The role of polyphenols on sugar release from carbohydrate rich foods, and the consequent impact on metabolic risk factors associated with type 2 diabetes

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Coe, S A, (2014), *The role of polyphenols on sugar release from carbohydrate rich foods, and the consequent impact on metabolic risk factors associated with type 2 diabetes,* PhD, Oxford Brookes University

The role of polyphenols on sugar release from carbohydrate rich foods, and the consequent impact on metabolic risk factors associated with type 2 diabetes

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Doctor of Philosophy

September 2014

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Abrreviations:

ANOVA, analysis of variance;	iNOS, nitric oxide synthase;
aPCK, atypical protein kinase C;	IR, insulin response;
AUC, area under the curve;	IRS1, insulin receptor substrate 1;
avCHO, available carbohydrate;	LD, low dose;
BAO, baobab;	LDL, low density lipoprotein;
BP, blood pressure;	NOX, nitric oxide;
C, control;	P13, phosphoinositide 3 kinase;
CON, control;	RDS, rapidly digestible starch;
CQA, caffeoyl quinic acids;	RES, resveratrol;
CRP, C-reactive protein;	RMR, resting metabolic rate;
CVD, cardiovascular disease;	RM-ANOVA, repeated measures ANOVA; species;
DBP, diastolic blood pressure;	ROS, reactive oxygen
dH$_2$O, distilled water;	sAUC, segmental AUC;
DIT, diet induced thermogenesis;	SBP, systolic blood pressure;
DNS, dinitrosalicylic acid;	SD, standard deviation;
EE, energy expenditure;	SDS, slowly digestible starch;
ECG, epicatechin-gallate;	SEM, standard error of the mean;
EGCG, epigallocatechin-gallate;	SGLT1, sodium dependent glucose transporter;
FCR, Folin-Ciocalteu;	SPSS, Statistical Package for Social Science;
FFQ, food frequency questionnaire;	TNF, tumour neurosis factor;
FMD, flow mediated dilution;	VAS, visual analogue scale
FRAP, ferric-ion reducing antioxidant power;	
GAE, gallic acid equivalents;	
GI, glycaemic index;	

GL, glycaemic load;

GLUT, glucose transporter;

GP, glycaemic profile;

GR, glycaemic response;

GSE, grape seed extract;

GTE, green tea extract;

HD, high dose;

HDL, high density lipoprotein;

HPLC, high performance liquid chromatography;

IAUC, incremental area under the curve;

IL, interleukin;

1. Abstract

In the United Kingdom from 1993 to 2011, the proportion of people who were overweight and obese increased from 58 to 65 % in men and from 49 to 58 % in women. There was also an increase in related comorbidities including type 2 diabetes, which is predicted to be the 7th leading cause of death by 2030. The modification of food is becoming an attractive option in obesity management and disease prevention with much interest recently focused on the role of plant extracts and secondary plant compounds such as polyphenols as potential functional food additives for improving glycaemic control.

The aim of this PhD was to assess the effect of a variety of polyphenol rich sources including green, black and white teas, and also green tea extract, grape seed extract, resveratrol, and baobab fruit extract, on starch digestion and on markers of glycaemia. Throughout an *in vitro* digestion procedure, green tea and all of the polyphenol rich extracts were found to reduce starch digestion from white bread, and this effect was dose specific. These same doses of green tea extract and baobab fruit extract added into white bread were shown to have no effect on reducing the glycaemic response in healthy subjects, and therefore there may be inconstancies between *in vitro* and *in vivo* methods. However, although having no effect on glycaemia, baobab addition to white bread was shown to reduce the postprandial insulin response. Conversely, baobab fruit extract consumed as a solution at higher doses in combination with white bread was found to reduce the postprandial glycaemic response. Therefore baobab fruit extract may show potential as a functional food additive for improving health, and more specifically for alleviating markers of abnormal glucose metabolism.

2. Aims and novelty of this PhD

White bread is a widely consumed major staple food which accounts for 75% of the bread consumed in the UK (Flour and Bread Consumption, 2014). However, white bread contains a high glycaemic index (GI) value and thus can elicit an elevated postprandial glycaemic response (GR; Englyst *et al.,* 1999). A potential strategy for lowering the GI could be to introduce compounds into the bread which reduce the degree to which the carbohydrate is digested. Polyphenols may show potential for reducing the amount of carbohydrate breakdown in a food, and therefore may possibly lower the GI and resulting postprandial blood glucose levels (Williamson, 2013).

This PhD was composed of a series of experiments in which the results of each study formed the basis of the next study. A variety of black, green and white teas and plant extracts including grape seed extract, green tea extract, baobab fruit extract and resveratrol, were selected due to their potential rich polyphenol content and beneficial effects on glucose homeostasis, based on previous literature (Baur *et al.,* 2006; Bryans *et al.*, 2007; Sapwarobol *et al.,* 2012; Tanko *et al.,* 2008; Yan *et al.,* 2012). The polyphenol content and polyphenol bioaccessibility of each tea and extract was assessed, and all sources were measured for their effect on *in vitro* starch digestion. The polyphenol sources with the greatest stability and potential for reducing sugar release *in vitro* were selected to measure their effects on markers of glycaemia in healthy humans. Therefore, the results from two years of *in vitro* work highlighted the potential of these polyphenol rich sources for reducing postprandial glycaemia *in vivo*. A comparison between *in vitro* and *in vivo* methods of starch digestion and resulting glycaemia was performed to determine if there was a correlation between the methods when studying the effect of polyphenols on starch degradation.

