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Berries and anthocyanins: promising functional food ingredients with postprandial glycaemia-lowering effects

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The prevalence of type 2 diabetes (T2D) is predicted to reach unprecedented levels in the next few decades. In addition to excess body weight, there may be other overlapping dietary drivers of impaired glucose homeostasis that are associated with an obesogenic diet, such as regular exposure to postprandial spikes in blood glucose arising from diets dominated by highly refined starches and added sugars. Strategies to reduce postprandial hyperglycaemia by optimising the functionality of foods would strengthen efforts to reduce the risk of T2D. Berry bioactives, including anthocyanins, are recognised for their inhibitory effects on carbohydrate digestion and glucose absorption. Regular consumption of berries has been associated with a reduction in the risk of T2D. This review aims to examine the evidence from *in vitro*, animal and human studies, showing that berries and berry anthocyanins may act in the gut to modulate postprandial glycaemia. Specifically, berry extracts and anthocyanins inhibit the activities of pancreatic α-amylase and α-glucosidase in the gut lumen, and interact with intestinal sugar transporters, sodium-dependent glucose transporter 1 and GLUT2, to reduce the rate of glucose uptake into the circulation. Growing evidence from randomised controlled trials suggests that berry extracts, purées and nectars acutely inhibit postprandial glycaemia and insulinaemia following oral carbohydrate loads. Evidence to date presents a sound basis for exploring the potential for using berries/berry extracts as an additional stratagem to weight loss, adherence to dietary guidelines and increasing physical exercise, for the prevention of T2D.

Anthocyanins: Type 2 diabetes: Postprandial hyperglycaemia: Functional food: Berries

Obesity is the primary cause of type 2 diabetes (T2D), although it has been estimated that 10 % of T2D patients are not overweight or obese[1], and most people with obesity do not develop T2D. Other risk markers that are loosely associated with excess body weight are likely to determine the progression to insulin resistance (IR) and eventually β cell failure. Elevated postprandial glycaemia has been implicated in the development of T2D[2] and represents a risk factor that can easily be targeted by dietary modifications. The concentrations of glucose in the blood following a meal containing a known amount of carbohydrate represents the sum total of the rate of digestion and absorption of glucose in the gut, as well as the rate of uptake from the circulation into the cells for oxidation or storage. An exaggerated postprandial glycaemic response to a standard carbohydrate load is indicative of a reduction in insulin secretion or sensitivity. Reducing the rate of delivery of glucose to the bloodstream by manipulating the carbohydrate type and/or meal composition is one way in which these adverse metabolic profiles might be ameliorated. High dietary glycaemic index and glycaemic load independently increases risk of T2D[3] (relative risk 1·4 and 1·3, respectively). Frequent elevated excursions in postprandial glucose concentrations are thought to increase risk of T2D and CVD by inducing oxidative stress and glycation of proteins, as reviewed by Blaak *et al.*[4]. Studies with acarbose (an

Abbreviations: HR, hazard ratio; IR, insulin resistance; PCA, protocatechuic acid; SGLT1, sodium-dependent glucose transporter 1; T2D, type 2 diabetes.
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inhibitor of α-glucosidase) show that reducing the rate of carbohydrate digestion can reduce the risk of progression to diabetes in participants with impaired glucose tolerance by 25 %[5], suggesting that dietary strategies to reduce the rate of carbohydrate digestion may have similar preventative effects. This review will examine the evidence for a similar action of berries and their anthocyanins on the rate of glucose absorption, including an analysis of mechanistic insights from cell studies and enzyme inhibition experiments, data from animal studies and an evaluation of the latest evidence from human dietary intervention studies.

What are anthocyanins?

Classification

Anthocyanins are a subclass of a large group of plant-based, bioactive compounds called polyphenols, which are named for having one or more aromatic rings with at least one hydroxyl group (a phenol) and are present in a wide range of plant-based foods such as fruit and vegetables, soya, chocolate, wine and tea[6]. Polyphenols are commonly classified in four major groups: flavonoids, phenolic acids, lignans and stilbenes. Flavonoids can be further broken down into subclasses, including anthocyanins, flavanones, flavonols and flavones, to name a few. Anthocyanins are often responsible for the bright and deep colours associated with certain fruit and vegetables such as grapes, berries, cherries, aubergine and red onion[7]. The six most abundant anthocyanin aglycones (anthocyanidins) are malvidin, petunidin, delphinidin, peonidin, pelargonidin and cyanidin. These anthocyanidins share a structure of 2-benzene rings (A and B rings) united by a heterocyclic ring (C ring; Fig. 1). As suggested by their name, aglycones are not bound to a sugar molecule. However, polyphenolic compounds mainly exist as O-glycosides in plants, where a sugar moiety resides most often at position 3 of carbon ring C (Fig. 1). Commonly linked sugars include glucose, galactose, arabinose and rutinose. For example, blackcurrants are high in delphinidin-O-3-rutinoside and cynanidin-O-3-rutinoside[8–10] (Fig. 1). Aside from sugars, anthocyanins may also be found acylated to aromatic and aliphatic acids. The variety of ways in which anthocyanins might be glycosylated or acylated has led to reports of up to 650 varieties of anthocyanins identified so far in flowers, fruit, vegetables and other types of plant material[11].

Main sources and dietary intake

Dietary intakes of anthocyanins are derived from a relatively narrow range of foods. The total anthocyanin content varies widely from 0·28 to 1480 mg/100 g in both fruit and vegetables. The main sources in the human diet are berries with blue, purple and orange/red pigments. Berries with the largest concentrations are elderberries, chokeberries, blackcurrants and blueberries with estimated contents in the range 160–1300 mg/100 g fresh weight[8,12–16] (Supplementary Online Table S1).

