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The glycemic index of pigmented potatoes is related to their polyphenol content

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Polyphenol extracts from coloured fruits and vegetables inhibit α-glucosidase *in vitro*, however it is not known whether this translates into an attenuation of blood glucose response *in vivo*. We examined this relationship in a GI study by feeding coloured potatoes to 9 healthy volunteers. We also examined the *in vitro* inhibitory activity of potato anthocyanin extracts on rat intestinal α-glucosidase. Potatoes (Purple Majesty; Red-Y38; Yukon Gold and Snowden) were fed with skin after cooking in a convection oven, using a random block design and 50 g available carbohydrate. Glucose was used as the standard and venous blood collected at 0, 15, 30, 45, 60, 90, 120 min. Areas under the curve (AUC) for glucose and insulin were calculated, and GI and Insulin Index derived. Neither AUC for blood glucose response nor insulin was significantly different among the various potatoes studied. Although the mean GI (±SE) values for the potato types varied (purple = 77.0 ± 9.0; red = 78.0 ± 14.0; yellow = 81.0 ± 16.0; and white = 93.0 ± 17.0), these differences were not significantly different. The mean (±SE) polyphenol content (mg GAE/100 g DW) was 234 ± 28; 190 ± 15; 108 ± 39; 82 ± 1 for purple, red, yellow and white potatoes, respectively. There was a significant inverse correlation between polyphenol content and GI of the potatoes ($r = -0.825$; $p < 0.05$; $n = 4$). *In vitro*, polyphenol extracts of red and purple potatoes inhibited α-glucosidase by 37.4 ± 2.2% and 28.7 ± 3.2%, respectively. The GI of coloured potatoes is significantly related to their polyphenol content, possibly mediated through an inhibitory effect of anthocyanins on intestinal α-glucosidase.

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Introduction

Type 2 diabetes mellitus (T2DM) is a significant global health problem, characterized by abnormal glucose tolerance and insulin resistance, and is often associated with significant long term complications, and poor quality of life.[1,2] T2DM is associated with diets high in calories and reduced physical activity; consequently many dietary interventions have been advocated for the management and prevention of T2DM.[3] The glycemic index (GI) of foods has been proposed as evidence based guidance in choosing carbohydrate rich foods on the basis of their post prandial blood glucose raising potential.[4,5] Potatoes have been characterized as being a medium to high GI food, although this varies among different varieties[6,7] and by cooking and processing methods[8–10] as well as starch digestibility.[9,11]

Potatoes are the third largest food crop worldwide following rice and wheat, and as such play a significant role in human health.[12] Potatoes with pigmented flesh are becoming more readily available in the market place and there is growing interest in their potential health benefits. Pigmented red and purple flesh potatoes contain two to three times more antioxidants than white-flesh potatoes.[13,14] The antioxidant properties of pigmented potatoes are accounted for by the presence of polyphenols, specifically anthocyanins, phenolic acids and carotenoids.[13,15] Further, consumption of purple and yellow pigmented potatoes has been associated with a reduction in inflammation and oxidative damage in healthy adult males when compared to white potatoes.[16] Despite their nutritional and antioxidant properties, potatoes have been implicated in contributing to T2DM due to their higher GI values; however, the GI of pigmented potatoes has not been previously determined.

Foods rich in polyphenols, especially anthocyanins and condensed tannins, have been associated with reduced glycemic response, with a negative correlation observed between polyphenol content of leguminous foods and non-leguminous cereals and blood glucose response.[17] Further, polyphenol-rich extracts from fruits have also been shown to inhibit the activity of α-glucosidase, the main enzyme responsible for intestinal starch digestion.[18] The effect of polyphenol content on the digestibility of starch in newer cultivars of pigmented potatoes and blood glucose response has not been studied. We reasoned that the GI of pigmented potatoes may be lower than that of white potatoes and that this may be related to the inhibitory effect of anthocyanins on intestinal α-glucosidase. Further, with pigmented potatoes emerging in the market place with increasing frequency, the availability of GI values would be

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beneficial in making informed dietary choices. The objectives of this study were to determine the GI values of commonly available pigmented Canadian potatoes, to define the relationship between potato polyphenol content and GI, and to assess the *in vitro* inhibitory effect of crude anthocyanin extracts from pigmented potatoes on intestinal α-glucosidase activity.

