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ORIGINAL CONTRIBUTION

Postprandial insulin and glucose levels are reduced in healthy subjects when a standardised breakfast meal is supplemented with a filtered sugarcane molasses concentrate

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Abstract
Purpose A phytochemical- and mineral-rich filtered sugarcane molasses concentrate (FMC), when added to carbohydrate-containing foods as a functional ingredient, lowers postprandial blood glucose and insulin responses. We hypothesised that this beneficial effect would also occur if FMC was administered as an oral supplement taken before a meal.
Methods This study measured the postprandial glucose and insulin responses elicited by different doses of FMC administered immediately prior to a standard breakfast to healthy subjects. Each subject was given three or five breakfast meals once, on different days. The composition of the meals was identical, except for the addition of either placebo syrup (test meal 1) or increasing doses of FMC (test meals 2–5).
Results The plasma glucose concentration curves were similar for the five test meals. Plasma insulin curves were lowered in a dose-dependent manner. Stratifying subjects based on age, BMI and insulin resistance showed greater effects of low doses of FMC on lowering insulin responses in those subjects with potentially greater insulin resistance. When insulin response is standardised to amount of carbohydrate in the meal/dose combination, the reduction in response is linear and inversely proportional to the FMC dose.
Conclusions FMC shows promise as an agent that can reduce insulin responses and lessen the load on the pancreatic beta cells.

Keywords Blood glucose · Insulin · Insulin resistance · Metabolic syndrome

Introduction

The role of carbohydrate metabolism and its influence on health is clearly recognised by health professionals [1–3]. There is increasing prevalence of so-called diseases of civilisation in developed populations where highly processed foods are consumed, and carbohydrate and other nutrient consumption is well beyond nutritional needs [4]. There is growing interest in the benefits of low glycaemic index (GI) diets as useful tools in addressing metabolic disorders, weight management and/or weight maintenance [5, 6]. A meta-analysis suggested the benefits of low GI diets are comparable to known pharmacological agents that target postprandial hyperglycaemia on medium-term glycaemic control among diabetic patients [7]. Moreover, a Cochrane Review assessment indicated low GI diets could lessen the risk of disease complications and improve the quality of life of diabetic patients [8].

Sugarcane (*Saccharum officinarum* L.) is primarily used for sucrose production, but is also used for a variety of other products such as dark molasses, rapadura, jaggery, rum and ethanol. Molasses, a dark brown by-product, is produced during the conventional processing of sugarcane

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juice and is a rich source of phytochemicals and minerals. As a by-product of cane sugar manufacture, molasses offers a low cost source of these beneficial compounds.

Sugarcane molasses is also rich in hydroxycinnamic acids, mainly coumaric, ferulic, chlorogenic acids and their derivatives, as well as the flavonoids apigenin, luteolin and tricin [9]. The polyphenols found in sugarcane molasses have been found to act as antioxidants [10, 11].

Phenolic compounds are a fundamental element of both human and animal diets [12] and are abundant in tea, red wine, fruit and chocolate [13]. The most widely studied classes of polyphenols are the flavonoids, catechins, tannins and procyanidins [14]. Traditionally, polyphenols have been considered antinutritive, as some forms reduce macronutrient digestibility [15]. More recently, it has been established that many phenolic compounds have potent antioxidant and free radical scavenging activity in vivo which provides numerous health benefits [13, 16–20].

Members of most classes of polyphenols and phenolic acids have been demonstrated to have effects on carbohydrate metabolism. Hanhineva et al. [21] extensively reviewed the literature concerning polyphenol effects on inhibition of α-amylase [22], α-glucosidase [23] and glucose transporter activity in the gut [24]; protection of pancreatic cells from cytokine toxicity and effects on insulin release [25]; improved glucose uptake into tissues [26]; induction of hepatic glucokinase activity [27]; inhibition of gluconeogenesis [28]; and activation of adenosine monophosphate-activated protein kinase [29].

A filtered sugarcane molasses concentrate (FMC) has previously been shown to reduce postprandial glucose and insulin responses, as measured by standard GI and Insulin Index tests, when incorporated into carbohydrate-rich food matrices [30]. The following study was designed to determine the effects of FMC on carbohydrate metabolism in a meal tolerance test (MTT) when taken as an oral supplement before a standard breakfast meal.

Materials and methods

Study design

This study used to test the effects of FMC on postprandial glucose and insulin levels was a randomised, single-blinded, crossover design such that every subject consumed three test meals on one occasion only in random order, completing a total of three test sessions. Each subject completed his or her test sessions on separate weekday mornings at a similar time of day, as close as possible to the time at which the subject normally ate breakfast. A subset of subjects completed a further two test sessions in random order after the completion of the first three sessions.

Table 1 Composition and nutrition information for filtered molasses concentrate (FMC)

	Average values
Component	
Moisture (g/100 g)	38.7
Energy [(calc) kJ/100 g]	942
Protein (N × 6.25, g/100 g)	2.1
Fat (g/100 g)	0.9
Sucrose (g/100 g)	29.0
Glucose (g/100 g)	5.4
Fructose (g/100 g)	6.0
Total sugars (g/100 g)	41.0
Soluble dietary fibre (g/100 g)	0.3
Total carbohydrate (by difference, g/100 g)	53.0
Ash (g/100 g)	6.1
Minerals	
Sodium (mg/100 g)	47
Calcium (mg/100 g)	535
Iron (mg/100 g)	7.3
Magnesium (mg/100 g)	218
Manganese (mg/100 g)	4.4
Potassium (mg/100 g)	2350
Zinc (mg/100 g)	0.34
Phytochemicals	
Polyphenols[a] (mg CE/100 g)	Minimum 1150
Flavonoids[b] (mg/100 g)	398
ORAC value Vit E equivalent[c] (total) (μmol/100 g)	19,970

CE catechin equivalents, *calc* calculated, *N* nitrogen, *Vit* vitamin

Reference methods for determination: [a] polyphenols [46]; [b] flavonoids [47]; [c] ORAC value [48]

FMC

FMC was developed to separate and concentrate naturally occurring low molecular weight phytochemicals and minerals present in sugarcane molasses. FMC is produced by sequential micro- and ultra-filtration of primary mill molasses. Molasses was diluted with water before filtration and the filtrate was subsequently concentrated in an evaporator to 60 % (w/w) solids minimum. No solvents are used during the extraction process, and the extraction is performed in a HACCP-certified manufacturing plant using a standardised manufacturing process. The dark brown liquid extract still contains sugars, but is less dense and less viscous than molasses and is readily pourable. Table 1 demonstrates a typical nutritional profile of FMC.