Previous studies have looked at the combination of polyphenol rich foods with carbohydrates and resulting glycaemic parameters (Clegg *et al.,* 2011; Johnston *et al.,* 2003; Törrönen *et al.,* 2013). This is the first study to investigate the effect of green tea extract and baobab fruit extract baked into white bread, on *in vitro* starch digestion and also on postprandial glycaemia and insulin response (IR) in humans. Therefore, the first part of this research focused on the chemistry and characteristics of different polyphenols using various *in vitro* assays. However the wider implications of this work are public health related, building up from what was learned at the molecular level to how this can bring benefits for improving metabolic abnormalities in the general population. The overall aim of this PhD was to lower the GR after the consumption of white

bread by the addition of extracts rich in total polyphenols, and therefore to construct potential functional foods for reducing the risk factors of diabetes and other metabolic diseases.

3. Introduction to polyphenols

3.1 Classes and characteristics

Polyphenols are plant secondary metabolites found abundantly in foods such as fruits and vegetables, and also in sources such as dark chocolate, tea and wine. These compounds are classified into four main groups depending on their structure as shown in Figure 3.1, and can be further broken down into subclasses as represented in Figure 3.2. Polyphenols can be more broadly classified as flavonoids or non-flavonoids, and are usually found in foods combined to sugars or organic acids (Landete, 2012). The parent flavonoid molecule is composed of two aromatic rings bound together by three carbon atoms, which form an oxygenated heterocycle and flavonoids are thus placed into subclasses based on the type of heterocycle involved (Spencer *et al.,* 2008; Figure 3.1). Non-flavonoid phenolics are more heterogeneous and can vary in chain length. The flavonoids are the most frequently found in the diet (Gharras, 2009), and over 4000 flavonoids have been identified with different foods containing their own unique mixtures of polyphenol compounds (Harborne & Williams, 2000). Some common characteristics of the different flavonoid subclasses are presented in Table 3.1.
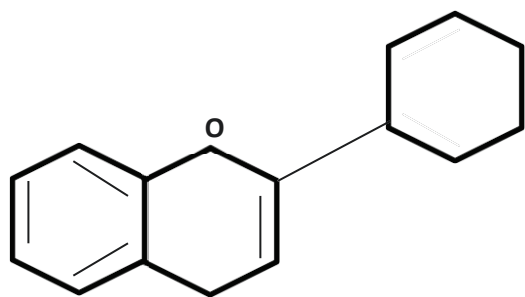
Flavonoids

