased only 16% postprandial insulin levels, neither white bread and corn arepa nor β -glucan arepa increased significantly postprandial plasma glucose. In conclusion this study shows that by addition of β -glucan, to corn products like arepa reduces glucose and insulin response.

Key Word: Insulin response, Glucose response, β -glucans, Arepa, precooked flour

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Introduction

Results from a relatively large number of surveys show that corn (*Zea mays*) is one of the most important cereal grains for the nutrition of large groups of the Latin American population, particularly for Mexico, Central America, Colombia and Venezuela. In South America is consumed in a number of ways as a decorticated, degermed precooked flour which is mainly used to make arepas. In Venezuela the consumption of precooked corn flour per capita was 80 g/d for the total population in 1994¹. Arepas are staple Venezuelan food and the most common food item; they are a special corn bread that substitutes white wheat bread. The chemical composition of the arepa shows low fibre (less than 2%) content, high carbohydrates (40.5-37%), high available starch (35-37%) with high amilopectina content^{2,3}. This composition makes the arepa a very high digestible preparation. The rate of digestion and absorption of different carbohydrates has differing effects on the postprandial rise of blood glucose and insulin levels. The absorption of carbohydrates is affected by many factors, such as the composition of the grain, particle size, amount and type of fibre, viscosity, availability of starch, amylopectin and amylose content and cooking/preparing methods⁴⁻⁶. The amylose to amylopectin ratio is a very important aspect to be consid-

ered because it is one of the factors been used to explain the differences observed, between glucose and insulin responses to various starchy foods. This ratio in most cereal starches is about 20:80⁷⁻⁹. Behall et al. in 1989 and Amelsvoort and Weststrate in 1992, have indicated that higher amylose content is accompanied by a lowered metabolic response^{10,11}. Results from in vitro studies show that a possible mechanism for lowered metabolic responses to high amylose starch is a lowered rate of amylolysis. The cause for reduced enzyme availability of starch in high amylose products is not clear, but could be related to the tendency of amylose to recrystallize or interact with lipids¹².

The glycemic index (GI) is a concept for ranking carbohydrate foods based on their effects on postprandial glycemic response. This is due to different rates of digestion and absorption of the carbohydrates. Low GI foods are those that release glucose to the blood at a slower rate. The therapeutic potential of low GI foods is not only for diabetes but also for dyslipidemia and prevention of type 2 diabetes mellitus¹³⁻²³. Most common starchy foods consumed in the Western diet such as bread, breakfast cereals, and potato products have high GI values and stimulate insulin secretion because the starch in most of these foods is rapidly digested^{6,24,25}. Different studies reveal that the GI for the arepa is superior than 72 with a starch hydrolysis index of 85 however, the arepas made with high amylose corn flour observed lower GI and starch hydrolysis index. The decreased metabolic response elicited by the high amylose corn products seems to be related to a decreased enzymatic digestion rate^{3,12,26}.

On the other hand, a 30–60% reduction in blood glucose peak can be achieved when β -glucans constitute 8–10% of the carbohydrates in cereals^{6,27,28}. β -glucan is linear, unbranched water soluble polysaccharide composed of 1-4-O-linked (70%) and 1-3-O-linked (30%) β -D-glucopyranosyl units. It is found in the cell walls of the bran layer and endosperm fractions of the whole seed of some cereals, like barley and oat grains. Different physiological effects of β -glucan in isolated form or as a constituent of oat and barley products are related to its viscosity. The ingestion of this type of soluble fibre, results in a slower rate of carbohydrate and lipid absorption, high transport of bile acids towards lower parts of the intestinal tract and high excretion of bile acids. Other positive health effects have been attributed to the β -glucans, including reduced plasma total and low-density lipoprotein (LDL)-cholesterol, weight management and improved gastrointestinal function^{6,29,30}.

Taking in account that the arepa is a very popular food preparation in Venezuela which has high glycemic response and the ingestion of this type of food could be a risk factor for the development of diabetes, atherosclerosis, and obesity; the addition of β -glucans, possibly will modify its original metabolic characteristics and it can be used in healthy subjects and also for those which requires a dietetic therapy for the treatment of dyslipidemias, diabetes or for prevention of type 2 diabetes mellitus without the transgression of food habits of the patients. Therefore, the aim of this study was to investigate the arepa consumption effects on glucose and insulin response, when β -glucans are added to this preparation.

