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Acute postprandial gut hormone, leptin, glucose and insulin responses to resistant starch in obese children: a single blind crossover study

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ABSTRACT

Introduction Resistant starch (RS) has beneficial effects on postprandial glucose metabolism in both animals and adults. Hitherto, there have been no studies in children of the acute metabolic and hormonal effects of RS-containing meals.

Objectives We aimed to compare serial plasma glucose, insulin, gut hormone, leptin profiles and satiety scores in obese children after meals containing variable amounts of RS.

Methods This was a single blind, non-randomised, crossover study of 20 obese children aged 10–14 years old without comorbidities. Three test meals containing rice (M1), rice cooked with coconut oil (M2), rice cooked in coconut oil with lentils (M3) were given in sequence after a 12-hour fast . Blood samples were analysed for glucose (PG), insulin, leptin, glucagon-like polypeptide (GLP) 1, ghrelin and peptide YY (PYY) at appropriate times between 0 and 180 min.

Results Meal M2 resulted in significantly lower postprandial glucose values compared with meal M1 (maximal incremental glucose, ΔC_{max}, p<0.05; area under the curve, ΔAUC_{0-3}, p<0.01) and meal M3 (maximal concentration, C_{max}, p<0.01; ΔC_{max}, p<0.001, and ΔAUC_{0-3}p<0.01). M2 also produced lower insulin values compared with M1 (p<0.05). Postprandial ghrelin was significantly higher after M1 compared with M3 (p<0.05). PYY, GLP1 and median satiety scores were not significantly different between the three meals.

Conclusion This study shows that M2, the meal containing RS alone, induced beneficial effects on acute postprandial glucose, insulin and ghrelin concentrations in obese children without diabetes. Acute postprandial satiety scores were not significantly affected by the three meals.

Trial registration number SLCTR/2020/007.

WHAT IS ALREADY KNOWN ON THIS TOPIC

⇒ Resistant starch (RS) improves postprandial plasma glucose and insulin profiles when given to healthy adults.
⇒ Consumption of a combination of RS and protein enhances satiety in them.
⇒ No studies have hitherto been done investigating the effects of RS combined with protein in children with and without diabetes (both obese and non-obese).

WHAT THIS STUDY ADDS

⇒ In obese children, the meal containing RS alone produced acute postprandial glucose, insulin and ghrelin responses which were metabolically advantageous.
⇒ Satiety was unaffected in this acute study when meals containing RS alone were compared to meals with RS and protein.

HOW THIS STUDY MIGHT AFFECT RESEARCH, PRACTICE OR POLICY

⇒ There is a need to examine the effects of RS and protein on postprandial metabolic profiles and satiety in a longer term study in obese children without diabetes.
⇒ The results would provide information about mitigating the adverse metabolic effects of childhood obesity and its prevention.

INTRODUCTION

The increasing prevalence of obesity in children and adolescents is a major cause for concern.[1] In Sri Lanka, the prevalence of childhood obesity is high with some regional variability.[2] Although multiple factors contribute to childhood obesity, lifestyle changes such as high calorie, high carbohydrate diets and reduced physical activity may play a significant role.[3] Serious attempts are currently being made to modify diets to alleviate risk.[4]

One such nutritional component amenable to change is resistant starch (RS). RS is resistant to alpha-amylase digestion and is fermented by the large intestinal microbiome to short chain fatty acids and other metabolites, leading to short-term and long-term metabolic benefits.[4 5] RS provides multiple health benefits including weight loss in subjects with diabetes and impaired glucose tolerance.[5–7] They may also reduce the incidence of large bowel disease.[4] A recent meta-analysis of studies of RS on glucose metabolism, insulin, peptide YY (PYY), glucagon-like peptide 1 (GLP-1) and leptin in adults showed mixed results (partly on account of the mixed populations studied, the variable duration of studies, etc); they produced beneficial effects on glucose and insulin homeostasis and variable results on appetite-related hormones.[8] The short-term response of gut hormones and satiety to RS

Table 1 Details of study subjects

	Males	Females
Number recruited	12	8
Median age (IQR) (years)	12 (10, 12)	12.5 (11.3, 13)
Median waist circumference (IQR) (cm)*	83.5 cm (76.5, 90.5)	91 cm (85.3, 98.25)
Median BMI (IQR) (kg/m^2)*	25.15 kg/m^2 (23.6, 27.3)	27.05 kg/m^2 (24.61, 29.6)
Acanthosis nigricans	12	8

There was no significant difference in median age, waist circumference and BMI in the 20 subjects who took part in this study. All of them had acanthosis nigricans, which is a skin marker of underlying insulin resistance.
*Greater than 90th centile compared with a standard age-matched and sex-matched population.[23 24]
BMI, body mass index.

containing meals has also been examined in adults and showed several beneficial changes.[5 7 9 10] However, the short-term postprandial effects of RS on gut hormones and satiety have not been studied in children.