Methods

Potatoes

Potato cultivars of Purple Majesty with purple skin and flesh, Y38 with red skin and flesh, Yukon Gold with white skin and yellow flesh, and Snowden with white skin and white flesh, were obtained as mature potatoes at fall harvest from the Elora Research Station, University of Guelph (Elora, Ontario, Canada – 43°41′N, 80°26′W) on a Conestoga silt loam soil in 2009.

Food analysis

Detailed proximate and dietary fibre analysis was performed commercially (Maxxam Analytics International Corporation, Mississauga, ON, Canada) using standard AOAC methods for total fat (AOAC 922.06), protein (AOAC 992.15), total dietary fibre (AOAC 985.29) and moisture (AACC 44-15A). Energy and total carbohydrates were derived and available carbohydrate was calculated as the difference between total carbohydrate and total dietary fibre.

Test foods

Fresh whole potatoes were washed in tap water, air dried, packaged into 50 g available carbohydrate portions and stored at −20 °C. On test days, the potatoes were quickly defrosted in a microwave oven, cut into 2 cm^3 cubes and baked in a convection oven for 40 min until soft when prodded with a fork. Potatoes were served with skin on, a pinch of salt and pepper and about 5 g of margarine, if desired. Flavourings remained constant for each participant, for all test foods. The potatoes as well as the standard glucose solution were served with 300 mL of water. Participants consumed the foods within 15 minutes.

GI study

This study was conducted according to the guidelines set by the Declaration of Helsinki and all procedures involving human participants were approved by the Canadian Shield Ethics Review Board. Written informed consent was obtained from all participants. The study protocol was based on the method of Wolever *et al.* (1991),[19] was approved by the Canadian Shield Ethics Review board and registered at http://www.clinicaltrials.gov (#NCT01053793). The following exclusion criteria were applied: BMI > 30 kg m^{-2}, history of drug abuse, pregnancy or lactation, allergy to potatoes, and abnormal fasting glucose (≥5.56 mmol L^{-1}), and nine healthy Caucasian adults (3 males, 6 females) were recruited. On the day prior to testing, participants were asked to refrain from alcohol, intense physical activity and high fibre foods at dinner. On each study day participants arrived at the human trial facility (Nutrasource Diagnostics Inc., Guelph, ON, Canada) in a fasted state (12 h). On the first visit participants were given a 50 g standard glucose solution. At subsequent study visits participants consumed one of the four potato varieties or a second standard glucose solution in a randomized block design. Before consumption of the test meal or standard a fasting blood sample was obtained using an indwelling catheter and additional blood samples collected at 15, 30, 45, 60, 90, and 120 min after the first bite was taken. Participants remained seated quietly during the test period. Whole blood samples were collected into lithium heparin tubes and an aliquot was stored at −80 °C pending glucose analysis on a YSI model 2300 STAT analyzer (Yellow Springs, OH), with fasting samples measured in duplicate. The remainder of the whole blood was centrifuged to obtain plasma which was stored at −80 °C pending analysis for insulin using a commercial ELISA kit and multi-level quality control material (Invitrogen, CA, USA).

