British Journal of Nutrition (2023), 129, 771–778 doi:10.1017/S0007114522000824

Dose–response efficacy of mulberry fruit extract for reducing post-prandial blood glucose and insulin responses: randomised trial evidence in healthy adults

David J Mela[1]*, Xiu-Zhen Cao[2], Santhosh Govindaiah[3], Harry Hiemstra[1], Ramitha Kalathil[3], Li Lin[2], Joshi Manoj[3], Tingyan Mi[2] and Carole Verhoeven[1]

[1]*Unilever R&D, Wageningen, the Netherlands*
[2]*Unilever R&D, Shanghai, People's Republic of China*
[3]*Unilever R&D, Bengaluru, India*

(Submitted 14 July 2021 – Final revision received 11 January 2022 – Accepted 4 March 2022 – First published online 11 March 2022)

Abstract

Extracts of mulberry have been shown to reduce post-prandial glucose (PPG) and insulin (PPI) responses, but reliability of these effects and required doses and specifications are unclear. We previously found that 1·5 g of a specified mulberry fruit extract (MFE) significantly reduced PPG and PPI responses to 50 g carbohydrate as rice porridge, with no indications of intolerance. The trials reported here aimed to replicate that work and assess the efficacy of lower MFE doses, using boiled rice as the carbohydrate source. Two separate randomised controlled intervention studies were carried out with healthy Indian males and females aged 20–50 years (n 84 per trial), with PPG area under the curve over 2 h as the primary outcome. Trial 1 used doses of 0, 0·37, 0·75, 1·12 and 1·5 g MFE in boiled rice and 0 or 1·5 g MFE in rice porridge. Trial 2 used doses of 0, 0·04, 0·12, 0·37 g MFE in boiled rice. In trial 1, relative to control, all MFE doses significantly decreased PPG (−27·2 to −22·9 %; all $P \leq 0{\cdot}02$) and PPI (−34·6 to −14·0 %, all $P < 0{\cdot}01$). Breath hydrogen was significantly increased only at 1·5 g MFE (in rice porridge), and self-reported gastrointestinal symptoms were uniformly low. In trial 2, only 0·37 g MFE significantly affected PPG (−20·4 %, $P = 0{\cdot}002$) and PPI (−17·0 %, $P < 0{\cdot}001$). Together, these trials show that MFE in doses as low as 0·37 g can reliably reduce PPG and PPI responses to a carbohydrate-rich meal, with no apparent adverse effects.

Key words: **Mulberry: Extract: Dose: Glycaemic response: Glucose: Insulin**

Lower post-prandial blood glucose and insulin responses (PPG and PPI, respectively) are associated with a reduced risk of development and progression of diabetes and cardiovascular diseases in healthy populations as well as those with (pre-) diabetes[1–4]. One potential approach to reduce the PPG and PPI responses to commercial foods is the use of natural sources of inhibitors of enzymes or transporters involved in carbohydrate digestion and uptake[5]. However, a potential side-effect of inhibiting carbohydrate digestion is that not just the rate but also the amount of carbohydrate absorbed might be reduced. Substantial inhibition of carbohydrate digestion or absorption could give rise to osmotic diarrhoea, and bloating and flatulence secondary to fermentation[6]. Rises in breath hydrogen (H_2) production > 10 ppm from baseline are commonly used as a relative indicator of carbohydrate malabsorption[7,8], along with any reported symptoms of gastrointestinal malaise.

We recently reported research testing the efficacy and tolerability of several well-characterised, commercially available plant extracts for reducing PPG and PPI responses to a meal of rice porridge containing 50 g available carbohydrate[9]. A dose of 1·5 g of a specified mulberry fruit extract (MFE) showed robust efficacy, with limited evidence of carbohydrate malabsorption (breath H_2), and no indications of adverse gastrointestinal effects. MFE has other attractive features as a candidate food ingredient: the fruit itself has a long history of safe consumption, the extract has acceptable sensory attributes and the main proposed active component (the alpha-glucosidase inhibitor 1-deoxynojirimycin, DNJ) shows high stability to thermal and oxidative stresses.

There is mixed body of evidence showing efficacy of DNJ-containing mulberry extracts for PPG, but this is almost exclusively using extracts from mulberry leaf. Because mulberry

Abbreviations: AUC, area under the curve; DNJ, 1-deoxynojirimycin; ITT, intention-to-treat; MFE, mulberry fruit extract; PP, per-protocol; PPG, post-prandial glucose; PPI, post-prandial insulin.

* **Corresponding author:** David J Mela, email djmela@djmela.eu

extracts differ in their sources and production methods, the DNJ levels (if reported) rather than total extract doses are the preferred basis for comparing exposures across studies. We observed similar efficacy of mulberry fruit and leaf extracts at doses containing ~8 mg DNJ, and statistically significant increases in H_2 were seen only for the leaf extract[9]. There are many reports of significant reductions in PPG or PPI resulting from mulberry leaf extract interventions with a DNJ dose content ≥ 9 mg[10–21], but DNJ doses of ~6 mg or less in some of those same studies have shown less consistent effects[10,13–15,20].

