Journal of Pharmaceutical Research International

32(30): 86-92, 2020; Article no.JPRI.62480
ISSN: 2456-9119
(Past name: British Journal of Pharmaceutical Research, Past ISSN: 2231-2919, NLM ID: 101631759)

Effect of Resistant Starch (RS) Rich Sorghum Food Consumption on Lipids and Glucose Levels of Diabetic Subjects

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Authors' contributions

This work was carried out in collaboration among all authors. Author TVH designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Author EJ managed the tabulation and arrangement of results of the study. Authors TPR and VTS managed the literature searches. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/JPRI/2020/v32i3030909
Editor(s):
(1) Dr. Jongwha Chang, University of Texas, USA.
Reviewers:
(1) Rania Reafaat Abdelkader Atia, Zagazig University, Egypt and Albaha University, Saudi Arabia.
(2) Keiron Audain, University of Zambia, Zambia.
Complete Peer review History: http://www.sdiarticle4.com/review-history/62480

Original Research Article

Received 24 August 2020
Accepted 30 October 2020
Published 27 November 2020

ABSTRACT

Resistant starches are an important class, which gives benefits of fiber without affecting the sensory characteristics. Few studies reported the beneficial effect of resistant starch supplementation in reduction of lipid and glucose levels in diabetic subjects. In the present study we investigated the Resistant Starch (RS) rich millet food on lipid and glucose levels in diabetic subjects. Supplementation of 65 g of RS rich *rawa* (broken sorghum) for 90 days, significantly reduced Body Mass Index (BMI), Fasting Glucose (FG), TC (Total Cholesterol) and LDL-C (Low Density Lipo protein) (p,0.05) in diabetic subjects (n=15), while a non-significant reduction was found in HbA1c, eAG (estimated Average Glucose), TG, HDL-C and VLDL-C. The study indicated that regular consumption of RS rich foods might be beneficial for the diabetic population. Studies in larger population further strengthen the present understanding.

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Keywords: Resistant starch; sorghum; lipids; HbA1c; diabetes.

1. INTRODUCTION

The number of people with diabetes rose from 108 million in 1980 to 422 million in 2014. Almost half of all deaths attributable to high blood glucose occur before the age of 70 years. Diabetes was the seventh leading cause of death in 2016 [1]. In 2019 approximately 463 million adults (20-79 years) were living with diabetes; by 2045 this will rise to 700 million [2]. Diet is an important preventive measure among others and has a significant effect on the prevention of macro and micro vascular complications.

Resistant starch (RS) is the part of starch molecule with its unique property of being resistant to enzymatic digestion and reaching the colon either unaffected or slightly effected. Hence, it is considered as a dietary fiber. There are 5 types of RS; RS1, RS2, RS3, RS4 and RS5 – which are inaccessible to digestive enzymes due to various reasons, among them RS3 is retrograded starch formed when starchy foods (*e.g*. potatoes, pasta) are cooked then cooled. Long-branched chains of amylopectin form double helices that cannot be hydrolyzed by digestive enzymes.

Currently, RS has gained more importance with its positive benefits on human health due to its prebiotic effects, laxation, hypocholesterolemic and hypoglycaemic effects there by reducing the risks of ulcerative colitis and colon cancer; besides its applications in improving the functional properties of foods. Use of resistant starch has been proposed as a probable management strategy for complications of obesity [3]. An RS3-containing bar decreased postprandial blood glucose and could play a role in providing improved metabolic control in type II diabetes (non-insulin dependent) [4]. Resistant starch rich (16%) *rawa* supplementation for 21 days to healthy subjects significantly improved glucose and lipid levels effects on lipid profile and glycemia in healthy individuals [5]. Supplementation of novel resistant starch (RS) made by complexing high-amylose maize starch VII (HA7) with palmitic acid (PA) incorporated bread was found effective in improving postprandial plasma glucose and insulin [6]. RS consumption was found beneficial on the body to prevent and treat obesity through mechanisms including synthesis and secretion of leptin (LP) and adiponectin (ADP) and improvement in intestinal flora in rats. Resistant starch developed in the extracted sorghum starch using cyclic autoclaving and cooling and addition of pullalanase enzyme [7].