Average anthocyanin intakes are difficult to establish. FFQ from a randomised controlled trial on the impact of adherence to UK dietary guidelines on markers of CVD risk (CRESSIDA study, *n* 161 middle-aged and older men and women)[17], were recently analysed by our group using a polyphenol database developed at University of East Anglia, UK[18]. Prior to randomisation to dietary intervention group, the geometric mean for estimated anthocyanidin intakes in the CRESSIDA study population was 18 mg/d, 95 % CI 15, 21 (arithmetic mean 27 mg/d, interquartile range 10, 35; ML Castro-Acosta and WL Hall, unpublished results). Mean estimates from FFQ and 24-h recalls in adult populations from different countries vary from 0·04 (food intake data from five major population nutrition surveys in Fiji)[19] to 215 mg/d[20], but with the majority of reports ranging between 18 and 43 mg/d[13,18,21–25].

FFQ may underestimate true anthocyanidin intakes since questions are not specific to individual fruits and averaged values are applied to groups of foods that may vary widely in their anthocyanin contents, for example ‘strawberries, raspberries, kiwi fruit’ are grouped together in one category to indicate frequency of consumption, and other anthocyanin-rich foods are not mentioned in the questionnaire. Food diaries may provide a more accurate representation of intake, but estimates represent short-term intakes rather than habitual consumption patterns, which could be particularly misleading for seasonally available foods such as berries. Research groups have created and validated FFQ to estimate dietary flavonoid intake in different populations[26–28], which should provide more reliable intake estimations for specific populations, although they remain unavoidably susceptible to bias due to self-reporting errors, portion size quantification and estimation errors resulting from the lack of data on polyphenol content in food[29]. At present, the most common databases employed to assess flavonoid intakes are the USDA[14,30,31] and Phenol-Explorer[8], which provide information on the content of thirty-five flavonoids in 506 food items and 502 polyphenols (of the four classes) in 459 food items, respectively. The USDA database expressed flavonoid content as aglycone equivalents exclusively while Phenol Explorer database expressed polyphenol content as aglycones, glycosides or esterified metabolites and also includes retention factors to calculate changes in content due to cooking process. Although the Phenol Explorer database offers a wealth of detailed data on the polyphenol composition of foods, it might still be considered a work in progress when considering the broad diversity of polyphenols in food and the remaining gaps in the food analytical literature.

New techniques for intake estimation have been examined; these include innovative technologies for measuring dietary intakes in epidemiological studies[32], as well as biomarker approaches. The use of metabolomic techniques to analyse phenolic metabolites in urine or plasma have a promising role in epidemiological studies[33,34]. Although current estimates of dietary anthocyanin intakes are limited, epidemiological studies suggest that higher consumption rates of berries and anthocyanins are associated with beneficial effects on risk factors related to vascular function and T2D.

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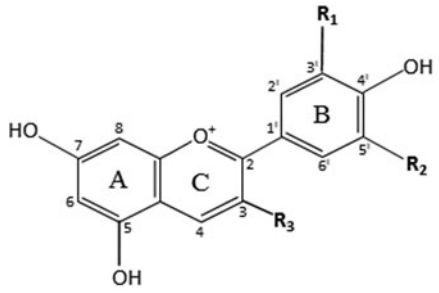

Anthocyanidin	R_1	R_2	R_3	Anthocyanin	R_3
Pelargonidin	-H	-H	-OH	Pelargonidin-3-O-glucoside	-O-glucose
Cyanidin	-OH	-H	-OH	Cyanidin-3-O-rutinoside	-O-rutinose
Delphinidin	-OH	-OH	-OH	Delphinidin-3-O-rutinoside	-O-rutinose
Peonidin	$-OCH_3$	-H	-OH	Peonidin-3-O-glucoside	-O-glucose
Petunidin	$-OCH_3$	-OH	-OH	Petunidin-3-O-glucoside	-O-glucose
Malvidin	$-OCH_3$	$-OCH_3$	-OH	Malvidin-3-O-glucoside	-O-glucose

Fig. 1. Structure of the most common anthocyanidins and anthocyanins found in berries.

Epidemiological studies

Prospective and cross-sectional studies in different populations have investigated associations between berry consumption and the risk of T2D, providing some support for a potentially protective effect arising from increased berry intakes.

Prospective cohort and cross-sectional studies

An inverse association between high consumption of berries and risk of T2D was observed in a Finnish cohort study (*n* 10 054 men and women), with a hazard ratio (HR) of 0·74 (95 % CI 0·58, 0·95) when comparing highest and lowest quartiles[35]. The Kuopio Ischaemic Heart Disease Risk Factor Study in Finnish middle-aged men (*n* 2682) reported that consumption of >59·7 g berries per d compared with <1·3 g lowered risk of T2D (mean follow-up 19 years), with a multivariable-adjusted HR 0·65 (95 % CI 0·49, 0·88). Importantly, total fruit or vegetable consumption had no statistically significant fully adjusted association with T2D risk, possibly signifying a more potent role of berries in modulation of risk[36].

High intakes of anthocyanins and anthocyanin-containing foods were significantly associated with a lower risk for T2D in US men and women (*n* 199 980, three cohorts), with a pooled HR 0·85 (95 % CI 0·80, 0·91) for the highest quintile of anthocyanidin intakes compared with the lowest. Cyanidin exhibited the strongest effect on T2D risk; HR 0·79 (95 % CI 0·72, 0·85), followed by malvidin, delphinidin, peonidin and petunidin. Blueberry intakes were most closely negatively associated with T2D risk (HR 0·77, 95 % CI 0·68, 0·87), as well as apple/pear intakes, followed by strawberry intakes[37,38]. In contrast, although lower T2D risk was observed when intakes of flavonol and flavan-3-ol were greater (*n* 2915; Framingham Offspring cohort, USA), no associations with anthocyanin intake were detected[39]. Furthermore, contrary to the aforementioned studies, there was no association between total flavonoid or anthocyanin intake and risk of T2D in the Iowa Women's Health Study prospective cohort (*n* 35 816 postmenopausal women, USA)[40].

