American Journal of Food and Nutrition, 2019, Vol. 7, No. 4, 158-165
Available online at http://pubs.sciepub.com/ajfn/7/4/6
Published by Science and Education Publishing
DOI:10.12691/ajfn-7-4-6

Development of Low Glycemic Index Rice and Its Effectiveness in the Regulation of Postprandial Glucose Response in Type 2 Diabetes

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Received November 11, 2019; Revised December 18, 2019; Accepted December 22, 2019

Abstract **Background/Objective:** To determine the glycemic index (GI) of RNR15048 rice variety and study its effectiveness in reducing postprandial blood glucose levels and the regulation of lipid profile in patients with type 2 diabetes. **Subjects/Methods:** The GI of RNR15048 rice was measured in 54 healthy subjects in the age group of 30-50 years. The dietary intervention study was conducted in 80 subjects with diabetes in the age group of 40-60 years where 40 test subjects replaced their regular rice with RNR 15048 rice variety for 3 months. Anthropometric and biochemical parameters, HbA1c, fasting blood glucose, lipid profile, insulin were determined before and after the study. **Results:** The glycemic index (GI) of RNR 15048 rice variety was observed to be low with GI of 51.72 ± 3.39. The dietary intervention study in type 2 diabetic subjects revealed significant decrease in fasting blood glucose (158.4 ± 9.30 vs 140.2 ± 8.87 mg/dL) and HbA1c levels (7.1 ± 0.34 vs 6.1 ± 0.33 %) with an increase in HDL levels (33.7 ± 1.29 vs 37.1 ± 1.83 mg/dL) while parameters such as body weight, blood pressure and insulin levels did not show any significant changes. **Conclusion:** Low GI rice is effective in the reduction of postprandial glucose response in type 2 diabetes and increase in plasma HDL levels and therefore useful in the management of type 2 diabetes and in the long term management of cardiovascular diseases.

Keywords: *blood glucose, glycemic index, glycosylated haemoglobin, low GI rice*

Cite This Article: Prasanthi Prabhakaran Sobhana, Jeyakumar Shanmugam Murugaiha, Panda Hrusikesh, Srinivas Epparapalli, and Damayanti Korrapati, “Development of Low Glycemic Index Rice and Its Effectiveness in the Regulation of Postprandial Glucose Response in Type 2 Diabetes.” *American Journal of Food and Nutrition*, vol. 7, no. 4 (2019): 158-165. doi: 10.12691/ajfn-7-4-6.

1. Introduction

Rice is one of the major staple foods consumed in most of the Asian countries and this contributes to the dietary glycemic load in Asian population. Diets which are higher in glycemic load implicated the development of various metabolic and chronic diseases such as diabetes and various cardiovascular diseases. Several studies have reported that higher intake of rice is strongly associated with type 2 diabetes [1,2,3]. Systematic reviews from various parts of the world have shown that the GI of various varieties of rice ranged between 48 to 93 and these variations in the GI are attributed to factors such as inherent starch characteristics (amylose:amylopectin ratio and rice cultivar); different physicochemical characteristics (morphology of the grain, water absorption, dietary fibre and macronutrient content); [4] post-harvest processing (particularly parboiling); and consumer processing (cooking, storage and reheating) [5,6]. Studies have also reported the beneficial effects on blood glucose responses upon the consumption of low-GI meals in healthy individuals as well as individuals with diabetes. Foods are categorised as low-GI (≤ 55), medium- GI (56-69), high-GI (≥70), based on their ability to raise blood glucose. The present study was aimed to check the GI of RNR15048 (Telangana sona), having low GI and its impact on HbA1c, when it replaced the regularly consumed rice in diabetic subjects.

2. Subjects and Methods

2.1. Study Approval

Human ethical approval was taken from the Institutional ethics committee of National Institute of Nutrition (IEC-NIN), ICMR Hyderabad, India for conducting the study.

