Mela et al., Effect of mulberry fruit extract on post-prandial glycemic and insulinemic responses to different rice types

SUPPLEMENTARY MATERIAL

Table S1. Inclusion and exclusion criteria.

Subjects who met the following criteria could be included in the study:

1. Willing and able to give consent to participate in the study in writing
2. Age >20 and <50 yr
3. Body Mass Index (BMI) ≥18.5 and <25 kg/m^2
4. Volunteer apparently healthy [No medical conditions which might affect study measurement, as judged by study physician or measured by questionnaire, and/or assessed by haematology, blood chemistry and urinalysis]
5. Willing to comply to study protocol
6. Agree to be informed by study physician about medically-relevant personal test results
7. Willing to refrain from drinking alcohol the day of and one day before blood withdrawals
8. Fasting blood glucose value >3.4 and <6.1 mmol/ litre (62-110 mg/dl)
9. Haemoglobin level within normal reference range as judged by the research physician
10. Literate

Subjects who met any of the following criteria were excluded from the study:

1. Employee of Unilever, Hindustan Unilever Limited, or Lambda Therapeutics Research
2. Smoking or consumption of tobacco in any form, and/or was smoking or consuming tobacco in any form within 6 months preceding the study and/or smoking or consuming tobacco in any form, during the study
3. Participated in any other biomedical study within 3 months before screening visit day for this study and/or participating in any other biomedical study during the study period
4. Alcohol intake > 120 ml/week
5. On a medically prescribed/slimming diet
6. Work in night shifts (between 23.00 and 6.00 hrs) in the week preceding or during the study
7. Use of any medication including traditional medicines, vitamins or tonics which might interfere with study measurements, as judged by the PI and/or study physician?
8. Engaging in intense exercise >10h/week (defined as exercise which induces sweating and causes sufficient breathlessness to limit conversation)
9. Reported weight loss/gain >10% of body weight in the 6 months preceding screening
10. Blood donation within 2 months prior to screening visit
11. Evidence of drug abuse based on urine analysis
12. Allergy to any food or cosmetics
13. Pregnant or planning pregnancy during the study period
14. Lactating or has been lactating within 6 weeks before pre-study investigation and/or during the study period

Selection, characteristics and preparation of test products

A systematic review of the literature showed that the main factors responsible for variation in post-prandial glucose responses to different rice types are amylose content, cultivar, and post-harvest processing and preparation.[1] In order to identify a limited set of rice types representative of a wider range of commonly consumed rices, 15 rice types commercially available on the Dutch and Indian market (Table S2) were selected based on a range of amylose content, cultivar, grain size and shape, and post-harvest processing.

Table S2. Rice types evaluated for possible use in trial.

Rice type	Origin	Grain size	Grain shape	Cultivar	Post-harvest processing
Pandan/Thai Jasmine	India	Long	Slender	Aromatic	Polished
Ketan/ Sanpathyong	Thailand	Long	Medium	Glutinous	Polished
Bora Saul	India	Medium	Medium	Glutinous	Polished
Sushi	Japan	Medium	Medium	Japonica	Polished
Arborio risotto	Italy	Medium	Bold	Arborio	Polished
Minikit	India	Long	Slender	Aromatic	Polished, Parboiled
Gobindobogh	India	Short	Medium	Indica	Polished
Basmati	India	Extra Long	Slender	Aromatic	Polished
Sanam	India	Short	Medium	Indica	Polished
Banskati (Banskarthi)	India	Long	Slender	Aromatic	Polished, Parboiled
Sona Masoori	India	Short	Medium	Indica	Polished
Idly	India	Short	Bold	Indica	Polished
Doodeshwar	India	Medium	Slender	Indica	Polished, Parboiled
Ambemohar	India	Short	Medium	Indica	Polished
Navara (Njavara)	India	Short	Medium	Indica	Unpolished

Measurements were made of relevant physical and chemical properties of the candidate rice varieties, and cluster analysis and biplots were used to visualize how the different rice variants are positioned with respect to each other in the multi-dimensional space defined by their properties. These approaches showed that Sona Masoori (SM), Bora Saul (BS), Gobindobogh (Gb), and Banskati (Bn) were classified into different clusters and were also well separated along the dimensions describing most of the variability. Therefore, the BS, Gb and Bn rice types were selected for use in clinical testing, in addition to the SM variety previously used.

The statistical procedures were carried out using data on starch digestibility (RDS, SDS, RS)[2] and chemical analyses (amylose, moisture and total starch contents) of the cooked rice varieties. The result of the Cluster Analysis is shown in Figure S1, which illustrates the separation of the final selected varieties in different clusters.