Recently, a high intake of anthocyanins was found to be associated with lower IR (Homeostatic model for assessment of insulin resistance; HOMA-IR) in a cross-sectional study using the Twins UK registry (*n* 1997 women, UK). Women reporting higher intakes of anthocyanidins by FFQ (quintile 1: 3·5 mg/d; quintile 5: 40 mg/d) had lower HOMA-IR scores and lower fasting serum insulin levels following adjustment for BMI, age, smoking, physical activity, diet, menopausal status and medication. More specifically, higher intakes of delphinidin, malvidin and petunidin were associated with lower HOMA-IR and insulin levels[41]. This supports longitudinal observations of T2D risk and suggests that anthocyanins may reduce T2D risk by modulating IR independently of BMI and other major dietary factors. Inconsistent findings from longitudinal studies might be due to limitations and errors inherent to dietary intake methodology. Overall, there are sufficient epidemiological data to support a likely relationship between greater intakes of berries, anthocyanin-rich foods and anthocyanins, and reduced risk of T2D in adult populations. The mechanistic effect of anthocyanins *in vitro* and *in vivo* systems will be discussed later.

Bioavailability of anthocyanins

Bioavailability of anthocyanins was formerly believed to be low (<2 %)[42], with levels in plasma varying from 1 to 592 nM following the consumption of an anthocyanin-rich meal[43], and up to millimolar values in the gut lumen[44]. However, it was demonstrated using a stable isotopically labelled anthocyanin that bioavailability may not be lower than other flavonoids; bioavailability of cyanidin-3-glucoside was estimated to be at least 12 % calculated from ^{13}C recovery in urine and breath[45]. Anthocyanin metabolites excreted in urine corresponded to 15 % of total intake when consuming 300 g raspberries in a low polyphenol diet[46]. Human studies have shown that the time to reach maximum concentrations in plasma varies between 0·5 and 4 h[42,43]. This is consistent with evidence showing that anthocyanins may be partly absorbed in the stomach before reaching the small intestine[47,48].

After ingestion, anthocyanins appear to permeate the stomach mucosa; proposed mechanisms include a bilitranslocase carrier and a saturable transporter, GLUT1[48–50]. Nevertheless, the majority of absorption and transformation occurs in the small intestine[51,52], although mechanisms are not entirely clear. Many flavonoid glycosides undergo hydrolysis of the sugar moiety

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by the membrane-bound enzyme, lactase phlorizin hydrolase with subsequent passive diffusion of the aglycone into the enterocyte. However, some anthocyanin glycosides, such as cyanidin-3-glucoside and cyanidin-3-galactoside, have shown resistance to lactase phlorizin hydrolase[53,54]. In fact, anthocyanins that are absorbed in the small intestine are more likely to be taken up into enterocytes intact, their metabolites then being formed in the small intestine after absorption[53,55]. Any deglycosylation within the gut lumen primarily occurs in the colon due to the action of gut microbiota, as reviewed by Fang[53].

Early studies suggested a role for sodium-dependent glucose transporter 1 (SGLT1) in the absorption of glycosides[56], but SGLT1 expressed in *Xenopus* oocytes does not transport flavonoid glycosides[57]. In enterocytes, intact glycosides may undergo the action of the cytosolic-β-glucosidase, which cleaves the sugar moiety and release the free aglycone[58]; aglycones then undergo phase II metabolism by sulfotransferases, methyltransferases and glucoronyltransferases, forming sulphated, methylated and glucuronidated metabolites[42]. Efflux of metabolites into the small intestine may occur via transporters inserted into the luminal membrane, such as multidrug resistance protein 2 and breast cancer resistance protein[59,60]. Phase II metabolites reach portal circulation via active transporters inserted in the basolateral membrane, such as multidrug resistance protein 3[59,60], studies have also suggested the action of GLUT2[52,58,61].

Once in the portal circulation, metabolites can reach the liver and undergo additional phase II metabolism before entering the systemic circulation[43], from where they are directed to several organs and tissues (e.g. adipose tissue, heart, eyes, cerebrum and kidneys) to exert their biological effects or to be metabolised and eliminated in urine[16,62]. Anthocyanin metabolites could be directed to the enterohepatic circulation for their subsequent excretion into the small intestine via bile for reabsorption or make their way to the large intestine to be transformed by microbiota and then reabsorbed or eliminated in faeces[16,47]. Unabsorbed anthocyanins reaching the large intestine may be converted to other metabolites by resident colonic bacteria, followed by absorption or excretion in the faeces[42,63,64]. Microbiota can degrade anthocyanins to phenolic acids and aldehydes by splitting the C-ring and modifying the remaining A and B-rings[62]. Some of the main metabolites of microbiota degradation are gallic acid, vanillic acid, homovanilic acid, protocatechuic acid (PCA), syringic acid and 4-hydroxybenzoic acid[55,65–68]. Despite knowledge of the high rate of anthocyanin degradation by gut microbiota there is still no consensus about the proportion absorbed into the systemic circulation[45,53,55]. (See Fig. 2 for the proposed mechanism of anthocyanins absorption).

Studies showing higher bioavailabilities of anthocyanins have been able to detect a broader spectrum of metabolites in blood and urine samples. For example, a study published in 2007 reported only the recovery of cyanidin-3-glucoside and PCA in the plasma following ingestion of 1 litre blood orange juice[65]. However, de Ferrars *et al.*[55] in 2013 reported twenty-eight total metabolites, seventeen phenolics and eleven anthocyanin conjugates in urine following consumption of an elderberry extract (500 mg mixed anthocyanins) consisting mostly of cyanidin glycosides, while plasma analysis discovered seventeen phenolics and four anthocyanin conjugates. Urine samples demonstrated high amounts of vanillic acid and conjugates and were more abundant in anthocyanin conjugates, while the plasma was highest in 4-hydroxybenzaldehyde and PCA-sulphate[55]. Isolated and ^{13}C-labelled cyanidin-3-glucoside was traced in healthy males, demonstrating a total of twenty-five different metabolites, including a range of cyanidin glucuronides and methyl compounds as well as aldehydes and phase II PCA conjugates[45]. The metabolism of raspberry anthocyanins produced eighteen detectable compounds in urine, including cyanidin-3-O-glucoside, peonidin-3-O-glucoside and sixteen phenolic metabolites, while in plasma nine anthocyanin metabolites were quantified including glucuronides and sulphated compounds[46]. Furthermore, not all metabolites were recovered, which is in concordance with more recent data that identified a total of thirty-six metabolites in serum, urine and faecal samples following ingestion of 500 mg labelled cyanidin-3-glucoside[69]. These studies raise the possibility that the health effects associated with berries and their anthocyanins may be in part attributable to metabolites of parent anthocyanin compounds. It is now apparent that parent anthocyanins maintain a relatively short half-life, whereas their metabolites, which includes phase I and II compounds, are active for longer and reach higher maximum concentrations[69].

