BLOOD GLUCOSE RESPONSE AND *GLYCEMIC INDEX* OF DIETS CONTAINING DIFFERENT SOURCES OF CARBOHYDRATE IN HEALTHY RATS

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ABSTRACT

Blood glucose response and *Glycemic Index* (GI) of diets containing different sources of carbohydrate including glucose, starch, amylopectin, bread, rice, whole wheat kernels (habbiyah), lentils, check peas, kidney beans, and mixtures of bread or rice with lentils, chick peas or kidney beans for healthy Sprague Dawley rats using glucose as standard were studied. Fifty five normal animals were housed individually to perform this study. Animals were fasted (overnight fasting) for 12 hours and tested for blood glucose at zero time before given in amount of test food containing 0.15 g carbohydrates. Blood glucose was determined after 15, 30, 45, 60, 90 and 120 minutes. Results showed that amylopectin based diet had the highest GI (96) comparing with lowest GI (44) for habbiyah based diet. Results also show that addition of lentils, check pea or kidney beans to the bread resulted in lowering the GI of the final meals from 70 to 46. However, addition of these legumes to the rice base diet did not enough reduce the GI. In conclusion, mixing different sources of carbohydrates might be change the blood glucose response and GI of the original foods.

INTRODUCTION

It has been known that both the amount of carbohydrates consumed and its source have different effects on postprandial blood glucose and insulin responses in healthy and diabetic subjects depending on the rate of digestion (Wolever and Bolognesi, 1996a and b and Jenkins *et al*., 2002) and the rate at which food is passed through the digestive tract or may be slowing the rate of nutrient absorption following ingestion of the diet (Liljeberg and Bjorck, 1998). Nevertheless, more recently, John and Vladimir (2004) stated that the source (type) of carbohydrate is of potential determinant of postprandial glucose and insulin responses which is associated with the treatment of diabetes. In addition, the type of dietary carbohydrate in healthy, physical active woman's diet influenced both postprandial blood glucose profile and blood sugar response to exercise (Clapp, 1998). The extent and duration of the blood glucose response depend on the rate of absorption, which in turn depends on factors such as gastric emptying as well as the rate of hydrolysis and diffusion of nutrients in the gut (FAO/WHO report, 1997). Nowadays, the effect of carbohydrates on blood responses is indicated by a parameter called Glycemic Index (GI). The Glycemic index is a physiologically based measure of the effects of carbohydrates on blood glucose levels. It is a parameter of the blood glucose change after eating a certain food compared to the change after eating a similar amount of glucose (Jenkins *et al*., 1981 and Wolever *et*

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al., 1991). In human, lower GI diets reduced both fasting blood glucose and glycated proteins independently of variance in available and unavailable carbohydrate intakes (Geoffrey *et al*. 2008). Furthermore, low-GI diet, which is high in dairy and fruit but low in potatoes and cereals, is associated with improved insulin sensitivity and lipid metabolism and reduced chronic inflammation (Du *et al*., 2008).

Although, the GI is a useful tool for classifying the impact of carbohydrates on the body, however it is not useful to get accredited on the GI values of individual foods, as the overall impact of a meal on GI is difficult to predict (Flint *et al*., 2004). As far as eating is concerned, the best way to maintain optimal control over the blood glucose is to choose dietary carbohydrates with a lower GI value and this may be accomplished by mixing the foods to make a new meal. Wolever and Jenkins (1986) found that the GI concept applies well to mixed meals containing fat and protein. In order to lower the carbohydrates load of the diets, it is better to raise intake of low GI foods such as legumes, whole cereals, fruits and vegetables or may be by substituting high GI foods for lower GI alternatives. However, the goal is not to eat low-GI carbohydrate at the prevention of all other, in which people try to include one low-GI carbohydrate choice per meal or to base at least two of their meals each day on low-GI choices (Brand-Miller, 1999). The aim of this study was conducted to estimate blood glucose response and GI of meals containing different sources of carbohydrates including glucose, starch, amylopectin, bread, rice, whole wheat kernels (habbiyah) and mixtures of bread or rice with lentils, chick peas, and kidney beans for healthy Sprague Dawley rats.

MATERIALS AND METHODS

Food samples: Glucose, wheat white four, corn starch, corn amylopectin, long grain rice, whole wheat kernels habbiyah, lentils, check pea, and kidney beans were purchased from local market in Mosul city- Iraq. Glucose was provided by Sameraa Drugs Company-Iraq, whereas corn amylopectin was provided by Fluka packed Switzerland. Grains were sorted and washed with tap water then dried in Cabinet dryer with hot air at 55°C for 48 hours. Grains were ground in Quadrume Junior Mill, Brabender-Germany and the flours were packed in polyethylene sacs and stored in the refrigerator until used in the next step.