Ethics, informed consent and trial registration

This study was conducted according to the guidelines laid down in the Declaration of Helsinki, and all procedures involving human subjects were approved by the

Human Research Ethics Committee of Sydney University. Written informed consent was obtained from all subjects. This trial was registered at www.anzctr.org.au as ACTRN12614001141639.

Subjects

A group of 38 healthy, non-smoking people aged between 18 and 35 years were recruited from the staff and student population at the University of Sydney. People volunteering to participate in the study were screened at the research centre and excluded if they were over- (BMI > 25 kg/m^2) or underweight (BMI < 18 kg/m^2), were dieting, had impaired fasting glucose (fasting capillary glucose >6.1 mmol/L; capillary glucose values are typically higher than venous values), were suffering from any illness or food allergy, or were regularly taking prescription medication other than standard contraceptive medication. The subject group consisted of 19 males and 19 females. The mean age of the subjects was 26.8 years (range 18.6–34.2 years), and the group's mean BMI score was 22.3 kg/m^2 (range 18.9–25.0 kg/m^2). These 38 subjects ingested the test meal and placebo or 2 doses of FMC (3 tests). Fifteen of the original 38 subjects were selected post hoc to further test two additional doses of FMC (5 tests total; 3 in first phase, 2 in extension phase). These fifteen subjects satisfied at least two of the following criteria selected as representing a higher risk of insulin resistance: age >30 years, BMI > 23.5 kg/m^2, fasting glucose >5.24 mmol/L, MTT-Matsuda Index (calculated from placebo meal) <10.5. These 15 subjects included 9 males and 6 females with a mean age of 30.2 (range 18.8–34.4 years) and BMI of 23.3 kg/m^2 (range 19.2–25.0 kg/m^2). Figure 1 illustrates the flow chart for the study population.

Meals and investigational products

The product tested in this experiment was FMC, given in a meal tolerance test (MTT) as a supplement consumed immediately prior to a standard breakfast meal. All 38 subjects tested meals 1–3, and a subset of 15 subjects also tested meals 4 and 5. The product was given to all subjects in two different doses, 8 and 22 g, and a sucrose-containing, dark-coloured placebo syrup was used as a control. The 15 subjects who were enrolled in the trial extension also subsequently ingested FMC doses of 40 and 60 g.

Each subject consumed the first three meals, in random order, each on a separate occasion with at least 1-day washout in between. The base meal consisted of 100 g of white bread, 12 g butter, 65 g scrambled eggs and 170 g orange and mango juice. Test meal one was supplemented (immediately prior to meal consumption) with 30 g of placebo syrup (meal 1), 8 g FMC + 22 g water (meal 2) or 22 g FMC + 8 g water (meal 3). The extra meals tested by 15 subjects, in random order, during the trial extension consisted of the same base meal supplemented with 40 g FMC syrup + 45 g water (meal 4) or 60 g FMC + 25 g water (meal 5). Nutritional information for the standard meal, the placebo and the FMC doses is shown in Table 2.

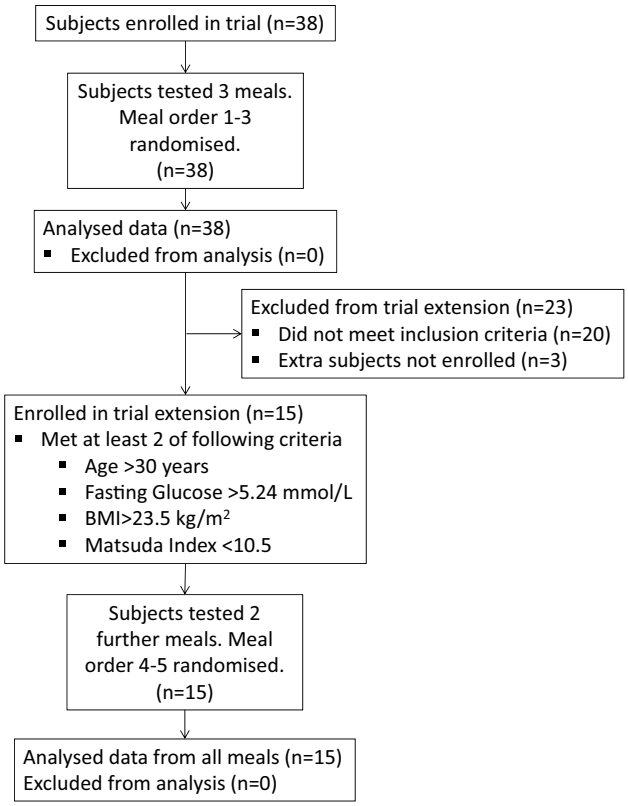

Fig. 1 Flow diagram for trial enrolment and analysis

Placebo and FMC syrups were visually similar dark-coloured liquids but differed in smell and taste. Subjects were blinded as to which syrups were investigational products and which was placebo.

Study procedures

The day before each test session, the subjects were to avoid unusual levels of food intake and physical activity and to refrain from consuming alcohol for the entire day. The night before the test session, they were required to eat a regular evening meal based on a low-fat, carbohydrate-rich food, other than legumes, and then fast for at least 10-h overnight, until the start of their test session the next morning. During the fasting period, the subjects were only allowed to drink water.