Stilbenes

Phenolic acids

14
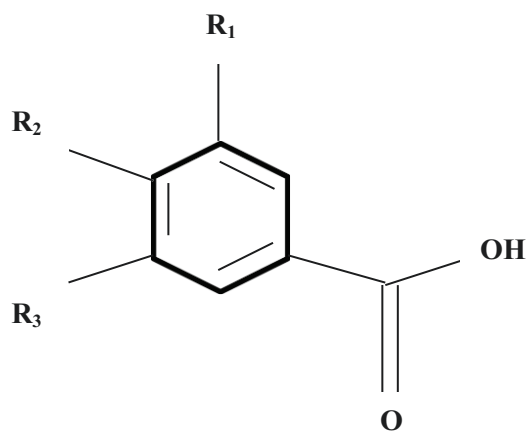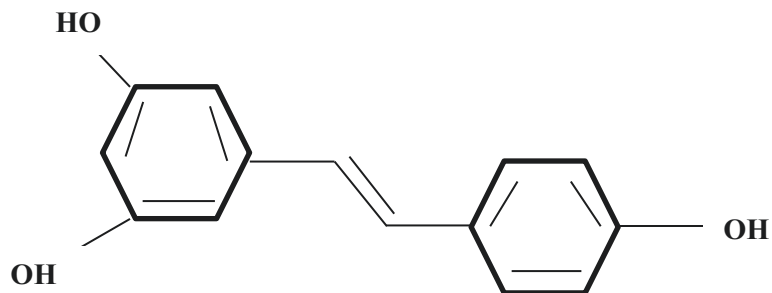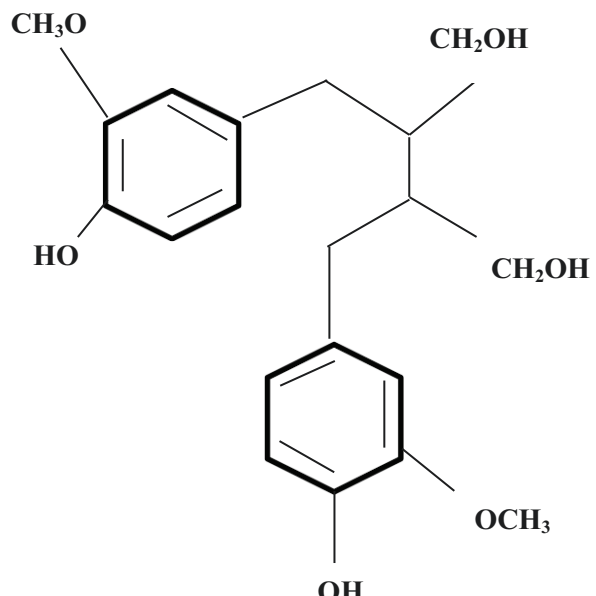
Figure 3.1 Basic structure of each polyphenol
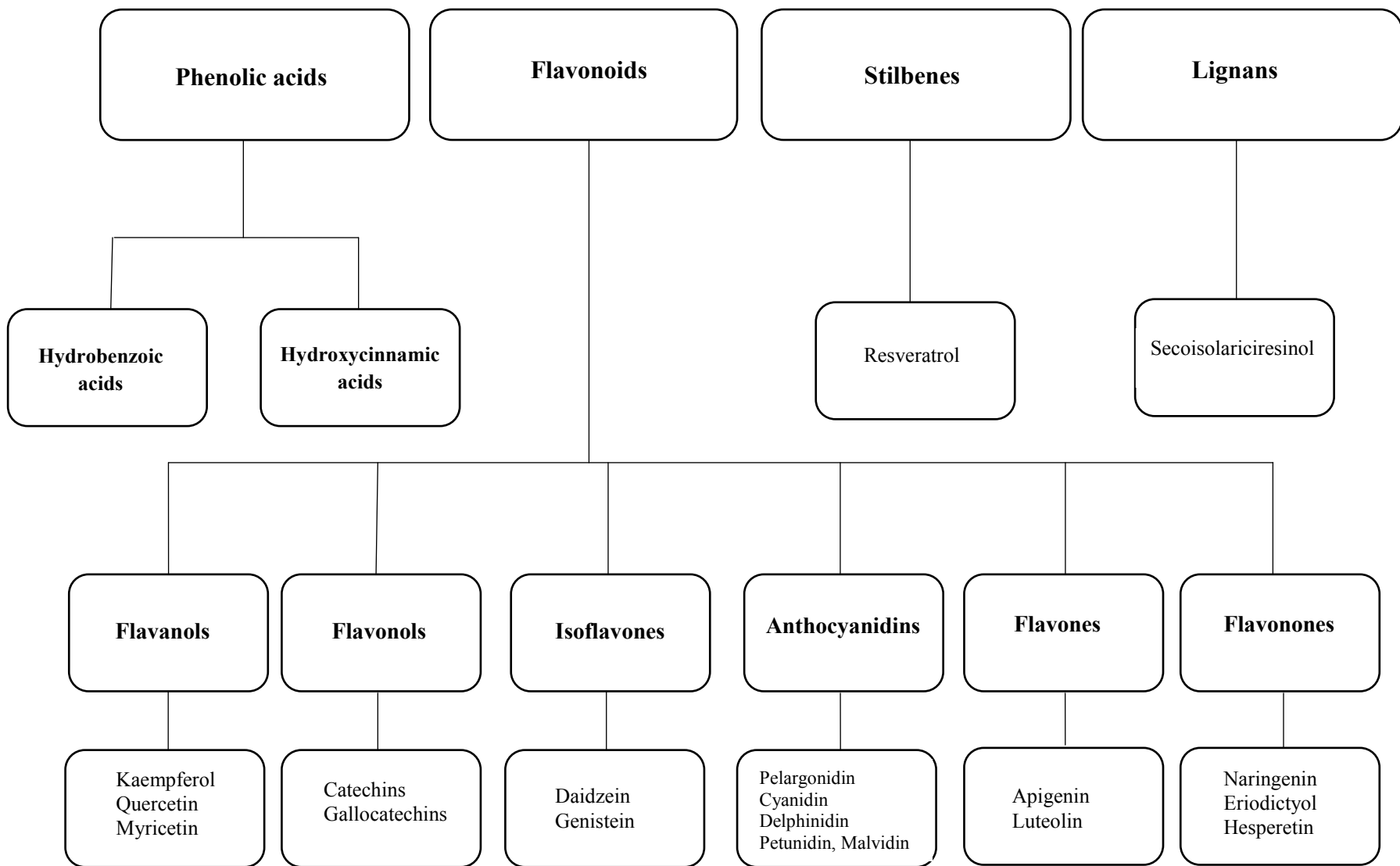
Figure 3.2 Polyphenol classes
and subclasses (Hardman, 2014)

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Table 3.1 Flavonoid characteristics