Berries and anthocyanins: modulation of glucose metabolism (*in vitro* studies)

Consumption of anthocyanin-rich foods has been associated with beneficial effects on metabolic biomarkers in human subjects, including postprandial concentrations of glucose, insulin, free fatty acids and gastrointestinal hormones such as glucose-dependent insulinotropic polypeptide and glucagon-like peptide-1[70–74]. There is increasing evidence for a potential role for dietary anthocyanins in glucose homeostasis, but there is a lack of understanding of the mechanisms by which these effects are exerted. To elucidate the mechanisms, different extracts and individual compounds have been tested using *in vitro* experiments. Enzymatic studies suggest that anthocyanins may inhibit digestive enzymes such as salivary and pancreatic α-amylases and α-glucosidases: sucrase and maltase. Studies using Caco-2 cells as a model of the small intestine and *Xenopus laevis* oocytes expressing glucose transporters, show sugar uptake inhibition by anthocyanin extracts and individual compounds.

Carbohydrate digestion and absorption

Carbohydrate digestion begins in the mouth by α-amylases that hydrolyse α(1,4)-glycosidic bonds of polysaccharides (e.g. starch), which are broken down

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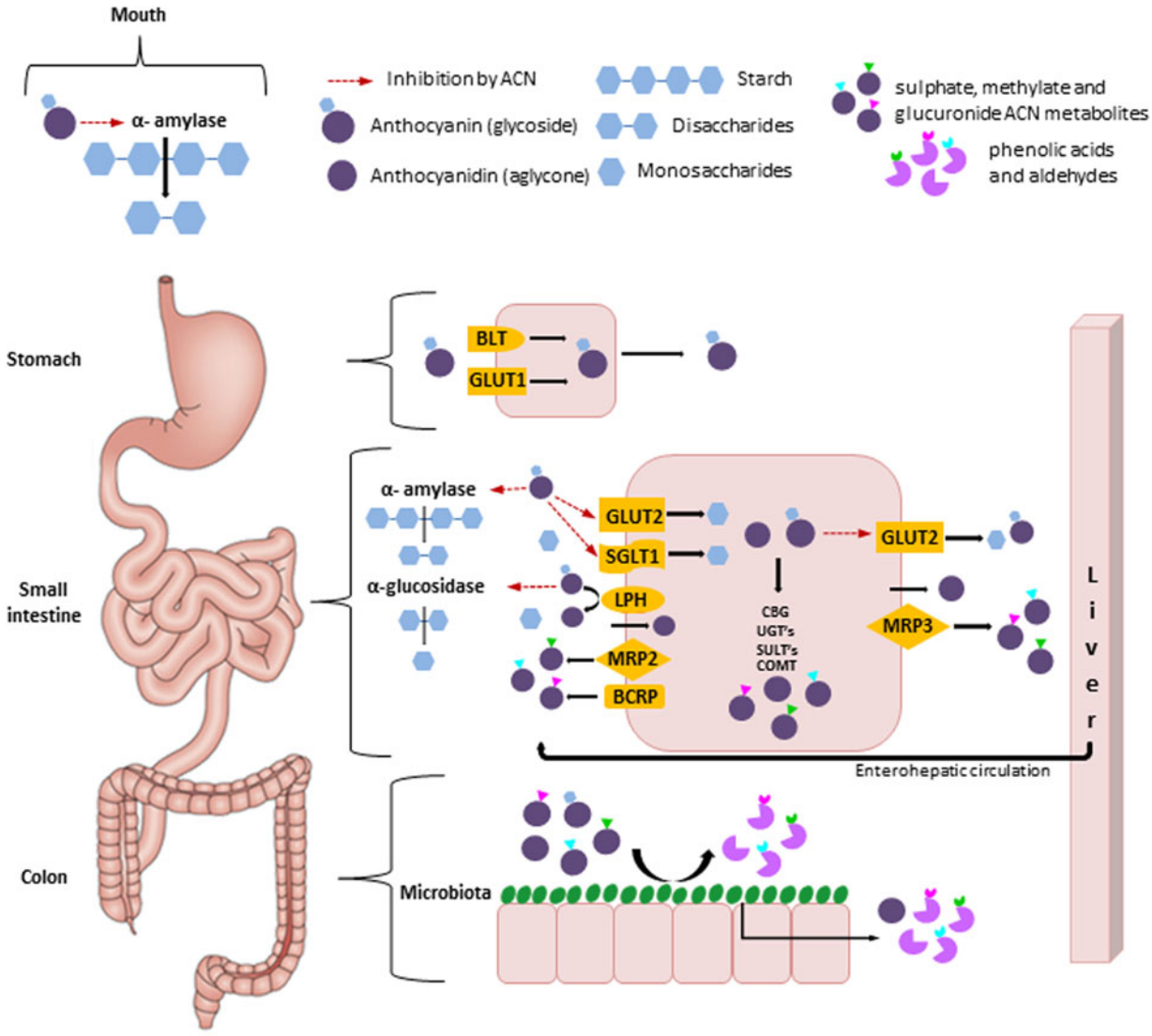

Fig. 2. Metabolism of carbohydrates and effects of anthocyanins on enzymes and glucose transporters. Adapted by permission in part from MacMillan Publisher Ltd: Nature Reviews Immunology[120], copyright 2015.

into smaller peptides, amylose and amylopectin[75]. In the small intestine, additional pancreatic α-amylases are secreted. α-Glucosidases act on sucrose and maltose, generating monomers of glucose and fructose that are absorbable by brush border cells of the small intestine (Fig. 2). Glucose may be transported by SGLT1 and GLUT2 at the apical membrane, the latter of which is primarily functional during high luminal glucose concentrations[76]. Higher free glucose in the gut lumen may influence the net uptake of glucose, contributing to a higher release of glucose into the bloodstream. Therefore, the ability to slow the rate of carbohydrate digestion and the release of free glucose may be important in managing postprandial hyperglycaemia.