2.2. Development of RNR 15048 Rice Variety (*Oryza sativa* L, sub sp. *indica*)

RNR15048 also known as Telangana sona or Chittimallelu was developed by crossing of two rice varieties namely MTU1010 (Female), which had high yielding characteristic and JGL 3855 (Male), which produces quality grain rice. The breeding method involved was pedigree method by which RNR15048 was developed with characteristics such as fine grain, high yielding quality, blast resistance, short duration for vegetative growth and suitable for cultivation in both kharif and rabi seasons. The rice variety produced short slender grains with better cooking qualities, less broken percentage, good head rice recovery (67%) and with high yield potential (~6.5 tonnes/hectare).

2.3. Nutrient Composition

The proximate composition, vitamins, kernel quality and fatty acid profile of RNR 15048 was determined and compared with another common variety BPT 5204. (Source: Release proposal of RNR 15048).

2.4. Morphological Study of Rice Grains by Scanning Electron Microscope (SEM)

The rice grains were studied for the morphological structure and arrangement and compared with other varieties, using SEM. For SEM analysis all the rice grains were cleaned thoroughly to remove extraneous particles and the whole grain was cut in transverse section and mounted on aluminium stub with double sided adhesive tape and coated with gold (600°A) in sputter coating unit E-1010 *(Hitachi Japan*) at high vacuum. It was then scanned, using scanning electron microscope model S3400N *Hitachi Japan* at 15kV. Pictures were taken in different magnifications 50X, 250X, 500X and 1000X.

2.5. Subjects for Oral Glucose Tolerance Test (OGTT) and GI Study

54 healthy volunteers (male and female) in the age group of 30-50 years participated for the initial GI study of RNR15048. They were given reference glucose, white rice and test samples which included cooked RNR 15048 rice along with a curry which was low in carbohydrate with a two day wash out period. The blood glucose response was studied at different time points (0 h, 15, 30, 45, 60, 90, 120 min) similar to that of reference glucose. The study purpose was briefly explained to each participant and their written consent of participation was obtained.

2.6. Subjects for Human Metabolic Study

80 diabetic subjects (male and female),who were in the age group of 40-60 years with history of diabetes for at least one year and who were free from other gastrointestinal disorders, thyroid problems and food allergies were included in the study. They were divided into control (40) and experimental group (40). The test group was provided with the low GI rice variety (RNR 15048). Each subject was given 20 kg rice/month for a period of three months to replace their regularly consumed rice variety. To achieve total compliance not only the subjects but also the entire family was supplied with the low GI rice variety. The control group were not supplied with rice but were instructed to continue with their regular diet pattern with rice as a major cereal.

2.7. Anthropometric Measurements

The heights and weights of the subjects were measured using standard stadiometer with an accuracy of 1 mm and standard weighing balance (SECA) with an accuracy of 100 g. BMI /Quetelet Index were calculated using the formula.

$$\mathbf{BMI}\left(\mathbf{kg}/\mathbf{m^2}\right)=\frac{Weight\left(kg\right)}{\left(\mathbf{Height}\left(\mathbf{m}\right)\right)^2}$$

Blood pressure was checked with fully automated one touch operated Omron HEM-7120.

2.8. Collection of Blood and Analysis of Biochemical Parameters

3mL each blood was collected by venipuncture from the subjects after overnight fasting into labelled BD vacutainer tubes. Fasting blood glucose and glycosylated haemoglobin (HbA1c) were determined by using accu-chek active glucometer and Alere affinion HbA1c kits. Samples were centrifuged at 4000 rpm for 10 min to separate serum/plasma, divided into aliquots and stored at -80°C in labelled vials until analysis. Lipid profile of plasma samples was determined using colorimetric based kits and insulin in serum, using ELISA kit. Friedewald’s formula was used to calculate low density lipoprotein (LDL) and very low density lipoprotein (VLDL).

2.9. GI Study Methodology

The method given by Brouns et al., 2005 was the protocol followed for the study. The participants were given instruction to visit the Institute after overnight (10-12 h) fasting. Details of the subjects were collected using a validated questionnaire which included their food pattern, nature of work; physical activity, health status and medication. Written consent was taken from all the participants who participated in the study. Oral Glucose Tolerance test was conducted for three consecutive days in the subjects prior to initiation of test food.