Chemical analysis: Approximate analysis was performed to determine moisture, protein, fat, fiber, ash and carbohydrates in the grain flours (Table 1). Moisture was determined according to the method of AOAC (1980) by using rapid moisture tester made by Brabander, Germany at 105°C until constant weight. Protein was determined according to the procedures mentioned by Pearson (1973) using Macrokjeldahl method. Fat was determined according to the procedures mentioned by Pearson (1976) using Soxhlet method. Crude fiber was determined according to the procedures mentioned by Pearson (1973). Ash determination was performed according to the method of AOAC (1980).

Balanced diets preparation: Ten balanced diets were prepared and formulated to contain 63.6g CHO from glucose, corn starch, corn amylopectin, bread , rice, Table (1): Chemical composition of Grain flours on dry weight bases (g/100g)

Foods	Components				
	Protein	Fat	Fiber	Ash	Carbohydrate*
Wheat white flour	12.96	0.86	0.44	0.84	84.9
Whole wheat kernels (Habbiyah)	13.56	1.20	1.92	1.22	82.1
Long grain rice	8.66	0.0	0.34	0.39	90.6
Lentils	28.59	1.5	2.0	2.4	65.5
Chickpea	22.97	5.4	3.63	2.68	65.3
kidney beans	24.79	1.98	5.29	4.1	63.8

*by difference**.** The numbers are average of three samples.

habbiyah, bread + lentils, bread + chickpea, bread + beans, and rice + beans according to the NAS/NRC (1978) (Table 2). All dry ingredients with corn oil were weighed, mixed, homogenized and kneaded electrically in a dough mixer with addition of deionized distilled water. They were formulated; finger shaped of about 5 g each piece, spread in large stainless steel trays and dried in a cabinet drier at 50ºC for 12 hours, then packed in plastic bags and kept frozen for final analysis.

Experimental animals: Fifty five Sprague-Dawley derived male rats (150-160g body weights) were housed individually in suspended mesh bottom and front stainless steel hanging cages of 25X22X20 cm (fecal collection trays underneath) in a controlled condition, between 20 - 25°C, 12 hours light and dark cycle. After two days of adaptation period, these animals were weighed and divided into 11 groups of five animals each, notably, with no statistical differences. Deionized distilled water was offered *ad libitum.* Animals were fasted (overnight fasting) for 12 hours and tested for blood glucose at zero time before given in amount of test food (Table 2) containing 0.15 g carbohydrates. Blood glucose was determined after 15, 30, 45, 60, 90 and 120 minutes. The same method was performed with the group giving 0.15 standard glucose dissolved in distilled water.

Measurement of Blood Glucose Response: Blood glucose was determined by using glucose tester Device made by Johnson and Johnson Co., Lifescon, USA. Blood samples were taken from Tail tipping. Glycemic Index (GI) for each diet was determined by calculation of Incremental Area Under two hours of blood glucose response or Curve (IAUC) for each diet and compared with the IAUC for glucose solution standard according to the method of Jenkins *et al*. (1981); Wolever and Jenkins (1986) and Wolever *et al*. (1991) which also reported by FAO/WHO (1997) using the following equation:

$$GI = \frac{\text{Incremental Area Under 2h blood glucose Curve for food}}{\text{Incremental Area Under 2h blood glucose Curve for glucose or white bread}} \times 100$$

Statistical Analysis: The complete randomized design (CRD) was used. Statistical difference was determined using Duncan's multiple range test at ($p<0.05$) by SAS Version (1989).

Table (2): Diets formulation and composition (g/100g).