Digestive enzymes: in vitro *studies*

Anthocyanins are highly bioactive molecules; however, defining their bioactivities *in vivo* can present many challenges. As previously mentioned, anthocyanins are thought to reach millimolar concentrations within the gut lumen and intestinal tissues, although only nanomolar concentrations are present in the blood stream[53]. The relatively higher concentrations in the gut tissues

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may provide sufficient potency for the effects observed within *in vitro* studies, which are discussed later. Many cellular models testing inhibition of digestive enzymes and intestinal transporter proteins by anthocyanins have found IC_{50} values within the range of micromolar concentrations, which are well within a physiologically feasible range.

Strawberry extracts from various species of Brazilian strawberry significantly inhibited α-glucosidase activity up to 70 % in a dose-dependent manner[77]. Although these effects were marked, it is difficult to pinpoint whether enzyme activity inhibition was attributable to specific anthocyanins or other polyphenols in the strawberry extract. Therefore, many *in vitro* studies focus on testing individual anthocyanins, as described later. The results of these studies are also displayed in Supplementary Online Table S2.

One of the first studies investigating the effects of cyanidin-3-galactoside, high in blueberries, lingonberries and cranberries, showed inhibition of both sucrase and maltase enzyme activity[78]. Comparable results were observed by the same authors for cyanidin-3-rutinoside, which is found in particularly concentrated amounts in blackcurrants[79]. Similarly, sucrase was inhibited to a larger extent than maltase, and cyanidin-3-rutinoside was significantly more potent compared with cyanidin-3-galactoside, which might be related to the disaccharide structure of rutinose[79]. Aglycone cyanidin also shows inhibition of sucrase activity, although to a much lesser extent than its glycosides, while cyanidin-3,5-diglucoside showed relatively no inhibition[80] and cyanidin-3-rutinoside is a more potent inhibitor of α-amylase[81]. These data highlight the potent effects of cyanidin glycosides on carbohydrate-digesting enzymes within the gut, and suggest that cyanidin glycoside-containing berry species, such as blackcurrant, blackberry and lingonberry[8], might have particularly potent postprandial glycaemia-lowering effects.

Separate extracts of strawberry, raspberry, blueberry and blackcurrant showed dose-dependent inhibition of α-amylase, with strawberry and raspberry (also rich in hydrolysable tannins, ellagitannins) demonstrating the most significant effects[82]. Anthocyanins present in high amounts in raspberries and strawberries include cyanidin and pelargonidin glycosides; however, they may also contain significant amounts of phenolic acids and other flavonoids[8]. Alternatively, blueberry and blackcurrant extracts were more potent α-glucosidase inhibitors in comparison with the other two extracts. By separating the anthocyanin-containing portion of the extract from the whole raspberry extract, McDougall *et al.*[82] demonstrated that the inhibitory effects on α-amylase are largely mediated by non-anthocyanins, while α-glucosidase activity is modulated by the anthocyanin-containing portion. In fact, in a separate study it was found that the inhibitory effects of rowanberries on α-amylase are primarily exerted by proanthocyanidins[83], a flavonoid subclass constituted of dimers, oligomers or polymers of catechins or epicatechins linked together, also known as condensed tannins[15]. This was further exemplified by the weak inhibition of α-glucosidase by a proanthocyanidin-rich rowanberry extract, signifying the specific importance of this group of compounds for α-amylase inhibition[84]. Both red and yellow raspberries significantly influenced α-amylase activity, suggesting that synergism between proanthocyanidins, which are more concentrated in yellow raspberries compared with red, anthocyanins, which are much higher in red raspberries, and other compounds such as ellagitannins, flavonols and hydroxycinnamic acids may occur to effect α-amylase inhibition[83]. More recently, the same authors provided significant evidence of anthocyanin-rich blackcurrant extract and rowanberry inhibition of α-glucosidase *in vitro*[84]. However, no synergistic effects were apparent after combining the extracts[84]. As this particular rowanberry extract was low in anthocyanins, it suggests they are not the sole compounds contributing to these effects, as observed for proanthocyanidins and α-amylase inhibition.

Acarbose is a competitive inhibitor of maltase and sucrase in the digestive tract and is a drug used in the management of T2D. European regulations suggest doses between 25 and 200 mg three times daily, depending on severity of disease[85]. Gastrointestinal side effects associated with acarbose have limited the success of the drug[86]. Administration of certain polyphenols may pose synergistic effects on sucrase and maltase activity[78–81] depending on doses of polyphenol and acarbose[78]. Together these studies suggest that acarbose acts via different mechanisms than some polyphenols, providing the synergistic inhibition observed, and may give insight into strategies to lower prescribed doses in acarbose treatment to diminish side effects associated with the drug.

Glucose uptake: in vitro *studies*

Following the release of glucose from sucrose and starch by digestive enzymes, glucose absorption may be further disrupted by interactions between berry anthocyanins and intestinal sugar transporters. The human cell line Caco-2, has been widely used as an *in vitro* model of the small intestine; the cell line obtained from a human colon adenocarcinoma, under culture conditions develops as a cell monolayer with characteristics of a mature enterocyte[87]. Johnston *et al.* tested the effect of polyphenols on glucose transport in the Caco-2 cell line. Although no berry-specific anthocyanins were tested, it was one of the first studies suggesting competitive inhibition of SGLT1 and inhibition of GLUT2 in a cellular model of the human intestinal lining[73]. Manzano and Williamson[61] monitored the rate of apical glucose uptake and basolateral GLUT2-mediated glucose transport, showing that strawberry extract is able to hinder translocation to a much larger extent within Na^+-free conditions (GLUT2), although transport was also inhibited during Na^+-dependent conditions (SGLT1 and GLUT2), suggesting that the inhibition of GLUT2 was greater than the inhibition of SGLT1. Strawberry extract (50–400 mg/ml) showed inhibition of total (SGLT1 and GLUT2) apical glucose uptake and basal transport in