Figure S1. Cluster Analysis based on characteristics of cooked rice varieties. Rice types selected for the trial are highlighted in boxes.

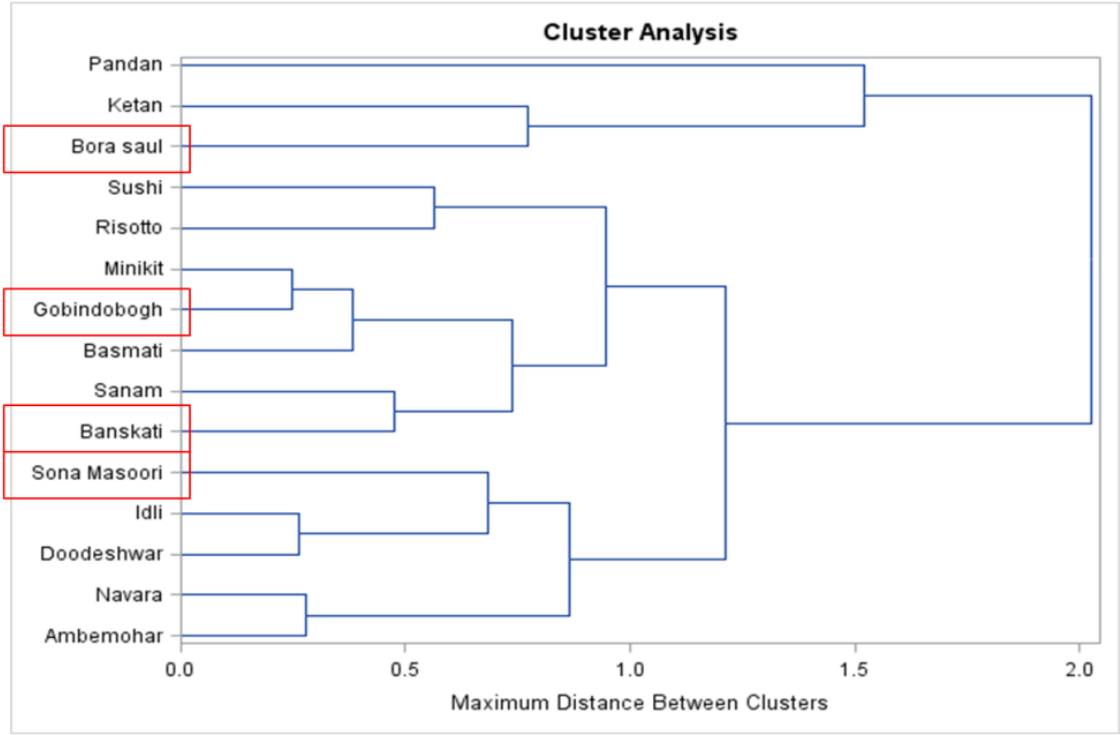


Table S3. Amount and macronutrient composition rice used to prepare one serving of each rice type.

Rice type	Uncooked rice per serving, g	Macronutrients per serving, g			
		Carbohydrate	Fat	Protein	Fibre
SM	64	49.9	<0.5	6	<0.5
BS	60	49.8	<0.5	4	<0.5
Gb	62	50.3	<0.5	5	<0.5
Bn	62	50.3	<0.5	4	<0.5

Blood collection, handling and analyses

On test days, an intravenous indwelling cannula was inserted in a forearm vein of the participants and 0.5 ml of normal saline solution injected to maintain the cannula patent for blood collection. After discarding the first 0.5 ml of normal saline containing blood from the tubing, 5 ml blood samples were collected into the syringe. Alternatively, if the cannula was blocked or there was difficulty in withdrawing blood through the cannula, blood samples could be withdrawn by a fresh vein puncture using a disposable sterile syringe and a needle at each time of collection.

From a 5 ml venous blood sample at each time point, a 3 ml aliquot was transferred to a tube with a clotting activator for measurement of serum insulin, and 2 ml into sodium fluoride tubes for plasma glucose. The blood samples for insulin and glucose were kept at room temperature or wet ice box, respectively, for no more than 45 min, and then centrifuged at 2500-3000 rpm for 10 minutes at ambient temperature. Proper clot formation was ensured before centrifugation for serum separation. Duplicate aliquots of plasma and serum were prepared for each endpoint and transferred within 15 min of separation for analysis (plasma glucose) or storage (serum samples), using gel packs to cool samples during transfer. The two aliquots of serum for insulin were stored at -20 °C for later analysis, along with one aliquot of plasma for re-analysis if required.