	Flavanols	Flavonols	Isoflavones	Flavones	Flavanones	Anthocyanins
Structure (D' archivio *et al.,* 2007)	Saturated 3 C chain with –OH on C3	Double bond between C2 and C3 with -OH on C3	-OH on C7 and C4	Double bond between C2 and C3	Saturated 3 C chain with O_2 on C4 and glycosylated by disaccharide on C2	Sugar at C3 or on A5', A7'
Subclasses (Higdon, 2005)	Catechin Epicatechin EGC, ECG, EGCG Theaflavin Thearubigin Proanthocyanidin	Quercetin Kaempferol Myricetin Isorhamnetin	Daidzein Genistein Glycitein Biochanin A Formononentin	Apigenin Luteolin	Naringenin Hesperetin Eriodictyol	Cyanidin Delphinidin Malvidin Pelargonidin Peonidin Petunidin
Common dietary sources (D' archivio *et al.,* 2007)	Green tea Red Wine Black Tea Dark Chocolate Berries Apricots	Green tea Black Tea Dark Chocolate Red Wine Onions (red) Curley Kale Leeks Blueberries Broccoli	Soyabeans Legumes	Parsley Celery	Citrus Orange Juice Tomatoes	Red wine Cereals Vegetables Fruits
Preferentially absorbed form	EGC and epicatechin better than + enantiomers (Peters *et al.,* 2010) (−) epicatechin and (+) catechin (Crozier *et al.,* 2010)	Glucosides of quercetin (Manach *et al.,* 2004) Kaempferol more than quercetin (Dupont *et al.,* 2004)	All generally well absorbed, 20-50 % of intake absorbed Genistein more than daidzein, aglycones, glucosides (Manach *et al.,* 2005)	β -glucoside of apigenin (Walle, 2004)	Aglycones (Manach & Donovan, 2004)	Acylated better than non- acylated aglycones Glucosides better than galactiosides Pelargonidin better than cyanidin (Walle, 2004)
Poorly absorbed form	Proanthocyanidins EGCG, ECG (-) catechins Gallated forms (Van Amelsvoort *et al.,* 2001)	Glycosides and rutin (Manach *et al.,* 2004)	Glycosides (Setchell *et al.,* 2001)	n/a	n/a	Glucosides (Passamonti *et al.,* 2002)

Absorption site	Procyanidins and thearubigins in colon by microflora All other in small intestine (Manach et al., 2005)	Rutin in colon by microflora Quercetin and isoquercitrin in small intestine (Mullen et al., 2006)	Small intestine Equol, dihydrodaidzein and dihydrogenistein in large intestine (Tamura et al., 2008)	n/a	Rutinosides in large intestine Aglycone and other forms in small intestine (Manach et al., 2003)	Small intestine (Manach & Donovan, 2004)
Active metabolites in plasma	Free form (77-90 % of time), glucuronic, sulphate, methylated (Manach et al., 2005; Ullmann et al., 2003)	Quercetin as methyl, sulfate, or glucu-ronic acid conjugates (Mullen et al., 2006) Kaempferol as glucuronides and free form (Dupont et al., 2004)	Aglycones, glycosides, sulfates or glucuronides (Hosoda et al., 2008)	Monoglucuronide of luteolin and free luteolin (Shimoi et al., 2000)	Hesperetin – monoglucuronide and diglucuronides, sulphated (Mullen et al., 2008)	O-methylation of B ring & glucuronidation, sulphation (Felgines et al., 2003)
Excretion	Galloylated in bile (Van Amelsvoort et al., 2001) EGCG low in urine, high in bile (Kohri et al., 2001) Most others in urine	Glucuronide in urine (Crozier et al., 2010)	Urinary (Crozier et al., 2010)	n/a	8.6% hesperitin and 8.8% naringenin in urine (Manach et al., 2005) Glucuronides (Mullen et al., 2008)	Low urine excretions, 80% monoglucuronides in urine (Felgines et al., 2003)

[1]C, carbon; OH, hydroxyl; O_2, oxygen, A, acylated; EGC, epigallocatechin; ECG, epicatechin-gallate; EGCG, epigallocatechin-gallate.

3.2 Polyphenol intake

Although the different classes and structures of polyphenols are well known, few researchers have succeeded in assessing the total amount of polyphenols consumed in different populations due to the difficulty in adequately performing these studies. Overall, the estimated average intake of polyphenols worldwide is thought to be around 1000 mg/ day, with a third of this intake resulting from phenolic acids (Landete, 2012). Japan has the highest level of flavonol intake compared to other countries, which is predominately due to their high intake of green tea (Skibola & Smith, 2000).   Polyphenol intake in the Spanish diet has been estimated between 2590 and 3016 mg/ day (Saura-Calixto *et al.*, 2007) and the intake of flavonoids in the United States is in the range of 500 to 1000 mg/ day. By performing 48-hour dietary recalls in 2007 Finnish adults, polyphenol intake was estimated at 863 ± 42 mg/ day, composed mainly of phenolics followed by proanthocyanidins and anthocyanins (Ovaskainen *et al.,* 2008). In the diet of 4942 French adults aged 45 - 60 years, 337 different polyphenols were determined with an average intake of 1193 ± 510 mg/ day (Pérez-Jiménez *et al.,* 2011). In the European Prospective Investigation into Cancer and Nutrition (EPIC) study, Denmark was found to have the highest phenolic acid intake of 1265.5 mg/ day in men and 980.7 mg/ day in women, with Greece having the lowest intake of 213.2 mg/ day and 158.6 mg/ day in men and women, respectively (Zamora-Ros *et al.,* 2013b). There was a great heterogeneity in phenolic acid intake across European countries, thus further studies need to consider the meaning and relevance of these results.