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the Caco-2 cell *in vitro* model with an IC_{50} = 324 mg/ml, a high concentration compared with physiological levels. Although the specific strawberry compounds responsible for these effects were not clear, HPLC analysis showed the extract was relatively high in pelargonidin-3-glucoside, which showed inhibition of glucose transport into and across the cell (IC_{50} = 802 μM)[61]. These results suggest that strawberry compounds may influence glucose transport across intestinal cells, thereby modulating the rate of glucose flux into the bloodstream. More recently, Alzaid *et al.*[88] demonstrated that acute exposure of Caco-2 cells to cyanidin, cyanidin-3-glucoside and cyanidin-3-rutinoside (100 μM each), significantly reduced total and facilitated (GLUT-mediated) glucose transport. Whole mixed berry extract (approximately 2 mM) also significantly inhibited uptake of glucose in this model[88]. In our *in vitro* study in Caco-2 cells, testing different concentrations of a highly purified blackcurrant extract (0·15–1·2 mM) we demonstrated acute inhibition on total glucose uptake with an IC_{50} = 0·3 mM; allowing for dilution in gastric juice, this could represent an ingestion of approximately 50 g fresh blackcurrant fruit (ML Castro-Acosta, WL Hall and CP Corpe, unpublished results).

Although the exact mechanisms for anthocyanin absorption are not clearly identified, GLUT2 and SGLT1 may play an important role. An *in vitro* study using Caco-2 cells treated with cyanidin-3-glucoside showed decreased absorption of the anthocyanin when specific inhibitors of GLUT2 and SGLT1 were added compared with without inhibitors; the same effect was observed in cells with decreased expression of GLUT2 and SGLT1, suggesting the involvement of both transporters in the absorption of cyanidin-3-glucoside[89]. Furthermore, expression of GLUT2 in the apical side of Caco-2 cells was decreased at the same time as glucose uptake was decreased following cyanidin-3-glucoside treatment[90]. Competition between glucose and anthocyanins for glucose transporters may represent a potential mechanism for inhibition of postprandial glycaemia.

Interestingly, longer-term exposure (16 h) of berry extracts (approximately 2 mM) to Caco-2 cells showed an inhibitory effect on both GLUT2 and SGLT1 expression, which was further exemplified at the protein level for GLUT2. However, the 16 h exposure showed minimal effects on total glucose uptake, with only facilitated (GLUT-mediated transport) uptake demonstrating a statistically significant reduction[88]. Caco-2 cell model pre-treated for 96 h with anthocyanin extract (200 μg/ml) increased the expression of GLUT2 but not SGLT1 and GLUT5. Acute glucose transport, mediated by GLUT2 was decreased in cells pre-treated with anthocyanin extract and malvidin-3-glucoside (200 μg/ml), pre-treatment with malvidin (200 μg/ml) showed no inhibitory effect[90]. In summary, the evidence for opposing results on glycoside and aglycone treatments suggests that the sugar moiety may interfere with GLUT2-mediated glucose transport, and up-regulation of GLUT2 gene expression may be relevant for longer-term glycaemic control.

In vitro studies using *Xenopus* oocytes to express either SGLT1 or GLUT2 under controlled conditions have shown an inhibitory effect of polyphenols and anthocyanins on glucose uptake. Pelargonidin and pelargonidin-3-glucoside inhibit glucose absorption (1 mM), with IC_{50} = 1·34 and 2·47 mM respectively, in oocytes expressing SGLT1[57]. In a separate *in vitro* study using *Xenopus* oocytes expressing GLUT2, Kwon *et al.*[91] were not able to detect an inhibitory effect on glucose uptake (10 mM) when testing delphinidin and cyanidin (0–300 μM), although their glycosidic forms were not tested and other flavonoids present in berries were observed to inhibit GLUT2-mediated glucose transport.

Overall, experiments using oocytes expressing individual sugar transporters and cultured intestinal cells have shown that berry extracts and individual anthocyanins may interfere with glucose transport from the gut lumen into the enterocyte, and also across the basolateral membrane into the blood. This may occur via inhibition of GLUT2 under postprandial glycaemic conditions, since this transporter is believed to be functionally important under conditions of high luminal glucose concentrations[76]. However, inhibition of SLGT1 activity may also play a significant role in berry polyphenol-mediated control of glucose homeostasis since SGLT1 is instrumental in the regulation of glucose-dependent insulinotropic polypeptide and glucagon-like peptide-1 secretion and up-regulation of GLUT2[92]. Extracts of berries used in the different studies may contain a range of different classes of polyphenols, and further *in vitro* studies testing individual anthocyanins are needed in order to pinpoint exact mechanisms for these intestinal transport effects.

Animal studies

A number of animal studies have demonstrated inhibitory effects of berry anthocyanins on the postprandial plasma glucose curve, fasting glucose and increases in insulin sensitivity. Mice consuming a very high-fat diet were changed to a low-fat diet supplemented with blueberry concentrate (providing an average of 4·4 mg blueberry anthocyanins/d) and showed a reduction in weight gain and increased glucose tolerance compared with the blueberry-free low- and high-fat controls, despite no differences in total food intake[93]. Conversely, no significant lowering of glycaemia was observed in mice receiving low- and high-fat diets with the addition of 3·25 mg blueberry anthocyanins/d[94], nor a range of freeze-dried berry powders[95,96]. Purified blueberry anthocyanins (0·49 mg/d) reduced fasting glucose levels in mice receiving a high-fat diet to levels comparable with a low-fat diet, and also reduced percentage body fat and body weight[97,98], and pure cyanidin-3-glucoside supplementation resulted in markedly lower fasting blood glucose concentrations after 3 weeks of high-fat diet compared with high-fat diet with no supplementation, as well as in diabetic mice consuming regular chow[99]. Cyanidin-3-glucoside was administered as 0·2 % of total diet, equivalent to 160–300 mg for every kg body weight/d, considered to be at the supra-physiological level[99]. The evidence as a whole suggests

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that blueberry anthocyanins and perhaps other berry anthocyanins may have potentially preventative effects on glycaemia and weight gain. However, the observed effects were specific to concentrated extracts and no differences were seen for mice receiving a freeze-dried powder or juice.

