Short Communication

The addition of raspberries and blueberries to a starch-based food does not alter the glycaemic response

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Abstract

It is now known that health benefits associated with diets rich in fruit and vegetables may be partly derived from intake of polyphenols. Berry polyphenols may influence carbohydrate metabolism and absorption and hence postprandial glycaemia. To date, studies related to polyphenol effects on the glycaemic response have been completed only in liquids using either monosaccharides or disaccharides. It remains to be determined whether berries known to be rich in polyphenols can reduce the glycaemic response (GR) to a solid polysaccharide meal. The aim of the present study was to investigate whether berries alter postprandial hyperglycaemia and consequently the GR to a starchy food. Blood glucose was tested on seven occasions, on three occasions using a reference food and on four occasions using pancakes supplemented with either raspberries or blueberries or control pancakes containing similar amounts of fructose and glucose. Results showed that there were no differences in GR (blueberry 51·3 (SEM 5·7); raspberry 54·7 (SEM 5·6); blueberry control 43·9 (SEM 4·2); raspberry control 41·8 (SEM 6·4)), GR area under the curve or satiety index between any of the tests. The present study indicates that the ability of berries to reduce blood glucose from starch-based foods is unsubstantiated.

Key words: Glycaemic response: Polyphenols: Berries: Starch

Polyphenols represent one of the most ubiquitous class of minor plant constituents from a simple phenolic molecule to highly polymerised complexes. Historically, polyphenols were considered as anti-nutritional factors. This was due to their significant effect on protein digestibility and quality due to the binding of tannins with proteins, notably in legumes[1]. However, recent interest in polyphenols has emerged from their antioxidant capacity and their potential in human health and well-being[2]. These health claims include reducing the risk of CVD, cancer and combating inflammation. In addition to the well-recognised role that polyphenols play in chelating with proteins, Thompson et al.[3] reported that polyphenolic compounds from legumes bind with the carbohydrate fraction, leading to a reduction in the glycaemic response (GR) of such foods. In contrast to the extensive research conducted on the influence of polyphenols on legume carbohydrates, little or no work exists on the role that the polyphenols from berries may have on starch metabolism. Most berries contain a variety of polyphenolic compounds, including anthocyanins, flavanols, phenolic acids, ellagitannins and proanthocyanins[4–7].

Several studies have examined the effect of polyphenols on the GR of monosaccharides and disaccharides[6]. The most recent study compared the consumption of a test meal of mixed berry purée with sucrose with a control of sucrose, fructose and glucose dissolved in water. A lower plasma glucose concentration was observed 15–30 min post-meal after the berry test meal in comparison with the control[7]. Consumption of cranberry juice sweetened with high-fructose maize syrup resulted in an altered (but not statistically significant) pattern of postprandial glycaemia compared with the similar amount of high-fructose maize syrup in water[4]. Research on the effect of coffee suggests that chlorogenic acid in coffee might have an antagonistic effect on glucose transport of sucrose[5] and a further attenuated GR to sucrose occurred in chlorogenic acid-enriched coffee[4]. This may be due to a shift in the site of glucose absorption to a more distal part of the intestine[8]. Finally, a 25 g glucose load combined with commercial apple juice compared with that consumed in water altered plasma concentrations of glucose, insulin, glucose-dependent insulinotropic polypeptide and glucagon-like peptide-1, suggesting

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delayed intestinal absorption of glucose[9]. To date, the effect of polyphenolic compounds on polysaccharides, notably starch with a greater degree of polymerisation, has not been explored nor has the effect of polyphenols on solid test foods.

The aim of the present study was to incorporate berries into a starch-rich food to lower the GR value for that food. We speculated that the polyphenols will bind with the starch fractions and also reduce the α-amylase activity *in vivo*, leading to a slow digestion of the starch component. This should result in a more sustained blood glucose response. This is the first study to examine the effect of berries, known to be rich in polyphenols, on the glycaemic response to a starch-based food.

Methods

Volunteers

The present study was conducted according to the guidelines laid down in the Declaration of Helsinki, and all procedures involving human volunteers were approved by the University Research Ethics Committee at Oxford Brookes University. Written informed consent was obtained from all volunteers. A total of twelve volunteers (three men and nine women; age 33 (SEM 13) years; height 1·68 (SEM 0·07) m; weight 64·8 (SEM 10·8) kg; waist circumference 78·0 (SEM 14·1) cm; fat mass 24·4 (SEM 10·7) %) were recruited by means of flyers and posters around the college campus. Exclusion criteria included those suffering from diabetes and those with a fasting blood glucose >6·1 mmol/l. Health questionnaires were provided during the participant's first visit, so that details could be obtained about their current health, diet and lifestyles.

All anthropometric measurements were made in the fasting state during the first test session. Height was measured using a stadiometer (Seca Limited, Birmingham, West Midlands, Middlesex, UK), body weight and body fat using the Tanita BC-418 MA (Tanita UK Limited, Yiewsley, UK). Waist circumference was measured midway between the lowest rib margin and the iliac crest.