Glucose was measured in plasma using the glucose oxidase-peroxidase method and reflectance photometry (Vitros 5.1 FS chemistry platform, Ortho Clinical Diagnostics, Raritan NJ, USA). Insulin was measured in serum using an immunoassay (Roche cobas e411, Roche Diagnostics GmbH, Mannheim, Germany). The inter- and intra-assay coefficients of variation for glucose were 3.8 and 6.6%, respectively, and the corresponding values for insulin were 0.4 and 1.3%.

Figure S2. Consolidated Standards of Reporting Trials (CONSORT) subject flow diagram.

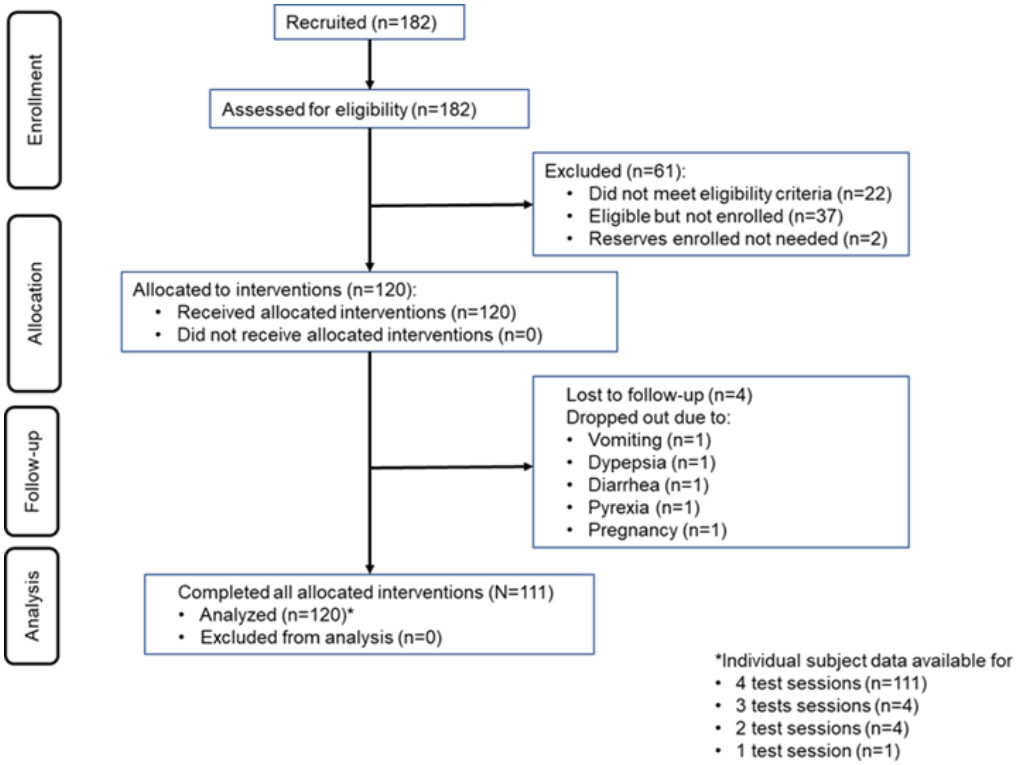


Table S4: Number and baseline characteristics of subjects randomized to and completing each specific treatment, for control (no addition) or with addition of mulberry fruit extract (+ MFE) to Sona Masoori (SM), Bora Saul (BS), Gobindobogh (Gb), and Banskati (Bn) rice types.

Treatment	Randomized, N	Completing treatment		
		N	Mean (SD) age, yr	Mean (SD) BMI, kg/m^2
SM control	60	56	36.3 (9.2)	22.5 (2.3)
SM + MFE	60	54	35.9 (9.2)	22.4 (2.3)
BS control	60	59	36.7 (8.5)	22.5 (2.2)
BS + MFE	60	60	36.4 (8.5)	22.5 (2.2)
Gb control	60	58	37.7 (8.3)	22.2 (2.1)
Gb + MFE	60	57	37.8 (8.2)	22.2 (2.2)
Bn control	60	55	38.4 (8.2)	22.3 (2.1)
Bn + MFE	60	57	38.4 (8.2)	22.3 (2.1)

Figure S3. Mean blood glucose responses to four rice varieties with and without the addition of 0.37 g mulberry fruit extract (MFE). ●-● Control; ○-○ Rice + MFE.

