# Polyphenol and fibre-rich dried fruits with green tea attenuate starch-derived postprandial blood glucose and insulin; a randomized, controlled, single blind, crossover intervention.

H. Nyambe-Silavwe and G. Williamson

School of Food Science and Nutrition, University of Leeds, Leeds, United Kingdom

The present work was supported by the Commonwealth Scholarship Commission U.K. (ZMSC-2012-593) and the National Institute for Scientific and Industrial Research (NISIR), Zambia.

Address correspondence to G Williamson, School of Food Science and Nutrition, University of Leeds, Woodhouse Lane, Leeds, Yorkshire LS2 9JT, United Kingdom. E-mail g.williamson@leeds.ac.uk. Telephone: +44 (0)113 3438380.

Abbreviations used: Polyphenol and fibre-rich food (PFRF); incremental area under the curve (IAUC); sodium dependent glucose transporter type 1 (SGLT1); glucose transporter type 2 (GLUT2); body mass index (BMI); ethylenediaminetetraacetic acid (EDTA); impaired glucose tolerance (IGT); 3,5-dinitrosalicylic acid (DNS).

Reprints will not be available from the author.

PubMed indexing: Nyambe-Silavwe, Williamson.

Running header: Polyphenol and fibre-rich foods attenuate post-prandial glucose

This study is listed in the ClinicalTrials.gov registry (www.clinicaltrials.gov) with ref no. NCT01994135.

## Abstract

Polyphenol and fibre-rich foods have the potential to affect postprandial glycaemic responses by reducing glucose absorption, and so decreasing the glycaemic response of foods when consumed together. A randomized, single blind crossover study was conducted on 16 healthy volunteers to test whether polyphenol and fibre-rich foods (PFRF) could attenuate post-prandial blood glucose in healthy volunteers when added to a source of carbohydrate (starch in bread). This is the first study to examine the effects of a meal comprised of components to inhibit each stage of the biochemical pathway leading up to the appearance of glucose in the blood. The volunteers were fasted and attended four visits: two control visits (bread, water, balancing sugars) and two test visits (single and double dose of the PFRF) where they consumed bread, water and PFRF. Blood samples were collected at 0 (fasted), 15, 30, 45, 60, 90, 120, 150 and 180 min post consumption. The PFRF components were tested for α-amylase and α-glucosidase inhibitory potential in vitro. Plasma glucose was lower after consumption of both doses compared to controls: Lower dose, change in incremental area under the curve (IAUC) =-27.4±7.5 % (mean ± SD) $p<0.001$; higher dose, IAUC=-49.0±15.3 %, $p<0.001$); insulin IAUC was also attenuated by -46.9±13.4% (mean ± SD; $p<0.01$). Consistent with this, the polyphenol components of the PFRF inhibited α-amylase (green tea, strawberry, blackberry and blackcurrant) and α-glucosidase (green tea) activities in vitro. The PFRF have a pronounced and significant lowering effect on postprandial blood glucose and insulin response in humans, due in part to inhibition of α-amylase, α-glucosidase and also glucose transport.



**Key words:** postprandial glucose**,** diabetes, α-amylase, α-glucosidase, polyphenols, fibre

## Introduction

Postprandial hyperglycaemia and high glycaemic index diets in humans play a major role in the development of type 2 diabetes [1; 2; 3; 4], and furthermore low glycaemic index diets show favourable changes in health markers such as Plasminogen activator inhibitor-1[5], glycated proteins [6; 7] and fasting blood glucose, especially in those with already elevated values [7]. Recent meta-analyses report that low carbohydrate and low GI diets have promising effects in diabetes management [8]. Strategies to reduce the glycaemic index of foods, even without altering the total carbohydrate content, are therefore of growing interest for reducing diabetes risk. The glycaemic index and response depend on several related factors, including the nature and amount of carbohydrate, the rate of carbohydrate digestion in the gastrointestinal tract, the rate of absorption of the resulting glucose, the insulin response to the absorbed sugar, and the intrinsic insulin sensitivity [9]. The presence of naturally-occurring polyphenols have been associated with low glycaemic index foods for many decades [10]. Fibre can also play a role in reducing hyperglycaemia, by delaying glucose absorption, increasing insulin secretion and sensitivity, and binding of bile acids [11]. In addition, soluble fibre attenuates postprandial glucose by increasing the viscosity in the gastrointestinal tract which disturbs carbohydrate breakdown and glucose absorption [12]. Possible mechanisms by which polyphenols may affect post-prandial glycaemia are the inhibition of carbohydrate digesting enzymes and glucose transporters, stimulation of pancreatic β-cells to secrete insulin, activation of insulin receptors, modulation of the release of glucose from the liver, and effects on intracellular signalling pathways and gene expression [13; 14]. The potential action of polyphenols can be compared to that of acarbose, an α-glucosidase and α-amylase inhibitor, which reduces diabetes risk [15]. The Study To Prevent Non-Insulin dependent Diabetes Mellitus (STOP-NIDDM) trial in impaired glucose tolerant (IGT) subjects showed a 36% risk reduction in the

progression to diabetes after treatment with acarbose [16]. The use of diet-related intervention either on its own or in combination with acarbose would be an alternative to the use of acarbose alone, which can lead to side effects such as flatulence, nausea and diarrhoea.

Some polyphenols inhibit starch-digesting enzymes (α-amylase and α-glucosidase), in addition to glucose transporters SGLT1 (SLC5A1) and GLUT2 (SLC2A2) [13]. Most intervention studies so far have focused on the effect of polyphenols with the endogenous carbohydrates already present in the food, but addition of polyphenols and fibre to reduce the glycaemic index of that food has not been fully explored, but is the normal way in which most foods are consumed i.e. as a total meal in combination with other foods. We therefore tested the hypothesis that a combination of components in the diet (polyphenols and fibre) capable of inhibiting the different stages of starch digestion would reduce postprandial blood glucose and insulin using a randomized, controlled, single blind, crossover intervention. The test diet consisted of an α-glucosidase inhibitor (green tea), α-amylase inhibitors (green tea, blackberry, blackcurrant and strawberry) and glucose transport inhibitors (apple peel and strawberry), with all fruits also providing fibre.