The relatively short half-lives of anthocyanins suggest that their phase I and II metabolites may contribute significantly to their sum effect on glucose homeostasis. Significant circulating metabolites include PCA and its metabolites vanillic and hippuric acid, as well as phloroglucinaldehyde and its metabolite ferulic acid[69,100]. In fact, some of these metabolites have been shown to be important for glucose uptake, insulin signalling and adipokine expression in *in vitro* studies in human and rat adipocytes[101–103]. Diabetic KK-A^y mice subjected to 5-week daily administration of concentrated bilberry extract (27 g/kg diet) showed a marked reduction in fasting plasma glucose and improved insulin sensitivity[104]. Interestingly, phosphorylated AMP-activated protein kinase-α and GLUT4 mRNA and protein levels were increased in both skeletal muscle and mesenteric adipose tissues. AMP-activated protein kinase-α acts as sensor of cellular energy status and when activated, stimulates processes to generate ATP, such as the translocation of GLUT4 transporters in order to increase cellular influx of glucose[105]. The significant increase in AMP-activated protein kinase-α was also observed in liver tissue, resulting in the down-regulation of glucose-synthesising genes. These data suggest that in addition to influencing carbohydrate digestion and absorption, circulating anthocyanin metabolites may act to increase insulin sensitivity.

In conclusion, there is significant evidence of a beneficial role of anthocyanins present in berry extracts in glycaemic control during fasting and postprandial states in mice, and the role of anthocyanins in the regulation of peripheral tissue gene expression warrants further investigation. The apparently favourable effects of berry polyphenols in animals are extended to human subjects in the following section.

Human studies

Research investigating the role of polyphenols in carbohydrate digestion and absorption in human subjects is largely dominated by randomised, placebo-controlled trials, considered the gold standard in scientific research. However, the varying types of berries and berry combinations, the methods of administering the treatments and the specifics of each study design bring a substantial amount of variation. Details of these studies are outlined in Table 1.

Edirisinghe *et al.*[106] tested the effects of a strawberry extract milk-based drink on plasma glucose and serum insulin levels. Although no changes in glucose were observed, insulin levels were significantly higher in the placebo arm. The lack of effect on glucose levels could be explained by the presence of milk proteins, which may compete for polyphenol binding[107,108]. Postprandial glucose and insulin responses were reduced in overweight subjects who consumed 0·47 g encapsulated bilberry extract (equivalent to a 50 g serving of bilberries) alongside 75 g Polycal liquid as an oral glucose tolerance test[109]. Interestingly, the most significant effects were observed at later time points (120, 150 and 180 min), in contrast with other studies[70,72].

Lingonberry and blackcurrant purées significantly lowered plasma glucose at 15 and 30 min, and increased plasma glucose after 60 min relative to control, post-ingestion of 35 g sucrose, and blackcurrant nectar showed comparable results[70]. Similar studies using a mixed berry purée of blackcurrant, strawberries, cranberries and bilberries demonstrated consistent time-dependent glucose and insulin responses to 35 g sucrose (reduced 15–45 min, increased at approximately 90 min), together with a borderline increase in plasma glucagon-like peptide-1 concentrations in the early postprandial phase[71,72]. Interestingly, these experiments, all by the same group, consistently demonstrate that berries inhibit the early phase of postprandial glycaemia, when sucrose is being hydrolysed by α-glucosidase into glucose and fructose, and glucose is being rapidly absorbed from the upper small intestine, whereas there is a compensatory increase in the later postprandial phase relative to control, suggesting a more protracted glycaemic response to sucrose ingestion when consumed with berries.

More recently, the same group reported that a blackcurrant, strawberry, bilberry and cranberry purée reduced postprandial glycaemia 0–30 min in response to white wheat bread consumption (50 g starch) by 32 %[110]. On the other hand, a study investigating the effect of blueberries and raspberries found no differences in postprandial glycaemia compared with control, although the low participant number, short washout period (1 d), use of a fingerprick capillary bloods with a portable glucose analyser, and possibly inadequate mastication of the whole berries are important limitations to consider[111]. Additional studies investigating the effects of anthocyanins on glycaemic responses to solid and liquid mixed meals containing starches and/or sucrose will further elucidate the potential effects of berry polyphenols on metabolic response.

The earlier mentioned studies investigate the acute response to the administration of anthocyanins; however, there may also be a longer-term effect as a consequence of daily consumption. Obese men and women administered a blueberry smoothie (668 mg anthocyanins; 1462 mg total phenolics) twice daily for 6 weeks demonstrated a positive effect on IR compared with a nutritionally matched blueberry-free smoothie[112]. Subjects followed an *ad libitum* diet, suggesting the addition of this amount of blueberries, about two cups fresh blueberries per d, even without dietary changes may influence the current state of IR, although it is possible that other dietary changes were made in a motivated study population.

Overall, the evidence base to date indicates an inhibitory effect of berry extracts or purées in the initial postprandial glycaemic response, suggesting that berry components such as anthocyanins and proanthocyanidins might be acting in the gut as a 'brake' on the rate

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Table 1. Summary of randomised controlled acute and chronic dietary intervention trials using berry meals