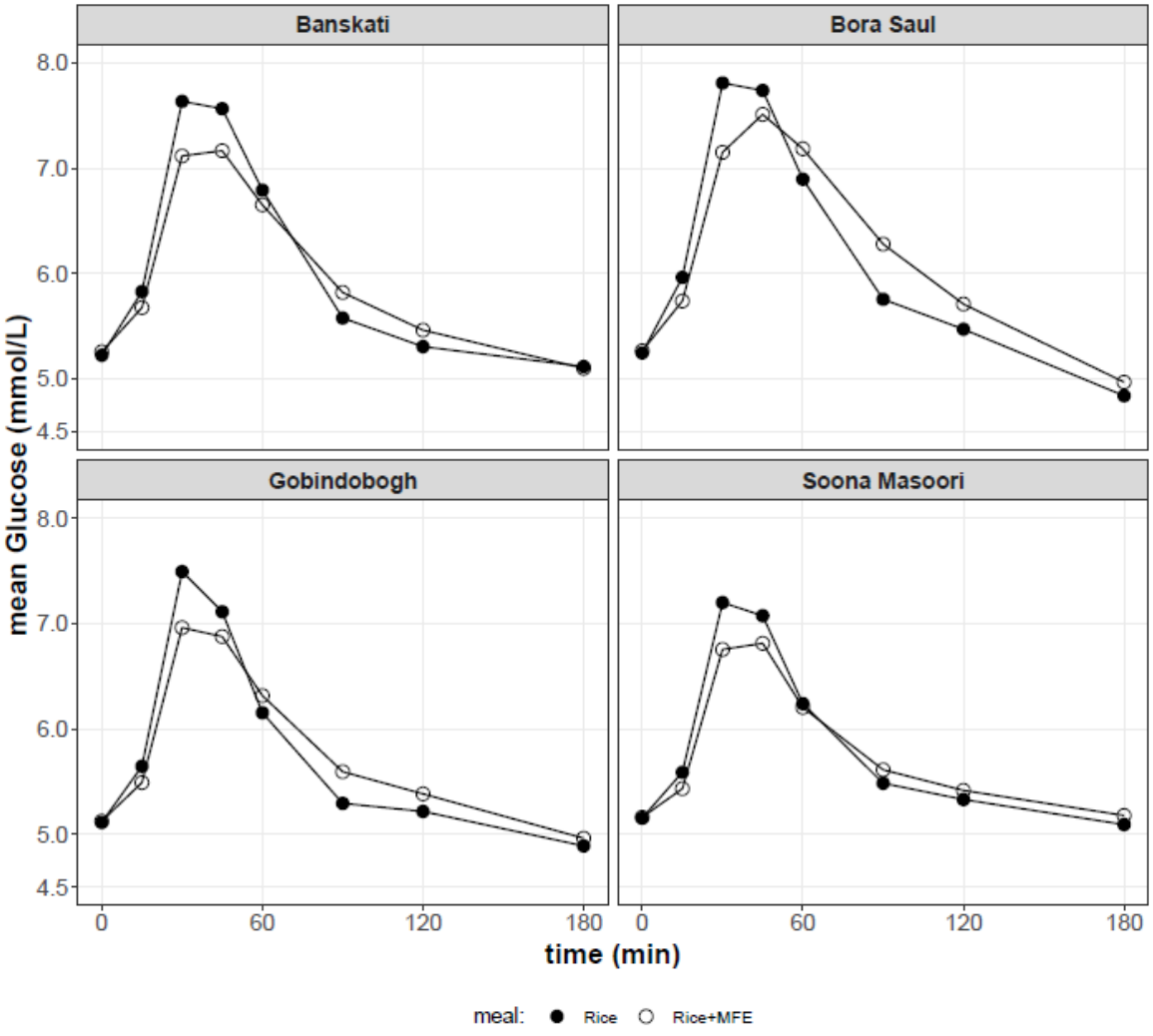