## Experimental methods

### Subjects

The recruitment of subjects was carried out at the University of Leeds, School of Food Science and Nutrition, Leeds, UK. Poster advertisements around the University of Leeds notice boards were used to recruit interested potential volunteers, who were then screened for fasting blood glucose (required to be between 3.9 and 5.9 mmol/L). They were then asked to assess themselves using criteria to ensure they could be classified as healthy and free of symptomatic disease. The eligibility criteria were: Aged 18-75, apparently healthy, not diabetic, not on long term prescribed medication, not pregnant nor lactating, and not on a special diet (e.g. for losing weight or fruit supplements). The preferred order of consumption was reference meal at the first and last visit, with randomized consumption of the high and low dose on their $2^{nd}$ and $3^{rd}$ visits. However, due to availability of some volunteers, who started late and required a break of 3 weeks after 2 visits, the order was changed for them to reference, high/low dose, reference, then low/high dose, in order to start with the control meal after a break (Figure 1). In total, 16 healthy volunteers aged 26 ± 4 y with BMI of 24 ± 3 kg gave their written informed consent and completed the 4 study visits as shown in Figure 1. The fasting plasma glucose and insulin concentrations were 4.8 ± 0.4 mmol/L and 24 ± 10 pmol/L respectively.

### Study design

A randomized, controlled, single blind, crossover intervention was carried out on a total of 16 healthy volunteers with the primary outcome of post-prandial blood glucose area under the curve. Due to the nature of the test meals, it was impossible to blind participants. However analysis of the plasma samples was blinded and was only unblinded after data analysis. Subjects were

cannulated to ensure comfortable collection of blood samples. Each participant had four visits, two of which were reference meals and two visits were test meals (single and double dose of PFRF, in a randomized pattern).

## Test meals

All meals contained 109.0 ± 1.2 g white bread (50 g available carbohydrate as analysed by the method of [17]). The higher dose consisted of 1 g green tea powder in 200 ml water, with 20 g each of apple peel, blackberry, blackcurrant and strawberry freeze-dried powders mixed with water to make a paste and spread on the bread. The reference meal included 0.8, 5.4 and 8.6 g of sucrose, glucose and fructose respectively dissolved in 200 ml water to standardize the amounts of sugars present in the extracts of the high dose. The volunteers consumed the reference meal on two of the visits to determine any variability in the measurements [18]. The lower dose of the test meal contained half the amount of fruits and green tea with half the amounts of balancing sugars dissolved in 200 ml water to equalize the amount of sugars present in all doses. A polyphenol and fibre-rich food (PFRF) containing polyphenols that are effective inhibitors of different stages of starch digestion and absorption was used in this study. It comprised of an α-glucosidase inhibitor (green tea) [19; 20; 21], α-amylase inhibitors (green tea, blackberry, blackcurrant and strawberry) [22; 23; 24] and glucose transport inhibitors (apple peel and strawberry) [25; 26; 27], with all fruits also providing fibre. The PFRF components were analysed for total polyphenol content, specific major polyphenols and for α-amylase and α-glucosidase inhibition in vitro.

## Materials

Human salivary amylase, rat intestine powder, sucrose, maltose, glucose, fructose and glucose hexokinase reagent were from Sigma-Aldrich. Co., Ltd., Dorset, UK. Freeze dried fruit extracts were from Healthy supplies, UK and green tea powder was from Nestle, Research Centre, Lausanne, Switzerland. The insulin immunoassay kit was from Mercodia AB, Sweden.

## Study protocol

The University of Leeds, Faculty of MaPS and Engineering Ethics Committee (MEEC) approved the study protocol (MEEC 12-037) and the protocol was registered with ClinicalTrials.gov, ID number NCT01994135. Each participant had one visit per week and hence did the study in 4 weeks with body weight and height measurements taken on the first visit. On each visit the cannula was inserted in the forearm of the subject. A fasting blood sample was taken and afterwards the volunteer consumed the meal and the timer started upon first bite or sip. The volunteers consumed the whole meal and blood was collected after 15, 30, 45, 60, 90, 120, 150 and 180 min. Neither harm nor side effects were incurred during the consumption of the meals. Blood samples were collected in fluoride/oxalate and ethylenediaminetetraacetic acid (EDTA) tubes for glucose and insulin measurements respectively and immediately placed on ice. The tubes were then centrifuged within 15 min at 4000 g at 4°C for 15 min. Thereafter, plasma was placed in storage tubes and stored at -80°C. Plasma glucose concentrations were determined using hexokinase linked to NADH oxidation (Sigma-Aldrich, UK) and insulin concentrations by immunoassay.

## Statistical analysis

The incremental areas under the glucose curves (IAUC) were calculated for each subject for each visit using the trapezoidal rule. Data was analysed using the two tailed paired T-test analysis and

results were confirmed by using the one factor repeated measures analysis of variance (ANOVA) between the two references, reference and dose 1, reference and dose 2 and between lower and higher dose. Sample size was determined by designing the trial to have 90% power to detect a clinical difference of 15% IAUC between test and reference meal. The study required 15 participants each for reference and test meal. Each participant being a control of themselves, a minimum of 15 participants was required.

Enzyme assays in vitro

Green tea and fruit extracts were tested separately in vitro to determine the inhibition of starch-digesting enzymes. Measurement of human salivary α-amylase inhibition was carried out as described previously [28]. Briefly, sugars were removed from the fruit extracts using oasis max 3cc cartridges. The sugar free extracts, in water, were used as the inhibitor stock for the experiments. The 500 µl assay volume consisted of 200 µl amylose or amylopectin, 50 µl PBS, 50 µl inhibitor and was started by adding 200 µl of 1.25U/mL human salivary α-amylase (Sigma-Aldrich. Co., Ltd., Dorset, UK). After 10 min incubation at 37 °C, the reaction was stopped by placing the tubes in a water bath at 100 °C. The tubes were cooled to room temperature and solid phase extraction (SPE) was used to remove polyphenols from the assay contents prior to the addition of 1 mL DNS, since some polyphenols can interact with DNS and so interfere in the reaction [28]. A plate reader was used to measure the absorbance at 540 nm and inhibition was calculated as a percentage of the control.

The inhibition of rat α-glucosidase method was adapted from [29]. The apparent $K_m$ for sucrose, iso-maltose and maltose were determined and calculated using the Lineweaver-Burk plot by using a chosen enzyme concentration and incubation times giving linear rates of reaction. $K_m$ values

obtained were 16, 6 and 3 mM for sucrose, iso-maltose and maltose respectively and these were subsequently used as the substrate concentrations in the assays. The assay consisted of substrate (200 µl of sucrose, iso-maltose or maltose), sodium phosphate buffer (50 µl, 10 mM), inhibitor or extra buffer (50 µl), and the reaction was started by adding 200 µl of acetone-derived protein intestinal extract from rat intestine (20 mg solid/mL for sucrose and iso-maltose, and 4 mg solid/mL for maltose). After incubation at 37 °C for 20 min, the reaction was stopped by placing the tubes in a water bath at 100 °C for 10 min. After cooling to room temperature, solid phase extraction was used to remove polyphenols and the resulting solution analysed for glucose at 340 nm in a plate reader using hexokinase, which catalyses NADH reduction. Inhibition was calculated as a percentage of the control.