2.10. Oral Glucose Tolerance Test

Glucose was taken as reference food for the study. 55 g of glucose (Glucon D with 10% moisture) was dissolved in 200 mL of water (containing 50 g of available carbohydrate) and given to the participants for oral glucose tolerance test (OGTT), which was performed for 3 consecutive days. Blood glucose in the samples of the subjects were measured using Accu check active blood monitoring system manufactured by Roche Diabetes care, Germany which contained the test strips and sterile lancets. Initially fasting capillary blood was taken before intake of glucose at 0 h and after consumption of glucose; blood was collected at different time points such as 15, 30, 45, 60, 90,120 min.

2.11. Preparation of Test Food and White Rice

The rice variety RNR 15048 and white rice was cooked with 1 part of rice and 2.5 parts of water in a pressure cooker for 9 minutes. The available carbohydrate in the rice varieties were determined to be 74.6% and 78.2%, so 67 g of raw rice RNR15048 and 64 g of white rice containing 50 g of available carbohydrate was cooked with 168 mL and 160 mL of water respectively. Each participant was given 226 g of cooked rice along with 25 g of curry made with leafy vegetable (Ambat chukka) which contained 0.3% of carbohydrate.

2.12. GI Calculation

The trapezoid rule was used for calculating the incremental area under curve for reference (glucose) and test foods [7]. The following equation was used for the calculation of GI [8].

$$\mathbf{GI} = \frac{IAUC\ of\ \ test\ food}{IAUC\ of\ \ reference\ food} x100.$$

2.13. Statistical Analysis

Data represents mean ± SE. The differences in the GI and IAUC were statistically analyzed by paired t test using SPSS software (SPSS, Inc., Chicago, IL). The effects of age, sex and body mass index on the GI and IAUC were assessed for the test foods. Statistical significance was set at $p < 0.05$.

3. Results

3.1. Nutrient Composition of RNR 15048

The nutrient composition of RNR 15048 in comparison with BPT 5204 is given in Table 1. The protein content of RNR 15048 in both brown rice and medium milled was higher by 0.5% compared to the brown and medium milled BPT 5204 while the fat and fibre content were not different. Further, the niacin content of RNR15048 was significantly higher as compared to BPT 5204, on the other hand no significant differences were observed in the riboflavin content. As per the American Heart Association the ratio of SFA: MUFA: PUFA recommended is 1:1:1 and in our study the rice RNR 15048 showed the ratio of SFA (Palmitic, Stearic acid): MUFA (Oleic acid): PUFA (Linoleic, α-Linolenic acid) as 25.14:39.7:35.16 respectively and the proportions of SFA: MUFA: PUFA being 1:1.58:1.39 while BPT 5204 showed a ratio of 21.88:41.86:36.26 respectively and the proportion being 1:1.91:1.65.

3.2. Scanning Electron Microscopy of Rice Kernels

The Figure 1 and Figure 2 reveals the scanning electron microscopic images of the rice RNR15048 in the perpendicular view and transverse sections of the kernel of the cultivar with the structural differences between RNR 15048 and locally available sona masuri rice. The scanning electron microscopic (SEM) images of the rice kernels of these cultivars in the peripheral view and transverse section are given in Figure 1. The scanning electron microscope studies of rice kernels revealed the structure of full grain and at the middle of cortex, the fractured grain contained starch granules which were packed compactly in para crystalline form within the cellular boundaries of the kernel. The entire cross section of rice kernel looked like an orthorhombic crystal form. The creamy hue of the kernel was due to the paracrystalline nature of starch granules, which were refringent in light; these kernels were graded as good quality rice. Under the electron microscope, normal rice kernel showed that the individual starch granule appeared in compact spherical clusters known as compound granules. The individual starch granules were polygonal in shape, which resulted from pressure against other granules within the compound granule. In test samples, the individual starch granules were not polygonal in shape so cells of granules were loosely fitted and centre region of cells were very freely moving. Figure 2 showed, the starch granules of low GI rice were semi polygonal in shape because of less pressure against other granules. At higher magnifications, the transverse section of all rice showed ultra structural changes in the low GI rice samples as compared to normal rice Figure 1a to Figure 2c.