Resistant starch is of considerable interest to the food industry as humans can tolerate relatively large amounts without the usual gastrointestinal symptoms, but also relating to the ease of use within food fortification producing high-fibre foods which may be acceptable to the consumer. As there were no studies on sorghum based resistant starch rich product developed through processing, the present investigation was undertaken to study the effect of resistant starch rich RS rich *rawa* on glucose and lipid profiles.

2. MATERIALS AND METHODS

2.1 Production of Resistant Starch (RS) Rich *rawa*

Sorghum grain was used for the preparation of RS rich *rawa*. The whole grain was subjected to thermal and enzymatic treatments to enhance the RS content as per a previous method for enzyme modification of starch and resistant starch production [8]. The process in brief: Dehulled sorghum grain was soaked in water overnight and autoclaved at 120°C for 30 minutes, then cooled at 50°C and 200 units of de-branching enzyme was added and again autoclaved at 95°C for 20 minutes, cooled and stored for 24 hours at 4°C and dried at 40°C to attain moisture content of 2-3%. The process results into de-branching of branched amylopectin and then rearranging into a linear chain and thus resisting the digestion. The resistant starch formation in the grain is due to starch retrogradation and termed as RS3. The grain was then converted into *rawa* in an impact mill. The RS rich rawa and sorghum rawa were subjected for sensory evaluation and found the later was better accepted than the former. The *rawa* thus obtained was packed in polyethylene bags and stored until further use.

2.2 Nutrient Analysis

Moisture, protein, carbohydrate, fat, ash content were estimated using standard methods [9]. Resistant starch was measured using a technique described by Englyst et al. [10] Carbohydrate content was calculated the difference (dry extract – (ash + lipids + proteins) method. Energy value was determined by adding lipid, carbohydrate and protein contents with the formula: (9 × Lipids) + (4 × Carbohydrates) + (4 × Proteins) [11].

2.3 Study Design

A pre and post-study was used. Type 2 diabetic patients (15 no) were selected to study the implications of RS rawa consumption of diabetics. Inclusion criteria; who are on drugs only for diabetes treatment. Age 25-60, BMI- 18-30, Non-smoking, were selected to the study from the selected subjects written consent was obtained. Exclusion criteria; BMI more than 30, kidney and cardiac problems, smoking and alcoholics. The parameters HbA1c, total cholesterol (TC), Triglycerides (TG), High Density Cholesterol (HDL-C), Low Density Cholesterol (LDL-C), Very Low Density Cholesterol (VLDL) were tested at zero day and at the end of 90^{th} day. BMI and FSB were measured at Zero, 30^{th}, 60^{th} and 90^{th} day.

2.4 Supplementation

The study was approved by the Technical Advisory Committee of the University in 2018 (MPIC/2018-01). A group of 20 diabetic subjects were selected with the help of a Medical doctor of the University Health Centre. They were taken informed consent. During the study 5 subjects discontinued due to the personal reasons. A 65 g of RS rich *rawa* was packed in sachets and given at every fortnight and asked to consume daily. Instructions were given to subjects about the cooking methods and in what form it should be taken. They were also informed to replace one of their meals with the RS rich *rawa*. The food was supplied for 90 days.

2.5 Physical and Biochemical Measurements

BMI was calculated using height and weight of the subjects as; Weight (kg)/ [Height (m)] [2] HbA1c and lipid levels were estimated by ion exchange (Fully automated HPLC using Biorad Variant II Turbo, NGSP certified) method. The estimated Average Glucose levels was calculated using the formula; eAG (mg/dl) =28.7 X A1C – 46.7 [12]. Cholesterol [13,14] LDL [15] triglycerides by glycerol phosphate oxidase-phenol amino antipyrine method by using enzymatic kit and HDL cholesterol by cholesterol oxidase/ phenol amino antipyrine method [16] were assessed. VLDL-C, TC/HDL-C, TG/HDL-C, LDL-C/HDL-C and non-HDL-C were calculated from TC.HDL and TG.