Total polyphenols by Folin assay

Extracts from the fruits were analysed for total polyphenols using the Folin assay [30], including a control for each sample to account for any interference from, for example, ascorbic acid, and data expressed as µg/mg gallic acid equivalents (GAE). To 15 ml falcon tubes, 1 mL of each solution (standards and samples) was added. The assay was conducted by adding 5 mL of the Folin-Ciocalteu reagent to all the samples and standards. The tubes were capped, votexed and 4 mL of sodium carbonate solution was added within 3-8 min from the addition of Folin-Ciocalteu reagent. The tubes were capped, votexed quickly and then placed in the water bath and incubated at 26 °C for 2 h. Absorbance readings at 765 nm were relative to a gallic acid standard curve.

Analysis of polyphenols by HPLC

The major polyphenols in the fruit samples and green tea were characterized using HPLC as described previously [27]. An Agilent 1200 SL system (Agilent Technologies, Dorset, UK)

196 equipped with a diode array detector (DAD) was used. It comprised of a binary pump, degasser,
197 column oven (35˚C) and well plate autosampler (5˚C). A Zorbax Eclipse plus C18 column (1.8
198 µm, 100 x 2.1 mm) and Agilent- Zorbax eclipse XDB-C18 (1.8 µm, 50 x 4.6 mm), both from
199 Agilent Technologies, Dorset, UK, were used for green tea and fruit extracts respectively. Other
200 parameters were 5 µL injection volume, at 0.5 ml/min flow rate with needle wash in flush point
201 for 3 s. For all analyses, ultrapure, nuclease free water (≥18.2 MΩ cm at 25˚C) from a Millipore
202 Q water purifying system (Millipore, Hertfordshire, UK) was used. For sample preparation, a
203 Genevac (EZ-2 plus model, Fisher Scientific Ltd, Leicestershire, UK) was used for centrifugal
204 evaporation. Polyphenols were identified by their retention times compared to authentic standards
205 and standard curves were used for quantification.

206 Sugar analysis by HPLC

207 Sugar quantification of the fruit extracts was conducted on a Shimadzu HPLC instrument equipped
208 with a model DGU-20 A5 degasser, a LC-20 AD XR pump system, a SIL-20 AC XR auto sampler
209 (Shimadzu), column oven, a diode array detector system (SPD-M20A) and a Shimadzu ELSD-
210 LTII low temperature evaporative light scattering detector. A sample volume of 10 µL was injected,
211 and separations were achieved on a Prevail Carbohydrate ES 5 µm column (250 mm x 4.6 mm;
212 GRACE, Lokeren, Belgium). The column was held at 20 $^{0}$C, and individual sugars were eluted
213 isocratically using a 1 mL/min flow of 75 % acetonitrile. Solutions of standard sugars prepared in
214 water (Millipore, HPLC grade) with concentrations between 0 and 10 mg/mL were used for the
215 calibration curve. The sugars were identified by their retention time characteristics at 40 °C.
216 Quantification was achieved using standard calibration curves obtained by plotting area versus
217 concentration ($r^2 > 0.98$). Data from the sugar analysis allowed balancing of glucose, fructose and
218 sucrose in the fruit in the control samples as indicated above.

219

220 Fibre estimation

221 The AOAC method [31] was used for fibre determination by Healthy Supplies, UK.

222

223

## RESULTS

### Polyphenol and sugar analysis

Total polyphenol contents of the fruits and green tea are shown in Table 1, and all of the data fell within the normal range as recorded in phenol explorer [32]. Sugar analysis of the PFRF gave a total of 1.3, 9.0 and 14.3 g/100g sucrose, glucose and fructose respectively and these values were used to balance the control meal. Fibre contents were 0.22, 0.53, 0.43 and 0.2 g/100g DW in apple peel, blackberry, blackcurrant and strawberry respectively.

### Post-prandial plasma glucose and insulin

Both the low and the high dose test meals containing PFRF showed a significant dose-dependent decrease in the glucose IAUC compared to the control meals (Figure 2), - 27.4±7.52 % (mean ± SD; $p<0.01$) and -49.0±15.3 % ($p<0.01$) respectively, with no significant difference between the two reference (control) meals. The peak glucose concentration was also significantly lower in both PFRF test meals compared to the reference meals. There was a reduction in insulin IAUC for the PFRF meal compared to the reference meal of - 46.9±13.4 % (mean ± SD) ($p<0.01$) (Figure 3). The PFRF meal also attenuated the peak postprandial insulin concentration, and any differences had disappeared by 120 min.

245 Inhibition of *α*-amylase *and α*-glucosidase activities

246 Green tea, and extracts from blackberry, blackcurrant and strawberry inhibited human salivary α-
247 amylase ($IC_{50}$ values = 0.009, 1.2, 1.5 and 2.5 mg dry powder /ml water (amylose as substrate);
248 0.025, 1.6, 1.7 and 3.9 mg/ml (amylopectin as substrate) (Figures 4 and Table 2). Green tea
249 inhibited maltase, sucrase and iso-maltase in vitro with $IC_{50}$ values of 0.02, 2.3 and 2.0 mg
250 solid/mL water (Table 2 and Figure 4C).

251

252

## Discussion

Consumption of foods rich in polyphenols and fibre (PFRF) together with bread resulted in a highly significant dose-dependent lowering of the glucose area under the curve (AUC), and an associated attenuation of insulin. Although there was substantial inter-individual variation, the cross-over design has minimised the consequences of this and the two curves obtained for the control meals were not significantly different. We propose that the effects of the test meals are most likely due to the results observed in the in vitro inhibition of human salivary α-amylase (mainly green tea, blackberry, blackcurrant and strawberry), α-glucosidase (green tea), and glucose transport (green tea, apple and strawberry [26; 27], and additionally also the effect of fibre [33; 34]. Although it is not possible to define the exact contribution of inhibition of the different steps (inhibition of α-amylase, α-glucosidase or glucose transporters) to the attenuation of blood glucose, we would speculate that partial inhibition of multiple steps is important to give the observed effect on the glycaemic response. These reductions can play a major long term role in the management or risk reduction of diabetes type 2, comparable to the drug acarbose, an α-glucosidase inhibitor [15], since high concentrations of postprandial glucose lead to insulin resistance, pancreatic exhaustion, glucose intolerance and an increased insulin demand [35]. A highly significant effect on blood glucose was observed at both doses, with the higher dose (~2-fold higher than the lower dose) leading to a doubling of the measured reduction in AUC. On the other hand, the peak glucose concentration was not further decreased by the higher dose.