However, there is great difficulty in determining more accurate predictors of polyphenol intake as it is unknown whether those polyphenols most abundant in foods are those most bioactive in the body. Furthermore, obtaining intakes of certain polyphenol rich foods such as fruits and vegetables can be complicated due to poor understanding around how much a 'portion' is and what constitutes a fruit or vegetable. Biomarkers of intake are thus hard to determine due to the variety of factors that influence polyphenol intake and bioactivity.

3.3 Changes in the polyphenol content of foods

Polyphenols exist in plants to act as defences against pathogens, oxidants and ultraviolet radiation (Beckman, 2000). Organic fruits and vegetables often have higher polyphenol contents because more polyphenols are produced in order to protect the plant against these foreign pathogens (Carris-Veyrat *et al.,* 2004). Plant variety is a major determinant of polyphenol composition, as is the environment it is grown in and the

conditions of storage (Spencer *et al.,* 2008). Polyphenols are very unstable and easily degraded by such things as light, air oxidation and heat. However, the bio-synthesis of flavonoids is stimulated by light, and for this reason phenolic compounds tend to accumulate in the outer parts of the fruit. Many flavonoids are found in the peels of fruits, vegetables and grains, and therefore refining or peeling will reduce the polyphenol content. Cooking, such as boiling or frying, can result in major losses of flavonoids. As flavonoids are highly soluble in water, cooking foods with a high surface area or ruptured cell wall in water can result in a substantial reduction in flavonoids that leach into the surrounding water (Rodriguesa *et al.,* 2009). Food processing can also reduce the polyphenol content as the plants are grinded thus causing enzymes to act on the polyphenol compounds (Manach *et al.,* 2004). Esterases, glycosidases, and decarboxylases all can alter polyphenol structure in foods through oxidation and this can influence the sensory quality of foods, either resulting in beneficial or harmful changes to the foods (Pandey & Rizvi, 2009). For example, in tea production oxidation enhances sensory quality, yet in fruits this oxidation process results in fruit browning.

Individual polyphenols can be affected differently by external stimuli. For example, epigallocatechin-gallate (EGCG) is unstable to auto oxidation and epimerization, with temperature, pH, oxygen and the level of associated antioxidants also affecting EGCG stability (Sang *et al.,* 2005). In a review by Ananingsih *et al.* (2013) investigating the stability of various catechins, stability was low at a pH < 4, increased at a pH of > 6, and most catechins were easily degraded by heat. The phenolic acid content of a plant tends to decrease during ripening, yet the anthocyanins in fruits and vegetables are found predominantly in the skins of plants and increase with colour intensity and fruit ripeness (D'Archivio *et al.,* 2007). These compounds are highly sensitive to changes in pH and may undergo ring opening or oxidation during sample extraction and thus when foods are measured for polyphenol content, anthocyanins may not always be detected (Pandy & Rizvi, 2009). Therefore due to the easily degradability of polyphenols, the analysis of these compounds in foods can vary greatly between different varieties of the same source depending on many external factors.

3.4 Bioaccessibility and bioavailability

Although much research has been completed assessing the total content of polyphenols in foods, there is confusion around the bioaccessibility and bioavailability of phenolic molecules in the digestive system, plasma and tissues (Saura-Calixto *et al.,* 2007). A main goal for future research is to determine not only which polyphenols are the most abundant in the diet and most efficiently absorbed, yet also which polyphenols produce active

metabolites and identification of those metabolites. Although polyphenols such as quercetin and genistein have been the most largely studied phenolic compounds due to their structural properties, they only constitute about 2-4 % of total polyphenol intake in Western diets and therefore their activity in the body is negligible (Scalbert & Williamson, 2000).

Bioaccessibility is defined as the amount of polyphenols that reach the intestinal border with the potential to be absorbed into the blood. The bioaccessibility of compounds is measured by *in vitro* methods and therefore has its limitations. In contrast, bioavailability refers to the amount of polyphenols that actually get absorbed and therefore have potential to exert their actions in the body. When ingested polyphenols reach the intestine, the chemical structure determines the rate and extent of absorption, and thus the content of polyphenol plasma metabolites. Some polyphenols are absorbed at the gastrointestinal level, whereas others are absorbed at the more distal parts of the intestine (Pandey & Rizvi, 2009; Table 3.1). It is thought that only approximately 5-10 % of total polyphenols are absorbed at the small intestine, with the remaining being metabolised by the colonic microbiota (Chiva-Blanch & Visioli, 2012). The nature and position of the conjugated groups can affect the biological properties of the compound, and is therefore important to determine for accurate analyses (Day, 2000a). During absorption, polyphenols may be transformed into aglycones or sugar conjugates rather than existing in the original form of the compound found in the food.   In order for diffusion across the intestinal brush border, the first step is the removal of sugar moieties from the polyphenol by glycosidases (Gharras, 2009). Following hydrolysis polyphenols are conjugated by methylation, sulfication, glucuronidation or a combination which is controlled by enzyme action. Flavonoids that are normally glycosylated include certain classes of flavonols, isoflavones, flavones and anthocyanins. Glucosides are more readily available from the small intestine than are other glycosides, and galloyl groups also influence the absorption, yet not to the same extent as glycosylation (Williamson & Manach, 2005).