2.6 Statistical Analysis

One-way repeated measurement ANOVA was used to test the significant effect of RS rich *rawa* supplementation by comparing pre and post results of all the parameters. Fishers Least Significant Difference was used to find out the difference between the pairs. All the tests were performed by using statistical software, Statgraphic centurion version 19.1.1.

3. RESULTS AND DISCUSSION

3.1 Nutritional Composition

The nutritional composition of the RS rich *rawa* is given in Table 1. The present nutritional value of sorghum was in within the range of the values reported [17,18]. However, there were some differences in the calcium and iron and zinc content, which can be attributable to the varieties used, the location grown, and practices used etc. The resistant starch (RS) contents in 49 sorghum genotypes and the effects of heat treatment using dry and wet heat on the grain and flour from two sorghum genotypes were investigated and reported loss of RS due to treatments. The results showed a wide variation in the RS contents of the genotypes analyzed. The RS mean values were ranged from 0.31 ± 0.33 g/100 g to 65.66 ± 5.46 g/100 g sorghum flour on dry basis. In the present study, heating and cooling cycles were used along with debranching enzyme to enable the formation of retrograded starch [19] which resulted in 38.1 g/100 g of sorghum rawa.

Table 1. Nutritional value of RS rich RS rich *rawa*

Nutrients /100 g	Value
Moisture(g)	5.23
Ash-(g)	2.53
Protein(g)	9.82,
Fat (g)	1.23.
Zinc(mg)	1.55
Iron (mg)	2.67
Calcium (mg)	32.55
Carbohydrates	81.19
Energy (Kcal)	375.11
Resistant starch(g)	38.1

3.2 Physical Measurement

The mean BMI 26.1 of the subjects from base line was gradually reduced at 30, 60 and 90 days, however, significant difference was observed only at 90 days with 2.88 per cent reduction. (P, 0.05) (Fig. 1a). Resistant starch rich *rawa* supplementation for 90 days helped in weight reduction, which is an important contributing factor for overall diabetic management.