A limited number of studies have reported the effects of isolated polyphenols or polyphenol-containing foods or extracts on post-prandial glycaemia [36; 37; 38; 39; 40; 41; 42; 43; 44; 45; 46; 47; 48; 49], but the results are mixed with only some studies reporting significant differences between test meal and reference meal, and sometimes only at one or two time points, possibly owing to the use of

different sugar sources, for example glucose [36; 41; 42; 44; 45; 46] or sucrose [47]. A limited number of starch-based interventions, using rice, pan cakes and white bread, have shown mixed results [38; 39; 40; 43; 48]. All of these studies above have not designed the study meal by considering the mechanism and including foods capable of attenuating the rate of each step of the digestive process. No significant difference was observed when a starch based meal (pancakes) was used together with 100 g berries as the source of polyphenols [38]. When used alone, green tea as the sole source of polyphenols also did not give a significant difference in the IAUC [43]. There was a significant difference in the IAUC when apple juice was used as a polyphenol source, clearly attributed to the inhibition of glucose transporters by polyphenols in apple, especially phlorizin [41]. Polyphenols and fibre (14.7g) [44], present in lingonberries, nulled the glycaemic effect of the endogenous sugars present in the lingonberries. In vitro, polyphenols, phenolic acids and tannins in strawberry and apple reduced glucose transport using Caco-2 intestinal cell monolayers by inhibiting the glucose transporters SGLT1 and GLUT2. Phlorizin contributed 52 % ($IC_{50}$ = 146 μM) and pelargonidin-3-O-glucoside ($IC_{50}$ = 802 μM) 26% to the total inhibition by apple and strawberry respectively [27]. These concentrations, together with those obtained for α-amylase and α-glucosidase inhibition (Table 2), were theoretically obtained in the gut lumen (Table 1) after taking into consideration calculated 3-fold dilution of consumed substances [14]. For example, the $IC_{50}$ value for α-amylase inhibition by green tea was 0.009 mg/ml in vitro, 3-fold dilution in vivo would require 0.027 mg/ml in the original sample, and the test meals contained 2.5 and 5 mg/ml in the low and high dose respectively. Hence we propose that polyphenols and fibre present in the PFRF act together by inhibiting α-amylase, α-glucosidase and glucose transporters and leading to the observed reduced glycaemic response in vivo. The observed reduction in postprandial blood glucose and insulin can play a major role in management and reducing the risk of type 2 diabetes, since hyperglycaemia is

a risk factor for developing insulin resistance, impaired glucose tolerance and consequently type 2 diabetes.

## CONCLUSION

Polyphenols and fibre present in fruits, together with a cup of green tea, have a pronounced lowering effect on postprandial glucose and insulin when consumed together with a starch food (bread), owing to inhibition of the different stages of starch digestion.

**Acknowledgements**:

The authors would like to thank the volunteers, Penny Rice (research nurse), David Tembo (assistance with sugar analysis) and the School of Food Science and Nutrition (University of Leeds) for meeting the research nurse's costs.

**Funding**:

Hilda Nyambe-Silavwe received a student grant from the Commonwealth Scholarship Commission U.K. (ZMSC-2012-593) and the National Institute for Scientific and Industrial Research (NISIR), Zambia for PhD funding. Neither body were involved in any way in the design, interpretation or writing up of the study.

**Conflict of interest:**

HN, no conflict of interest. This work did not receive funding from a commercial organisation, but GW has recently, or currently, received other research funding from Nestle and Florida Department of Citrus, and conducted consultancy for Nutrilite, USA, and Suntory, UK.

**The authors' responsibilities were as follows:**

Nyambe: design of study, carried out study and in vitro work, data analysis, writing the paper

Williamson: design of study, data analysis, writing the paper

**Table 1**

| Extract | Total polyphenols (µg/mg GAE) fresh weight basis ± SD | Specific polyphenols by HPLC | mg/g ± SD |
|---|---|---|---|
| **Green tea** | 541± 25 | (−)-epigallocatechin gallate | 199.8 ± 6.7 |
| | | (−)-epigallocatechin | 124.4 ± 9.3 |
| | | (−)-epicatechin gallate | 34.4 ± 1.9 |
| | | (−)-epicatechin | 23.3 ± 2.4 |
| **Apple peel** | 217± 3 | Phlorizin | 1.82 ± 0.03 |
| | | Quercetin-3-O-rhamnoside | 1.13 ± 0.02 |
| **Blackberry** | 295 ± 3 | Cyanidin-3-O-glucoside | 7.01 ± 0.08 |
| **Blackcurrant** | 303 ± 0 | Cyanidin-3-O-rutinoside | 1.04 ± 0.03 |
| **Strawberry** | 315 ± 2 | Pelargonidin-3-O-glucoside | 4.5 ± 0.1 |

Total polyphenol contents and specific polyphenol contents of green tea and extracts from the tested fruit as analysed by Folin assay and HPLC. Values are mean ±SD (n=3).

**Table 2**

| **Enzyme** | **$IC_{50}$ (mg/ml powder)** | | | |
|---|---|---|---|---|
| | Green tea | Blackberry | Blackcurrant | Strawberry |
| **Amylase (amylose)** | 0.009±0.001 | 1.22±0.02 | 1.5±0.1 | 2.47±0.31 |
| **Amylase (amylopectin)** | 0.025±0.001 | 1.57±0.21 | 1.7±0.1 | 3.85±0.05 |
| **Maltase** | 0.02±0.01 | > 4 | > 4 | > 4 |
| **Iso-maltase** | 2.02±0.01 | > 4 | > 4 | > 4 |
| **Sucrase** | 2.31±0.02 | > 4 | > 4 | > 4 |

Experimental $IC_{50}$ values for human salivary α-amylase using amylose and amylopectin as substrates and α-glucosidase using maltose, sucrose and iso-maltose as substrates for green tea and freeze-dried fruits (n=3).