Study design

The reference foods were tested three times and each test meal was tested on one occasion in a random order on separate days, with at least a 1 d washout period between measurements to minimise carry-over effects. The volunteers were advised to keep their lifestyles, body weight and medication constant and to follow their habitual diet throughout the study period.

Glycaemic response testing

The protocol used to measure the GR was adapted from that described by Wolever[9] and Brouns et al.[10], and is in line with procedures recommended by the FAO/WHO[11]. The experiments began in the morning following a 12 h overnight fast. Fasting blood glucose values were obtained from a fingertip capillary blood drop using a Unistik

3 single-use lancing device (Owen Mumford, Woodstock, Oxford, UK) at −5 and 0 min. An average of these two values was then taken as the fasting blood glucose level. The volunteers were given the test meal, which they were required to consume within 15 min. The time was set at 0 as soon as the first bite was in the mouth and the following blood samples were taken at 15, 30, 45, 60, 90 and 120 min. Blood glucose was analysed using a Hemocue Glucose 201 + analyser (HemoCue Limited, Dronfield, Derbyshire, UK) calibrated to plasma-equivalent glucose concentrations.

Each subject was required to complete three oral glucose tolerance tests as the reference food, before they began the berry trials, to assess their glucose response before participation in the study. The oral glucose tolerance test involved a 55·68 g loading dose of glucose powder (50 g available glucose; Lloyds, Coventry, West Midlands, UK) dissolved in 200 ml of water, followed by a further 50 ml of water. Postprandial blood glucose response was measured at 15, 30, 45, 60, 90 and 120 min.

Test meals

The test meal was pancakes containing either raspberries or blueberries or control pancakes containing an equivalent amount of fructose and glucose to achieve a similar carbohydrate profile of the berries. The berry pancake batter contained free-range eggs, plain flour, semi-skimmed milk and either 50 g of raspberries or blueberries (all Tesco, Cheshunt, Hertfordshire, UK). A further 50 g of berries accompanied the meal. In total, the meal contained 100 g of berries. The control pancakes contained 4·88 g glucose (Lloyds) and 4·95 g fructose (Tate and Lyle, Annesley, Notts, UK) or 2·65 g glucose and 2·88 g fructose, respectively, representing the carbohydrate content of the blueberries and raspberries. A 200 ml glass of water was served with both meals. The raspberry pancakes and raspberry control pancakes consisted of 1408 kJ (333 kcal), 14 g protein, 7 g fat and 50 g available carbohydrate. The blueberry pancakes and blueberry control pancakes consisted of 1324 kJ (313 kcal), 13 g protein, 7 g fat and 50 g available carbohydrate.

Calculation of the glycaemic response

For the reference food and each pancake meal, the incremental GR area under the curve (AUC), ignoring the area beneath the baseline, was calculated geometrically[11]. To determine the GR for each pancake meal, the AUC for each subject are expressed as a percentage of the mean AUC for the reference food eaten by the same subject.

Satiety index

Satiety ratings were obtained every 15 min for 60 min and every 30 min for the subsequent 60 min. This involved the volunteer rating of their hunger on a 10-point scale. A satiety index AUC was also calculated geometrically[11].

Statistical analysis

A difference in the mean AUC of 10 mmol/l and a mean standard deviation of 5 with a power of 0·9 and an α of 0·05 indicated that a sample size of twelve was required. Data preparation and statistical analysis were carried out using the Statistical Product and Service Solutions software (version 14.0.1, 2005; SPSS, Inc., Chicago, IL, USA). Data are presented as means and standard deviations or means with standard errors of the mean. The levels of intra-individual variation of the three reference (glucose) tests were assessed by determining the CV. Before statistical analysis, the normality of the data was tested using the Shapiro−Wilk statistic. Where data were skewed (i.e. GR−AUC min), non-parametric tests were used. Repeated-measures ANOVA, with Bonferroni correction, or Friedman one-way ANOVA (non-parametric) was used to compare the AUC and GR between the pancake test samples. Statistical significance for all tests was set at $P < 0·05$; all data are means with their standard errors of the mean unless otherwise stated.

Results

All volunteers completed the study. Hence, data are presented for twelve volunteers. The FAO/WHO[11] recommends that to determine the GR of a food, tests should be repeated in six or more subjects.

The mean intra-individual variation in GR to the three reference tests was 22 (SD 7)% CV. This value is consistent with previously reported data in healthy subjects[12]. The inter-individual variation in GR to the reference tests was 35 % CV.