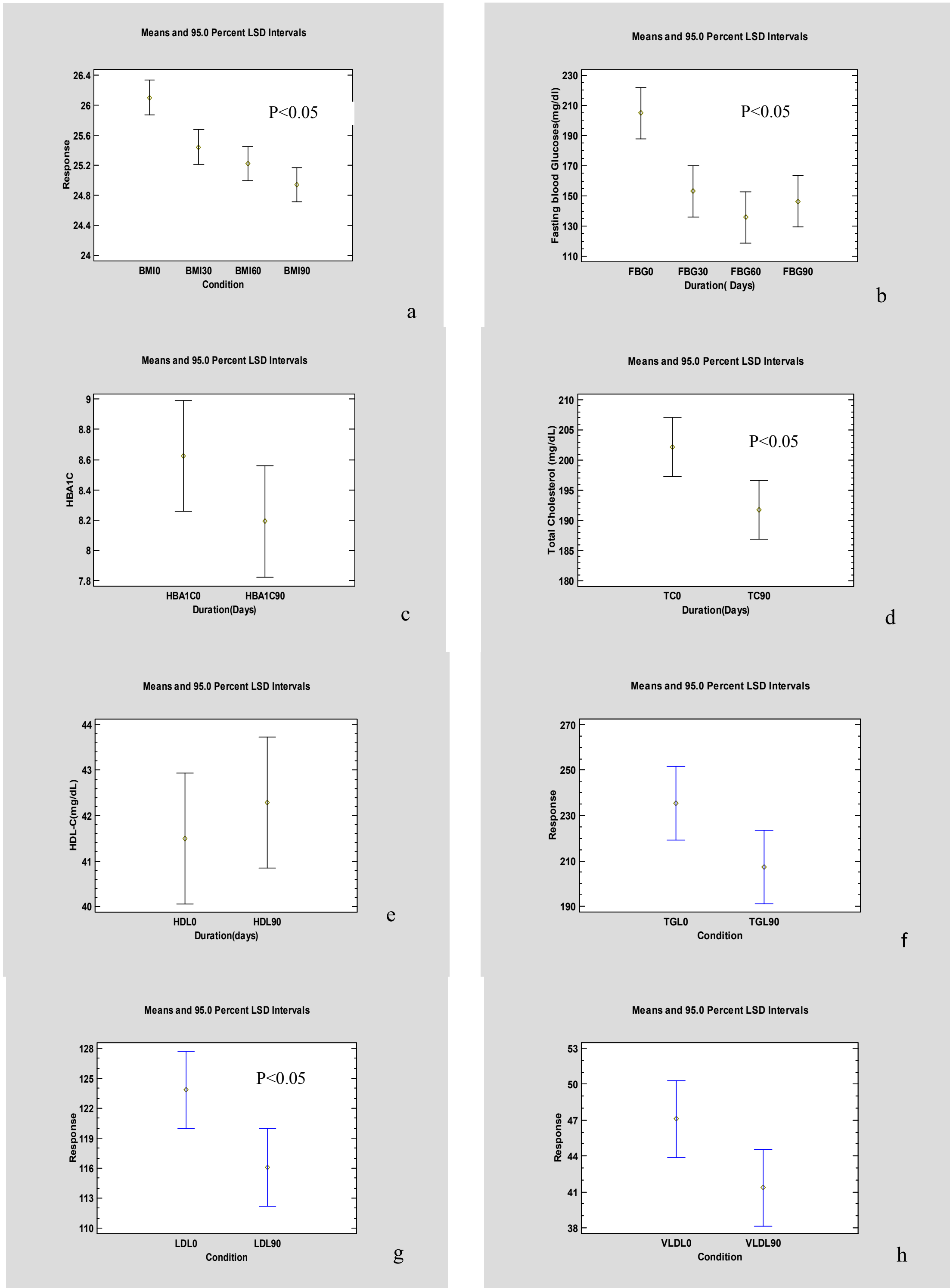

Fig. 1. Effect of RS rich *rawa* supplementation on BMI (a), fasting glucose (b) HbA1c (c) TC (d), HDL-C (e), TG (f), LDL-C (g) and VLDL-C (h)

Table 2. Effect of RS rich RS rich *rawa* on ratios of lipid fractions and eAG

Duration (days)	T-C/ HDL-C	TG/ HDL-C	LDL-C/HDL-C	Non-HDL-C	eAG(mg/dl)
0	4.86	5.67	2.98	160.6	200.06
90	4.54	4.91	2.75	149.5	188.35
Difference	0.32	0.76	0.23	11.10	11.71

3.3 Glucose Profile

Fasting Blood Glucose (FBG) was also significantly reduced during the study from baseline 205 mg/dl to 153.0 mg/dl (30 days). A further decrease was observed from 30 days to 60 days (153.0 to 133.2 mg/dl) and an increase to 146 g/dl at 90 days which was not statistically significant (p, 0.05) (Fig 1b). A non-significant reduction was observed in HbA1c from 8.62 to 8.19 percent (0.6% reduction) (Fig. 1.c) A higher reduction was observed with consumption of 80 g of foxtail millet diabetic diet by diabetic volunteers in HbA1c (19.14%) and fasting glucose (13.5%) [20]. This can be attributed to the differences in the grain type. Grain sorghum muffins with 50 g of starch found to be effective in controlling glucose levels in prediabetics [21]. HbA1c levels plays a greater role in maintaining the balance between hypoglycemia and hyperglycemia state which is more important to the diabetic individual to monitor regularly to avoid the further complications associated with the diabetes. The base line estimated average glucose of 200.06 (mg/dl) was reduced to 188.35 (5.85% reduction) (Table 2). The estimated average glucose (eAG) converts the diabetic patient's HbA_1c percentage point into an average blood glucose level in the units of measure seen by the patient on glucose meters for daily self-monitoring (mg/dL). Glycated or glycosylated hemoglobin (HbA_1c) levels have been used in planning and assessing the management of diabetic patients for the past couple of decades. Clinical trials have established the correlation between HbA_{1c} and the development of diabetes complications and patient outcomes [22]. In the present study there is a reduction in 11.71 mg/dl from 0^{th} day to 90^{th} day, indicating some beneficial effect of RS food on glucose concentration.