The present research was intended to replicate our earlier observation of efficacy of MFE in lowering the PPG and PPI responses to rice porridge and to determine the efficacy and tolerance of lower levels of MFE in boiled rice. The research was carried out as a series of two independent trials, in order to capture the full range of potentially efficacious doses.

Method

General

This report describes two independent dose–response trials, the second trial design based on the results of the first. Both trials were primarily designed to test efficacy and tolerance of 3 dose-levels of MFE for reducing the PPG and PPI responses to a carbohydrate load from boiled rice, relative to a control with no extract added. The primary objective was to identify the minimal effective dose of MFE (added to boiled rice) that achieves a statistically significant reduction in venous PPG positive incremental area under the curve over 2 h ($+iAUC_{2\ h}$), relative to the control. Secondary objectives were to test the effects on PPI total area under the curve over 2 h ($tAUC_{2\ h}$) and (only in trial 1) breath H_2 and tolerance to the extracts. In addition, trial 1 also included test arms assessing these same outcomes for 1·5 g MFE added to a rice porridge control, as a direct replication of our previous research[9].

The clinical phases were executed at Lambda Therapeutics Research Ltd (LTRL) from 24 September to 19 October 2012 and from 12 November to 5 December 2013, for trials 1 and 2, respectively. They were conducted according to the guidelines laid down in the Declaration of Helsinki, and all procedures involving human subjects were approved by the Independent Ethics Committee – Aditya (Ahmedabad, India), protocol numbers FDS-NAA-0720 (trial 1) FDS-NAA-0335 (trial 2). The research was explained verbally as well as on the informed consent form, and signed written consent was obtained from each subject before protocol-specific procedures were carried out. Each subject was given opportunity to inquire about details of the study and was informed of their right to withdraw from the research at any time. Instructions and self-report interviews and data collection were all undertaken in the native language of participants. The trials were registered at ClinicalTrials.gov with identifiers NCT04256746 (trial 1, retrospectively) and NCT01955447 (trial 2, prospectively).

Participants

Potential participants were recruited from a database of healthy volunteers at LTRL. Overweight and older subjects were excluded to minimise the risk of recruiting individuals with (undiagnosed) impaired glucose tolerance. Subjects were therefore selected in the age range 20–50 y, with a BMI in the range of 18·0–25 kg/m^2. The complete inclusion and exclusion criteria are described in the Online Supplementary Material, Table S1.

Potential participants were screened in two sessions prior to the start of the interventions. The study flow schedule detailed in Online Supplementary Table S2 was identical for both trials, except that there were no measures of gastrointestinal symptoms or H_2 in trial 2.

Eighty four male and female subjects were to be randomised into each trial. Subjects who dropped out before the first treatment were replaced, while those who dropped out after participating in any of the treatments were not replaced. When a subject decided to withdraw, or failed to attend a session, efforts were made to perform all planned assessments and record the reasons. Subject baseline characteristics are shown in Table 1. With the exception of five subjects in trial 1 (three with a BMI of 18·3, two with a BMI of 25), all subjects in both trials had a BMI within the 'normal' range of 18·5–24·9 kg/m^2 according to the WHO guidance (WHO Expert Consultation, 2004).

Design, allocation to treatments and blinding

Trial 1 used a balanced incomplete block design, with each subject randomly allocated to a treatment sequence in which they received four of the seven test products as one product per week over 4 weeks, in one of four cohorts. Trial 2 used a balanced complete block design, with each subject randomly allocated to a treatment sequence in which they all received all four test products as one product per week over 4 weeks, in one of four cohorts.

In both trials, each subject visited the site on same day of the week on all of their four treatment visits. Allocation of subjects to treatment days, as far as possible, maintained the same ratio of males to females. The randomisation schemes were computer-generated at the test site, and not accessible to personnel involved in the collection, monitoring, revision or evaluation of adverse events, nor to clinical laboratory or other personnel who could have an impact on the outcome of the study, until after the end of the clinical and analytical phases. Persons involved in the preparation and coding of test products were not involved in any other aspects of the trials. Other study team personnel and subjects were blinded to the identification of specific treatments, which varied only slightly in colour and flavour.

Interventions

The commercially available MFE used in both trials (batch No. MF-DC-KQ-111207, Draco Natural Products Inc.) contained 0·5 % (w/w) of DNJ and was packaged in pre-weighed and

Table 1. Baseline characteristics including mean and standard deviation (SD) age, weight and BMI of participants

			Age, years		Weight, kg		BMI, kg/m^2	
	n	Males/Females	Mean	SD	Mean	SD	Mean	SD
Trial 1	84	42/42	33·2	6·6	58·0	8·6	22·6	2·1
Trial 2	84	45/39	34·2	7·8	57·0	7·2	22·1	2·2

coded sachets. We previously confirmed the *in vitro* bioactivity of this source of MFE for α-glucosidase inhibition and efficacy of this dose for reducing PPG and PPI when added to rice porridge[9].