Plasma antioxidant status

The oxygen radical antioxidant assay (ORAC) and ferric reducing antioxidant power (FRAP) assays were used to determine total antioxidant capacity of serum as outlined by Li *et al.*[20,21]

Polyphenol analysis

Total polyphenol content (TPC) of ground freeze dried potato powders was determined in triplicate using an adapted 96-well micro plate Folin–Ciocalteu method[20,21] and expressed as milligram gallic acid equivalent (GAE)/100 g dry weight basis (mg GAE/100 g DW). Crude anthocyanin rich extracts were prepared from 50 g ground freeze dried potato powders that were double extracted with 500 mL acidified methanol (85% MeOH: 14% H_2O: 1% HOAc) and partially purified using flash chromatography (Isolera One, Biotage, Sweden, AB). Methanol was removed by rotary evaporation and the resulting extracts were freeze dried and stored at −20 °C until use. Total anthocyanin concentration (TAC) was determined in triplicate using a modified pH differential method[20,21] and expressed as mg cyanidanin-3-glucoside equivalent/100 g (mg CyGE/100 g DW). All general purpose chemicals were of analytical or HPLC grade and were purchased from Sigma-Aldrich Chemical Co. (St Lois, MO).

α-Glucosidase inhibition assay

Experiments were carried out with crude α-glucosidase from rat intestinal powder (Sigma I1630: 25 mg mL^{-1} in 0.1 M phosphate buffer, pH 6.9) as previously outlined.[18] Anthocyanin extracts were dissolved in buffer and serial dilutions prepared. Acarbose (Sigma A8980) was used as a positive control. The assay was carried out in a 96-well microplate and contained: 100 μL of extract, acarbose or buffer and 100 μL of enzyme solution. The reaction was initiated by the addition of 100 μL of enzyme substrate (5 mM *p*-nitrophenyl α-D-glucopyranoside; Sigma N1377), and monitored at 405 nm at 30 °C for 10–20 minutes. Assays were performed in triplicate on three separate days for each extract. The reaction rates of the assays with and without extracts were compared, and the α-glucosidase inhibitory activity determined.

Data analysis

The incremental AUC for blood glucose response, excluding area below fasting, was calculated for each potato tested by each participant using the trapezoid method.[19] GI values were derived by expressing the AUC as a percentage of the mean AUC for the reference food; the mean of the resulting values for each participant was used to determine the food GI. The incremental area under the blood insulin response curve was calculated and the insulin index (II) derived in the same manner as for GI values. Data were entered into Excel, 2007 (Microsoft Corporation, Washington, USA), verified and then imported to STATA[23] (version 10.0, STATA Corporation, Texas, USA). Differences in AUC, II and glycemic index were compared by potato type and time using two-way ANOVA, with post-hoc analysis when applicable. Spearman rank correlation was used to determine the relationship between potato GI and polyphenol content. Differences were considered statistically significant at $p < 0.05$.

Results

Table 1 shows the nutritional composition of 50 g available carbohydrate portions of the test meals. As eaten, the polyphenol content of purple and red pigmented potatoes were at least twice as high as that of yellow and white potatoes, respectively. Corresponding total anthocyanin (TAC) for purple and red pigmented potato powders were 152 and 179 (mg CyGE/100 g DW); no significant amount of anthocyanin was detected in the yellow and white potatoes.

Participants (3 males; 6 females) involved in the GI study were all Caucasian with ages ranging from 23 to 36 years with a mean (±SE) BMI of 25.2 ± 1.1 Kg m^{-2} and normal fasting blood glucose levels (4.8 ± 0.2 mmol L^{-1}). The mean blood glucose AUC following consumption of purple, yellow, red, and white potatoes and standard glucose solution were (mmol × min L^{-1}): 90 ± 17, 100 ± 30, 101 ± 26, 116 ± 27 and 125 ± 23, respectively. One way repeat measures ANOVA revealed that among the potatoes, blood glucose response at different time points was similar, but this differed significantly from the glucose standard (Fig. 1). At 15 min all potato types showed significantly lower blood glucose values ($p < 0.005$) than the glucose standard; however at 120 min white, red, and yellow potato types had significantly higher blood glucose values than the glucose standard ($p < 0.001$).