**Figure 1**

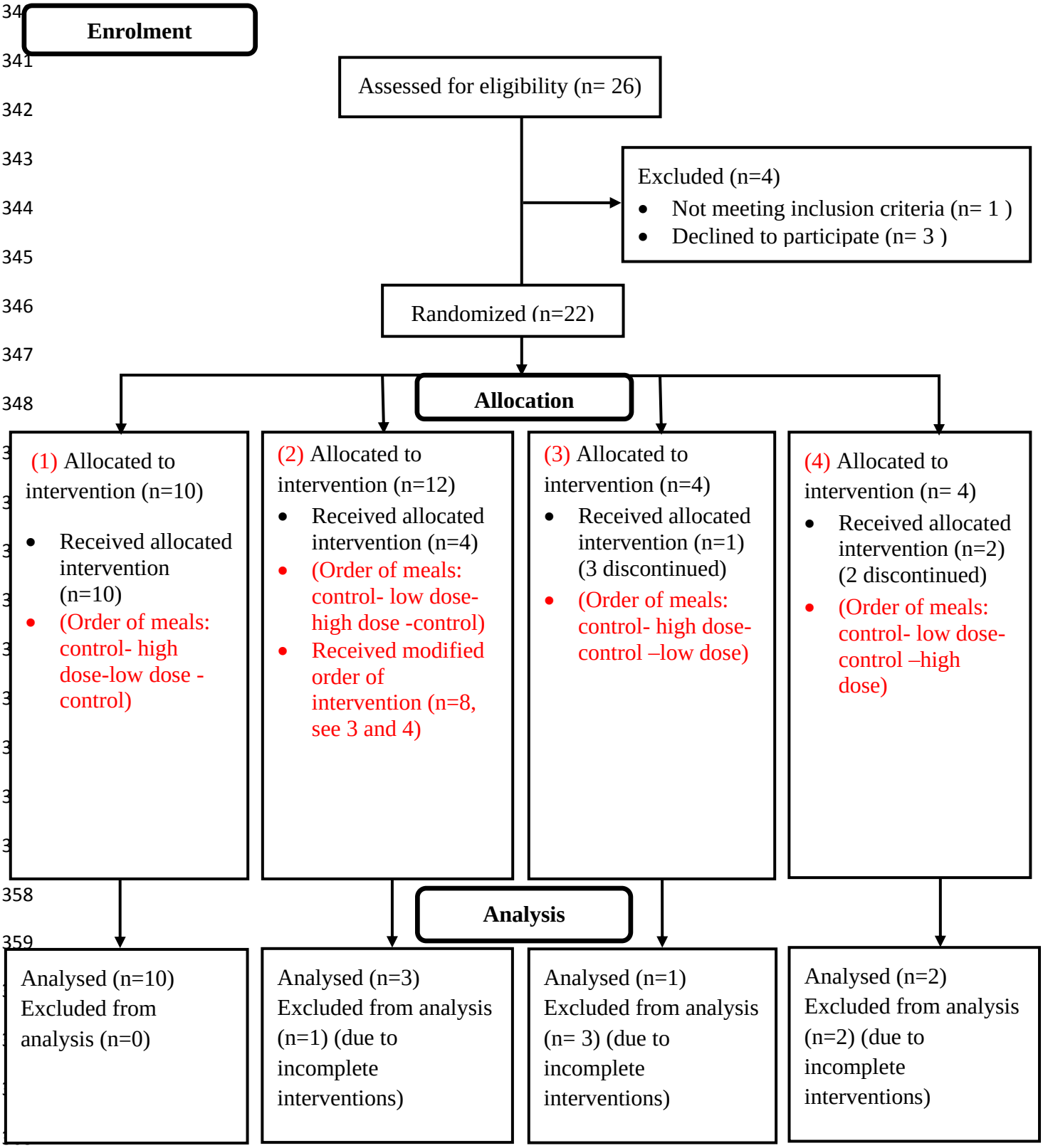

Enrolment
Assessed for eligibility (n= 26)
Excluded (n=4)
Not meeting inclusion criteria (n= 1 )
Declined to participate (n= 3 )
Randomized (n=22)
Allocation
(1) Allocated to intervention (n=10)
Received allocated intervention (n=10)
(Order of meals: control- high dose-low dose - control)
(2) Allocated to intervention (n=12)
Received allocated intervention (n=4)
(Order of meals: control- low dose- high dose -control)
Received modified order of intervention (n=8, see 3 and 4)
(3) Allocated to intervention (n=4)
Received allocated intervention (n=1) (3 discontinued)
(Order of meals: control- high dose- control –low dose)
(4) Allocated to intervention (n= 4)
Received allocated intervention (n=2) (2 discontinued)
(Order of meals: control- low dose- control –high dose)
Analysis
Analysed (n=10) Excluded from analysis (n=0)
Analysed (n=3) Excluded from analysis (n=1) (due to incomplete interventions)
Analysed (n=1) Excluded from analysis (n= 3) (due to incomplete interventions)
Analysed (n=2) Excluded from analysis (n=2) (due to incomplete interventions)

**Figure 2**

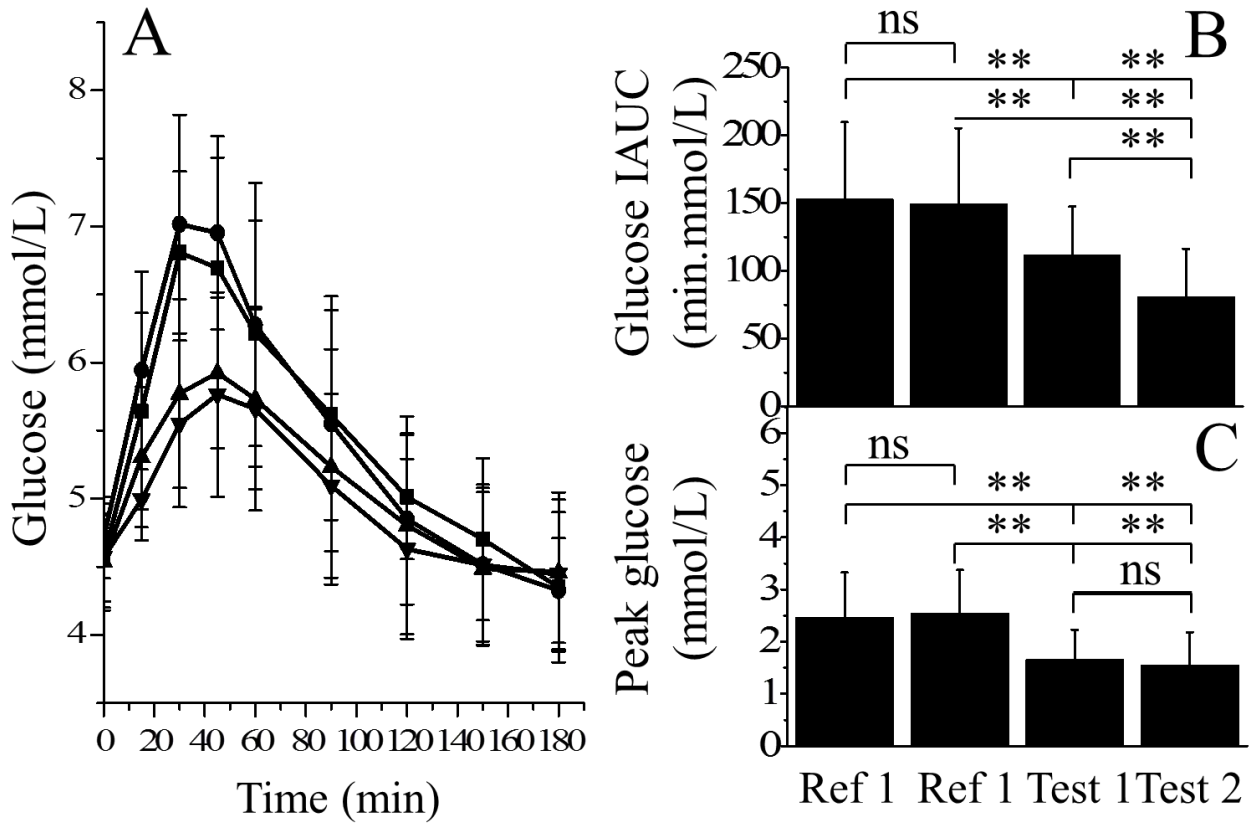

A
Glucose (mmol/L)
Time (min)
B
Glucose IAUC (min.mmol/L)
C
Peak glucose (mmol/L)
ns
**
Ref 1
Ref 1
Test 1
Test 2