3.3. Assessment of Glycemic Index of RNR 15048 Rice

The GI of RNR15048 rice was assessed in 54 healthy subjects with mean age of 43.8±9.8 years and BMI of 23.7±3.2 kg/m^2. The GI study of RNR15048 was conducted at two different time points, initially in the year 2016 in 44 healthy individuals, further the GI of the same rice was conducted in 10 healthy individuals in the year 2018. The IAUC and GI for RNR 15048 rice, white rice and reference glucose were determined in 54 healthy subjects and it was observed that RNR15048 rice significantly had lower GI (51.72±3.39) against white rice (73.4±7.79) and the reference glucose with GI of 100. In Figure 3 the blood glucose response of low GI rice is compared with white rice and the reference glucose, thereby indicating decrease of blood glucose concentration due to the consumption of low GI rice.

Table1. Proximate and vitamin composition of RNR 15048 and BPT 5204

Sample	Protein (%)	Fat (%)	Carbohydrates (%)	Energy (Kcal)	Niacin (B3) (mg/100g)	Riboflavin(B2) (mg/100g)
RNR15048(Brown rice)	8.76	2.00	76.89	360.60	4.07	0.45
BPT 5204 (Brown rice)	8.23	2.00	74.15	347.52	3.37	0.50
RNR 15048(Medium milling)	8.08	0.80	79.33	356.84	2.26	0.27
BPT 5204 (Medium milling)	7.65	0.80	80.84	361.16	1.88	0.28

Source: (Release proposal of RNR15048).