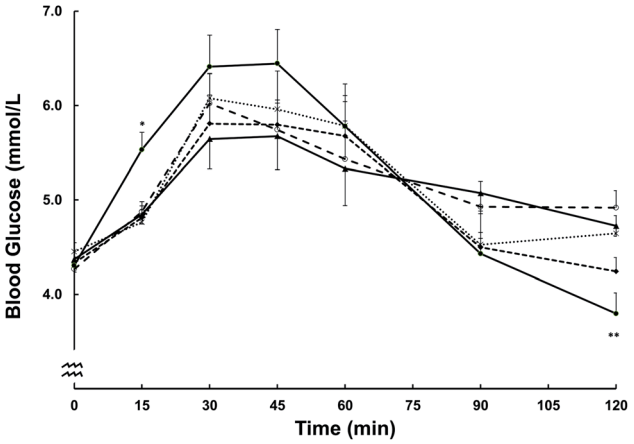

Fig. 1 Blood glucose response elicited from different colour potatoes. Values are means with standard errors represented by vertical bars. Standard glucose = ●; yellow potato = × ; white potato = ○; purple potato = ◆; red potato = ▲, *- at 15 min, mean blood glucose after consumption of glucose standard significantly different from all 4 potato types ($p < 0.01$). **- at 120 min, mean blood glucose after consumption of glucose standard significantly different from red ($p = 0.007$), yellow ($p = 0.013$) and white potatoes ($p = 0.001$).

The blood insulin response after consumption of potatoes, shown in Fig. 2, was similar to the glucose response curve with insulin levels peaking around 45 min. One way ANOVA on ranks indicated a significant difference in the blood insulin AUC between the glucose standard and red potato ($H = 10.2$; df = 4; $p = 0.039$). However, there were no significant differences in the blood insulin concentration between the glucose standard and potato types at any of the time points. The insulin index (%) for red, purple, white, and yellow potatoes were: 52 ± 7; 76 ± 10; 78 ± 14 and 81 ± 10, respectively; these were not significantly different by one-way ANOVA.

The mean (±SE) glycemic index values were 77 ± 9, 78 ± 14, 81 ± 16 and 93 ± 17 for purple, red, yellow and white potatoes, respectively (Table 1). Neither blood glucose AUC nor glycemic index differed significantly among the potato types when assessed either by repeat measures ANOVA or by one way ANOVA on ranks.

Plasma antioxidant status (Fig. 3A and B) as measured by ORAC remained relatively unchanged following the consumption of the different potatoes however, the pattern observed with FRAP suggested a trend towards decreasing antioxidant status.

Table 1 Nutritional composition of test meals (per 50 g available carbohydrate)

Food	Weight (g)	Energy (KJ)	Fat (g)	Protein (g)	Total CHO (g)	Fibre (g)	Moisture (%)	Anthocyanin (mg CyGE)[a]	Polyphenol (mg, GAE)[b]	Glycemic index (%)
Purple potato (Purple Majesty)	289.0	1020	0.3	6.3	53.8	3.8	78.5	16.4 ± 1.5	145 ± 17	77 ± 9
Red potato (Y38)	375.9	1056	0.5	7.8	54.1	4.1	82.8	15.4 ± 1.2	123 ± 10	78 ± 14
Yellow potato (Yukon Gold)	267.4	1027	0.3	7.2	54.0	4.0	76.3	nd	68 ± 25	81 ± 16
White potato (Snowden)	274.4	1029	0.3	7.4	53.5	3.6	76.8	nd	52 ± 1	93 ± 17

[a] Total anthocyanin (milligram cyanidanin-3-glucoside equivalent/100 g dry weight (mg CyGE)/100gDW). [b] Total polyphenol (milligram gallic acid equivalent/100 g dry weight (mg GAE/100 g DW)), nd = not detected.