**Figure 3**

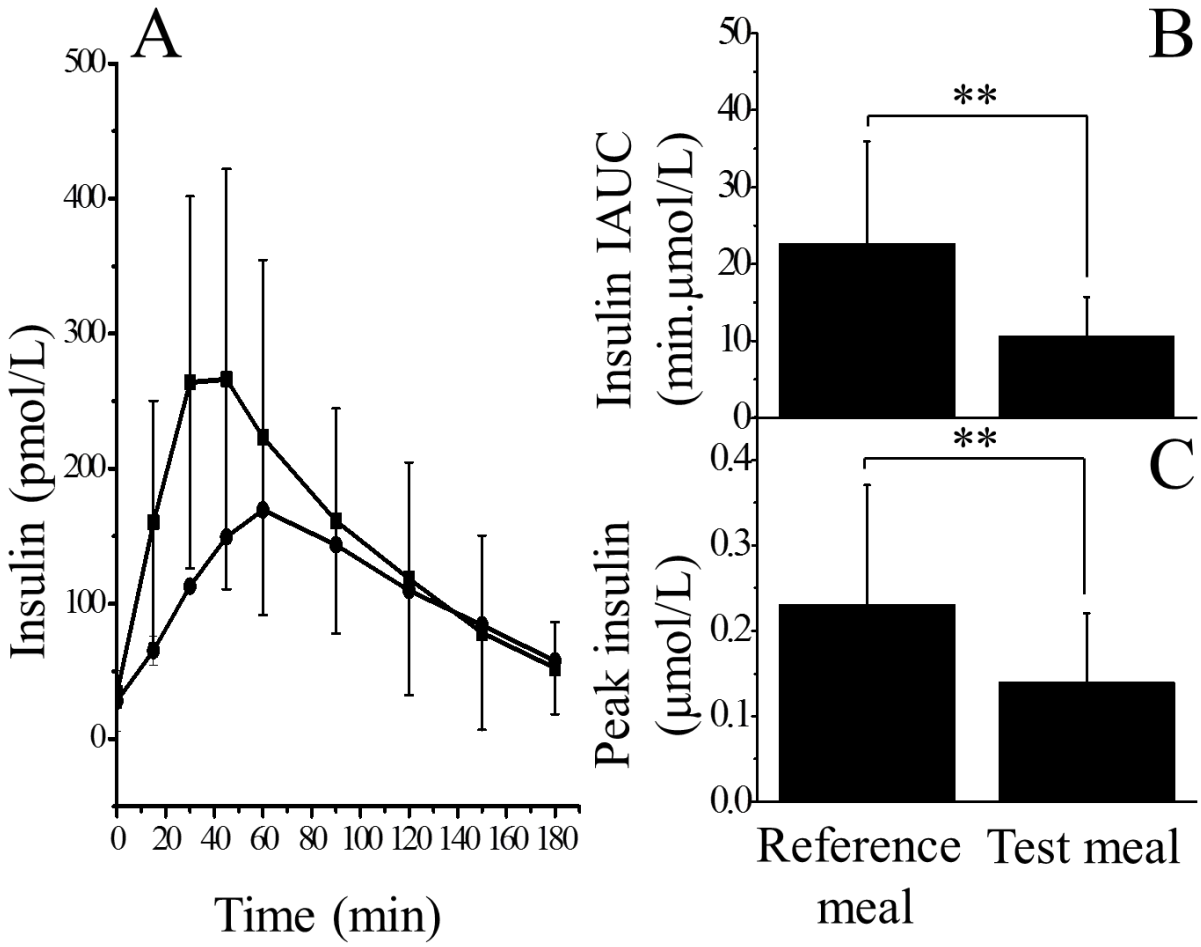
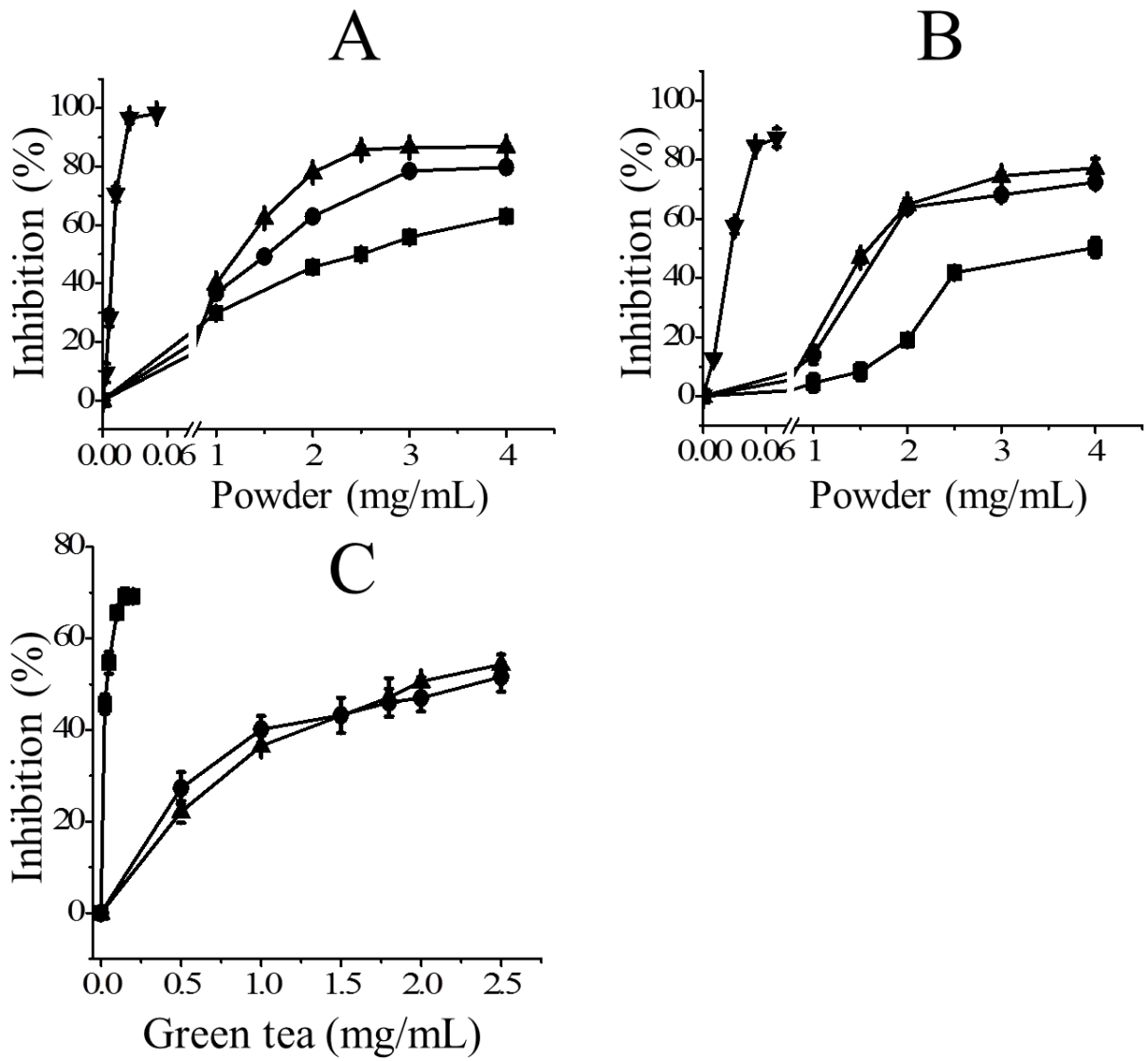