References	*n* (sex), status, country	Subjects age		Design	Dietary intervention	Food/ACN (dose/d)	Effects	Quality assessment
		Mean	SD					
Stull *et al.* [112]	Thirty two (M + F), insulin resistant, USA	51	3	DB P; Chronic (6 week)	freeze-dried BB powder	668 mg ACN	– stimulation of IS	+
Törrönen *et al.*[71]	Twelve (M + F), healthy, Finland	54	15	SB CO; acute	purée of BC, BIB, CB and SB	150 g	– ↓plasma glucose in early stages of post prandial glucose curve	Æ
Edirisinghe *et al.*[106]	Twenty four (M + F), overweight, USA	51	15	SB CO; acute	freeze-dried SB powder	82 mg ACN	↓ insulin at 60 and 180 min post meal and improved IS in post prandial state	+
Clegg *et al.*[111]	Twelve (M + F), healthy, Finland	33	13	CO; acute	RB- or BB-containing pancakes	100 g berries	– no effect on postprandial glucose response	+
Törrönen *et al.*[72]	Twelve (M + F), healthy, Finland	58	11	SB CO; acute	purée of BC, BIB, CB and SB	150 g	– glucose curve normalised	+
Törrönen *et al.*[70]	Twenty (F), healthy or overweight, Finland	57	10	SB CO; acute	BB purée and nectar, LB purée and nectar	150 g	– normalised postprandial glucose curve by purée and less-so by nectar	+
Hoggard *et al.*[109]	Eight (M), T2D, UK	62	5	DB CO; acute	BIB extract	169 mg ACN	– 18 % ↓plasma glucose iAUC following OGTT	+
Törrönen *et al.*[110]								
Study 1	Fifteen (F), healthy, Finland	48	14	SB CO; acute	purée of SB, BIB or LB	150 g	– SB purée may improve glycaemic response	+
Study 2	Thirteen (F), healthy, Finland	50	12	SB CO; acute	purée of RB, CLB or CHB	150 g	– no significant effects on blood glucose	+
Study 3	Twenty (F), healthy, Finland	57	12	SB CO; acute	purée of SB, BIB, CB + BC	150 g	↓ early postprandial hyperglycaemia; ↑ glycaemic profile	+

Quality assessment was based on the method recommended by the American Dietetic Association[113]. Studies were assessed and given one of the following three scores: Minus (–), indicating that the study scored poorly on at least six out of ten points; Neutral (Æ), indicating that the study was not exceptionally strong (scores were poor on points relating to validity/reliability of outcomes, whether the intervention was described in enough detail, whether selection of study participants was free from bias, and whether study groups were comparable and methods of randomisation was described and unbiased); and Plus (+), indicating that the study was of very high quality, i.e. scores were high on the previous 4 points and also high on at least one other point. ACN, anthocyanins; M, males; F, females; USA, United States of America; SB CO, single-blind cross-over; SB, strawberry; T2D, type 2 diabetes mellitus; UK, United Kingdom; DB CO, double-blind cross-over; BIB, bilberry; iAUC, incremental area under the curve; OGTT, oral glucose tolerance test; BB, blueberry; LB, lingonberry; BC, blackcurrant; CB, cranberry; RB, raspberry, CLB, cloudberry; CHB, chokeberry; CO, cross-over. DB P, double-blind parallel; IS, insulin sensitivity.

of glucose absorption, but not the total amount. However, the majority of the study designs involve administering whole berries or berry purées, making it difficult to pinpoint the relative effects of the polyphenols *v.* other components such as fibre, which might delay the rate of glucose absorption by slowing down gastric emptying. It is impossible to distinguish the relative contributions of the berry anthocyanins, proanthocyanidins, ellagitannins, flavonols, and phenolic acids from the human studies published to date. The *in vitro* experiments using Caco-2 cells and controlled expression of glucose transporters in *Xenopus* oocytes may help to shed light on these questions in order to optimise the efficacy of mixed berry extracts intended for nutraceutical or functional food applications. Further randomised controlled trials using alternative study designs may also address the relative contributions of anthocyanins compared with other berry polyphenols, for example comparing high anthocyanin blackcurrants with low anthocyanin greencurrants, or using powdered berry extracts concentrated in polyphenol content and containing minimal concentrations of other nutrients/non-nutrients.

Conclusions and future directions

As a whole, it is apparent from these studies that there is a role for berry anthocyanins, as well as other berry polyphenols, in regulating digestion and absorption of carbohydrates. Gastrointestinal interactions, in combination with post-absorptive effects of anthocyanins and their metabolites (such as changes in gene expression), would be expected to result in a sum normalisation of the postprandial glucose curve *in vivo* if berries were habitually consumed with meals, as suggested by both animal and human studies. Further mechanistic insights might be made using additional *in vitro* studies focussing on structural relationships between anthocyanins and the enzyme or transporter in question, and may highlight possible synergistic effects of different anthocyanins or polyphenols, increasing the potential to exploit these mechanisms in the improvement of postprandial hyperglycaemia. Incorporation of individual or blended berry extracts into novel food or drink products, such as no added sugar fruit drinks, cereal bars, wholegrain crackers, breads and pasta, offers a potential avenue of functional food development that could target consumers (healthy or with T2D) who are interested in controlling their blood glucose concentrations. A more gradual and sustained insulinaemic response could potentially increase satiety during intermeal intervals[4] and a lowered postprandial glycaemia is likely to protect the optimum functionality of pancreatic β cells, the vascular endothelium and hepatic lipid metabolism. However, there may be significant technical challenges for the food and drink industry in formulating products with the desired characteristics in terms of physical and chemical stability[114], bioavailability[107] and palatability[115]. Anthocyanin contents vary among species and cultivars, and are subject to genetic, agricultural and environmental factors as well as storage and processing conditions[116–118]. Processing and cooking (freezing, cutting, slicing, heating, etc.) can alter the cellular structure provoking transformation and/or degradation in the anthocyanin content[119]. Most phenolic compounds are known and even prized (particularly in the case of tea, coffee and wine) for their astringency and bitterness, but these properties are not always desirable in other foods so additional ingredients can sometimes be added to overcome these sensory challenges, for example, gums and pectins[120]. However, it is possible that addition of polysaccharides would enable the polyphenols to bind to these instead of the digestive enzymes, thus diminishing their glycaemia-lowering effects, and considerable efforts would be required by the food industry to develop acceptable novel products fortified with berry extracts. In conclusion, berry anthocyanins and other polyphenols are not the sole answer to preventing T2D, but a greater understanding of their potential in controlling blood glucose levels provides nutritionists, dietitians and other health professionals with another brick in the dam against the predicted tidal wave of T2D.

Supplementary material

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Conflict of Interest

None.

Authorship

M. L. C. A. and G. N. L. G. wrote the first draft of the paper C. P. C and W. L. H. modified it. All authors read and approved the final draft.

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