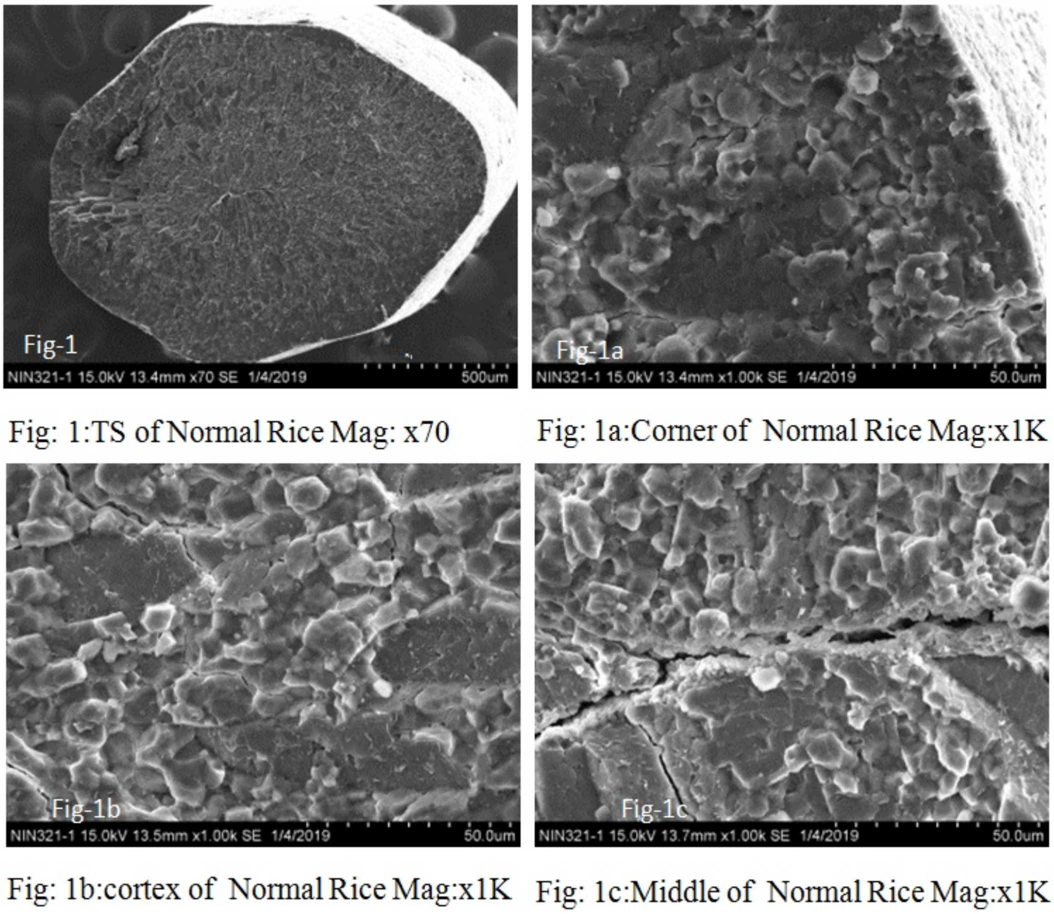

Figure 1. Scanning electron micrographs of normal and low GI rice samples

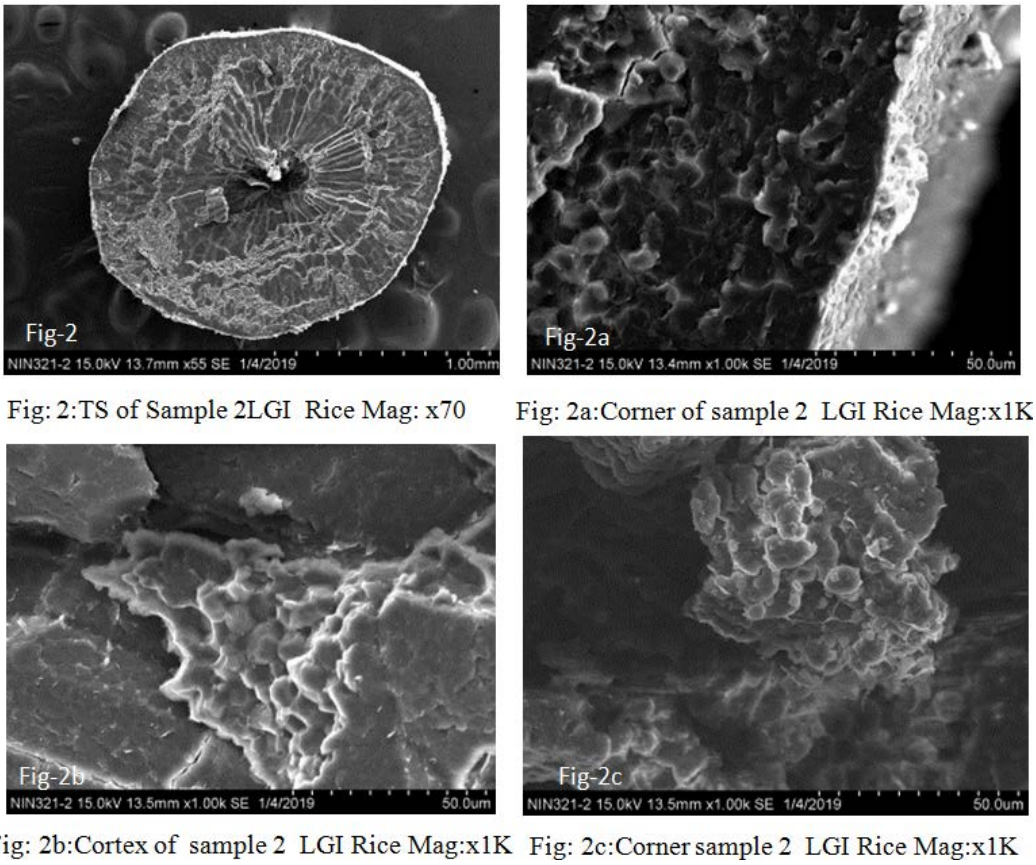

Figure 2. Scanning electron micrographs of normal and low GI rice samples

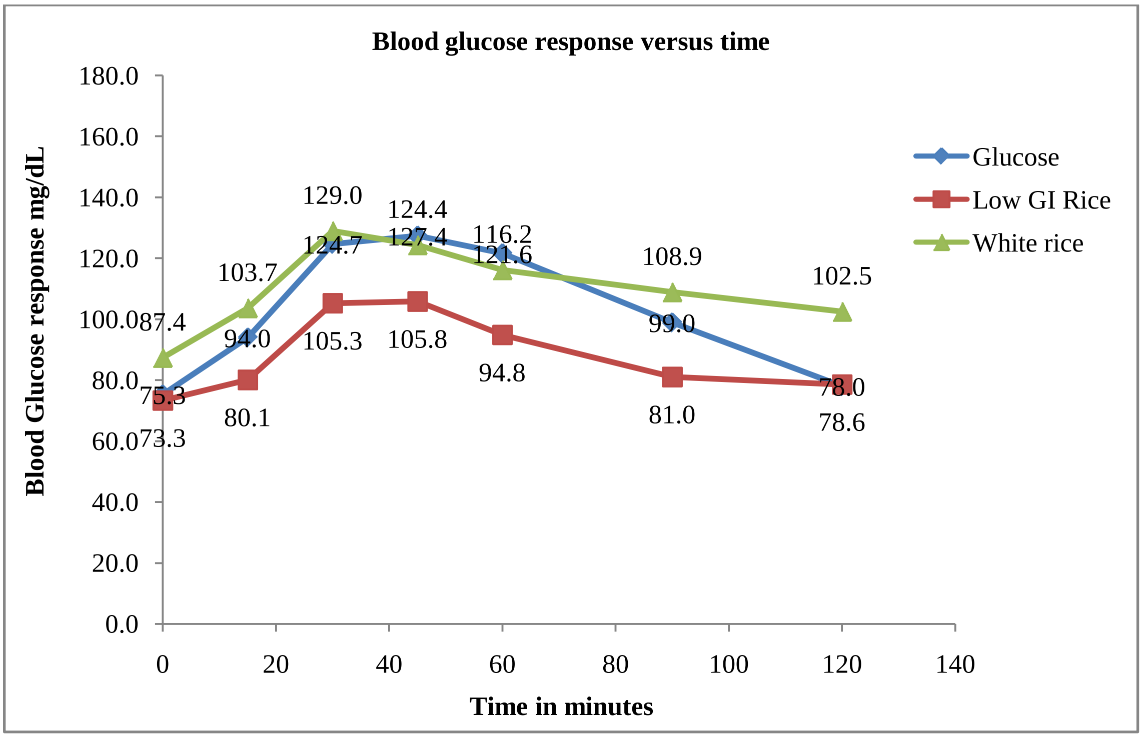

Figure 3. Mean blood glucose response of low- GI rice, white rice and reference food (glucose)

Table 2. Baseline comparison of control and experimental group

Parameter	**Control (n=47)**	**Test (n=47)**	**p value**
Age (years)	50.5 ± 1.08	51.9 ± 1.09	0.38
Height (m)	1.62 ± 0.013	1.63 ± 0.013	0.70
Body weight (kg)	70.98 ± 1.54	70.30 ± 1.69	0.77
BMI (kg/m^2)	26.53 ± 0.45	26.47 ± 0.52	0.93
HbA1c (%)	7.50 ± 0.19	6.95 ± 0.35	0.17

Values are expressed as Mean ± SE. $p \leq 0.05$ considered significant.