A
Insulin (pmol/L)
500
400
300
200
100
0
0 20 40 60 80 100 120 140 160 180
Time (min)
B
Insulin IAUC (min.µmol/L)
50
40
30
20
10
0
**
C
Peak insulin (µmol/L)
0.4
0.3
0.2
0.1
0.0
**
Reference meal
Test meal

**Figure 4**


A
Inhibition (%)
100
80
60
40
20
0
0.00 0.06 1 2 3 4
Powder (mg/mL)
B
Inhibition (%)
100
80
60
40
20
0
0.00 0.06 1 2 3 4
Powder (mg/mL)
C
Inhibition (%)
80
60
40
20
0
0.0 0.5 1.0 1.5 2.0 2.5
Green tea (mg/mL)

## Figure legends

### Figure 1

Participant flow diagram. Block randomization was used to generate the allocated sequences which were assigned to participants codes. Sequences were automatically allocated to participants according to participant codes.

### Figure 2

Average glucose curves (A) after consumption of reference, test meal dose 1 and test meal dose 2 for 16 volunteers. There is a significance difference between IAUC of reference meals and test meals (B) as well as between the peak rise in glucose concentration (C). **p>0.01 (**)**, **no significant difference (ns)**

### Figure 3

Average Insulin curves (A) after consumption of reference and test meal dose 2 and for 16 volunteers. There is a significance difference between insulin IAUC of reference meal and test meal (B) as well as between the peak rise in glucose concentration (C). **p>0.01 (**)**, **no significant difference (ns)**

### Figure 4

Inhibition of human salivary α-amylase by green tea (▼), freeze dried strawberry (■), blackcurrant (●) and blackberry (▲) using amylose as substrate (A) or amylopectin as substrate (B) and inhibition of maltase (■), sucrase (▲) and iso-maltase (●) by green tea (C).

# REFERENCES


1. Hodge AM, English DR, O'Dea K *et al.* (2004) Glycemic index and dietary fiber and the risk of type 2 diabetes. *Diabetes Care* **27**, 2701-2706.
2. McKeown NM, Meigs JB, Liu S *et al.* (2004) Carbohydrate nutrition, insulin resistance, and the prevalence of the metabolic syndrome in the Framingham Offspring Cohort. *Diabetes Care* **27**, 538-546.
3. Salmeron J, Manson JE, Stampfer MJ *et al.* (1997) Dietary fiber, glycemic load, and risk of non—insulin-dependent diabetes mellitus in women. *JAMA* **277**, 472-477.
4. Steven J, Ahn K, Juhaeri *et al.* (2002) Dietary fiber intake and glycemic index and incidence of diabetes in African-American and white adults. *Diabetes Care* **25**, 1715-1721.
5. Järvi AE, Karlström BE, Granfeldt YE *et al.* (1999) Improved glycemic control and lipid profile and normalized fibrinolytic activity on a low-glycemic index diet in type 2 diabetic patients. *Diabetes Care* **22**, 10-18.
6. Brand-Miller J, Hayne S, Petocz P *et al.* (2003) Low–Glycemic Index Diets in the Management of Diabetes A meta-analysis of randomized controlled trials. *Diabetes care* **26**, 2261-2267.
7. Livesey G, Taylor R, Hulshof T *et al.* (2008) Glycemic response and health—a systematic review and meta-analysis: relations between dietary glycemic properties and health outcomes. *Am J Clin Nutr* **87**, 258S-268S.
8. Ajala O, English P, Pinkney J (2013) Systematic review and meta-analysis of different dietary approaches to the management of type 2 diabetes. *Am J Clin Nutr* **97**, 505-516.
9. Augustin L, Franceschi S, Jenkins D *et al.* (2002) Glycemic index in chronic disease: a review. *Eur J Clin Nutr* **56**, 1049-1071.
10. THOMPSON LU, YOON JH (1984) Starch digestibility as affected by polyphenols and phytic acid. *J Food Sci* **49**, 1228-1229.
11. Scazzina F, Siebenhandl-Ehn S, Pellegrini N (2013) The effect of dietary fibre on reducing the glycaemic index of bread. *Brit J Nutr* **109**, 1163-1174.
12. Dikeman CL, Fahey Jr GC (2006) Viscosity as related to dietary fiber: a review. *Crit Rev Food Sci Nutr* **46**, 649-663.
13. Hanhineva K, Torronen R, Bondia-Pons I *et al.* Impact of Dietary Polyphenols on Carbohydrate Metabolism. *Int J Mol Sci* **11**, 1365-1402.
14. Williamson G (2013) Possible effects of dietary polyphenols on sugar absorption and digestion. *Mol Nutr Food Res* **57**, 48-57.
15. Hanefeld M, Schaper F, Koehler C (2008) Effect of acarbose on vascular disease in patients with abnormal glucose tolerance. *Cardiovas Drugs and Ther* **22**, 225-231.
16. Chiasson J-L (2006) Acarbose for the prevention of diabetes, hypertension, and cardiovascular disease in subjects with impaired glucose tolerance: the Study to Prevent Non-Insulin-Dependent Diabetes Mellitus (STOP-NIDDM) Trial. *Endocr Pract* **12**, 25-30.
17. Englyst HN, Kingman SM, Cummings JH (1992) Classification and measurement of nutritionally important starch fractions. *Eur J Clin Nutr* **46**, S33-S50.
18. Brouns F, Bjorck I, Frayn KN *et al.* (2005) Glycaemic index methodology. *Nutr Res Rev* **18**, 145-171.
19. Honda M, Hara Y (1993) Inhibition of rat small intestinal sucrase and α-glucosidase activities by tea polyphenols. *Biosci Biotechnol and Biochem* **57**, 123-124.
20. Kamiyama O, Sanae F, Ikeda K *et al.* (2010) In vitro inhibition of alpha-glucosidases and glycogen phosphorylase by catechin gallates in green tea. *Food Chem* **122**, 1061-1066.
21. Matsui T, Tanaka T, Tamura S *et al.* (2007) alpha-glucosidase inhibitory profile of catechins and theaflavins. *J Agric Food Chem* **55**, 99-105.