Lentils, which contain RS, are a common component of Sri Lankan diets and are a rich source of proteins, minerals and vitamins.[11] We chose lentils to enhance the protein content in one of our test meals, as it was a natural, easy to use, readily available method, making these interventions practical and easy to use in domestic situations.

The aims of our study were as follows:

1. Primary aim: to compare plasma glucose, insulin, ghrelin, GLP-1, leptin and PYY responses to test meals with variable RS and protein content in obese children without diabetes, aged between 10 and 14 years.
2. Secondary aim: to compare satiety scores in these subjects following each test meal.

METHODS

Study setting

The study was conducted in the Endocrinology and Diabetes Unit, Lady Ridgeway Hospital for Children, Colombo 8, Sri Lanka, between December 2019 and June 2020. The study was registered in the Sri Lanka Clinical Trials Registry (SLCTR/2020/007) and was funded by Dr Stella de Silva research grant of Sri Lanka College of Paediatricians.

Participants, inclusion and exclusion criteria

Twenty consecutive obese children were recruited after obtaining written informed consent from their parents/guardians (table 1). The sample size was based on a previous study on adults[9] and was done for pragmatic reasons in the absence of previous data from children.

1. *Inclusion criteria*: (1) Children of both sexes; (2) between 10 and 14 years of age; (3) with a body mass index (BMI) of over the 95th centile for age and sex (BMI between +2 and +3 SD, WHO normative data)[12]; (4) normotensive (<95th centile for height, sex and age)[13]; (5) non-diabetic (HbA1c <5.7%); (6) with lipid profiles and liver enzymes (aspartate transaminase and alanine transaminase) within the reference range[14]; and (7) liver parenchyma showing only normal or stage I fatty liver appearances on ultrasound scanning.[15]
2. *Exclusion criteria*: (1) Children with extreme obesity (BMI more than +3 SD WHO normative data)[12]; (2) pre-diabetes or diabetes (HbA1C ≥5.7%); (3) total cholesterol ≥200 mg/dL, low-density lipoprotein ≥130 mg/dL; (4) hypertension—blood pressure ≥95% centile for height, sex and age[13]; (5) stage II and III fatty liver disease[15]; (6) children with chronic diseases; (7) those with egg or dhal (lentil) allergy and (8) practicing vegetarians.

Study design and methods

This was a single blind, non-randomised, crossover study. Each meal was given in consecutive order (study subjects blinded to its contents), with a 1-week washout period (figure 1). All subjects consumed a standard 280 calorie meal consisting of 'Suduru samba' rice (one cup), one boiled egg and two tablespoons of a carrot curry, between 1900 and 2000 hours on the day before the investigation. This meal was designed to minimise effects on the test meal the following day.[7] This meal and all other meals on the day before the test meal were consistent with the diet prescribed for obese children and they were strongly encouraged not to deviate from it. Physical activity was restricted for 24 hours before the test meal.

The subjects attended after a 12-hour fast and had a venous cannula inserted half hour before sampling. After collecting fasting blood samples, the subjects consumed the test meal within 15 min and remained in a seated position for 3 hours thereafter.

Blood sampling and meal content supply

The RS content of test meals 1 and 2 (Megazyme, K-RARPS 11/18) was analysed by the Department of Biochemistry, University of Sri Jayewardenepura, Sri Lanka (table 2). The RS content of test meal 3 could not be analysed as all university laboratory facilities were closed initially as part of the country's COVID-19 response and subsequently because of recent extreme civil unrest and have remained so to date. We have therefore estimated its nutritional content (table 2) from published nutritional assessment data (online supplemental appendix).[16]

The contents of the test meals were designed to vary their RS content (higher RS in meals 2 and 3 vs meal 1) and protein