The subjects reported to the research centre in a fasting condition the next morning. On arrival, the investigators checked that the subjects had complied with the

Table 2 Macronutrient composition of breakfast meal and doses

Test food	Portion size (g)	Energy from product (kJ)	Protein (g)	Fat (g)	Available carbohydrate (g)	Sugar (g)	Fibre (g)
Standardised meal	347 g	1999	17.3	18.1	59.1	18.4	6.6
Placebo syrup	30 g	95	0	0	5.7	5.7	0
8 g FMC	8 g FMC + 22 g water	75	0.2	0.1	4.2	3.4	0
22 g FMC	22 g FMC + 8 g water	207	0.6	0.2	11.7	9.2	0.1
40 g FMC	40 g FMC + 45 g water	377	1	0.4	21.2	16.8	0.1
60 g FMC	60 g FMC + 25 g water	565	1.5	0.5	31.8	25.2	0.2

FMC filtered molasses concentrate

experimental conditions described above. The subjects then warmed a hand in hot water, after which two fasting finger-prick blood samples of ≥0.7 ml (−5 and 0 min) were obtained, using a non-reusable lancet (Safe-T-Pro®, Boehringer Mannheim Gmbh, Germany). After the second fasting sample (0 min) was obtained, the subjects were seated at a large table in a quiet room and served one of the breakfast meals, including the pre-meal syrup dose, which they consumed within 12 min. Additional blood samples were collected at 15, 30, 45, 60, 90 and 120 min after eating had commenced. The subjects were required to remain seated during their test sessions, and only minimal movement was allowed (visiting the rest rooms or walking a couple of metres to the blood sampling area). During each test session, the subjects were monitored by research staff to ensure they complied with the test conditions.

Each blood sample was centrifuged for 45 s immediately after collection. The plasma layer of the sample was then transferred into a labelled, uncoated tube, and was immediately placed in a freezer. The plasma samples were stored in the freezer at −20 °C until their glucose and insulin concentrations were analysed.

Measurement of plasma glucose concentrations

The glucose concentrations of plasma samples were analysed in duplicate using a glucose hexokinase enzymatic assay (Roche Diagnostic Systems, Sydney, Australia) and an automatic centrifugal spectrophotometric analyser (Roche/Hitachi 912®, Boehringer Mannheim Gmbh, Germany) with internal controls. If the duplicate values differed by more than 0.3 mmol/L, the sample was reanalysed twice, and the most similar concentrations were used to calculate an average plasma glucose concentration for that sample. The two fasting plasma samples of each test session were averaged to provide one baseline glucose concentration. A 2-h plasma glucose curve was then constructed for each subjects' test sessions. Incremental area under the curve (iAUC) was calculated using only the area above the baseline (fasting) glucose value.

Measurement of plasma insulin concentrations

The concentration of insulin in plasma samples was analysed using a solid-phase antibody-coated tube radioimmunoassay kit (Coat-a-Count® Insulin RIA kit, Diagnostic Products Corporation, Los Angeles, CA, USA) with internal controls. The two fasting blood samples were averaged to provide one baseline insulin concentration. A 2-h plasma insulin curve was then constructed for each subject's test sessions.

Calculation of a modified Matsuda Index based on meal tolerance test measurements

The standard Matsuda Index is a measure of insulin sensitivity based on plasma glucose and insulin levels at a specific time during an oral glucose tolerance test (OGTT). The MTT-Matsuda Index used for this study was calculated using measurement of plasma glucose and insulin responses to the test meal and placebo supplement, calculated as described [31]:

$$10{,}000/\mathrm{sqrt}((\text{fasting plasma glucose} \times \text{fasting plasma insulin}) \times \text{mean plasma glucose concentration} \times \text{mean plasma insulin concentration})$$

As this study evaluates glucose and insulin responses to this test meal (rather than glucose syrup used in standard OGTT), MTT-Matsuda Index values are only relevant for internal comparisons of this study. MTT-Matsuda Index values were only used for calculation of insulin sensitivity to the placebo meal for subgrouping of subjects; no comparisons were made of MTT-Matsuda Index values between meals.

Standardisation of meal carbohydrate content

The carbohydrate endogenous to FMC means that the meal/dose combination for each treatment contained different amounts of carbohydrate. Standardisation of results to the level of carbohydrate consumed was calculated for a

reference amount, arbitrarily set at the carbohydrate level in the placebo/meal combination. Based on the findings of Lee and Wolever [32], a response factor based on the curvilinear glucose responses and linear insulin response to increasing carbohydrate levels was included in the standardisation. As all the meals had a carbohydrate content between 63 and 91 g, the response factor was set at 0.4 for glucose values and 1 for insulin values and the standardisation calculated as follows:

(carbohydrate content in placebo meal)/[(difference between carbohydrate content in FMC dose meal and carbohydrate content in placebo meal) × response factor + carbohydrate content in placebo meal] × (study measure)

Statistical analyses

The sample size of 38 subjects for the first part of the study was selected to allow detection of an effect size of 0.35 in postprandial glucose iAUC (22 g FMC vs. placebo) with 80 % power and two-sided alpha of 0.05, allowing for 10 % attrition. Post hoc selection of 15 participants for the extended trial was based on "at greater risk" (of developing insulin resistance/glucose intolerance) selection criteria rather than a sample size calculation (see below), but this sample size results in a 94 % power to allow detection of an effect size of 0.35 in postprandial insulin iAUC.

Meal order was randomised for the three meals in the first part of the study, with the six possible meal order combinations reasonably balanced (5, 5, 6, 7, 7 and 8 subjects in each combination). Order effects were determined by adding a code specific for meal order into the ANOVA. There were no significant period or order effects in either the glucose or insulin responses in the first part of the study. Meal order was also randomised for the two meals in the extension part of the study, and the two possible meal order combinations were balanced (7 and 8 subjects in each combination). There were no significant period or order effects in either the glucose or insulin responses in the extension part of the study. Although these meals (4 and 5) were not randomised within the meal 1–3 testing, results from all tests for these subjects were combined for ANOVA as there were no discernible crossover or period effects in either part of the study.