On test days, subjects consumed the study product after an overnight fast and were not allowed to drink water 1 h prior to study product administration. For the primary objective of the trials, we tested the efficacy of several doses of MFE in a standard boiled rice (Sona Masoori rice, Aksshatta Golden Harvest LLP). Each serving of boiled rice was prepared by adding 140 ml water to 63 g dry rice (~50 g available carbohydrate), and then cooking in an automatic rice cooker. The rice was removed and allowed to cool for 5 min before mixing in MFE. In trial 1, the MFE doses added to boiled rice were 0, 0·37, 0·75, 1·12 and 1·5 g. In trial 2, the doses were 0·04, 0·12 and 0·37 g MFE. The decision to use a logarithmic rather than linear dose range in trial 2 was based on further consideration and *in silico* modeling of the likely dose–response kinetics.

Only in trial 1, as a 'positive control' and to replicate the results of a previous study[9], we also tested MFE in the same dose and rice porridge source used previously (the rice portion from Knorr 'cup Jok'; Unilever Thai Holdings Limited, Thailand). Each serving of rice porridge was prepared by adding 300 ml of boiling water to 60 g rice (~50 g available carbohydrate) and allowing it to cool to approximately 60°C, when 0 or 1·5 g MFE was stirred in. To equate the meal size and total water intake of the boiled rice and rice porridge treatments, subjects consumed these together with 360 and 200 ml of water, respectively, within a 15-min period.

If any subject was not able to finish consuming a test product meal within 15 min, the 15 min blood sample was taken and they finished consuming the meal immediately after blood sampling. Time of consumption of meal was recorded. If any subject was unable to consume the full quantity of the meal within 30 min, they were excluded from the study. Subjects were not allowed to consume any food after test product meal administration, except the lunch that was provided after the last blood sample had been collected. Subjects were not allowed to drink water for 1 h prior to consuming the test meal. They were then allowed a maximum additional 500 ml water for the rest of the time until blood sampling was completed.

Data and sample collection

Blood draws. On test days, an intravenous indwelling cannula was inserted in a forearm vein of the subjects 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. Two consecutive baseline blood samples, with a gap of maximum 5 min, were collected within a period of 15 min before the test product ingestion. From commencement of eating, subsequent samples were collected 15, 30, 45, 60, 90, 120 and 180 min. The actual time of collection of each blood sample was recorded immediately after blood collection, and variation of ±1 min was considered acceptable for each time point of blood sampling. The time of the baseline sampling, study product administration and subsequent blood samplings were kept constant, with a maximum allowable variation of ± 30 min between visit days. Time points outside these allowed ranges as above were documented as protocol deviations. In all such instances, appropriate time corrections for the actual time of sample collection were incorporated at the time of data analysis.

Breath hydrogen sampling (trial 1 only). Subjects in trial 1 exhaled into the H_2 monitor at −20, +65, +125, +185, +245, +305, +365 and +425 min relative to ingestion of the test product.

Gastrointestinal discomfort and defecation self-reports (trial 1 only). A questionnaire for gastrointestinal discomfort was completed on paper by subjects in trial 1 at baseline (before the blood sampling) and +4 h 30 m. Intensity of nausea, flatulence, bloating and pain were rated as 'none' (= 0), 'mild' (=1), 'moderate' (=2) or 'severe' (=3).

A variation of ±5 min for breath sample analysis and questionnaire administration were considered acceptable, and no protocol deviations were recorded within this allowed range.

Analytical procedures

All analyses were carried out by LTRL at the clinical test facility.

From the 5 ml venous blood samples at each time point, a 3 ml aliquot was transferred to a tube with a clotting activator for measurement of serum insulin, followed by 2·0 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 a maximum 30–45 min, and then centrifuged at 2500–3000 rpm for 10 min 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). Insulin was measured in serum using an immunoassay (Roche cobas e411, Roche Diagnostics GmbH).

Breath H_2 (trial 1 only) was directly measured in breath exhaled into a H_2 monitor (Gastrolyzer2™, Bedfont Scientific Ltd).

Statistical analyses

Statistical analyses were carried out according to a pre-specified plan. No interim analyses were planned or performed, and treatment assignments were revealed only after blind review of the data following the clinical data collection phase. The blind review was undertaken by the principle investigator, statistician

and principle sponsor contact, and the treatment code was broken only after a hard lock of the data was agreed following this blind review.