The results showed that there was no difference in GR (blueberry pancake 51·3 (SD 5·7); raspberry pancake 54·7 (SD 5·6); blueberry control 43·9 (SD 4·2); raspberry control 41·8 (SD 6·4)%) or GR−AUC (blueberry pancake 84·1 (SD 16·7); raspberry pancake 88·0 (SD 17·5); blueberry control 69·2 (SD 12·1); raspberry control 68·3 (SD 12·6) mmol × min/l) between any of the tests ($P > 0·05$) (Fig. 1).
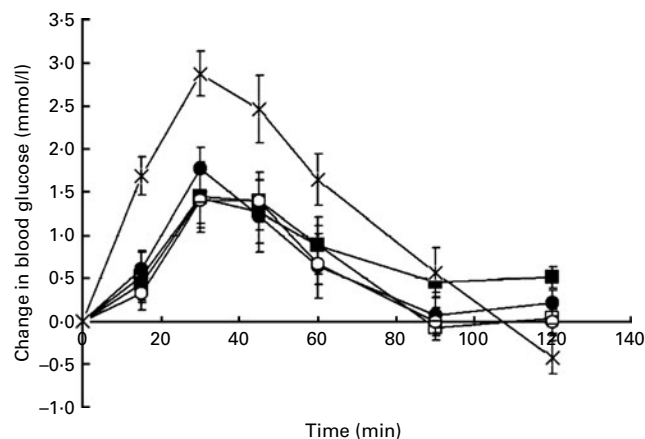
Fig. 1. Temporal curves of the blood glucose response (n 12) following consumption of the same quantity (50 g) of available carbohydrate of blueberry (—●—) raspberry (—■—), blueberry control (—○—) and raspberry control (—□—) pancake compared with glucose solution (—✕—).

There was also no difference in the satiety index AUC between the four pancake meals (blueberry 264 (SD 37); raspberry 314 (SD 38); blueberry control 223 (SD 38); raspberry control 254 (SD 32)).

Discussion

The reported impact of polyphenols on GR observed in previous studies may be due to the testing of carbohydrates as monosaccharides or disaccharides. The present results suggest that when such polyphenols are added to starch-based foods, they do not elicit any reduction in GR, as the phenolic compounds are unable to bind with the amylose and amylopectin fractions. This is the first study to examine the effect of polyphenols on starch digestion and to examine the carbohydrate−polyphenol relationship *in vivo* within a solid starch-based test food. Previous *in vivo* studies have been concentrated on liquid-based nutrient intake such as juices, coffee and purées containing monosaccharides and disaccharides.

While the polyphenol content of the test meal was not examined in the present study, analysis on these berries has been reported elsewhere[13]. In blueberries, fifteen anthocyanins were detected, totalling 8010 nmol/g; this is 84 % of the total antioxidant activity. From *in vitro* analysis, inhibition of α-glucosidase by berries was related to their anthocyanin content[14]. There was also a relatively large amount of quercetin glucosides, which inhibits glucose uptake into brush-border membrane vesicles[15−20]. Most antioxidant activity was attributed to delphinidin-3-O-galactoside, cyanidin-3-O-galactoside and delphinidin-3-O-arabinoside, petunidin-3-O-galactoside, malvidin-3-O-galactoside and malvidin-3-O-arabinoside. In raspberries, eight anthocyanins were detectable, totalling 885 nmol/g. Cyanidin-3-O-sophoroside was the major anthocyanin followed by the three compounds: cyanidin-3-O-(2″-O-glucosyl)rutinoside, cyanidin-3-O-sambubioside and cyanidin-3-O-glucoside. The berries also contained the ellagitannins; lambertianin C and sanguin H-6 along with trace amounts of ellagic acid derivatives and quercetin conjugates.

Polyphenol-rich extracts of blueberries, blackcurrants, strawberries and raspberries have been shown to inhibit α-glucosidase activity[14,21−24] and glucose transport[20]. Zhanga et al.[20] investigated the extracts of various cultures of raspberries and their effects on starch-digesting enzymes *in vitro*. They showed that raspberry extracts were inhibitors of α-glucosidase but not α-amylase. McDougall & Stewart[25] asserted that α-glucosidase activity was inhibited, in particular, by polyphenols diacylated and acylated anthocyanins. It was thought that the acylated anthocyanins are more stable at intestinal pH than their deacylated counterparts and hence have the greater effect.

Some studies have been able to replicate findings from *in vitro* experiments. Torronen *et al.* found lower plasma glucose concentration 15−30 min post-meal after the berry test meal consisting of a berry purée with 35 g sucrose compared with sucrose, glucose and glucose liquid as controls. However, they were unable to find differences in the AUC between the berry purée and control, indicating that

the effect was not sufficient enough to influence the overall glucose response. In the present study, no differences in GR were observed between the berry and the control pancakes containing the same quantity and types of sugars. This may be due to several reasons. First, previous research in this area has been conducted on monosaccharides and disaccharides, not on a starch-based food. Second, as the berries were partially served in the pancakes and partially served on the side, the berries may not have been masticated sufficiently to allow for the release of the polyphenols and their complete chelation with the carbohydrates. Research indicates that berry polyphenols lower the GR by working as enzyme inhibitors as they have a similar structure to the normal substrate of the enzyme (in the case of α-glucosidase maltose). The future challenge is to explore how best berry polyphenols can be used not only as antioxidants but also as phytochemicals capable of lowering the GR to complex carbohydrates.

Acknowledgements

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