22. Forester SC, Gu Y, Lambert JD (2012) Inhibition of starch digestion by the green tea polyphenol, (-)-epigallocatechin-3-gallate. *Mol Nutr Food Res* **56**, 1647-1654.
23. McDougall GJ, Shpiro F, Dobson P *et al.* (2005) Different polyphenolic components of soft fruits inhibit alpha-amylase and alpha-glucosidase. *J Agric Food Chem* **53**, 2760-2766.
24. McDougall GJ, Stewart D (2005) The inhibitory effects of berry polyphenols on digestive enzymes. *BioFactors* **23**, 189-195.
25. Hossain SJ, Kato H, Aoshima H *et al.* (2002) Polyphenol-induced inhibition of the response of Na+/glucose cotransporter expressed in Xenopus oocytes. *J Agric Food Chem* **50**, 5215-5219.
26. Kobayashi Y, Suzuki M, Satsu H *et al.* (2000) Green tea polyphenols inhibit the sodium-dependent glucose transporter of intestinal epithelial cells by a competitive mechanism. *J Agric Food Chem* **48**, 5618-5623.
27. Manzano S, Williamson G (2010) Polyphenols and phenolic acids from strawberry and apple decrease glucose uptake and transport by human intestinal Caco-2 cells. *Mol Nutr Food Res* **54**, 1773-1780.
28. Nyambe-Silavwe H, Villa-Rodriguez JA, Ifie I *et al.* (2015) Inhibition of human α-amylase by dietary polyphenols. *J Funct Foods* **19**, 723-732.
29. Adisakwattana S, Charoenlertkul P, Yibchok-anun S (2009) alpha-Glucosidase inhibitory activity of cyanidin-3-galactoside and synergistic effect with acarbose. *J Enzyme Inhib Med Chem* **24**, 65-69.
30. Singleton VL, Orthofer R, Lamuela-Raventos RM (1999) Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocalteu reagent. In *Oxid Antioxid, Pt A*, vol. 299, pp. 152-178 [L Packer, editor].
31. International A (2005) *Official methods of analysis of AOAC International*: AOAC International.
32. Neveu V, Perez-Jimenez J, Vos F *et al.* (2010) Phenol-Explorer: an online comprehensive database on polyphenol contents in foods. *Database* **2010**, bap024.
33. Anderson JW, Baird P, Davis RH *et al.* (2009) Health benefits of dietary fiber. *Nutr Rev* **67**, 188-205.
34. Weickert MO, Pfeiffer AF (2008) Metabolic effects of dietary fiber consumption and prevention of diabetes. *J nutr* **138**, 439-442.
35. Willett W, Manson J, Liu S (2002) Glycemic index, glycemic load, and risk of type 2 diabetes. *Am J Clin Nutr* **76**, 274S-280S.
36. Bryans JA, Judd PA, Ellis PR (2007) The effect of consuming instant black tea on postprandial plasma glucose and insulin concentrations in healthy humans. *J Am Coll Nutr* **26**, 471-477.
37. Chai Y, Wang M, Zhang G (2013) Interaction between Amylose and Tea Polyphenols Modulates the Postprandial Glycemic Response to High-Amylose Maize Starch. *J Agric Food Chem* **61**, 8608-8615.
38. Clegg ME, Pratt M, Meade CM *et al.* (2011) The addition of raspberries and blueberries to a starch-based food does not alter the glycaemic response. *Brit J Nutr* **106**, 335-338.
39. Coe SA, Clegg M, Armengol M *et al.* (2013) The polyphenol-rich baobab fruit (Adansonia digitata L.) reduces starch digestion and glycemic response in humans. *Nutr Res* **33**, 888-896.
40. Hlebowicz J, Darwiche G, Bjorgell O *et al.* (2007) Effect of cinnamon on postprandial blood glucose, gastric emptying, and satiety in healthy subjects. *Am J Clin Nutr* **85**, 1552-1556.
41. Johnston KL, Clifford MN, Morgan LM (2002) Possible role for apple juice phenolic, compounds in the acute modification of glucose tolerance and gastrointestinal hormone secretion in humans. *J Sci Food Agric* **82**, 1800-1805.
42. Johnston KL, Clifford MN, Morgan LM (2003) Coffee acutely modifies gastrointestinal hormone secretion and glucose tolerance in humans: glycemic effects of chlorogenic acid and caffeine. *Am J Clin Nutr* **78**, 728-733.
43. Josic J, Olsson AT, Wickeberg J *et al.* (2010) Does green tea affect postprandial glucose, insulin and satiety in healthy subjects: a randomized controlled trial. *Nutr J* **9**.

511 44. Linderborg KM, Jarvinen R, Lehtonen HM *et al.* The fiber and/or polyphenols present in lingonberries
512 null the glycemic effect of the sugars present in the berries when consumed together with added
513 glucose in healthy human volunteers. *Nutr Res* **32**, 471-478.
514 45. Makarova E, Gornas P, Konrade I *et al.* (2015) Acute anti-hyperglycaemic effects of an unripe apple
515 preparation containing phlorizin in healthy volunteers: a preliminary study. *J Sci Food Agric* **95**, 560-568.
516 46. Schulze C, Bangert A, Kottra G *et al.* (2014) Inhibition of the intestinal sodium-coupled glucose
517 transporter 1 (SGLT1) by extracts and polyphenols from apple reduces postprandial blood glucose levels
518 in mice and humans. *Mol Nutr Food Res* **58**, 1795-1808.
519 47. Torronen R, Sarkkinen E, Niskanen T *et al.* (2012) Postprandial glucose, insulin and glucagon-like
520 peptide 1 responses to sucrose ingested with berries in healthy subjects. *Brit J Nutr* **107**, 1445-1451.
521 48. Tsujita T, Takaku T (2008) Mechanism of the Inhibitory Action of Chestnut Astringent Skin Extract on
522 Carbohydrate Absorption. *J Nutr Sci Vitaminol* **54**, 416-421.
523 49. Tsujita T, Takaku T, Suzuki T (2008) Chestnut astringent skin extract, an alpha-amylase inhibitor,
524 retards carbohydrate absorption in rats and humans. *J Nutr Sci Vitaminol* **54**, 82-88.

525

526