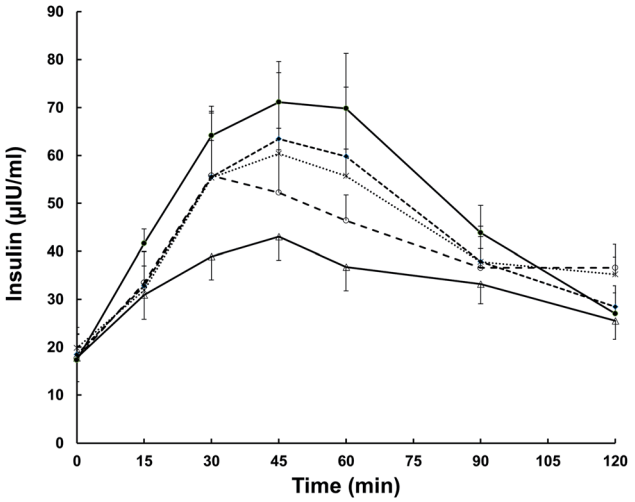

Fig. 2 Insulin response elicited from different colour potatoes* values are means with standard errors represented by vertical bars. Standard glucose = ●; yellow potato = × ; white potato = ○; purple potato = ◆; red potato = △, * blood insulin AUC between the glucose standard and red potato is significantly different ($H = 10.2$; df = 4; $p = 0.039$).

Polyphenol

Overall, the highest polyphenol concentration was found in purple potato, whereas the glycemic index of this potato was lowest compare to the other potato varieties (Table 1). Ranked correlation showed that there was a significant inverse relationship between the total polyphenol content and glycemic index among different coloured potatoes ($r = -0.825$; $n = 4$; $p < 0.05$). Crude anthocyanin-rich extracts from red potato and purple potato, at a concentration of 1.67 mg mL^{-1}, inhibited the *in vitro* action of rat intestinal α-glucosidase by (mean and SE) 37.4 ± 2.2% and 28.7 ± 3.2%, respectively. The same concentration of acarbose, a synthetic α-glucosidase inhibitor used to manage postprandial blood glucose levels, resulted in 100% inhibition.

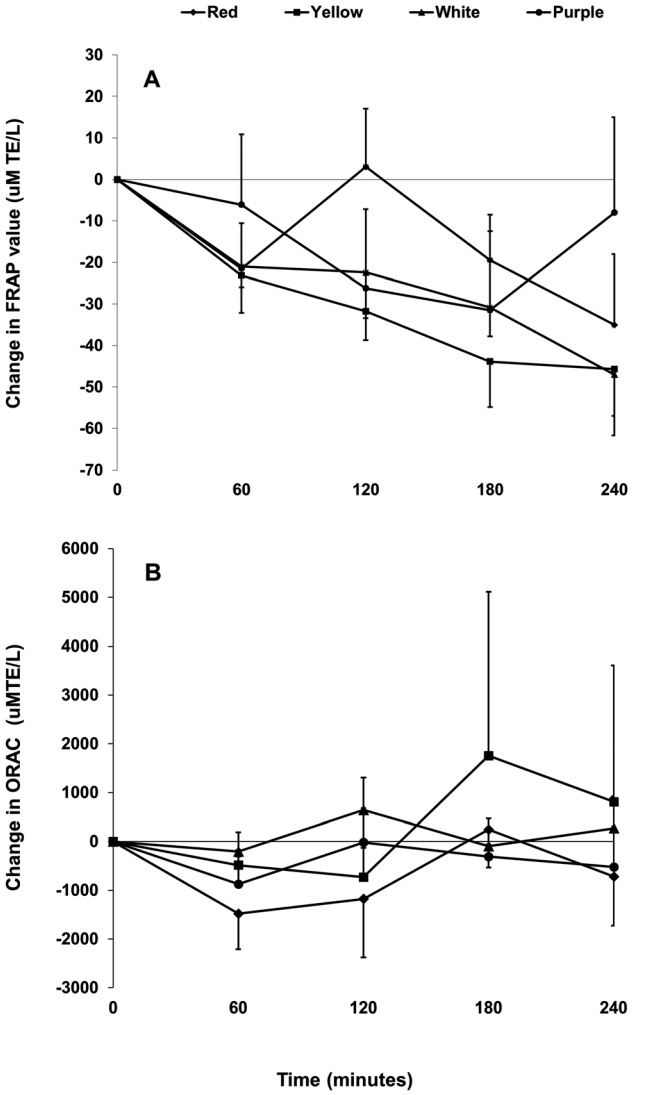

Fig. 3 (A) Plasma ferric reducing antioxidant power (FRAP) values and (B) oxygen radical absorbance capacity (ORAC) following consumption of potatoes. Mean and SEM; $n = 3$.