Formulation design

A corn meal arepa (CA) was prepared by the laboratory staff using a standardize method (64g of flour and 134.4 mL of water) to obtain a 110 g final product with 50 g of carbohydrates, 0.7 g of lipids and 4.6 g of proteins. For this purpose, commercial precooked white corn meal flour with 77.7 carbohydrates, 1.1 lipids and 7.2% of proteins content was used. This preparation was later modify by partial substitution of precooked corn flour for 15, 20 and 30 g of β -glucans (c-trim 20), resulting in three different formulations: Arepa with β -glucans A (BAA), arepa with β -glucans B (BAB) and arepa with β -glucans C (BAC); with 50 g of carbohydrates and 115, 125 and 130 g of weight respectively. The commercial β -glucans (c-trim 20) was supplied by the National Center for Agricultural Utilization Research, Peoria, Illinois. A mixture of corn meal or corn meal with β -glucans and water was prepared, followed by kneading for 5 min until soft dough was achieved. Round breads (arepas) of this mixture were cooked in a special toaster for 7 min and allowed to cool.

Sensory test

In order to select the more accepted formulation a sensory test was carry out. For this purpose a total of 20 untrained panelists were recruited and a 9-point hedonic scale ranging from 1= dislike extremely to 9 = like extremely was used to evaluate overall acceptance of the formulations (arepas)³¹. For the assessment, the arepa samples were placed into plastic cups and coded with 3 digit random numbers. The 3 samples consisting of BAA, BAB and BAC were tested on same days and presented to panelists at random, in order to avoid interaction between samples during the test day. The sensory test was replicated three times on different weeks. Every time the samples were presented with water and paper ballots on a plastic tray. All sensory evaluations were conducted under white fluorescence light at a room temperature of 21 ± 1 °C. Panelists were instructed to consume the whole sample and rinse their mouths with water between samples, to minimize any residual effect.

Subjects

Fourteen healthy medical students between 18 to 22 years old (20.1 ± 1) gave written consent to participate in the study according to the Helsinki ethical guidelines. All subjects were evaluated at the Metabolic and Endocrine Research Center "Dr. Félix Gómez", at the Faculty of Medicine, University of Zulia. A detailed background clinical history (which included family history of heart disease, diabetes mellitus, hypertension, dyslipidemia and obesity) was carried out; a physical examination and laboratory test were performed to each patient in order to discard cardiovascular disease possibilities and confirm a healthy state. Height, weight, skinfolds thickness, waist circumference, and blood pressure measurements were carried out as well. Body mass index (BMI) was also determined. Total cholesterol, HDL cholesterol, triacylglycerides and glycemia, of subjects before entering the protocol were normal and were determined using commercial kits (Human Gesellschaft für Biochemica und Diagnoses Mbh).

LDL cholesterol was calculated by the Friedwald formula³². The bioimpedance (BIA) was performed with a TANITA BIA body fat analyser which incorporates weighing scales as it quantifies weight and bioimpedance (TBF-401, TANITA Co. Tokyo, Japan). For height measuring balance height meter of DETECTO 140 kg balance device was used. Utilization of any medication was an exclusion criteria. Approval of the study was given by the Ethics Committee of the "Dr. Félix Gómez" Research Center at the Universidad of Zulia, Venezuela.

Evaluation of postprandial glucose and insulin responses

Three different meals (white wheat bread, corn flour arepa and arepa with β -Glucans) were served as breakfasts to the fourteen subjects, in random order, after an overnight fast on three separate occasions, with at least a one-week interval. The bread (WB) and the corn flour arepa (CA) were used as references. The arepa with β -glucans (BAB) given to the participants was previously selected (the more accepted formulation from BAA, BAB or BAC). The test meals were fed in order to provide 50 g of carbohydrate and were served with 250 mL of water.

Basal and postprandial blood glucose concentration was determined with a glucose oxidase method (Human Gesellschaft für Biochemica und Diagnoses Mbh) and ELISA method was used to measurement basal and postprandial insulin levels on serum (DRG Instruments GmbH Germany).

Statistical analyses

All the statistical analyses on the resulting data were carried out using the SPSS software version 10.0 for Windows (SPSS Inc., Chicago, IL) and are shown as arithmetic mean $\pm$ standard deviation (SD). In order to select the more accepted formulation an ANOVA test was used. One way ANOVA test was carry out as well to compare glucose and insulin responses between test meals and between basal and postprandial (glucose and Insulin) levels of each meal (WB, CA and BA). Differences were considered significant when p value was <0.05 .

Chemical composition of the three arepas formulations in witch corn flour was partially substituted for 15, 20 and 30 g of c-trim 20 (β -glucans) is shown in Table 1. All the resulting formulations had 50 g of carbohydrates. The 15, 20 and 30 g of c-trim 20 represents 13%, 16% and 23% of the total arepa weigh respectively.

Table 2 presents the mean sensory score defined by panelists for the three formulations (arepas with β -glucans) evaluated. As can be observed was not significant difference between formulation BAA and BAB, but it was significant between BAA and BAC ($p<0.001$) and BAB and BAC ($p<0.001$). These results indicate that both formulations with different amount of β -glucans had the same acceptance by the panelist. The formulation BAB was selected to carry out the evaluation of postprandial glucose and insulin responses on the base of its β -glucans constitution.