Table 3. Impact of low GI rice on anthropometric and biochemical parameters

Parameter	**Initial**	**Final**	**p value**
Anthropometric parameters			
Weight (kg)	70.30 ± 1.69	70.40 ± 1.73	0.560
BMI (kg/m^2)	26.47 ± 0.53	26.49 ± 0.52	0.749
Fat%	32.69 ± 1.43	34.09 ± 1.37	0.000
Blood pressure (mm/Hg)			
Systolic	130 ± 3.52	133 ± 3.74	0.379
Diastolic	86 ± 2.43	85 ± 2.21	0.760
Biochemical parameters			
Fasting Blood Glucose (mg/dL)	158.4 ± 9.30	140.2 ± 8.87	0.010
Fasting Insulin (µIU/mL)	10.72 ± 0.87	11.17 ± 0.89	0.566
HbA1c (%)	7.06 ± 0.34	6.09 ± 0.33	0.002
Lipid profile			
Total cholesterol (mg/dL)	165.1 ± 5.20	168.8 ± 5.53	0.309
Triglycerides (mg/dL)	165.8 ± 17.81	155.24 ± 11.99	0.380
High density lipoprotein (mg/dL)	33.73 ± 1.29	37.06 ± 1.83	0.016
Low density lipoprotein (mg/dL)	98.22 ± 4.84	100.73 ± 4.92	0.372
Very low density lipoprotein(mg/dL)	33.16 ± 3.56	31.05 ± 2.40	0.380

Values are expressed as Mean ± SE. $p \leq 0.05$ considered significant.

Table 4. Summary of comparison between treatments

Physical and plasma biochemical parameters	Control group Mean difference (Initial-Final)	Test group Mean difference (Initial-Final)	Mann Whitney U test (*P* value)
Anthropometric parameters			
Body weight (kg)	-0.07	0.11	0.95
BMI (kg/m^2)	-0.38	0.21	0.92
Blood pressure (mm/Hg) Systolic	-0.89	2.82	0.43
Blood pressure (mm/Hg) Diastolic	1.79	-0.55	0.35
Biochemical parameters			
Fasting Blood Glucose (mg/dL)	3.73	-18.21	0.009
Fasting Insulin (µIU/mL)	-1.15	0.45	0.36
HbA1c (%)	-0.07	-0.97	0.012
Lipid profile			
Total cholesterol (mg/dL)	1.04	3.73	0.35
Triglycerides (mg/dL)	28.2	-10.56	0.008
High density lipoprotein (mg/dL)	2.81	3.33	0.69
Low density lipoprotein (mg/dL)	-6.78	2.52	0.006
Very low density lipoprotein (mg/dL)	6.74	-2.11	0.006

Means differences (n=40) between final and initial measurements (Delta value) of control and treatment group was subjected to nonparametric 2-independent Mann Whitney U Test.

3.4. Human Metabolic Study

The clinical intervention study was conducted in 80 diabetic subjects with 40 in the control group and 40 in the experimental group and the baseline comparison between control and test group is given in Table 2 which showed no significant difference between control and test group subjects. However, in the experimental group, the low-GI rice RNR15048 replaced their regular rice and its impact on anthropometric and biochemical parameters was assessed. The initial and final anthropometric measurements, blood glucose, plasma glycosylated haemoglobin, total cholesterol, HDL cholesterol and triglyceride concentrations of 40 diabetic subjects are given in the Table 3 and it revealed significant decrease in the HbA1c and fasting blood glucose concentration ($p < 0.05$) and significant increase in plasma HDL cholesterol concentration after consumption of RNR15048 rice for a period of 3 months. No significant changes were observed in the body weights or BMI and blood pressure except fat% increased significantly at the end of 3 months after consumption of low GI rice. In Table 4, the summary of the comparison of final and initial (Delta values) of the control and experimental group is given.