Diets		Glucose	Starch	Amylopectin	Bread	Rice	Habbiyah	60% Bread+ 40% Lentils	60% Bread+ 40% Chickpea	60% Bread+ 40% Beans	60% Rice+ 40% beans
Ingredients, g/100g	CHO	63.6	63.6	63.6	74.9	70.2	78.2	83.6	83.9	82.9	81.9
	Casein	17.9	17.9	17.9	7.56	12.39	5.74	0	1.7	1.81	3.06
	Corn oil	6.5	6.5	6.5	5.86	5.9	5.56	5.54	4.02	4.61	5.36
	Cellulose	5.0	5.0	5.0	4.67	4.56	3.5	4.03	3.39	3.67	2.67
	Vitamins mixture[1]	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0
	Minerals mixture[2]	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0
Determined, g/100g	Protein	16.8	16.7	16.7	16.7	16.6	16.6	16.8	16.5	16.9	16.9
	Fat	6.4	6.4	6.5	6.6	6.4	6.4	6.5	6.6	6.6	6.5
	Fiber	5.0	5.0	5.0	5.0	5.1	5.0	5.0	5.1	5.1	5
	Ash	3.8	3.9	3.9	4.4	4.1	5.1	5.0	5.2	5	5.7
Energy, Kcal/Kg		3792	3788	3797	3806	3784	3748	3801	3798	3814	3805

1 The *vitamins mixture* was prepared (g/kg) as follows: alpha-tocopherol 50 g, inositol 5.0 g, menadione 1.25 g, vitamin A concentrate 4.5 g, retinyl acetate 200,000 I.U., vitamin D concentrate 0.25 g, calciferol 400.000 I.U., niacin 4.5 g, riboflavin 1.0 g, pyridoxine-HCl 1.0 g, thiamine-HCl 1.0 g, ascorbic acid 45.0 g, pantothenate 30.0 g, biotin 0.02 g, folic acid 0.09 g, and vitamin B_{12} 0.00135g and the weight was completed to 1 kg by adding starch.

2 The *minerals mixture* was prepared (g/kg) as follows:
KCl 296.7 g, $MnSO_4.H_2O$ 12.7 g, $CoCO_3.6H_2O$ 0.7 g, $MgCO_3$ 121.0 g, $CuSO_4$ 1.6 g, KI 0.8 g, $Na_2MoO_4.2H_2O$ 0.1 g, $ZnSO_4.7H_2O$ 28.0 g, $FeSO_4.7H_2O$ 30.0 g. The weight was completed to 1 kg by adding starch.

RESULTS AND DISCUSSION

Blood Glucose response and Glycemic Index (GI) of diets containing different sources of carbohydrate namely, glucose, starch, amylopectin, bread, rice, habbiyah, lentils, check peas, kidney beans, and mixtures of bread or rice with lentils, chick peas, and kidney beans for healthy Sprague Dawley rats using glucose as standard are shown in Table (3) and Figure (1). Results show that there was a sharp increase in blood glucose responses for glucose as standard and for glucose and amylopectin as a meals to reach a relative high mean value (5.13 mmol/L) 15 min after the beginning of the animals feeding of the meals. Fifteen minutes later (at 45 min), the glucose responses were begun to slow down to reach the highest values of 5.63 and 6.4 mmol/L for glucose and amylopectin meals respectively (Figure 1). Table (3) shows that there were significant ($P<0.05$) differences in blood glucose responses between the different meals made from different foods comparing to the glucose standard. The mean values of IAUC for some meals such as amylopectin (195 mmol.min/L) was significantly ($P<0.05$) high comparing with the other meals. However, some meals like glucose, starch and bread were developed a relative high IAUC values (144, 140 and 142 mmol.min/L, respectively) comparing with other meals of low IAUC (90 to 97 mmol.min/L) (Table 3). These values of IAUCs would yield corresponding respective high GI values of 96, 80, 70 and 68 for amylopectin, glucose, bread and starch meals fed to the rats. The GI value for amylopectin meal was significantly ($P<0.05$) high comparing with GI values for glucose, bread and starch meals. However, the respective decreasing low GI values of 49, 48, 48, 46, 46 and 44 for rice, bread + lentils, bread + chickpea, bread + beans, rice + beans and habbiyeh meals fed to the animals. These results show that GI values for amylopectin, glucose, bread and starch meals were significantly ($P<0.05$) high comparing with other GI values.

Results in Table (3) and Figure (1) also show that addition of lentils, check pea and kidney beans to the bread resulted in lowering the GI of the final meals from 70 to 46. However, addition of these legumes to the rice base diet did not enough reduced the GI even no more than 3 degrees. Therefore, mixing different sources of carbohydrates might be change the glycemic response of the original foods. Kabir *et al*. (1998) found that meals containing more corn amylopectin fed to the rats resulted in high GI value (107) comparing with lower value (67) of mung bean meals which contains low percent of amylopectin. Comparable results of blood glucose response and GI values were shown with those results found in the same laboratory by Thannoun and Al-Kubati (2005a) in human subjects such as white bread (69) and habbiyah (40).