Repeated-measures analysis of variance was calculated with time (7) and treatment (3 or 5) as the within-subject factors. Between subject factors included risk factors for metabolic syndrome/insulin resistance: BMI ($\leq$ or >23.5 kg/m^2), age ($\leq$ or >30 years), MTT-Matsuda Index group (<10.5 or >10.5) and fasting glucose levels (<5.243 or $\geq$5.243 mmol/L). Cutoffs were selected to compare the top "at greater risk" tertile (for the development of insulin resistance/metabolic syndrome, $n = 12$) to the rest of the subjects ($n = 26$). In some analyses, the data were standardised to equal carbohydrate loads as described above, as the amount of carbohydrate varied. Primary endpoints were changes in 2 h or peak glucose and insulin responses. Secondary endpoints were the time points at which glucose and insulin changed if the primary analysis was significant. No adjustment was made for these secondary endpoints. Bivariate correlation was conducted using Pearson's correlation coefficient. Analyses were performed with SPSS 10.0 for Windows (SPSS Inc, Chicago, Ill). Significance was set at $p < 0.05$ for iAUC and peak glucose comparisons and $p < 0.01$ for time-by-treatment comparisons (6 comparisons after $T = 0$ is set at baseline) to reduce chances of type I errors.

Results

Postprandial glucose responses to two FMC doses versus placebo

Postprandial glucose responses to the placebo, 8 g and 22 g FMC doses before a standardised breakfast meal are shown in Fig. 2a ($n = 38$). Using repeated-measures ANOVA, there was no significant effect of treatment on glucose responses.

In post hoc analysis, when all treatments were normalised to equal amounts of available carbohydrate (see "Materials and methods", Fig. 2b), there is a time-by-treatment effect ($p = 0.03$). The normalised change value at 30 min was significantly lower ($p < 0.01$) with the 22-g FMC dose (2.18 mmol/L) compared to the placebo (2.55 mmol/L). The normalised change value at 45 min with 22 g FMC (1.39 mmol/L) was also significantly lower than placebo (1.71 mmol/L, $p < 0.01$). Peak change values, when adjusted for carbohydrate, showed a significant treatment effect ($p < 0.001$) with the peak value in response to the 22-g FMC dose (2.42 mmol/L) significantly lower than the placebo (2.66 mmol/L; $p < 0.02$) treatment (Table 3).

Postprandial insulin responses to two FMC doses versus placebo

Postprandial insulin responses to the placebo, 8 g and 22 g FMC doses before a standardised breakfast meal are shown in Fig. 2c ($n = 38$), and responses standardised to carbohydrate level are shown in Fig. 2d. There is a treatment effect for both absolute values ($p < 0.01$) and carbohydrate standardised values ($p < 0.005$) as well as a time-by-treatment effect with standardised values ($p < 0.0001$). The 22-g dose of FMC resulted in a significant reduction in peak insulin response compared to placebo, 252 v