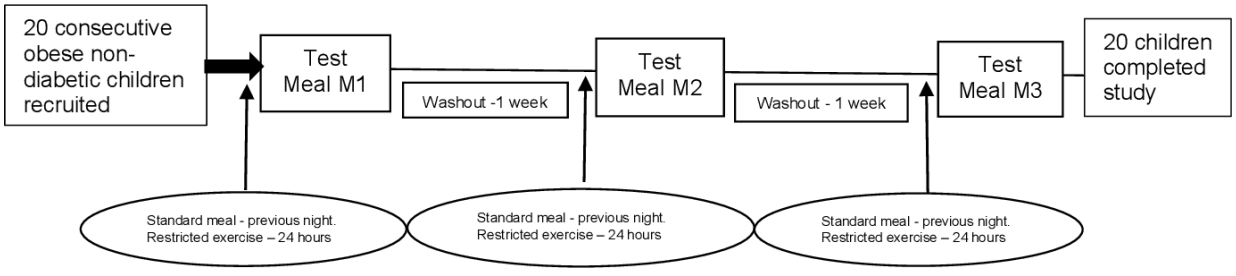

Figure 1 Trial plan.

Table 2 Nutritional composition of test meals

Test meal (200 g)	Resistant starch (g)	Carbohydrate (g)	Protein (g)	Energy (kcal)
M1 (200 g rice)	1.44*	39.9*	4.5	197.4
M2 (200 g rice+oil)	3.98*	42.9*	4.5	199.8
M3 (180g rice+oil+20 g lentils)	3.76†	42.6†	5.85†	205.4

The three test meals were isocaloric and isovolumetric (one standard cupful). Test meals 2 (M2) and 3 (M3) had a higher resistant starch content, while M3 had a higher protein content compared with the other two—the composition of M3 was derived from published nutritional assessment data.[16]
*Measured using the 'Megazyme' assay (Ireland).
†Calculated from nutritional composition data.[16]

content (higher protein content in meal 3 vs meals 1 and 2) as follows:

1. Test meal 1 (M1): Cooked Suduru samba rice 200 g (one standard cupful).
2. Test meal 2 (M2): Suduru samba cooked with coconut oil (100 g of rice cooked with 3 g of coconut oil)–cooked rice 200 g (one standard cupful).
3. Test meal 3 (M3): Suduru samba cooked with coconut oil (100 g of rice cooked with 3 g of coconut oil) served with 20 g of cooked red lentil–cooked rice 180 g+lentils 20 g (one standard cupful).

The test meals were cooked on the day before the test, refrigerated for 12 hours and heated in a microwave oven on the day of the test. Meals were consumed within 15 min with 10 g of onion 'sambol' (onion salad with salt and chillies) and 200 mL of plain water. The onion sambol contained no RS and its calorific content was minimal.

Height and weight measurements

Height was measured to the nearest 0.1 cm using a stadiometer and weight to the nearest 0.1 kg using a bioelectrical impedance analysis scale (Seca GmbH, Germany, Series No. 5769102177778).

Biochemical assays

Insulin was measured using a chemiluminescent immunoassay (Invitron IV2-001, Invitron, Monmouth, UK). Cross-reactivity with C-peptide and proinsulin was less than 1.5% and assay sensitivity was 1.5 pmol/L. Active ghrelin (Millipore, Merck, UK), PYY (Millipore), total GLP-1 (Millipore) and leptin (R&D Systems, Abingdon, UK) were all measured using ELISA assays. Assay sensitivity for ghrelin was 15 pg/mL; PYY was 6.5 pg/mL; GLP-1 was 1.5 pmol/L and leptin was 7.8 pg/mL.

In non-obese individuals, blood glucose is expected to peak around 60–90 min after a carbohydrate-containing meal and returns to preprandial levels within 180 min. Glucose homeostasis will be abnormal in children with obesity, and varying degrees of postprandial hyperglycaemia may occur. However, RS is expected to improve PYY, GLP1 and gut hormones in a metabolically advantageous manner, and will improve insulin sensitivity and therefore is expected to improve glucose homeostasis.