An early study by Lipinski and others (2012) proposed that compounds should have a molecular weight of no more than 500 Daltons, less than five hydrogen bond donors and no more than ten hydrogen bond acceptors in order to be absorbed into the body. EGCG contains a large number of hydrogen bond acceptors and donors, and therefore is not well absorbed despite showing high activity *in vitro* (Lee, 2013). Proanthocyanins can have a wide range of molecular weights with varying degrees of polymerization (Guyot *et al.,* 2001). As polymerization increases, degradation decreases in the intestinal tract and overall absorption of the polymer and complex compounds decreases (Rios *et al.,* 2003). Quercetin is promising for its health benefits yet has a low bioavailability *in vivo*; however combining polyphenol compounds with other sources may influence their degree of bioavailability (Guo *et al.,* 2013). For example, in the study by Guo *et al.* (2013) consumption of the aglycone

quercetin in combination with a high fat breakfast was shown to enhance absorption of quercetin, possibly due to the micellarisation at the small intestine.

The two most common methods of evaluating polyphenol intake and bioavailability are through the plasma and urine (Vetrani *et al.,* 2014). A high concentration of polyphenol metabolites in the urine generally corresponds to a high absorption of polyphenols from the small intestine, however this can be misleading as many phenolics are eliminated in the bile, and thus urine evaluation can result in an underestimation of polyphenol bioavailability. Metabolites that have been extensively conjugated are more likely to be excreted by means of bile, whereas small conjugates are more likely to exit via urine (Pandy & Rizvi, 2009). Urinary excretion is generally high for flavanones from citrus fruits (Manach *et al.,* 2004) and for isoflavones (Setchell *et al.,* 2003), while very low for flavonols such as quercetin and its glycosides (Graefe *et al.,* 2001; Table 3.1). However, due to the vast structural changes that take place, there is great limitation for determining accurate biomarkers of polyphenol intake.

3.5 Factors affecting polyphenol bioaccessibility and bioavailability

By performing a simulated *in vitro* digestion, it is possible to assess the changes in polyphenol bioaccessibility throughout the digestive process. For example, Ryan and Prescott (2010) tested various fruit juices and found that certain polyphenols became more bioaccessible as they changed form throughout digestion. The conclusion was that this increase may be due to the change in structure of certain phenols such as the anthocyanins. This was further highlighted in a later study by Wootton-Beard *et al.* (2011) which found the antioxidant and polyphenol content of 23 vegetable juices to increase in bioaccessibility into the gastric phase and then decrease into the duodenal stage of digestion. Bermudez-Soto *et al.* (2007) also found that polyphenol bioaccessbility increased after the gastric phase of digestion yet decreased after the duodenal phase, and the alkaline pH in the duodenum was thought to be responsible for the degradation. Heat treatment is generally considered harmful for polyphenols, however it may also allow for the formation of new structural groups giving polyphenols enhanced productivity (Kim *et al.* 2010). For example Kim *et al*. (2010) found that compared to the fresh form, thermally processed tannic acid had a 67% higher plasma antioxidant capacity.

The food matrix is another important issue to consider as the consumption of polyphenols in combination with other compounds can influence the degree of degradation. For example, Rios and others (2002) found that *in vivo* the food medium was shown to buffer the pH of the

intestine and thus compounds such as proanthocyanins were not subject to as high a degree of hydrolysation as they would otherwise be in isolation. In the study by Seeram *et al.* (2005), pomegranate juice was found to have greater bioactivity in colon cancer cell lines compared to its purified polyphenols, and it was concluded from this that synergism and multi factorial effects were greater compared to any single compound on anti-proliferative activity. However, other studies show conflicting results. Bermudez-Soto *et al.* (2007) found that the pure compound quercetin-3-rutinoside from chokeberry was more stable when incubated alone than in the chokeberry juice. Therefore, depending on the type of polyphenols and the food medium, and also the specificity of the method of analysis used, the digestion process may enhance or decrease the amount of polyphenols absorbed and thus the polyphenol activity in the body.