3.4 Lipid Profile

The lipid fractions of the subjects before (0 day) and after the study (90 day) is presented in Fig. 1 (d, e, f, g and h). Among the lipid parameters TC and LDL significantly (p, 0.05) reduced during the study from 202.1 to 191.7 mg/dl and 123.8 to 116.1 mg/dl respectively. A non-significant reduction was observed in HDL-C (1.3%), TG (5.2%) and VLDL-C (3.2%). The TC/ HDL-C ratio, TG/ HDL-C ratio and LDL-C/HDL-C changed from 4.86, 5.67 and 2.98 to 4.54, 4.91 and 2.75 respectively (Table 2). Higher reduction was observed in the TG: HDL-C ratio than other ratios. The non-HDL-C also reduced by 11.1 units during the study, indicating a positive effect of RS food on the lipid levels in the blood. TG/HDL-C ratio ≥2.5 was strongly associated with an increased risk of long-term major adverse cardiac event. (MACE) [23]. The LDL-C/HDL-C ratio is especially accurate at predicting risk among those who also had elevated triglyceride levels [24].

Diet certainly effects the lipid levels at different levels depending upon the presence of lipid lowering compounds such as dietary fiber, antioxidants etc. In a previous study, supplementation of millet based diet for 90 days, reduced total cholesterol, triglyceride and very-low-density lipoprotein cholesterol concentrations by 13.25, 13.51 and 4.5% respectively in the patients with type 2 diabetes [20]. A significant reduction was seen in the case of serum cholesterol (4.41%), serum LDL (11.22%), serum triglycerides (5.11%) and VLDL (4.74%). Serum HDL was significantly increased by 14.98% with finger millet product supplementation [25] Consumption of 80 g of foxtail millet food for 90 days resulted in reduction of total cholesterol triglyceride and very-low density lipoprotein concentrations by 13.25 and 13.51 4.5 percent respectively [20].

Diabetics exhibit a typical pattern of lipoproteins, known as diabetic dyslipidemia or atherogenic dyslipidemia, consists of moderate elevation in triglyceride levels, low HDL cholesterol values, and small dense LDL particles. This lipoprotein pattern is associated with insulin resistance and is present even before the onset of diabetes. LDL cholesterol levels in type 2 diabetic subjects are generally similar to those found in the general population. However, they are highly atherogenic because of their enhanced susceptibility to oxidative modification and increased uptake by the arterial wall. At triglyceride levels > 132 mg/dl, the occurrence of small LDL particles becomes common. On the whole, 30-40% of patients with diabetes have triglyceride levels > 200 mg/dl and 10% have triglycerides > 400 mg/dl [26]. American heart association and American College of Cardiology guidelines stated that LDL cholesterol, HDL cholesterol, and triglyceride levels as < 100, > 40 in men/> 50 in women, and < 150 mg/dl as goals for reducing risk of progression in to CVD in diabetic individuals, moreover the primary treatment goal should emphasis on reducing LDL- C proceeding to secondary goal as uprisal of HDL-C and third as triglyceride lowering [27].

To achieve desirable changes in the other components of lipid profile with the consumption current RS rich *rawa* might require longer duration of supplementation, however as the lipid levels were not adversely effected the RS rich *rawa* consumption can be helpful for the diabetic population.

4. CONCLUSION

This study revealed that consumption of sensorially accepted RS rich food significantly improved fasting glucose, total cholesterol and low-density lipoproteins. However, other fractions of lipids, lipid ratios and HbA1c concentration exhibited non-significant improvements, without any adverse effects. Hence from this study it can be concluded that regular consumption of RS rich *rawa* not only provides the abundant nutrients but also health benefits for diabetic population. The food might also be helpful for the prediabetics for prolonging the time to reach diabetic stage.

CONSENT AND ETHICAL APPROVAL

As per university standard guideline participant consent and ethical approval has been collected and preserved by the authors.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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Peer-review history:
The peer review history for this paper can be accessed here:
http://www.sdiarticle4.com/review-history/62480