Satiety scores

A visual analogue scale for satiety for children was used for the qualitative assessment of satiety at 15, 60, 120 and 180 min after ingestion of each test meal.[17]

Statistical methods

All data were tested for normality. Where normally distributed, data are presented as the mean±SD and compared using a repeated measures analysis of variance (ANOVA). Where not normally distributed, the data were log transformed, and if normally distributed, the data presented as the geometric mean±SD and again compared with repeated measures ANOVA. Data that remained non-normally distributed following log transformation were presented as median (IQR, ie, 25th–75th centiles) and compared using Friedman's test with Wilcoxon's signed-rank test to compare the individual meals. Plasma glucose, ghrelin, GLP1, leptin and PYY concentrations were distributed normally, and summary values were expressed as mean±SD. However, plasma insulin concentrations were not distributed normally, and they were log transformed before further analysis and were expressed as median (IQR). The change in maximal concentration was calculated as the difference between maximal concentration (C_{max}) and fasting concentration (ie, C_{max}–Fasting). Area under the curve (AUC) was calculated using the trapezoidal rule and reports a single, integrated result taking into account multiple timepoints and varying times to peak (including the '0' minute timepoint). The incremental AUC ($\Delta AUC_{0-x\ hours}$) was calculated as the total area above the baseline concentration (ie, $AUC_{0\text{-}x\ hours}$–(baseline concentration multiplied by x hours)). Appropriate comparisons were made using t-tests (parametric) or Mann-Whitney tests (non-parametric). P values <0.05 were interpreted as statistically significant.

RESULTS

Study population

We recruited 12 male and eight female children aged 10–14 years whose anthropometric parameters were above the 90th centile for a standard age-matched and sex-matched reference population, as shown in table 1.[18 19]

Test meals

The composition of the isocaloric and isovolumetric test meals M1–M3 was described earlier and in table 2. The RS content and total carbohydrate content of M2 and M3 was higher than in M1. The total protein content of M3 was higher than M1 and M2.

Plasma glucose, insulin and ghrelin responses to mixed meals

Test meal M2

(1) The postprandial incremental change in maximal concentration (ΔC_{max}) and area under the curve (ΔAUC_{0-3}) for plasma glucose was significantly lower after M2 compared with M1 (p<0.05 and <0.01, respectively). (2) Postprandial area under the curve (AUC_{0-3}) and $\Delta AUC_{0\text{-}3}$ for plasma insulin after M2 were significantly lower compared with M1 (both p<0.05) (table 3).

Test meal M3

(1) Postprandial C_{max} and AUC_{0-3} for plasma glucose were significantly higher after M3 compared with M1 (both p<0.05). Postprandial C_{max}, ΔC_{max}, AUC_{0-3} and ΔAUC_{0-3} for plasma glucose were all significantly higher after M3 compared with M2 (p<0.01, <0.001, <0.01 and <0.01, respectively). (2) The incremental plasma insulin response (ΔAUC_{0-3}) was higher after M3 compared with M1 (p<0.05). (3) Postprandial plasma ghrelin response as indicated by area under the curve 0–2 hours (AUC_{0-2}) was significantly lower after M3 compared with M1 (p<0.05).

Table 3 Plasma glucose, insulin, ghrelin, leptin, PYY and GLP-1 after test meals

Analyte	Meal 1	Meal 2	Meal 3	P value*
Plasma glucose (mg/dL)				
C_{max}	95.0 (86.5–101.25)	93.5 (88.0–98.5)	100.0 (93.0–108.75)*,***	**0.003**
ΔC_{max}	12.0 (6.75–20.5)	3.0 (0.0–11.75)*	16.0 (8.25–21.25)****	**0.001**
$AUC_{0–3\ hours}$	268±31.2	262±29.6	280±34.2*,***	**0.001**
$\Delta AUC_{0–3\ hours}$	21.7±17.88	1.5±27.83**	24.0±22.59***	**0.0001**
Plasma insulin (pmol/L)				
C_{max}	184.1 (150.6–327.3)	181.1 (129.7–297.5)	268.8 (163.9–374.5)	0.086
ΔC_{max} †	95.0±151.8	140.8±161.80	105.6±160.1	0.121
$AUC_{0–3\ hours}$ †	551.2±538.17	472.0±408.74*	584.6±482.32	**0.013**
$\Delta AUC_{0–3\ hours}$ †	121.1±246.11	91.6±164.09*	140.3±256.14*	**0.004**
Plasma ghrelin (pg/mL)				
C_{max}	97.3 (55.8–117.98)	75.3 (63.13–142.60)	66.9 (50.8–92.05)	0.116
ΔC_{max}	0.0 (0.0–16.27)	0.0 (0.0–11.73)	0.0 (0.0–4.28)	0.304
$AUC_{0–2\ hours}$	144.3±60.9	140.2±78.1	100.2±48.1*	**0.044**
Plasma leptin (pg/mL)				
C_{max}	32 680±13 650.8	34 221±13 220.0	39 257±17 174.7	0.196
ΔC_{max}	407 (0–4699.8)	731 (0–4301.0)	3637 (0–11 433.5)	0.551
$AUC_{0–3\ hours}$	57 277±24 564.8	59 887±25 194.1	60 851±31 094.8	0.830
Plasma PYY (pg/mL)				
C_{max}	105.2±28.13	109.4±28.30	108.0±25.98	0.438
ΔC_{max}	23.6 (23.75)	10.4 (29.78)	28.4 (36.25)	0.987
$AUC_{0–3\ hours}$	179.0±34.91	183.3±44.77	185.5±43.33	0.770
Plasma GLP-1 (pmol/L)				
C_{max}	24.5±10.98	21.1±7.62	23.6±11.01	0.107
ΔC_{max}	2.3 (8.07)	0.5 (3.48)	3.5 (6.40)	0.245
$AUC_{0–3\ hours}$	36.3±17.12	32.8±11.66	34.8±17.48	0.207