3.6 Analysis of polyphenols

To date, there is a lack of standardised analytical methods to quantify the polyphenol content of foods. Current methods include the Folin-Ciocalteu (FCR) method to estimate total phenols, where as individual compounds such as cholorogenic acid, quercetin or catechins can be estimated by chromatographic techniques (Spencer *et al.,* 2008). The FCR method is a standardised method for measuring the polyphenol content of a sample (Prior *et al.,* 2005), and estimates phenol content by measuring reducing capacity based on an electron transfer based assay (Phipps *et al.,* 2007). The method is sensitive and precise yet is slow and not standardised, and lacks specificity with many substances able to interfere with measurements. For example, sulfites and sulphur dioxide react with the FCR reagent and may therefore become an issue when measuring total polyphenol content of sources such as wine (Singleton & Rossi, 1965). Also, there is a tendency for overestimation when using the FCR method due to ascorbic acid being included in the total reducing power value. However, underestimation of polyphenol content is frequent when using chromatography because proanthocyanidin polymers and oxidised polyphenols may escape detection (Scalbert & Williamson, 2000).   The high performance liquid chromatography (HPLC) analysis and the liquid chromatography coupled with mass spectrometry (LC-MS) are the main methods used to analyse individual polyphenol contents and measure the compounds both qualitatively and quantitatively (Motilva *et al.,* 2013). A validation study of an HPLC analysis found it to be precise and sensitive, with good separation and quantification of compounds without sample preparation (Canas *et al.,* 2003); however, analysis time can be long and samples require a high resolution (Motilva *et al.,* 2013).

Hundreds of studies have used HPLC-MS and other methods to determine the individual polyphenol profiles of different food sources (Cacciola *et al.,* 2012). Information on the polyphenol content of a range of different fruits, vegetables and other foods is now available to the public. Polyphenol Explorer is the first comprehensive database to have a detailed record of the polyphenol content of select foods from a meta-analysis of studies (Neveu *et al.,* 2012). Therefore, research into the polyphenol content of foods, and also the structural changes that take place throughout digestion is progressing highlighting the potential variety of fates that these compounds may have in health.

4. **The potential of polyphenols as functional components in health**

4.1 Polyphenol rich diets and health: an introduction

Food and beverages rich in polyphenols are thought to be beneficial for sustaining long term health. A high fruit and vegetable consumption specifically has been extensively associated with a reduced risk of chronic disease (Liu, 2003). Fruits and vegetables are well known for their high micronutrient content, and are frequently acknowledged for being high in antioxidants and different fruits and vegetables contribute significantly to the intake of polyphenols in the diet worldwide (Scalbert & Williamson, 2000). The British Nutrition Foundation (BNF) recommends a minimal consumption of five servings of fruit and vegetables a day for disease risk reduction, however the average consumption in the UK is far below this level (BNF, 2014). Based on data from the Health Survey for England between 2001 and 2008, it has been proposed that five servings may not be an adequate intake to prevent chronic disease, and thus it has been recommended that the UK population should aim for seven plus servings a day. This increase in fruit and vegetable consumption may significantly increase overall polyphenol intake.

The consumption of a polyphenol rich diet may simply protect against disease because these healthful foods replace other 'non healthy' foods such as those rich in saturated fat and refined sugars (Chiva-Blanch & Visioli, 2012). Also, those who consume an overall healthy diet are more likely to have other healthy lifestyle behaviours, such as performing regular exercise, being non-smokers and consuming low amounts of alcohol. In a 12-year cohort study of 807 people, total urinary polyphenols were assessed for their association with overall mortality. Those in the highest tertile of total urinary polyphenols at baseline were shown to have the lowest mortality rate at follow up, thus indicating that high polyphenol intake may be associated with longevity (Zamora-Ros *et al.,* 2013a).

One mechanism in which polyphenols may slow down the ageing process is thought to be due to their antioxidant potential (Harmen, 2006). The increase in antioxidant capacity after consuming polyphenol rich products could be a result of their effects on endogenous antioxidants, their reduction on the absorption of pro-oxidants or simply due to their own reducing potential (Scalbert *et al.,* 2005). However, previously thought to work predominately as antioxidants in the body polyphenols are now thought of more for their other health promoting properties. Few polyphenols contain the right free hydroxyl structure to actually work as antioxidants and an insufficient amount may be absorbed to contribute to the overall antioxidant status (Bors *et al.,* 2001). Many foods and beverage products with extracts of single polyphenolic compounds are being marketed for their antioxidant potential, which is solely based on *in vitro* data and therefore products are being labelled and promoted before there is sound evidence for their actual wide variety of health benefits (Lee, 2013).

4.2 Functional Foods

The idea of a functional food was initiated in Japan in the 1980s when the Ministry of Health and Welfare took action to promote foods that were designed to prevent and treat chronic disease in the ageing population (Arai, 1996). These foods were termed Food for Specified Health Use (FOSHU) and by definition refer to foods that contain ingredients that are physiologically active and beneficial for health. In the UK, functional foods are not as advanced and there is still a lack of knowledge for consumers around what a functional food is and how these foods can be beneficial for health (Annunziata & Vecchio, 2011). This confusion revolves around the limited science supporting the role of functional components in relation to disease reduction. The EU regulations specify that in order to contain health claims a food must adhere to a set of nutrient requirements backed by clear and substantiated scientific evidence (EFSA, 2014). The consideration of polyphenols as functional additives is part of a rapidly growing area of nutritional science.