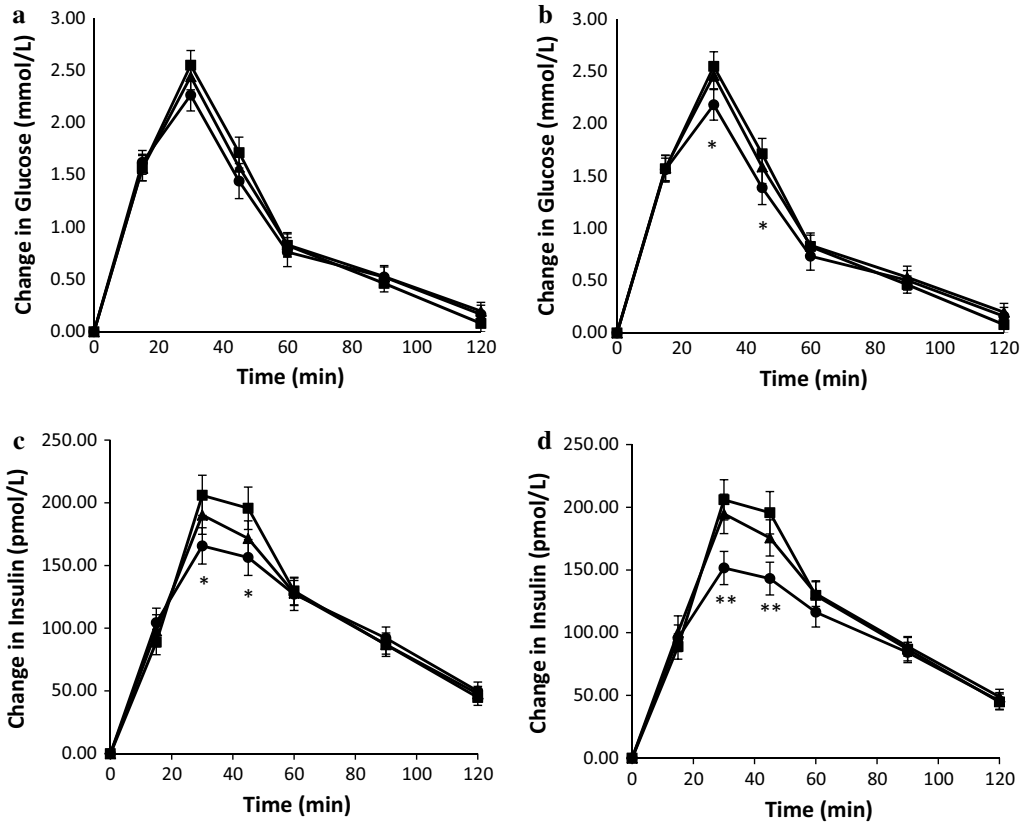

Fig. 2 Glucose and insulin responses to filtered molasses concentrate (FMC). **a** Plasma glucose change over 2-h period (mean ± SEM; $n = 38$) after ingestion of standardised breakfast meal with a syrup supplement taken before meal: placebo (*squares*), 8 g of FMC (*triangles*) or 22 g of FMC (*circles*). **b** Plasma glucose change standardised for carbohydrate ingestion (arbitrarily adjusted to 64.8 g, amount ingested during placebo test) over 2-h period (mean ± SEM; $n = 38$) after ingestion of standardised breakfast meal with a syrup supplement taken before meal: placebo (*squares*), 8 g of FMC (*triangles*) or 22 g of FMC (*circles*). **c** Plasma insulin change (mean ± SEM; $n = 38$) over 2-h period after ingestion of standardised breakfast meal with a syrup supplement taken before meal: placebo (*squares*), 8 g of FMC (*triangles*) or 22 g of FMC (*circles*). **d** Plasma insulin change standardised for carbohydrate ingestion (arbitrarily adjusted to 64.8 g, amount ingested during placebo test) over 2-h period (mean ± SEM; $n = 38$) after ingestion of standardised breakfast meal with a syrup supplement taken before meal: placebo (*squares*), 8 g of FMC (*triangles*) or 22 g of FMC (*circles*). *$p < 0.01$, **$p < 0.001$

Table 3 Glucose and insulin incremental area under the curve (iAUC) and peak change in response to differing doses of filtered molasses concentrate (mean ± SEM)

	Absolute values ($n = 38$)			Values adjusted for carbohydrate consumption[a] ($n = 38$)		
	Placebo	8 g FMC	22 g FMC	Placebo	8 g FMC	22 g FMC
Fasting glucose (mmol/L)	4.94 ± 0.06	5.03 ± 0.07	4.99 ± 0.06			
Glucose iAUC (mmol/L min)	123.6 ± 8.7	124.8 ± 8.1	119.2 ± 8.8	123.6 ± 8.7	124.8 ± 8.1	119.2 ± 8.8
Peak change glucose (mmol/L)	2.66 ± 0.12	2.61 ± 0.11	2.52 ± 0.12	2.66 ± 0.12	2.63 ± 0.12	2.42 ± 0.11*[,b]
Fasting insulin (pmol/L)	29.66 ± 1.58	28.31 ± 1.51	30.87 ± 1.55			
Insulin iAUC (pmol/L min)	13,554 ± 846	13,103 ± 810	12,766 ± 951	13,554 ± 846	13,414 ± 829	11,684 ± 870**
Peak change insulin (pmol/L)	251.6 ± 16.1	230.7 ± 13	211.1 ± 12.9**	251.6 ± 16.1	236.2 ± 13.3	193.2 ± 11.8**

[a] Response standardised to carbohydrate ingested during placebo/meal by the formula: (carbohydrate content in placebo meal)/[(difference between carbohydrate content in FMC dose meal and carbohydrate content in placebo meal) × response factor + carbohydrate content in placebo meal] × (study measure)

[b] Significantly different from placebo, * $p < 0.05$; ** $p < 0.01$

211 pmol/L ($p < 0.02$). When standardised to equivalent carbohydrate, the treatment effect was greater ($p < 0.001$; Table 3). Insulin resistance risk factors for BMI and age interacted with treatment (both $p < 0.01$). There was a time-by-treatment interaction with MTT-Matsuda Index ($p < 0.001$).

When subjects were grouped into the "at greater risk" tertile ($n = 12$) based on age, BMI or MTT-Matsuda Index, there were significant treatment and/or time-by-treatment effects on insulin response as compared with the remaining subjects. The remaining 26 subjects displayed no reduction in insulin response when treated with 8 g FMC compared to placebo, while the "at greater risk" tertile demonstrated up to 19 % reduced peak insulin response and up to 15 % reduction in incremental area under the curve (iAUC) for insulin response. The reduced insulin responses were almost identical for all "at greater risk" groups, for both FMC doses of 8 and 22 g (Table 4). Repeated-measures ANOVA on groups after fasting glucose-level stratification did not show an interaction with treatment or time-by-treatment in either subgroup, but the "at greater risk" group had a significantly lower iAUC for 22 g (12,841 pmol/L min) relative to placebo (14,325 pmol/L min, $p < 0.02$), which was not seen in the lower-risk group.

Trial extension: glucose responses with two additional FMC doses

Postprandial glucose and insulin responses to placebo, 8 g, 22 g, 40 g and 60 g of FMC were measured for those subjects with a greater risk of insulin resistance enrolled in the trial extension ($n = 15$, Fig. 3a). Repeated-measures ANOVA showed a treatment effect ($p < 0.05$) and a time-by-treatment effect ($p < 0.005$) on glucose responses. There was a dose-dependent increase in glucose response at 15 min relative to placebo which reached significance for the 40 and 60 g FMC doses (both $p < 0.001$) and an increase relative to placebo with 40 g FMC dose at 120 min ($p < 0.01$).

Post hoc adjustment for relative levels of carbohydrate ingested showed a significant time-by-treatment effect ($p < 0.001$) with the 22-g and 60-g dose value reduction compared to placebo of borderline significance at 30 min ($p = 0.016$ and $p = 0.013$, respectively). At 45 min, the 60-g dose value was significantly lower than placebo ($p < 0.005$) and 22 g dose value reduction was of borderline significance ($p = 0.018$). The 40-g dose had a borderline significant increase in glucose response at 15 min ($p = 0.014$). Peak glucose responses were also reduced, with 22 g (2.42 mmol/L, $p < 0.05$) and 60 g doses (2.35 mmol/L, $p < 0.05$) relative to placebo (2.67 mmol/L) (Fig. 3b)

Trial extension: insulin responses with two additional FMC doses

There were strong treatment and time-by-treatment interactions for insulin responses to FMC (both $p < 0.0001$). At 30 min, all doses of FMC resulted in significantly lower insulin responses than placebo. At 45 min, all FMC doses except the lowest (8 g) resulted in significantly lower insulin responses than placebo, and at 60 min, the highest FMC dose (60 g) had a significantly lower insulin response than placebo (Fig. 3c). After standardisation for carbohydrate content, additional significance was determined for the 40-g FMC dose at 60 min and the 60-g FMC dose at 90 min (Fig. 3d). All doses had a significantly lower peak insulin change than placebo ($p < 0.01$). Despite a greater than 50 % increase in the amount of available carbohydrate in the 60-g FMC dose/standardised meal combination than the placebo, there is a 39 % reduction in the insulin incremental area under the curve (iAUC) and a 48 % reduction in peak insulin response. After standardisation for carbohydrate content, there was a 57 % reduction in insulin iAUC and a 63 % reduction in peak insulin response.