The primary outcome measure in both trials was venous plasma glucose $+iAUC_{2\ h}$. Power calculations were based on an average $+iAUC_{2\ h}$ of 166 mmol.min/l, an effect size of 25 %, 80 % power, four comparisons for boiled rice (Dunnett multiple comparison adjustment), one comparison for rice porridge (*t* test, trial 1 only) and an adjusted overall error rate of 0·20, resulting in trial size estimate of eighty-four subjects.

The following procedures were agreed prior to de-blinding. All available results from all subjects were included in analyses if they completed at least one of the treatments. If baseline data were missing for a subject visit, the mean of the baseline values from the other visits for that subject would be used as the baseline for intention-to-treat (ITT) and per-protocol (PP) analyses. If only one of the baseline measurements was available, that would be used for both ITT and PP analyses. If a single post-prandial data point was missing for a measurement, the AUC would be calculated without that point. The visit for that subject would be included in the ITT but not the PP set. If a data point was omitted from the analysis due to measurement error, the AUC would be calculated without that point and the visit for that subject would be included in the ITT but not the PP set.

For the pre-specified analyses of glucose and insulin, data at all available time points from baseline through 120 min were used, and the values at 180 min analysed separately as an exploratory endpoint. The AUC values for PPG and PPI were calculated as we have previously described[9].

Statistical comparisons were only made between the appropriate control and the other test products (i.e. four statistical comparisons for boiled rice, one comparison for rice porridge). Dunnett's test was used to adjust for the multiple comparisons, using an overall significance level of 0·10.

In general, the AUC were not normally distributed as was determined by box-plots in a preliminary analysis. All statistical analyses therefore used log(AUC) as the response variable. The outcomes of the analysis were then backtransformed to the normal scale. PPG and PPI were tested using a linear mixed model, where log(AUC) = baseline + subject_baseline + weight + sex + visit + treatment + error. In this model, baseline is the mean baseline value for that visit for that subject and subject_baseline is the mean baseline score over all visits for the subject. This latter term is included to avoid possible bias in the estimates of the product effect due to the use of a mixed model and the inclusion of a different baseline value at each visit. Visit is the number of the visit (i.e. 1 to 4) and is a categorical variable. Inclusion of sex and weight as covariates was stipulated in the protocol. The error terms were assumed to be normally distributed.

Changes in H_2 concentrations in trial 1 were calculated by subtracting the lowest H_2 concentration of the first three breath samples (–20, +65 or +125 min) from all subsequent breath samples. The lowest of these three values was used as the baseline nadir value. An increase of 10 ppm or more in H_2 excretion from the basal nadir value was considered a 'positive' (physiologically relevant) increase in H_2[8]. Statistical analysis of the proportion of subjects with one or more positive H_2 breath readings was based on logistic regression, taking into account the design of the study (cross-over design) and using baseline readings as covariates.

Trial 1 exploratory analyses (summarised by descriptive statistics only) included maximum glucose concentration (Cmax, the maximum observed concentration at any time point) and time to maximum glucose response (Tmax). An exploratory trend analysis was also proposed to assess the relationship between MFE dose and PPG for boiled rice (using linear, quadratic and cubic contrasts). Trial 2 exploratory measures were limited to 3 h PPG and PPI.

All *P*-values are two-sided, with $P < 0{\cdot}05$ used as the criterion for statistical significance.

Results

Analysis populations

Online Supplementary Figures S1 and S2 describe the flow of subject numbers through the recruitment, screening and intervention phases of trials 1 and 2, respectively.

Trial 1 had an incomplete block design, and Online Supplementary Table S3 shows that the characteristics of the subsets of subjects receiving each treatment/dose differed only trivially. In trial 1, only one subject failed to complete any test sessions (unable to eat the test meal within 30 min); eighty-three subjects provided sufficient data to be included in the analyses. Seventy-eight subjects completed the entire study and all test sessions. As very few data points were excluded from the PP analysis in blind review, and thus ITT and PP groups almost identical, the PP analysis is reported.

As trial 2 had a full cross-over design, subject characteristics are essentially the same for all doses (Table 1). Seventy-nine of the eighty-four subjects entering the study consumed the control product and at least one other treatment, and seventy-seven subjects completed all test sessions and treatments. As there were only trivial deviations from the defined protocol, only a per-protocol analysis was generated.

Efficacy and tolerance

In trial 1, all doses of MFE significantly reduced 2 h AUC responses for both PPG and PPI, in boiled rice and rice porridge (Table 2 and 3). The effect sizes were consistent with a dose–response effect in the range 0·37 to 1·12 g MFE in boiled rice for PPI. However, the proposed exploratory trend analysis of the relationship between MFE dose and PPG response for boiled rice was not performed because all dose levels of MFE produced a similar response.