4. Discussion

In this study, RNR15048 (Telangana sona) was tested and found to be having low GI (51.72±22.50), which upon regular consumption for a period of 3 months reduced glycosylated haemoglobin (HbA1c) levels in diabetic subjects thereby suggesting its potential in the maintenance of blood glucose. The GI value and the digestibility of foods are known to depend on various factors such as the presence of high dietary fibre [9], processing techniques [10], physicochemical and cooking properties such as alkali spreading value and gel consistency [11], nutritional factors, cooking time, the presence of macronutrients and the ratio of amylose and amylopectin [12,13,14]. Hence, some of these factors may be responsible for the low GI of RNR15048 rice variety. Various studies as given in the International GI table [15] have reported that the GI for brown rice ranged from 45 to 87, while some studies have reported a GI ranging from 54 -121. Studies conducted in Japan with a short grain rice Japonica showed moderate GI of 68 [16] while in another study [17] eleven rice varieties have been reported to be of high GI and one was moderate GI rice and the reason for these variations in the GI could be due to the various factors mentioned above. In the present study, the GI of the rice variety RNR 15048 was observed to be lower, which could be attributed to the variations in the chemical nature of the starchy polysaccharides-amylose and amylopectin, structural pattern, cooking quality etc. Studies have proven that higher amylose fractions (28%) results in lower GI in rice [18] which in turn produced lower blood glucose and insulin response however contrary to this, in the present study despite lower amylose content (20.72 %) of the rice RNR 15048, the GI was found to be low. The consumption of rice with high amylose content reduced the rate of digestion which could be due to the formation of amylose-lipid complexes thus reducing the susceptibility to enzyme action and slowing the digestion rate [19]. Most of the rice varieties that are generally consumed contained 20% amylose and 80% amylopectin, which was also true for the low GI rice in the present study, hence there could be other reasons for the low GI of RNR 15048. Thermal treatment is also known to lower the GI [20], especially in parboiled rice where retro gradation leads to the formation of resistant starch which in turn elicits lower glycemic responses [21]. Studies have suggested that low GI foods improve blood glucose responses by maintaining glycemic control. The process of gelatinisation is also known to influence the GI value of rice. The GI value is reported to be higher, when the degree of gelatinisation is higher, which in turn depends on factors such as the cooking temperature, time, volume of water and the presence of sugar, acid, protein and fat [22]. It has been reported that the digestion and absorption of food can be delayed if the viscosity of soluble fiber is higher [23]. In the present study the supplementation of the low GI rice showed reduction in the glycemic response thereby suggesting that by altering the glycemic index and the glycemic load in one meal, the overall food consumption in the subsequent meal also can be reduced [24,25] and the quality of carbohydrate consumed can be improved [26]. The SEM analysis of our low GI rice clearly showed differences in the structural arrangement of starch granules when compared with the normal rice, indicating that the rate of digestibility and the response to glucose is affected by the structure of the grain as reported in previous studies [27]. Further, some studies have also reported that the structural properties of rice and the type of dietary fibre had profound effects on lowering the blood glucose levels [27].

In conclusion, it can be reported from the study that the low-GI rice RNR15048 can be a useful therapeutic diet that helps in the reduction of blood glucose and elevation in plasma HDL cholesterol levels in patients with Type-2 diabetes.

Acknowledgments

The authors wish to thank the Director Dr.R.Hemalatha, National Institute of Nutrition, ICMR for her support in conducting the study. The authors also acknowledge Mr.Madhusudanachary for the microscopic study using SEM, Mr Shaik Nasar Vali and Mr.M.Srinivas for their support during blood drawing and gratefully acknowledge the kind cooperation of the participants of this study.

Disclosure

The authors have no financial or personal relationships that could pose a conflict of interest and declare no potential conflict of interest.

Author Contributions

PPS-conducted the study, extracted, analysed, compiled results and drafted the report and manuscript; JSM-interpreted the data on biochemical parameters; PH-conducted the study and collected the data; SE-conducted the study; DK-conceived, designed and supervised the complete clinical trial, reviewed protocol and screened potentially eligible studies, contributed to the data extraction, statistical analysis, interpretation and finalisation of the report and manuscript.

Highlights

- The study demonstrated the development of RNR15048 rice and determination of GI in healthy participants which is effective in the management of type 2 diabetes.
- The human intervention study in type 2 diabetic subjects with low GI rice RNR15048 showed clinically and statistically significant reduction in HbA1c and fasting blood glucose levels and elevated HDL cholesterol levels.
- RNR15048 may be used as an alternative for regulating glucose levels in type 2 diabetic patients.

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