With post hoc calculation to standardise the amount of carbohydrate consumed with each test meal, the reduction in insulin iAUC is an almost perfectly linear dose response between 8 g and 60 g of FMC (Fig. 4). The reduction in insulin iAUC between placebo and 8 g FMC has a steeper gradient than the rest of the curve and is also significantly different to those subjects not included in the extended study (Fig. 4).

Adverse events

There were no adverse events reported by any subject for any dose of FMC.

Discussion

In absolute terms, there was no effect of FMC on postprandial glucose response (Fig. 2a); however, when corrected for the amount of carbohydrate consumed (see "Materials and methods") by subjects on each visit, there were small treatment and time-by-treatment effects of FMC in reducing overall 2-h postprandial glucose response (Fig. 2b). The effect of FMC was only significant with the 22-g dose of FMC.

Preclinical studies in mice and cats with diets supplemented with one of several sugarcane molasses extracts, including FMC, consistently show an increase in faecal energy which has been determined to be the result of increased carbohydrate excretion (unpublished results). This finding suggests that FMC inhibits uptake of

Table 4 Insulin responses of subgroups to filtered molasses concentrate

	All subjects ($n = 38$)				"At greater risk" groups								
	Treatment	Mean	SEM	% Change[a]	Age > 30 years ($n = 12$)			BMI > 23.5 kg/m^2 ($n = 12$)			Mat. Ind. < 10.5 ($n = 12$)		
					Mean	SEM	% Change	Mean	SEM	% Change	Mean	SEM	% Change
Peak insulin response (pmol/L)	Placebo	251.6	16.1	–	283.8	33.3	–	302.7	36.0	–	320.2	26.8	–
	8 g FMC	230.7	13.0	−9	228.5*,[b]	30.8	−19	241.7	24.2	−20	255.4*	31.5	−20
	22 g FMC	211.1**	12.9	−9	205.7**	28.4	−10	220.1*	25.1	−9	234.2***	24.8	−8
Insulin iAUC (pmol/L min)	Placebo	13,554	846	–	15,551	1737	–	16,014	1753	–	18,092	1326	–
	8 g FMC	13,103	810	−3	13,202	1847	−15	13,568*	1407	−15	15,596	1914	−14
	22 g FMC	12,766	951	−3	12,616**	1892	−4	13,463*	1889	−1	15,288*	1692	−2
					Reciprocal groups								
					Age ≤ 30 years ($n = 26$)			BMI ≤ 23.5 kg/m^2 ($n = 26$)			Mat. Ind. > 10.5 ($n = 26$)		
					Mean	SEM	% Change	Mean	SEM	% Change	Mean	SEM	% Change
Peak insulin response (pmol/L)	Placebo				233.2	17.4	–	224.4	14.8	–	219.9	17.1	–
	8 g FMC				231.3	13.2	−1	223.9	16.0	0	219.3	12.2	0
	22 g FMC				213.2	14.0	−8	206.6	15.2	−8	200.0	14.9	−9
Insulin iAUC (pmol/L min)	Placebo				12,632	909	–	12,418	872	–	11,459	797	–
	8 g FMC				13,058	851	+3	12,889	1005	+4	11,953	711	+4
	22 g FMC				12,835	1108	−2	12,444	1104	−3	11,602	1097	−3

FMC filtered molasses concentrate, *Mat. Ind.* MTT-Matsuda Index

[a] % Change, change relative to preceding dose level (8 g relative to placebo; 22 g relative to 8 g)

[b] Significantly different from placebo, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.005$