Discussion

Potato consumption is significant in many different cultures globally[12] so it is important to provide evidence based guidelines on the choice of potatoes. Potatoes have long been regarded as being a high GI food, but more recently the increasing availability of potatoes with pigmented flesh has generated interest in their potential health benefits. Pigmented potatoes contain significant amounts of antioxidant due to the presence of anthocyanins,[14,15,24] and their consumption is associated with a reduction in inflammation and oxidative damage.[16] Given that anthocyanin extracts from fruits have been shown to inhibit intestinal α-glucosidase *in vitro*,[18] it was hypothesized that pigmented potatoes would have lower *in vivo* GI values compared to white potatoes.

In the present study, consumption of the various pigmented potatoes resulted in blood glucose AUC that were not significantly different. However, compared with the glucose standard all potato varieties showed significantly lower blood glucose values 15 min after consumption. At 120 min white, red, and yellow potatoes had significantly higher blood glucose values than the glucose standard, indicating that the potatoes were digested slower, as expected. Among the potatoes studied the resulting GI values were not significantly different. Importantly, the potatoes used in this study had GI values ranging from mid-high (purple) to very high (white). Variations in the GI of potatoes have been accounted for by differences in the starch fractions and digestibility,[9–11] and by cooking and storage methods.[8,10,24]

According to Foster-Powell *et al.* (2002),[25] differences in GI values of the same type of foods may be explained by methodological differences in processing, in determining the digestible carbohydrate content of the test foods and in GI testing, and inherent botanical differences. The varieties of potatoes used in the present study had similar total dietary fibre levels so their digestible carbohydrate content was similar. The GI testing method used in the present study is widely used and utilized key

improvements in human GI studies as recommended by Wolever *et al.* (2008).[26] Further, the GI values obtained in the present study for Red, White and Yellow are similar to values obtained for same-day cooked and eaten, peeled, boiled or baked potatoes.[8,10]

Most GI studies on potatoes have used boiling as the preferred cooking method. In the present study frozen potatoes were thawed *via* microwave, cooked with skin in a convection oven and consumed immediately. Fernandes *et al.* (2005)[7] reported that the mean AUC elicited by day-cooked Russet Burbank potatoes did not differ significantly whether baked in a microwave or conventional oven. In addition, Soh and Brand-Miller (1999)[10] found no differences in GIs when they compared cooking methods (peeled and boiled; peeled, boiled and mashed; peeled and microwaved; peeled and baked). However, the effect of freezing whole potatoes and microwave thawing must be considered since ice crystal formation during initial freezing could have disrupted cells walls and physically separate starch from amylases through shrinking of gelled starch granules and cell distortion.[27] On the other hand, microwave heating increases hydration and consequently reverses retrogradation of starch. Mulinacci *et al.*[24] have shown that microwave heating of potatoes results in a higher level of resistant starch in both pigmented and white varieties. In the present study there could have been some resistant starch formation in the cooked potatoes, although this would have occurred in all potato types. It would have been ideal to use fresh potatoes in this study and it is likely that their GI values would have been lower, given the cell wall disruption and starch separation associated with freezing. However, this approach would have led to variation in the batch of potatoes studied.