M2 elicited significantly lower postprandial plasma glucose excursions compared with M1 (ΔC_{max}*, $\Delta AUC^{**}_{0–3\ hours}$) and M3 ($C_{max}$***, ΔC_{max}, $\Delta AUC_{0–3\ hours}$). M2 also produced significantly lower postprandial plasma insulin AUC_{0-3} compared with M1 and M1 produced higher ghrelin $AUC_{0–2}$ responses compared with M3. There were no significant changes to the postprandial leptin, GLP-1 and PYY responses following the meals (*ΔC_{max}=incremental maximum concentration; ***C_{max}=maximum concentration).
*p<0.05 vs meal 1; ** p<0.01 vs meal 1; ***p<0.01 vs meal 2; ****p<0.001 vs meal 2.
*P values derived for repeated measures ANOVA reporting differences in the means of the three meals.
†Geometric mean±SD.
ANOVA, analysis of variance; AUC, area under the curve; GLP, glucagon-like polypeptide; PYY, peptide YY.

Plasma leptin, PYY and GLP-1 responses to mixed meals

There were no differences in the postprandial responses of plasma leptin, PYY and GLP-1 to the mixed meals in relation to C_{max}, ΔC_{max} and AUC.

Satiety scores after test meals

Satiety scores changed as expected after each test meal. M3 elicited the highest score at 180 min postprandially, compared with M2 and M1. However, the difference in scores between meals was not significant (p=0.09) (figure 2).

DISCUSSION

This is the first study investigating acute postprandial plasma glucose, insulin and satiety hormone responses to meals containing RS in children with normal glucose metabolism. It shows the test meal containing RS alone (M2) produces significantly lower postprandial plasma glucose excursions compared with the meal with lower RS (M1) and the meal with similar RS but a higher protein content (M3), and produced acute postprandial glucose, insulin and ghrelin responses which were metabolically advantageous. There were no significant acute postprandial changes to PYY, GLP-1 and leptin profiles. Satiety scores were affected by the three isocaloric isovolumetric meals but were not significantly different in this acute study (figure 2).

Rice is the staple food in Sri Lanka. Furthermore, coconut 'milk' and coconut oil are also used very commonly to prepare food. There is unpublished evidence to suggest that the addition of coconut oil during cooking and cooling of rice increases its RS content by as much as 10 times.[20] During this process, amylose lipid complexes are formed which undergo crystallisation during cooling. Cooling and subsequent reheating further increases RS content.[21] It is also

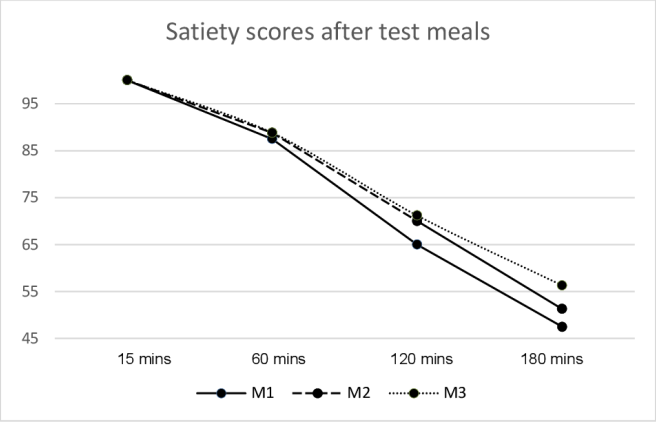

Figure 2 Satiety scores after test meals.

known that the addition of protein to RS enhances satiety.[6] We therefore chose coconut oil to enhance RS content in M2 and lentils to enhance protein content in M3 which are natural, easy to use, readily available.