It was well established that different carbohydrate foods elicit a wide spectrum of plasma glucose responses when eaten without other foods (Collier *et al*. 1986). In medium term studies of diabetes replacing high GI carbohydrates such as white bread and potatoes with low GI forms such as whole grain and legumes will improve glycemic control and, among person treated with insulin, will reduce hypoglycemic episodes (Willett *et al*., 2002). Recently, Geoffrey *et al*. (2008) found that lower glycemic index (GI) diets reduced both fasting blood glucose and glycated proteins independently of variance in available and unavailable

Table (3): Blood Glucose response, Incremental Area Under the Curve (IAUC) and Glycemic Index for different carbohydrates meals fed to healthy rats.

Diet	Blood Glucose Response, mmol/L							IAUC mmol.min/L	GIg [1]	GIwb[2]
	Time minutes									
	0	15	30	45	60	90	120			
Glucose	3.53 ab	5.13 a	5.50 abc	5.63 abc	5.00 abc	4.60 bc	3.96 bcd	144 b	80 b	114
Starch	3.56 ab	4.43 b	4.83 cd	5.06 c	5.03 abc	4.86 ab	4.43 ab	140 b	68 b	97
Amylopectin	3.63 ab	5.13 a	5.86 ab	6.40 a	5.36 a	5.10 a	4.73 a	195 a	96 a	136
Bread	3.26 ab	4.50 b	4.76 d	4.80 c	4.83 bcd	4.43 bcd	3.76 cd	142 b	70 b	99
Rice	3.56 ab	4.5 b	5.23 bcd	5.00 c	4.00 g	3.90 ef	4.06 bc	100 c	49 c	70
Habbiyah	3.63 ab	4.43 b	5.03 cd	4.96 c	4.50 deg	4.16 cf	3.86 cd	90 c	44 c	62
60% Bread+ 40% lentils	3.23 b	4.20 b	4.63 d	4.76 c	4.10 fg	3.66 f	3.70 cd	98 c	48 c	68
60% Bread+ 40% Chickpea	3.30 ab	4.13 b	5.06 cd	4.70 c	4.20 efg	3.83 ef	3.50 d	97 c	48 c	68
60% Bread+ 40% Beans	3.43 ab	4.06 b	5.26 bcd	4.93 c	4.20 efg	3.93 def	3.50 d	93 c	46 c	65
60% Rice+ 40% beans	3.66 a	4.30 b	5.16 cd	5.06 c	4.60 cde	4.23 cde	3.90 cd	95 c	46 c	66
Glucose standard	3.46 ab	5.13 a	5.9 a	5.6 b	5.26 ab	5.13 a	4.73 a	204 a	100 a	100

The numbers are average of five values of five animals.
Same letters in the column indicate that there is no significant differences at ($P<0.05$).
GIg : Glycemic index when the standard is glucose solution. (GIb X 0.7 Foster-Powell and Brand Miller, 1995).
GIb : Glycemic index when the standard is glucose solution. (GIb X 1.42 Foster-Powell and Brand Miller, 1995).

carbohydrate intakes. Generally, differences in blood glucose responses in human subjects and animals for different foods and among the same group of food may be related to various factors such as chemical composition or the components of the food, components and the nature of the carbohydrates, dietary fiber, method of food processing and presence of any substances act as inhibitors of enzymatic digestion (Tovar *et al*., 1992; Bjorck *et al*., 1994; Behall *et al*., 1999; Darabi, *et al*., 2000 and Thannoun and Al-Kubati, 2005a and 2005b). However, there is evidence indicating that adding foods of low glycemic index may reduce the glycemic impact of other (challenges with single) carbohydrate foods. Mixing two sources of carbohydrate such as cereals of higher GI value and legumes of lower GI value resulted in changing the chemical composition including amylase vs amylopectin, protein and fiber contents of the resulting meals.

The rates of digestion and uptake of carbohydrates in a mixed meal can be varied by the meal’s composition, so far, affecting the GI value may be toward the beneficial in reducing the risk of some nutritional disorders such as diabetes, Although, the relative glycemic effects of mixed meals can be predicted from the GI of their carbohydrate components (Collier *et al*., 1986).