Table 1. Chemical composition of the formulations (arepas with β -glucans)

	FORMULATIONS		
	BAA	BAB	BAC
Corn Flour (g)	57.00	54.00	51.00
Carbohydrates (g)*	44.30	41.96	39.60
Lipid (g)*	0.63	0.59	0.56
Proteins (g)*	4.10	3.88	3.67
c-trim 20 (g)	15.00 (13%)	20.00 (16%)	30.00 (23%)
β -glucans (g)**	3.14	4.18	5.23
Carbohydrates (g)**	6.29	8.38	10.48
Lipid (g)**	1.08	1.44	1.80
Proteins (g)**	4.05	5.40	6.75
Total Weigh (g)	115.00	125.00	130.00

BAA= Arepa with β -glucans formulation A

BAB= Arepa with β -glucans formulation B

BAC= Arepa with β -glucans formulation C

*Carbohydrates, lipids and proteins from Corn Flour (from Tabla de Composición de Alimentos, Venezuela)³³

** β -glucans, carbohydrates, lipids and proteins from c-trim 20

Table 2. Mean sensory score for formulations acceptance

Formulations	Sensory score
BAA	6.52 $\pm$ 0.8 ^a
BAB	6.02 $\pm$ 1.0 ^b
BAC	3.71 $\pm$ 0.7 ^{ab}

Sensory score are presented as arithmetic mean $\pm$ standard deviation (SD)

Similar letter indicate statistical difference. a, b = $p<0.001$

The anthropometric characteristics and the biochemical parameters of the subjects at the time of entry into the study are shown on Table 3. The daily caloric intake is presented as well, which was adequate to the age, according to the Venezuelan population recommendations³⁴.

Basal blood glucose and serum insulin concentration as well the glucose and insulin levels at postprandial stage after three meals consumption are shown in Table 4. Significant difference was found when postprandial glucose from CA and BAB were compared ($p<0.04$) and between postprandial insulin levels from WB and BAB ($p<0.03$). After the consumption of WB, CA or BAB basal and postprandial plasma glucose were compared but no significant difference was possible to establish however, the insulin levels increased on a significantly way after the WB ($p<0.006$) or CA ingestion ($p<0.001$) but subsequent to the BAB intake not significantly change was observed on insulin levels

Table 3. Baseline Characteristics of the study group

Characteristics/Parameters	Mean $\pm$ SD	(n=14)
Age (years)	20.1 $\pm$ 1.0	
Weight (kg)	71.0 $\pm$ 7.5	
Height (cm)	174.1 $\pm$ 5.7	
BMI (kg/m ²)	23.0 $\pm$ 1.4	
Impedance (Ω)	507.0 $\pm$ 39.3	
Body Fat (%)	14.7 $\pm$ 3.0	
Lean body mass (kg)	58.7 $\pm$ 7.0	
Waist circumference	79.0 $\pm$ 5.2	
Caloric intake (kcal)	2850.6 $\pm$ 230	
Fasting Glucose (mg/dL)	81.0 $\pm$ 8.8	
Postprandial Glucose (mg/dL)	78.0 $\pm$ 7.2	
Total Cholesterol (mg/dL)	143.6 $\pm$ 30.2	
Triacylglycerides (mg/dL)	71.2 $\pm$ 24.0	
HDL cholesterol (mg/dL)	40.2 $\pm$ 5.9	
VLDL cholesterol (mg/dL)	14.2 $\pm$ 4.9	
LDL cholesterol (mg/dL)	89.1 $\pm$ 29.8	
Fasting Insulin (μ U/mL)	14.8 $\pm$ 7.1	
Postprandial insulin (μ U/mL)	31.4 $\pm$ 13.7	

Data are shown as arithmetic mean $\pm$ standard deviation (SD)

Table 4. Postprandial glucose and insulin responses to the three different meals

	White Bread (WB)	Corn Arepa (CA)	β -glucans Arepa (BAB)
n	14	14	14
Fasting Glucose (mg/mL)	81.8 $\pm$ 7.7	81.8 $\pm$ 7.5	87.9 $\pm$ 11.1
Postprandial Glucose (mg/mL)	82.2 $\pm$ 8.1	84.9 $\pm$ 8.4a	82.6 $\pm$ 10.1a
Treatment difference (%)	$\uparrow$ 0.5	$\uparrow$ 3.8	$\downarrow$ 6.0
p	NS	NS	NS
Fasting Insulin (μ U/mL)	13.7 $\pm$ 5.7	13.7 $\pm$ 4.4	11.8 $\pm$ 3.3
Postprandial Insulin (μ U/mL)	23.4 $\pm$ 8.9b	20.8 $\pm$ 9.2	13.7 $\pm$ 2.8b
Treatment difference (%)	$\uparrow$ 70.1	$\uparrow$ 51.8	$\uparrow$ 16.1
p	<0.006	<0.001	NS