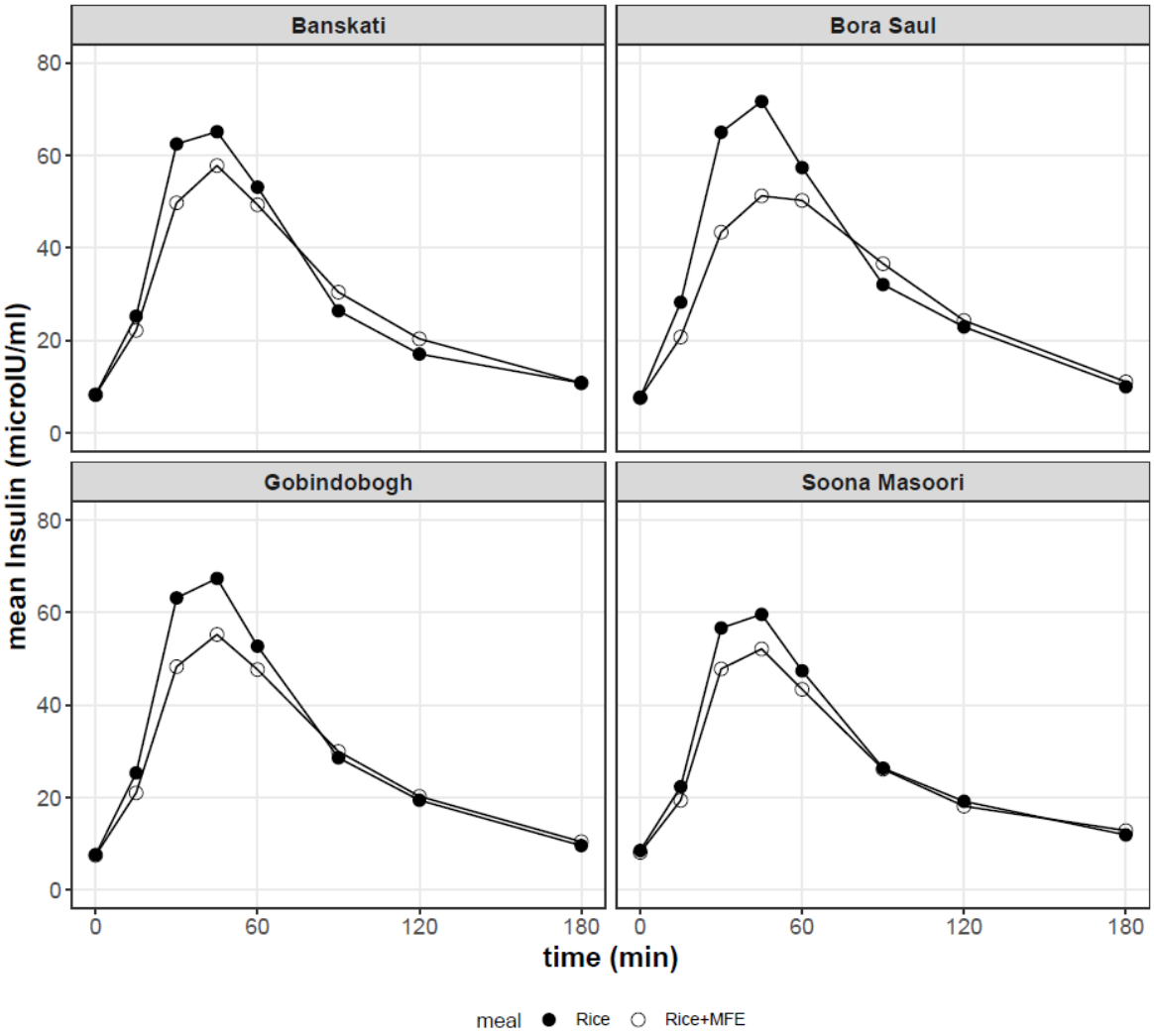