Data for the trial 1 exploratory variables (Cmax, Tmax, and PPG and PPI over 3 h) are presented in Online Supplementary Tables S4–S6. There was a general dose–response trend for a lower Cmax and longer Tmax with higher MFE doses in boiled rice, and effects in the same direction are also seen for MFE added to rice porridge. The PPG responses over 3 h were significantly reduced by all MFE doses ($P < 0{\cdot}05$) with the exception of 0·75 g MFE in boiled rice ($P = 0{\cdot}093$), and doses produced similar mean effect sizes. Three-hour PPI responses were significantly reduced by all

British Journal of Nutrition

Table 2. Trial 1: plasma glucose positive incremental area under the curve over 2 h (+$iAUC_{2\,h}$, min.mmol/l) following consumption of rice porridge (RP) or boiled rice (BR) alone or with additions of mulberry fruit extract (MFE)

		Glucose +$iAUC_{2\,h}$ adjusted for baseline, min.mmol/l		Percent difference from control		
Intervention	*n*	Mean	Lower, upper 95 % CI	Mean	Lower, upper 95 % CI	Adjusted *P*-value *v.* control
BR Control	47	125·3	108·3, 144·9			
BR + 0·37 g MFE	44	96·6	83·3, 112·0	−22·9	−37·1, −5·6	0·021
BR + 0·75 g MFE	46	95·1	82·1, 110·1	−24·1	−37·9, −7·3	0·011
BR + 1·12 g MFE	45	90·8	78·4, 105·2	−27·5	−40·6, −11·5	0·002
BR + 1·50 g MFE	47	91·2	78·9, 105·4	−27·2	−40·3, −11·3	0·002
RP Control	45	154·8	133·6, 179·3			
RP + 1·5 g MFE	44	115·3	99·4, 133·7	−25·5	−36·2, −13·0	0·002

Table 3. Trial 1: serum insulin total area under the curve over 2 h ($tAUC_{2\,h}$, min.mIU/l) following consumption of rice porridge (RP) or boiled rice (BR) alone or with additions of mulberry fruit extract (MFE)

		Insulin $tAUC_{2\,h}$ adjusted for baseline, min.mmol/l		Percent difference from control		
Intervention	*n*	Mean	Lower, upper 95 % CI	Mean	Lower, upper 95 % CI	Adjusted *P*-value *v.* control
BR Control	47	4807	4432, 5214			
BR + 0·37 g MFE	44	4134	3806, 4491	−14·0	−22·4, −4·7	0·006
BR + 0·75 g MFE	46	3907	3601, 4240	−18·7	−26·5, −10·1	<0·001
BR + 1·12 g MFE	45	3543	3263, 3846	−26·3	−33·4, −18·4	<0·001
BR + 1·50 g MFE	47	3571	3293, 3874	−25·7	−32·8, −17·8	<0·001
RP Control	45	7143	6579, 7754			
RP + 1·5 g MFE	44	4673	4302, 5075	−34·6	−39·0, −29·2	<0·001

Table 4. Trial 2: plasma glucose positive incremental area under the curve over 2 h (+$iAUC_{2\,h}$, min.mmol/l) following consumption of boiled rice (BR) alone or with additions of mulberry fruit extract (MFE)

		Glucose +$iAUC_{2\,h}$ adjusted for baseline, min.mmol/l		Percent difference from control		
Intervention	N	Mean	Lower, upper 95 % CI	Mean	Lower, upper 95 % CI	Adjusted *P*-value *v.* control
BR Control	77	119·5	108·0, 132·3			
BR + 0·04 g MFE	79	104·1	94·2, 115·1	−12·9	−22·3, −2·3	0·094
BR + 0·12 g MFE	78	105·1	95·0, 116·2	−12·1	−21·6, −1·4	0·130
BR + 0·37 g MFE	77	95·1	86·0, 105·2	−20·4	−29·0, −10·8	0·002

MFE additions ($P < 0·01$), with a dose–response trend in effect sizes.

In trial 2, statistically significant reductions in PPG or PPI were observed only with 0·37 g MFE (Table 4 and 5). PPG effect sizes were similar for the two lower doses, while PPI responses were consistent with a dose–response effect in the range tested. Similar patterns were observed for the corresponding exploratory 3 h PPG and PPI AUC values. Changes in PPG over 3 h for 0·04, 0·12, and 0·37 g MFE relative to control were −12·2 (95 % CI − 22·2, −1·2; $P = 0·151$), −9·2 (95 % CI − 19·5, 2·5; $P = 0·369$) and −17·5 % (95 % CI − 26·8, −6·9; $P = 0·017$). The corresponding results for PPI were −2·2 % (95 %CI − 7·5, 3·4; $P = 0·826$), −4·7 % (95 % CI − 9·8, 0·8; $P = 0·314$) and −13·4 % (95 % CI − 18·1, −8·4; $P < 0·0001$).

The mean PPG and PPI responses per time point over 3 h for each test meal in both trials are shown in Online Supplementary Fig. S3, and the overall dose–response profile for glucose (+) $iAUC_{2\,h}$ and insulin $tAUC_{2\,h}$ in both trials is illustrated in Online Supplementary Fig. S4.