A study on GI values for commercially available potatoes in Great Britain also showed that there was no significant difference in GI values between different potato types, although the GI values ranged from 56 to 94.[6] There were no pigmented potatoes in that study and the variation was accounted for by differences in the texture of the potato types: waxy texture produced low GI, whereas floury potatoes had high GI values.[6] It has been suggested that the latter could be partially explained by an increased amylopectin content of the floury, more mature potatoes.[10] Fernandes *et al.*[7] reported a GI of 89 for boiled red potatoes and showed that precooking and reheating, or consuming cold potatoes resulted in a lowering of the GI, which was accounted for by an increased formation of resistant starch. It is possible that the GI values obtained in the present study may be even lower if the pigmented potatoes were consumed cold or after precooking and reheating. It has been suggested that the glycemic responses to consumption of carbohydrate foods is also influenced by particle size and the presence of other macronutrient components, including fat, protein and dietary fibre.[28] However, the role of bioactive minor food components such as polyphenols, particularly anthocyanins, on blood glucose response and glycemic index has not been explored.

It is well known that anthocyanin-rich extracts from fruits and vegetables exhibit varying but significant *in vitro* inhibitory activity towards α-glucosidase, the main enzyme responsible for intestinal starch digestion.[18,22] However, few human studies have explored the relationship between the polyphenol content of foods and glycemic response *in vivo*. In a GI study that examined various leguminous and non-leguminous foods, Thompson *et al.*[17] reported a negative correlation between GI and polyphenol content. Results from the present study show that within a given food crop GI values are inversely related to the polyphenol content. Further, results of *in vitro* studies show that anthocyanin extracts from the pigmented potatoes display significant inhibition of α-glucosidase, and offers an explanation for the lower blood glucose response and GI values observed for pigmented potatoes. Many of these *in vitro* studies have reported that cyanidin 3-glucoside is a potent inhibitor of α-glucosidase.[17,29] In the present study, no attempt was made to identify the individual anthocyanin(s) that may account for α-glucosidase inhibition. However, Li *et al.*[21] have shown that the major anthocyanins in Purple Majesty and Y38 varieties of potatoes (used in the current study), were petunidin and pelargonidin, respectively. As such, it is unlikely that the inhibition of α-glucosidase by anthocyanin extracts from these potatoes is due to cyanidin 3-glucoside.

Several studies have shown that purple potatoes contain more total antioxidant capacity, total phenolics and total anthocyanins content than yellow, and white potatoes.[13,15,24,30] Further, Kaspar *et al.*[16] have shown that consumption of purple potatoes is associated with a reduction in inflammation and oxidative damage. In the present study, we found that there was very little change in plasma ORAC and FRAP. This suggests that the GI values obtained were independent of the antioxidant effects of polyphenols. Kasper *et al.*[31] reported that although consumers ranked the aroma and appearance of white and yellow potatoes higher than purple there were no significant differences in overall acceptance of the potato cultivars. It appears that consumers may be willing to consume pigmented potatoes, which are perceived to be beneficial to health.[31] Taken together with the lower GI values, the available evidence suggests that pigmented potatoes (purple and red) may be healthier choices than white potatoes. These findings could lead to greater awareness of consumers and breeders to increase the availability and usage of pigmented potatoes.

Conclusion

The GI values derived for the potatoes studied could be used to guide choices of potatoes in order to lower the overall GI and glycemic load of the diet. Further, with pigmented potatoes emerging in the market place with increasing frequency the availability of GI values would be beneficial in making informed dietary choices. Among the four potatoes studied, there was a highly significant inverse correlation between polyphenol content and *in vivo* GI suggesting a possible inhibitory effect on intestinal α-glucosidase. Compared to white potatoes, anthocyanin rich extracts from the pigmented potatoes displayed moderate inhibitory activity towards intestinal α-glucosidase *in vitro*. Further research is necessary to identify the mechanism by which polyphenol rich starchy foods influence blood glucose response.

Conflict of interest statement and contributions

All authors declare no conflict of interest. DDR conceptualized the study, managed the human trial, analyzed the data and prepared the final manuscript. AW, EP contributed to the human trial, laboratory analyses and data analysis; TS assisted with data analysis and manuscript preparation. RT oversaw analyses of polyphenols and contributed to the final manuscript.

Abbreviations

GI	Glycemic index
AUC	Area under the curve
II	Insulin index
GAE	Gallic acid equivalent
TAC	Total anthocyanin
CyGE	Cyanidanin-3-glucoside equivalent.

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