Data are shown as arithmetic mean $\pm$ standard deviation (SD)

Treatment difference (%) = [(postprandial $\times$ 100/fasting) - 100]

Postprandial represents the mean of absolute value in 2 hours

NS= No significant difference was observed

Similar letter indicate statistical difference (a= p<0.04; b= p<0.03)

Discussion

The postprandial insulin response to a β -glucans (BAB) intake was lower than the response observed to WB with similar amount of available carbohydrate. Postprandial glucose response to a BAB meal was also lower than to a CA meal. On the other hand, the insulin level enhance after ingestion of BAB was only 16% and the insulin level at postprandial stage was not significantly different when was compared with the basal level. However, the observed increase on insulin after WB and CA consumption was 70.1% and 51.8% respectively and in both cases, the levels detected at postprandial stage were significantly differ-

ent to the basal levels. These results are similar to the ones of Behall et al.; who in two separate studies found that consumption of β -glucans improves postprandial plasma glucose and insulin responses in men and women^{35,36}. In turn, Makelainen et al. testing four different products with three different amounts of β -glucan, obtained the same result in glucose and insulin responses⁶. Yokoyama et al. also reported a lower glucose and insulin response to a paste enriched with β -glucans³⁷.

The improvement on insulin response could suggest that fibre present on BAB slowed down the carbohydrates absorption in the intestine and the release of glucose into the bloodstream. Relating to this, Battilana et al. stated that after a meal containing β -glucans, the absorption of carbohydrates is decreased or delayed, conclusion that was supported by results of Lifschitz et al.^{30,38}. Water-soluble β -glucans exert their effects mainly by increasing viscosity in the small intestine. In the intestine the β -glucans absorb fluids and contribute to viscosity during digestion, resulting in an extended digestion period. When digestion is delayed, blood sugar increases more slowly, causing a low insulin response. However the effect has been established, the way of the actions causing the effect is not yet fully understood^{38,39}.

To this respect, two hypotheses have been proposed; one establishes that in the intestine, food is incorporated in the viscous β -glucans solution, making it more difficult for enzymes to degrade the food components and causing lower digestion. Evidence from in vitro studies suggests that dietary fibre can alter the activity of pancreatic amylase. The effects on enzyme activity were attributed to pH changes, ion exchange properties, enzyme inhibitors and absorption. Nevertheless, rather than a chemical enzyme-fibre interaction, the presence of fibre, through its particulate viscous nature has been suggested to impede enzyme-substrate interactions. Further, the presence of fibre in a form that restricts starch gelatinization or the access of the hydrolytic enzymes to starch can slow the rate of its digestion⁴⁰. The another hypothesis, indicate that the β -glucans form a protective layer along the intestinal wall that acts as viscous barrier or unstirred layer, slowing absorption of nutrient from intestine. In both hypothesis, the viscosity is the key role assigned to the recognized functional properties of β -glucans since the β -glucans absorb fluids and adds viscosity during the digestion period⁶.

Moreover, other effects of soluble fibre have been explored with the purpose to explain the lower insulin postprandial response to this type of fibre. A few studies have investigated the effects of dietary fibre on postprandial glucose-dependent insulintropic polypeptide (GIP) and glucagon-like-peptide 1 (GLP-1). These two incretin hormones were shown to be potent determinants of the postprandial insulin release that occurs after increases in blood glucose and therefore are also essential in the regulation of postprandial glycemia. Controlled studies in humans are scanty and discrepant, but most of them established that dietary fibres like the β -glucan seem to reduce GIP and to augment GLP-1 responses, probably because of decreased carbohydrate absorption, however the high postprandial response of GLP-1 to the β -glucan remains unexplained⁴¹⁻⁴³.

In conclusion, this study showed that by the addition of β -glucan, to corn products like arepas, produced favorably low metabolic responses, when comparing with the originals. Moreover, the results obtained from the addition of 20 g of c-trim 20 (correspond to 16% of the arepa weight and contain 4.18 g of β -glucans) to a preparation with a high glycemic and insulin response as the arepa, make this traditional preparation a therapeutic alternative for glucose intolerant, diabetic and obesity patients. The substitution of some corn flour for β -glucans in foods that contain a large proportion or, are made of corn flour is not only important from the nutrition standpoint but also for its therapeutic potential; may be a practical means of controlling postprandial glycemic and insulin response, could be helpful to control different diseases and also considered suitable to prevent various disorders. β -glucans can be incorporated into a wide variety of innovative food products improving the diet of the general population.

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