Figure S4 (A-D). Mean serum insulin responses to four rice varieties with and without the addition of 0.37 g mulberry fruit extract (MFE). ●-● Control; ○-○ Rice + MFE.



EXPLORATORY OUTCOMES

Table S5. Baseline-adjusted values for plasma glucose positive incremental area under the curve response over 3 hours (+$iAUC_{3hr}$) following addition of mulberry fruit extract (MFE) to Sona Masoori (SM), Bora Saul (BS), Gobindobogh (Gb), and Banskati (Bn) rice types and all types combined, relative to control.

Treatment	N	Mean +$iAUC_{3hr}$ (lower, upper 95% CI), min.mmol/L	% Difference, MFE vs control	
			Mean (lower, upper 95% CI)	p-value
All types combined control	228	117.1 (104.6, 131.0)		
All types combined + MFE	228	105.5 (93.7, 118.9)	-9.9 (-14.4, -5.1)	0.010
SM control	56	104.5 (90.4, 120.9)		
SM + MFE	54	95.4 (80.8, 112.8)	-8.7 (-21.9, 6.8)	0.253
BS control	59	141.2 (122.5, 162.8)		
BS + MFE	60	130.0 (110.7, 152.8)	-7.9 (-20.6, 6.9)	0.277
Gb control	58	114.4 (99.1, 132.0)		
Gb + MFE	57	103.1 (87.5, 121.6)	-9.9 (-22.6, 5.0)	0.181
Bn control	55	110.2 (95.3, 127.5)		
Bn + MFE	57	95.6 (81.1, 112.6)	-13.3 (-25.7, 1.1)	0.069

Table S6. Baseline-adjusted values for serum insulin total area under the curve response over 3 hours ($tAUC_{3hr}$) following addition of mulberry fruit extract (MFE) to Sona Masoori (SM), Bora Saul (BS), Gobindobogh (Gb), and Banskati (Bn) rice types and all types combined, relative to control.

Treatment	N	Mean $tAUC_{3hr}$ (lower, upper 95% CI), (min.microU/L)	% Difference, MFE vs control	
			Mean (lower, upper 95% CI)	p-value
All types combined control	221	4927 (4640, 5232)		
All types combined + MFE	220	4555 (4289, 4837)	-7.6 (-10.2, -4.9)	<0.001
SM control	55	4559 (4203, 4944)		
SM + MFE	51	4260 (3921, 4629)	-6.5 (-14.1, 1.7)	0.116
BS control	58	5717 (5282, 6188)		
BS + MFE	57	5134 (4741, 5561)	-10.2 (-17.2, -2.6)	0.009
Gb control	57	4997 (4613, 5412)		
Gb + MFE	56	4574 (4221, 4957)	-8.5 (-15.6, -0.7)	0.034
Bn control	51	4463 (4109, 4849)		
Bn + MFE	56	4280, (3948, 4639)	-4.1 (-11.9, 4.3)	0.328

Overlap of 95% confidence intervals between fice types

The percentage overlap of confidence intervals (CI) is calculated as (overlap of CI/mean width CI) x 100%. The means of two independent groups of similar size may be statistically significant different when their 95% confidence intervals do not overlap or overlap up to 25-30% (p-value ≤ 0.05).[3; 4] Tables S7 and S8 show a large overlap in the observed CIs, in every case above the threshold of 30%. On this basis, there is no justification to perform formal post-hoc analyses (which clearly will not reject the null hypothesis of equality of the two means at a p-value >0.05).

Table S7: Overlap of confidence intervals from comparisons between Sona Masoori (SM), Bora Saul (BS), Gobindobogh (Gb), and Banskati (Bn) rice types for the primary outcome, percent change in post-prandical glucose incremental area under curve over 2 hours (PPG $iAUC_{2hr}$) following addition of mulberry fruit extract.

Comparison	Difference in mean % change in PPG $iAUC_{2hr}$	Overlap of 95% confidence intervals	Mean width of 95% confidence intervals	% overlap of 95% confidence intervals
SM vs BS	1.0	26.0	27.0	96.5
SM vs Gb	0.2	27.0	27.2	99.4
SM vs Bn	4.3	22.3	26.7	83.7
BS vs Gb	0.8	25.8	26.6	97.0
BS vs Bn	5.3	20.8	26.1	79.7
Gb vs Bn	4.5	21.8	26.3	82.9

Table S8: Overlap of confidence intervals from comparisons between Sona Masoori (SM), Bora Saul (BS), Gobindobogh (Gb), and Banskati (Bn) rice types for the secondary outcome, percent change in post-prandial insulin total area under the curve over 2 hours (PPI $tAUC_{2hr}$).

Comparison	Difference in mean % change in PPI $iAUC_{2hr}$	Overlap of 95% confidence intervals	Mean width of 95% confidence intervals	% overlap of 95% confidence intervals
SM vs BS	4.4	10.9	15.4	71.0
SM vs Gb	1.9	13.8	15.7	88.2
SM vs Bn	2.9	13.5	16.4	82.0
BS vs Gb	2.5	12.3	14.9	82.6
BS vs Bn	7.3	8.2	15.6	52.6
Gb vs Bn	4.8	11.1	15.9	69.8

References

1. Boers HM, Seijen ten Hoorn J, Mela DJ (2015) A systematic review of the influence of rice characteristics and processing methods on postprandial glycaemic and insulinaemic responses. *Br J Nutr* **114**, 1035-1045.

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4. Austin PC, Hux JE (2002) A brief note on overlapping confidence intervals. *J Vasc Surg* **36**, 194-195.