Breath H_2 production and gastrointestinal symptoms were measured only in trial 1. The percent of subjects with breath H_2 production ≥ 10 ppm above any of three different defined baseline points increased with the MFE dose above 0·37 g in boiled rice (Table 6). However, this was similar to control with the lowest MFE doses in boiled rice, and only significantly more likely to occur with 1·5 g MFE in rice porridge. Scores for gastrointestinal discomfort were low for all treatments, never rated above 'mild' and not reliably associated with dose of MFE ingestion (Online Supplementary Table S7). The analysis of aggregated gastrointestinal complaint data showed no significant difference from control for MFE added to boiled rice (all odds ratios $P > 0·50$) or rice porridge ($P > 0·20$).

Table 5. Trial 2: serum insulin total area under the curve over 2 h ($tAUC_{2\ h}$, min.mmol/l) following consumption of boiled rice (BR) alone or with additions of mulberry fruit extract (MFE)

		Insulin $tAUC_{2\ h}$ adjusted for baseline, min.mmol/l		Percent difference from control		
Intervention	*n*	Mean	Lower, upper 95 % CI	Mean	Lower, upper 95 % CI	Adjusted *P*-value *v.* control
BR Control	77	4587	4348, 4839			
BR + 0·04 g MFE	79	4457	4226, 4701	−2·8	−8·2, 2·9	0·714
BR + 0·12 g MFE	79	4261	4040, 4495	−7·1	−12·3, −1·6	0·069
BR + 0·37 g MFE	78	3808	3610, 4016	−17·0	−21·6, −12·1	<0·001

Table 6. Trial 1: percentage subjects with one or more breath hydrogen (H_2) readings ≥ 10 ppm above baseline (baseline = nadir of readings at −20, 65 and 125 min) following consumption of boiled rice or rice porridge alone (control) or with additions of mulberry fruit extract (MFE)

Intervention	*n*	Percent subjects with H_2 readings ≥ 10 ppm above baseline	Odds ratio	Lower, upper 95 % CI	adjusted *P*-value *v.* control
Boiled rice					
Control	47	8·5	1·00		
+0·37 g MFE	44	4·5	0·49	0·07, 3·65	0·84
+0·75 g MFE	46	13·0	1·46	0·31, 7·01	0·95
+1·12 g MFE	45	17·8	2·31	0·51, 10·60	0·56
+1·50 g MFE	46	23·9	3·91	0·89, 17·25	0·15
Rice porridge					
Control	45	11·1	1·00		
+1·5 g MFE	44	52·3	12·50	4·00, 39·06	<0·001

Adverse events

In trial 1, three adverse events were reported across all subjects and test days. One subject reported an upper respiratory tract infection, not considered to be related to the test meals. One subject vomited ~1 h after consumption of the rice porridge with 1·5 g MFE, and another ~5 h after boiled rice with 1·5 g MFE. These adverse events were considered to be possibly related to the test meals.

In trial 2, five adverse events were reported across all subjects and test days. Three subjects vomited within 1 h after consumption of the test meals, two after the reference boiled rice with no MFE and one after boiled rice with the lowest MFE dose. These adverse events were considered to be possibly related to the test meals. One subject reported dizziness unlikely to be related to the test meals, and another subject reported a spider bite.

Discussion

Together, these trials have shown that doses of MFE as low as 0·37 g, containing ~2 mg DNJ, produced a consistent reduction in the PPG and PPI response to a realistic carbohydrate load from boiled rice, with no apparent evidence of malabsorption or intolerance. MFE doses lower than this did not produce significant reductions in PPG. Doses of 0·75 and 1·12 g had effects on PPG similar to 0·37 g, and somewhat larger effects on PPI, with no statistically significant carbohydrate malabsorption as reflected in breath H_2. The highest dose tested (1·5 g MFE, containing ~8 mg DNJ) produced the greatest reductions in PPG and PPI, replicating our previous results, but this was accompanied by evidence of carbohydrate malabsorption, which was particularly apparent when combined with rice porridge. However, there was no evidence of any adverse gastrointestinal symptoms at any dose, nor adverse events likely related to the MFE. As the lowest doses in trial 1 showed no evidence of malabsorption or intolerance, outcomes of interest in trial 2 were limited to PPG and PPI.

The data here for 0·37 g MFE, showing reductions of PPG in the range of 10–30 % relative to a reference product, are roughly in line with previous studies mainly using extracts of mulberry leaf and substantially higher DNJ levels[10,11,13,15,19,20,20–22]. However, direct comparisons are not possible due to differences in the carbohydrate sources and loads, as well as the subject populations and specific extracts used. Nevertheless, we are not aware of any study showing consistent efficacy with any mulberry extract at this low level of DNJ.