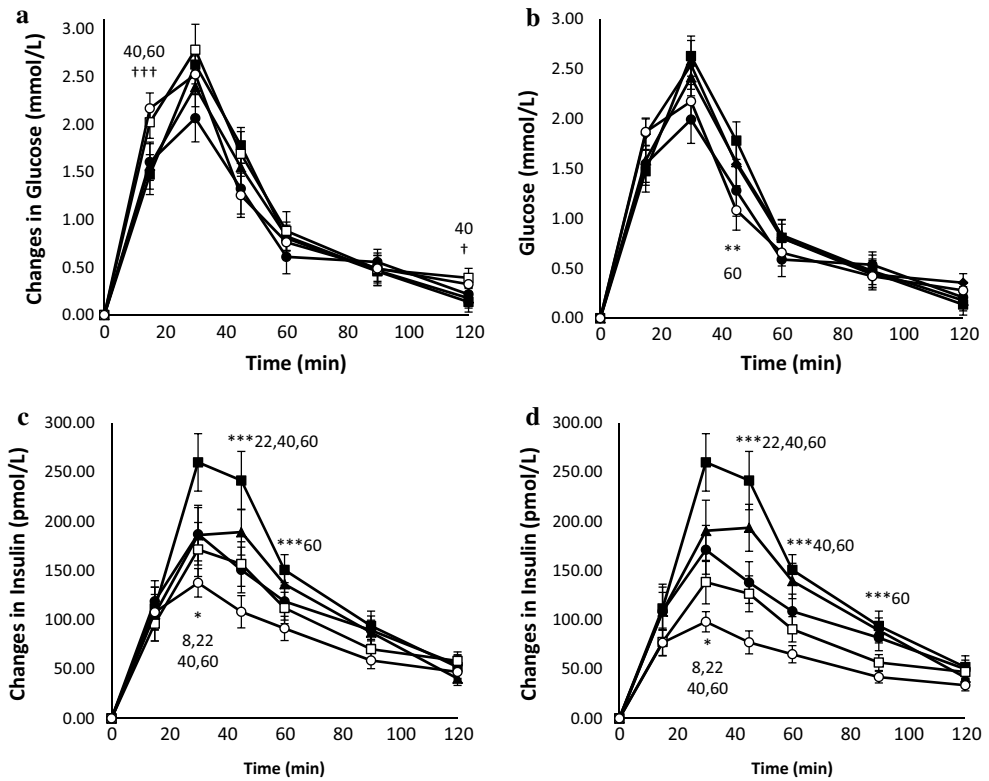

Fig. 3 Glucose and insulin responses to filtered molasses concentrate (FMC) in subjects undertaking extended trial. **a** Plasma glucose change over 2-h period (mean ± SEM; $n = 15$) after ingestion of standardised breakfast meal with a syrup supplement taken before meal: placebo (*closed squares*), 8 g of FMC (*closed triangles*), 22 g of FMC (*closed circles*), 40 g of FMC (*open squares*) or 60 g of FMC (*open circles*). **b** Plasma glucose change standardised for carbohydrate ingestion (arbitrarily adjusted to 64.8 g, amount ingested during placebo test) over 2-h period (mean ± SEM; $n = 38$) after ingestion of standardised breakfast meal with a syrup supplement taken before meal: placebo (*squares*), 8 g of FMC (*closed triangles*), 22 g of FMC (*closed circles*), 40 g of FMC (*open squares*) or 60 g of FMC (*open circles*). **c** Plasma insulin change (mean ± SEM; $n = 38$) over 2-h period after ingestion of standardised breakfast meal with a syrup supplement taken before meal: placebo (*squares*), 8 g of FMC (*closed triangles*), 22 g of FMC (*closed circles*), 40 g of FMC (*open squares*) or 60 g of FMC (*open circles*). **d** Plasma insulin change standardised for carbohydrate ingestion (arbitrarily adjusted to 64.8 g, amount ingested during placebo test) over 2-h period (mean ± SEM; $n = 38$) after ingestion of standardised breakfast meal with a syrup supplement taken before meal: placebo (*squares*), 8 g of FMC (*closed triangles*), 22 g of FMC (*closed circles*), 40 g of FMC (*open squares*) or 60 g of FMC (*open circles*). Doses with significantly lower values than placebo at a specific time point are shown with the least significant p value for all effective doses ($^{*}p < 0.01$, $^{**}p < 0.005$, $^{***}p < 0.001$). Significantly higher values than placebo at a specific time point are shown with the least significant p value for all effective doses ($^{\dagger}p < 0.01$, $^{\dagger\dagger\dagger}p < 0.001$)

carbohydrates. Several possible mechanisms include delaying digestion by inhibition of digestive enzymes, delaying gastric emptying or inhibiting intestinal glucose transport.

Earlier studies have shown FMC does not inhibit starch digestion in a simulated gastrointestinal digestion system, nor does it inhibit salivary α-amylase, pancreatic α-amylase or α-glucosidase (unpublished results). Additionally, the results described in Wright et al. [30] which demonstrate the linearity of glycaemic response reduction regardless of whether the carbohydrate is present as a starch (in a bread product, for example) or as a simple sugar (high-fructose corn syrup, etc.) support the finding that FMC does not extensively inhibit breakdown of complex carbohydrates. Also supporting this finding is that there were no adverse gastrointestinal events often seen in pharmacological treatments for reducing blood glucose levels, such as acarbose, which inhibit these digestive enzymes [33].

In the present study, the significant increases in glucose responses of the subjects at 15 min for the two highest FMC doses (40 and 60 g) also suggest that FMC does not physically delay gastric emptying which is seen with some other interventions used to lower glycaemic responses, such as a protein or D-xylose preload [34, 35].

Therefore, a more likely explanation for the lower postprandial glucose and insulin levels observed in this study would be the result of less glucose being transported across the intestinal layer; the fact that we do not see a lowering of glucose response concomitant with the lowered insulin response is likely to be the result of lower GLP-1/GIP-stimulated insulin release resulting from reduced glucose transit from the gut. Other researchers have shown that

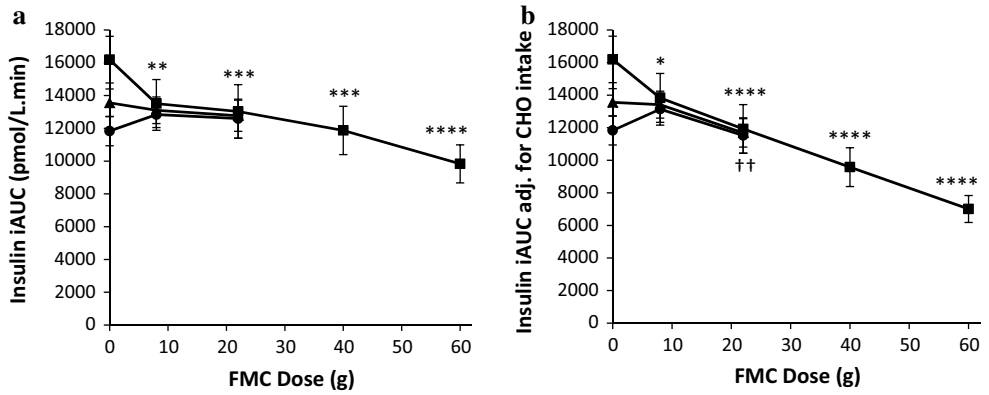

Fig. 4 Insulin responses to filtered molasses concentrate (FMC). Insulin incremental area under the curve (iAUC) responses are compared between "at greater risk" subjects included in the extended study ($n = 15$, *squares*), those not included ($n = 23$, *circles*) and all responses from original three meals ($n = 38$, *triangles*). **a** Insulin iAUC (mean ± SEM) plotted against amount of FMC ingested. **b** Insulin iAUC standardised by the amount of available carbohydrate in each meal/dose combination (arbitrarily adjusted to 64.8 g, the amount of carbohydrate in the placebo test; mean ± SEM) and then plotted against the amount FMC ingested. *Adj.* adjusted, *CHO* carbohydrate. Doses with significantly lower values than placebo with the extended study subjects ($n = 15$) are indicated with $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$, $^{****}p < 0.0001$. Doses with significantly lower values than placebo with all subjects ($n = 38$) are indicated with $^{\dagger\dagger}p < 0.01$