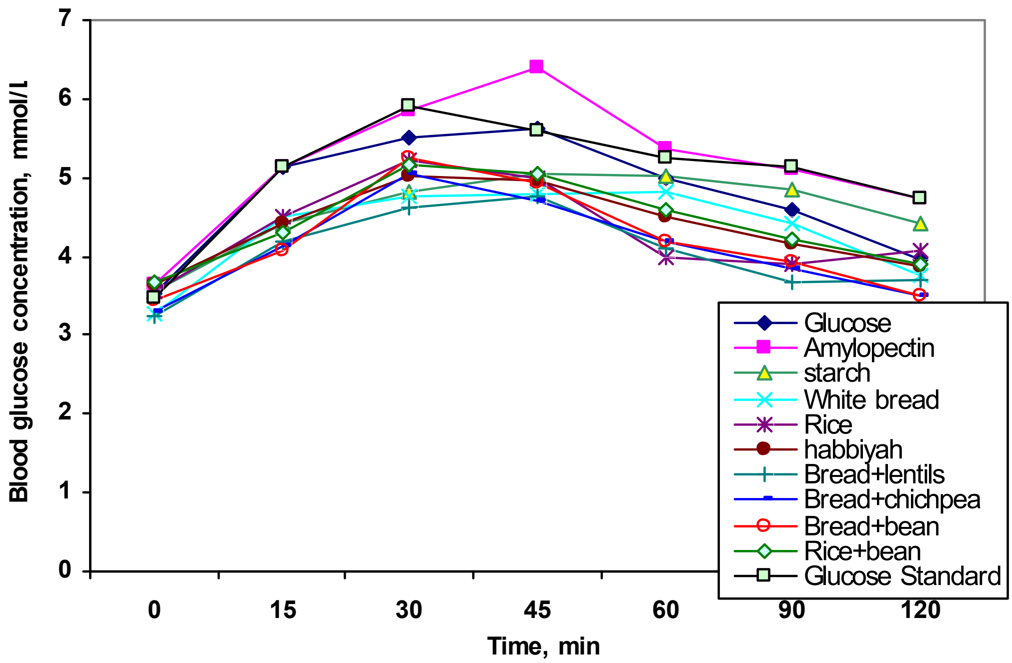

Figure (1): Blood glucose response and Glycemic Index for meals of different carbohydrate sources

It has been known that for individual foods with different GI, both the source and amount of carbohydrate consumed are important determinants of postprandial glucose and insulin responses in normal subjects (Wolever and Bolognesi, 1996a).Moreover, in other study the same researchers (Wolever and Bolognesi, 1996b) concluded that also both source and amount of carbohydrate determine the glucose and insulin responses of lean, young, and normal subjects after different mixed meals with variable GI. Further, blood glucose and insulin responses were differed with different sources of carbohydrates meals in healthy and diabetic (Jenkins *et al*., 1981 and Wolever *et al*., 1991). Many studies showed that the blood glucose response and GI were changed with consuming different meals mixtures (Goddard *et al*., 1984; Behall *et al*., 1989 and Weststrate and Van Amelsvoort, 1992). However, in this study, differences in carbohydrates sources in the meals resulted in different blood glucose responses in rats using glucose as standard. Furthermore, the meals which contain high percentage of amylopectin had given high GI value comparing with other meals. Goddard *et al*. (1984) and Byrnes *et al*. (1995) found that starchy meals containing high percent of amylopectin digest and absorb more comparing with those containing high percent of amylose, so they resulted in high blood glucose and insulin responses in fasting rats. They stated that feeding rats meals containing high percent of amylopectin resulted in increasing the insulin secretion. In 52 weeks experiment performed by Higgins *et al*. (1996) on rats fed meal containing high percent of amylopectin, gradual increase in insulin resistance was detected on week 12 until week 29.

Over the past 3 decays, the GI of the most common carbohydrate foods has been determined and among the foods that produced low GI values are legumes and whole cereal grains (Jenkins *et al*., 1988; Foster-Powell and Brand-Miller, 1995 and Foster-Powell *et al*., 2002). Further, the GI of dried legumes has been found to be the lowest among starchy foods (Bornet *et al*., 1987 and Tovar *et al*., 1992). Specifically, it was concluded in this laboratory that legumes such as lentils, chickpea and kidney bean elicited very low GI, whereas whole broad bean and broad bean kernels gave lower medium GI values in human (Thannoun and Al-Kubati, 2005b). The amylose contents of the legumes were higher (28-36 %) when compared with lower percent (15-30 %) for most of the other carbohydrate foods such as cereals. Darabi *et al*. (2000) mentioned that the nature of the starch in the legumes may influence their starch digestibility. High amylose starch has been shown to be digested far more slowly than the high amylopectin starch. It has been previously noticed that amylopectin of the starch has been shown to be more digested than amylose (Behall *et al*., 1988 and 1989). For this reason, in mixed meal, when starches with different amylose-amylopectin ratios were incorporated into a meal, the one with the high amylopectin starch showed higher GI than that of the low amylopectin starch for normal and diabetic rats (Kabir *et al*., 1998). In other words, higher amylose vs amylopectin percent in the foods decreases the digestion of the total starch and consequence decreasing the GI values (Thannoun and Alkubati, 2005a and b).