The present results indicate a level and specification of MFE that was both efficacious and well-tolerated when consumed with a digestible starch source. Even doses of this MFE as low as 0·04 and 0·12 g had modest absolute effects on PPG, which suggests that the lowest consistently effective dose in practice may be between 0·12 and 0·37 g. There is a wide range of DNJ concentrations across different sources and varieties of fresh mulberries[23], but based on a typical DNJ level of ~50 ug/g, the lowest consistently efficacious dose here of 0·37 g MFE would equate to about 20 g of fresh mulberry fruit.

The absence of any apparent effect on breath H_2 with 0·37 g (or other low doses) of MFE suggests no physiologically meaningful carbohydrate malabsorption with this exposure. In contrast, there are a number of previous reports of significantly increased breath H_2 following extracts of mulberry leaf with DNJ contents that were either unspecified[21] or more than twofold higher than the level (~8 mg DNJ) at which increases in H_2 were seen in our previous trial[9] and Trial 1 here[17,18,24]. Zhong *et al.*

observed increased breath H_2 following a mixture of teas containing only 5 mg 'DNJ-type compounds', but the complexity of the product mixture they used precludes attribution of the effects to the mulberry (or DNJ) component specifically.

There are a number of limitations to further extrapolation from this research. Most importantly, the results reflect the specific doses and source of MFE used here, and it is possible that variations in processing or components other than DNJ may influence efficacy of other mulberry extracts. The results are also limited to very basic rice 'meals'. Although the putative mode of action (DNJ inhibition of alpha-glucosidase) may be effective for many carbohydrate sources, this would need to be shown with more complex sources of carbohydrates or in the presence of other meal components. Lastly, these trials have been conducted in healthy Indian populations with a relatively low body weight. It would be valuable to establish the efficacy in populations with a higher prevalence of overweight or (pre-) diabetes.

These results directly replicate and add to previous data confirming the efficacy of relatively low levels of MFE for reducing the PPG and PPI responses to rice (Mela *et al.*). It suggests that MFE could be used to achieve these benefits as a commercial food ingredient, provided it meets other technical, safety and consumer acceptance criteria. Given the putative mode of action, the results probably also apply to other MFE sources with similar specifications, and other sources and preparations of readily digestible starch, but this should be explicitly confirmed. In addition to further exploring the effective conditions of use, future research may also consider the sustained effects or impact of the lowering of PPG and PPI by MFE on markers of disease risk in sensitive populations.

Acknowledgements

The authors wish to acknowledge the helpful contributions of Vishi Bansal (resources), Leo van Buren (methodology), Tanvi Kadam (project administration), Praful Lahorkar (resources), Suman Majumder (resources), Dr Anisha Pargal (supervision) and Zhang Zhang (resources).

Funding for this research came entirely from Unilever, a food and beverage manufacturer. Subject recruitment and contact, trial execution, clinical chemical analyses and all raw data collection and recording were carried out by Lambda Therapeutics Research Ltd acting as a contracted research organisation, independent of any direct Unilever involvement other than financial support and the sourcing and provision of test products. Unilever employees were additionally responsible for the study design, material selection and characterisation, statistical analyses and preparation of the manuscript. All authors were employees of Unilever at the time this research was designed and conducted. The following authors have since changed their professional affiliations. D. J. M., H. H.: No current affiliation. S. G.: G. S. K., Bengaluru, IN. L. L.: Philips Research China, Shanghai, CN. C. H. J. V.: Clover Biopharmaceuticals, NL.

All authors made substantial contributions to the conception and interpretation of the research. D. J. M., X-Z. C., R. K., L. L., J. M. and T. M. were responsible for the selection and specification of materials and methods. S. G. and C. V. were primarily responsible for protocol implementation and quality assurance and the interface between Lambda Therapeutics Research Ltd and Unilever. H. H. was responsible for the study design and statistical analyses. D. J. M. was primarily responsible for drafting the manuscript, with input from all other authors. All authors have seen and approved the manuscript and agreed that any questions related to the accuracy or integrity of any part of the work are appropriately investigated, resolved and documented.

There are no conflicts of interest.

Supplementary material

For supplementary material/s referred to in this article, please visit https://doi.org/10.1017/S0007114522000824