insulin responses to differing amounts of carbohydrate have a greater linearity than blood glucose responses [32]. If we accept the explanation for inhibition of glucose intestinal transport by FMC, this should theoretically result in a linear dose–response reduction in insulin response. Indeed, disregarding the differential insulin responses of the "at greater risk" and other subjects to the placebo meal, the FMC-mediated dose–response reductions in adjusted (for carbohydrate load) insulin iAUC from 8 g to 60 g are highly linear (Fig. 4b). Some of the phenolic compounds identified in FMC, including ferulic acid, have been shown to have this activity in in vitro assays [36].

Despite the young average age (26.8 years) of the study population and the absence of overweight subjects in the study, the insulin responses are readily stratified and grouped to show differing responses. Comparing the upper tertile of subjects ("at greater risk") to the rest (reciprocal groups), there are significant differences in insulin responses with groupings based on age (≤ or >30 years), BMI (≤ or >23.5 kg/m^2) and MTT-Matsuda Index (< or >10.5) (Table 4). This post hoc analysis performed on the data from the first part of this study (placebo, 8 g and 22 g FMC) suggests a potential improvement in insulin sensitivity. In Table 4, actual (not carbohydrate adjusted) insulin responses are shown comparing the "at greater risk" groups to the reciprocal groups. In the "at greater risk" groups, 8 g of FMC causes a 19–20 % reduction in peak insulin response compared to placebo, and a 14–15 % reduction in insulin iAUC. The reciprocal, potentially more insulin sensitive, groups essentially had little or no response to the 8-g FMC dose. Interestingly, all six subgroups, as well as the whole 38-subject population, had an 8–10 % reduction in peak insulin response when comparing the 8- and 22-g doses of FMC, likely due to the inhibition of intestinal glucose transport which action is presumed to be independent of sensitivity to insulin. The potential improvement in insulin sensitivity is also demonstrated in Fig. 4 where the at-risk (more insulin resistant) subjects ($n = 15$) included in the extended study (40 g and 60 g FMC doses) had an almost 40 % higher insulin response to the placebo meal than those less insulin-resistant subjects not included in the extended study ($n = 23$), while the insulin iAUC for 8 g FMC was similar for both groups.

Polyphenols have been shown to have various possible roles in improving insulin sensitivity. Numerous flavonoids and phenolic acids have been shown in in vitro studies to increase the uptake of glucose into peripheral tissue cells [37, 38]. They have been shown to enhance or bypass insulin signalling via numerous mechanisms: activation of insulin-dependent and insulin-independent signalling pathways such as AMP kinase [38, 39] and PI-3 kinase [39] as well as mimicking insulin and activating the insulin receptor [40–42].

It would be of interest to determine the active phytochemical(s) in FMC on both glucose and insulin metabolism. It is possible the metabolic effects observed are the polypharmacological action of several phytochemicals and/or minerals rather than only one. Mertens-Talcott and Percival [45] demonstrate this synergistic polypharmacological effect with ellagic acid, quercetin and resveratrol, three polyphenols found together in red wine.

This study was limited to solely determine the acute effects of FMC on postprandial glucose and insulin levels to healthy subjects. One of the confounding issues with studying FMC is the differing amounts of carbohydrate in different doses. Alternative study designs might

match placebo and FMC doses to the same amount of carbohydrate or instead utilise a carbohydrate-free placebo to determine whether FMC doses lower glucose and insulin levels when compared to the test meal itself. Further studies on more insulin-resistant subjects, such as those with metabolic syndrome or prediabetes, would be designed to determine whether FMC does have greater effects on postprandial glucose and insulin control in insulin-resistant subjects. Future studies, unlike the present one, could include biochemical measures to verify the hypotheses on mechanism of action. Gastric emptying and/or intestinal glucose transport inhibition can be assayed with addition of non-metabolisable markers into the test meal. Analysis of insulin production by C-peptide and incretion assays should also be performed. Another possibility is that FMC is causing inhibition of hepatic glucose production in subjects presenting in a fasted state. This inhibition of hepatic glucose production has previously been shown for phenolic compounds including epigallocatechin gallate, found in green tea [43]. Further studies using euglycaemic clamps and tracers of hepatic glucose production [44] would likely be required to verify an effect of FMC on the reduction in basal glucose production. Determination of any insulin-sensitising effects of FMC would require a longer-term study testing insulin responses to a standardised OGTT, for example, before and after a subchronic treatment. Measuring acute improvements in insulin sensitivity by immediate FMC supplementation would be confounded by the restriction of glucose intestinal transport, meaning that insulin release would be in response to differing amounts of glucose appearance in the blood.

In conclusion, there are two main findings from this study:

1. Despite increasing levels of ingested carbohydrate, higher FMC doses lower postprandial insulin responses by up to 50 % in measured responses (60 % when adjusted for carbohydrate load) in a linear fashion, consistent with a reduction in glucose transport across the intestine.
2. A low FMC dose (8 g) only reduces postprandial insulin responses in those subject groups with higher insulin responses to the placebo meal, possibly suggesting an additional beneficial effect for those subjects with increased insulin resistance.

How FMC directly and acutely affects insulin sensitivity remains to be determined, but the lowering of the insulin response by up to 50 % demonstrates the potential of FMC to reduce the metabolic stress on the pancreatic β cells of subjects with insulin resistance syndromes such as prediabetes, diabetes and polycystic ovarian syndrome.

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Compliance with ethical standards

Conflicts of interest This study was conducted under contract by the Sydney University Glycaemic Index Research Service and financed by Horizon Science. Horizon Science retained the services of the Baker IDI Heart and Diabetes Institute for the scientific input of Peter Clifton towards this study and manuscript.

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