RS remains resistant to alpha-amylase digestion during its passage through the small intestine. But in the large intestine, the gut microbiome ferments it and produces short chain fatty acids,[22] which in turn produce important effects on postprandial glucose and gut hormone profiles (GLP-1 and PYY) and satiety.[23] Some effects such as the effects on satiety are not observed in the immediate postprandial period as they take several hours to mature and the findings of this study on satiety scores are consistent with this.

Similar studies in adults have shown conflicting results. Some have shown results similar to our study with reduced postprandial plasma glucose and insulin profiles[5 11 24–26] but others have shown reduced plasma insulin profiles but no effect on plasma glucose.[27] The acute effects on GLP-1, PYY and leptin after RS were similar to our study.

The effects on satiety of this acute study were minimal—no significant changes at the end of 180 min following M1, M2 or M3, consistent with results in studies on adults.[28] Studies of longer duration with RS are needed to demonstrate the beneficial effect on satiety in children.

Our study has several shortcomings: (1) the single blind, non-randomised crossover design with its inherent disadvantages for example, the potential for 'carryover effects' although small; (2) the restricted timepoints examined for each biochemical analyte due to financial constraints and prevalent COVID-19 pandemic-related factors—we would prefer to have measured these analytes at multiple further timepoints within the 180 min, giving a higher degree of validity to the results; and (3) the lack of a formal biochemical analysis for contents of meal 3 because of the above reasons—the only option available to us was to use data from previous analysis of a similar meal.

This study also has several advantages: (1) this is the first such study of obese children as far as we are aware; (2) the use of sensitive assays measuring gut hormones in sequential order after meals; and (3) the easy applicability of the results of this study to practical day-to-day settings—clearly, this would be after further studies in larger groups of obese children.

CONCLUSIONS

This study demonstrates for the first time the beneficial effects of RS on acute postprandial metabolic and hormonal profiles in obese children without diabetes. M2, the meal containing RS alone, produced glucose, insulin and ghrelin responses which were of a metabolically advantageous nature compared with M1 and M3 in these children. Future long-term studies with RS in children (both obese and non-obese) should be done to demonstrate potential beneficial effects on metabolic profiles and gut hormone responses.

Correction notice This article has been corrected since it first published. The open access licence type has been changed to CC BY. 17th May 2023.

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Contributors JS, NA, DAGHdS, SE, EJ and LP designed the study. GD, SL, SE and EJ carried out laboratory analyses. LP drafted the initial drafts of the paper, but all authors contributed to subsequent versions. The final version of the manuscript submitted was approved by all authors. NA and DAGHdS are accountable for the integrity of this study.

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Competing interests None declared.

Patient consent for publication Not applicable.

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Blood sample collection and meal content supply

Venous blood samples (2 mL) were collected directly into EDTA-coated tubes for insulin, leptin, PYY, GLP1 (0, 120 and 180 minutes) and ghrelin (0 and 120 minutes). 1 mL of blood was mixed with AEBSF (4-2Aminoethyl–benzene-sulfonyl fluoride) at a concentration of 1mg/1mL to prevent degradation of ghrelin. Samples were centrifuged immediately at 2000g at 4°C, plasma separated and acidified with hydrochloric acid and stored at -20°C. Venous blood (1 mL) was collected in EDTA + sodium fluoride tubes for plasma glucose analysis at 0, 60, 120 and 180 minutes. Resource constraints, the prevailing COVID-19 related conditions, prevented more frequent sampling.

Test meals – Test meals were prepared with “Suduru Samba”, rice with the highest RS content, obtained from an identifiable single plot of land (Chemical Industries, CIC Colombo, Sri Lanka). The same supplier provided red lentils from one consignment and coconut oil with an iodine value of 10 (CIC, Colombo, Sri Lanka)