References

1. Blaak E, Antoine JM, Benton D, *et al.* (2012) Impact of postprandial glycaemia on health and prevention of disease. *Obes Rev* **13**, 923–984.
2. Ceriello A & Colagiuri S (2008) International diabetes federation guideline for management of postmeal glucose: a review of recommendations. *Diabetic Med* **25**, 1151–1156.
3. Livesey G, Taylor R, Livesey H, *et al.* (2013) Is there a dose-response relation of dietary glycemic load to risk of type 2 diabetes? Meta-analysis of prospective cohort studies. *Am J Clin Nutr* **97**, 584–596.
4. Thomas D & Elliott E (2009) Low glycaemic index, or low glycaemic load, diets for diabetes mellitus. Cochrane Database Syst Rev 2009, Cd006296.
5. El-Abhar HS & Schaalan MF (2014) Phytotherapy in diabetes: review on potential mechanistic perspectives. *World J Diabetes* **5**, 176–197.
6. Goldstein R, Braverman D & Stankiewicz H (2000) Carbohydrate malabsorption and the effect of dietary restriction on symptoms of irritable bowel syndrome and functional bowel complaints. *Isr Med Assoc J* **2**, 583–587.
7. Bond JH & Levitt MD (1977) Use of breath hydrogen (H2) In the study of carbohydrate absorption. *Am J Digestive Dis* **22**, 379–382.
8. Strocchi A, Corazza G, Ellis CJ, *et al.* (1993) Detection of malabsorption of low doses of carbohydrate: accuracy of various breath H2 criteria. *Gastroenterology* **105**, 1404–1410.
9. Mela DJ, Cao X-Z, Dobriyal R, *et al.* (2020) The effect of 8 plant extracts and combinations on post-prandial blood glucose and insulin responses in healthy adults: a randomized controlled trial. Nutr Metab 17, 1–12.
10. Asai A, Nakagawa K, Higuchi O, *et al.* (2011) Effect of mulberry leaf extract with enriched 1-deoxynojirimycin content on postprandial glycemic control in subjects with impaired glucose metabolism. *J Diabetes Investig* **2**, 318–323.
11. Banu S, Jabir NR, Manjunath NC, *et al.* (2015) Reduction of postprandial hyperglycemia by mulberry tea in type-2 diabetes patients. *Saudi J Biol Sci* **22**, 32–36.
12. Chiasson J-L, Josse RG, Gomis R, *et al.* (2002) Acarbose for prevention of type 2 diabetes mellitus: the stop-niddm randomised trial. *Lancet* **359**, 2072–2077.
13. Chung HI, Kim J, Kim JY, *et al.* (2013) Acute intake of mulberry leaf aqueous extract affects postprandial glucose response after maltose loading: randomized double-blind placebo-controlled pilot study. *J Funct Food* **5**, 1502–1506.

British Journal of Nutrition

14. Kimura T, Nakagawa K, Kubota H, *et al.* (2007) Food-grade mulberry powder enriched with 1-deoxynojirimycin suppresses the elevation of postprandial blood glucose in humans. *J Agric Food Chem* **55**, 5869–5874.
15. Lown M, Fuller R, Lightowler H, *et al.* (2017) Mulberry-extract improves glucose tolerance and decreases insulin concentrations in normoglycaemic adults: results of a randomised double-blind placebo-controlled study. *PLOS ONE* **12**, E0172239.
16. Nakamura M & Hashiguchi-Ishiguro M (2010) Suppressive effect of extractive from leaves of morus alba with α-glucosidase inhibitors on human postprandial blood glucose elevation induced by starchy foods. *Jpn J Nutr Diet* **68**, 351–358.
17. Nakamura M, Nakamura S & Oku T (2009) Suppressive response of confections containing the extractive from leaves of morus alba on postprandial blood glucose and insulin in healthy human subjects. *Nutr Metab* **6**, 29.
18. Nakamura S, Hashiguchi M, Yamaguchi Y, *et al.* (2011) Hypoglycemic effects of morus alba leaf extract on postprandial glucose and insulin levels in patients with type 2 diabetes treated with sulfonylurea hypoglycemic agents. *J Diab Metab Doi* **10**, 2155–6156.
19. Chaiyasit K, Thivasab D, Khusivilai W, *et al.* (2012) The study of morusalba extract (10 % 1–deoxynojirimycion) on plasma glucose in thais. *Diagn Ther Study* **1**, 85–90.
20. Thaipitakwong T, Supasyndh O, Rasmi Y, *et al.* (2020) A randomized controlled study of dose-finding, efficacy, and safety of mulberry leaves on glycemic profiles in obese persons with borderline diabetes. *Complementary Ther Med* **49**, 102292.
21. Mudra M, Ercan-Fang N, Zhong L, *et al.* (2007) Influence of mulberry leaf extract on the blood glucose and breath hydrogen response to ingestion Of 75 G Sucrose By type 2 diabetic and control subjects. *Diabetes Care* **30**, 1272–1274.
22. Thondre PS, Lightowler H, Ahlstrom L, *et al.* (2021) Mulberry leaf extract improves glycaemic response and insulaemic response to sucrose in healthy subjects: results of a randomized, double blind, placebo-controlled study. *Nutr Metab* **18**, 41.
23. Wang Z, Dai F, Tang C, *et al.* (2021) Quantitative determination of 1-deoxynojirimycin in 146 varieties of mulberry fruit. *Int J Food Prop* **24**, 1214–1221.
24. Zhong L, Furne JK & Levitt MD (2006) An extract of black, green, and mulberry teas causes malabsorption of carbohydrate but not of triacylglycerol in healthy volunteers. *Am J Clin Nutr* **84**, 551–555.

British Journal of Nutrition