In addition to be a good source of protein, legumes are also good source of fiber than other starch foods. As the fibers are constituents of the plant cell walls, it can be supposed that the fiber content of a particular food might influence the rate of carbohydrate digestion through the resistance induced by the cell wall to swelling of the starch granule during cooking (Wursch *et al*., 1989). Fibers reduce insulin secretion by slowing the rate of nutrient absorption following ingestion of the diet and also may reduce both the postprandial hyperglycemia and the insulin or antidiabetic agent requirements of diabetics in addition to helps to increase the rate at which food is passed through the digestive tract (Thannoun and Al-Kubati, 2005b). It was clear from the Table (2) that the test diets for animals were intended to be identical of normal mixed meals and were formulated to vary the source and the amount of carbohydrate and this resulted in nutrients content variation such as amylose-amylopectin ratio, fibers protein and may be the fat. Therefore the results emerged differences in blood glucose responses and GIs. Al-kubati (2003) concluded that the amount of carbohydrate alone was not significantly related to the glucose response in normal subjects. However, our results showed that both the amount and source of carbohydrate consumed by the rats had to be taken into account to might have a link between the components of the mixed formulated meals and blood glucose response and GI. These findings were in agreement with early results found by Wolever and Bolognesi (1996a and b). Despite of controversial on these results, positive findings have found to suggest that the lower dietary GIs were of potential importance in the prevention or management of diabetes. Recent results found by Barclay *et al*. (2007) suggested that a dietary pattern characterized by high fiber and low-GI carbohydrates is sustainable over time and may represent an effective strategy to prevent diabetes. In which their results supported the hypothesis that the type or quality of carbohydrate plays an important role in the etiology of type 2 diabetes.

استجابة كلوكوز الدم والمؤشر الكلوكوزي لبعض أنواع الأغذية الحاوية على مصادر مختلفة من الكاربوهيدرات في الجرذان السليمة

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ألخلاصة

تم دراسة استجابة كلوكوز الدم والمؤشر الكلوكوزي لبعض الأغذية الكاربوهيدراتية و خلائطها في الجرذان السليمة. تم تحضير عشرة من الأغذية الموزونة احتوت على ٦٣.٦ غم/ ١٠٠غم كاربوهيدرات مصدرها أغذية مختلفة وهي الكلوكوز والنشا و الأميلوبكتين و الخبز الأبيض و الرز و الحبية و العدس و والحمص و الفاصوليا. قدر المؤشر الكلوكوزي لهذة الأغذية باستخدام الجرذان البالغة تراوحت اوزانها بين ١٥٠-١٦٠ غم وبواقع ٥ حيوانات لكل مجموعة. تم قياس سكر الدم بعد مدة ١٢ ساعة صوم عبر الليل قبل إعطائها الأغذية الموزونة والتي احتوت على ٠.١٥٠ غم كاربوهيدرات وعلى فترات ١٥ و ٣٠ و ٤٥ و ٦٠ و ٩٠ و ١٢٠ دقيقة. وأستخدم محلول سكر الكلوكوز كمعيار قياسي. أشارت النتائج إلى أن أعلى مؤشر كلوكوزي (٩٦) حصل عندما كان مصدر الكاربوهيدرات في الغذاء هو الأميلوبكتين مقارنة بأقل مؤشر كلوكوزي (٤٤) حصل عندما كان مصدر الكاربوهيدرات في الغذاء هو الحبية. اشارت النتائج أيضا إلى إن إضافة العدس و الحمص و الفاصوليا كمصدر للكاربوهيدرات الى الخبز أدى الى انخفاض المؤشر الكلوكوزي للخبز من ٧٠ إلى ٤٦. في حين كان تأثير ذلك قليلا عند إضافة أحدها إلى الرز. أمكن الأستنتاج أن اعتماد أكثر من مصدر للكلربوهيدرات في الغذاء قد يؤدي إلى تغيير استجابة كلوكوز الدم والموشر الكلوكوزي في الوجبات الغذائية.

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