4.3 Establishing a recommended daily intake

There are a variety of conflicts that arise when considering polyphenols as functional food ingredients, including their potential toxic effects. Unlike the micronutrients in the diet, there is currently no recommended daily intake of polyphenols to maintain optimal health (Chiva-Blanch &

Visioli, 2012). Obtaining polyphenols exclusively through food and beverage sources is unlikely to lead to toxicity, even in high polyphenol consuming countries. However, taking isolated polyphenol supplements in addition to consuming a polyphenol rich diet may increase overall intake to very high levels. Polyphenols are generally consumed from the diet in milligrams per day, whereas taking supplements can result in the ingestion of grams of polyphenols (Skibola & Smith, 2000).

Although polyphenols are known for their antioxidant role, they can also work as pro-oxidants at higher doses. *In vitro* work has highlighted the potential of flavonoids in causing adverse reactions such as mutations and cytotoxicity if consumed in high amounts (Halliwell *et al.,* 2008).   In a review by Schönthal (2011) the adverse effects of polyphenol rich green tea at various doses were highlighted, including such things as liver damage and potential interactions with certain medications. Other compounds such as the isoflavones have been shown to have both protective and adverse effects on breast cancer at doses obtained from the diet, due to their oestrogen like nature (Dong & Qin, 2010). However, toxicity studies in humans are limited due to the ethical issues associated with these types of designs, making a clear upper limit difficult to determine.

Research performed in *in vitro* and in animal models is beneficial for considering these dose response studies for polyphenols and their influence on health, however when working with these models several conflicts may arise making it hard to translate results to human models (Yang *et al.,* 2001). For example, administered doses of phenolics are often much higher in animal models than those used in humans. Also, in obvious terms animals are not humans, and therefore the causative disease factors in animals are not necessarily the same for human pathologies. Even epidemiological human studies, although the best method for looking at real-life effects of dietary interventions, consist of confounding factors that make true cause-effect relationships hard to determine. However, considering the pitfalls polyphenols have still shown significant promise in some of the most prevalent chronic diseases in the Western culture.

4.4 Synergism

A potential strategy when considering the development of functional foods may be to combine different polyphenols in an attempt to produce synergistic effects compared to the isolated compounds. The literature is promising for the *in vitro* effects of polyphenol synergism on various disease parameters including risk factors of chronic inflammation and cancer, yet there are no well-designed human studies to date. For example,

the polyphenol compounds quercetin and resveratrol were found to reduce the inducible nitric oxide synthase (iNOS) gene and the production of NO in combination, and therefore may prove beneficial for reducing chronic inflammation (Man-Ying Chan *et al.*, 2000). Quercetin combined with catechin produced a significant inhibition of collagen induced hydrogen peroxide production, which did not occur with either polyphenol alone (Pignatelli *et al.*, 2000). Resveratrol and black tea polyphenols and also kaempferol and quercetin significantly suppressed tumour growth in synergism compared to any of the compounds in isolation (Ackland *et al.,* 2005). Also, Bastianetto *et al.* (2009) found that in combination, the polyphenols in red wine including resveratrol, quercetin and catechin reduced reactive oxygen species (ROS) levels. Therefore, although these studies are promising, polyphenol synergism in relation to health needs to be determined in human studies before more conclusive results can be drawn.

4.5 The role of polyphenols in inflammation

Polyphenol rich sources have shown promise in the prevention and treatment of some of the most common disease states throughout the developed world, including pathological inflammation and cardiovascular disease (CVD). Chronic inflammation plays a significant role in a number of disease pathologies, and involves the excessive release of ROS and pro inflammatory cytokines which can cause damage to host tissues (Medzhitov, 2008). Epidemiological evidence suggests that foods rich in polyphenols such as fruit, olive oil and nuts can reduce various markers of chronic inflammation (Salas-Salvadó *et al.,* 2008). Individual polyphenols may also reduce risk as found in the Nurse's Health Study in which total flavonoids were associated with a reduction in inflammatory markers, with the flavonoids found in citrus fruit specifically associated with a reduction in interleukin (IL)-8 (Landberg *et al.,* 2011). Results from the National Health and Nutrition Examination Survey (NHANES) study further support this role as total flavonoid intake was inversely related to C-reactive protein (CRP) concentration (Chun *et al.,* 2008). Of the individual flavonoids, flavonols, anthocyanidins and isoflavones were inversely associated with CRP, and specifically quercetin, kaempferol, malvidin, peondidin, daidzein and genistein. Most studies determining the role of polyphenols for reducing inflammatory markers have been performed *in vitro,* with few randomised controlled trials to date. Of the human trials performed, resveratrol (Ghanim *et al.,* 2011) and blueberries (Riso *et al.,* 2013) have shown promising results, black and green tea (Basu *et al.,* 2011; de Maat *et al.,* 2000; Fukino *et al.,* 2005; Ryu *et al.,* 2008) were less effective in reducing chronic inflammation and cocoa consumption showed conflicting results (Mathur *et al.,* 2002; Monagas *et al.,*

2009). Therefore more well-designed studies are needed to confirm the optimal foods, compounds and doses for reducing various markers of pathological inflammation.

4.6 Polyphenol consumption and risk of CVD

Atherosclerosis is a chronic inflammatory disease consisting of pathological lesions which can lead to complications such as myocardial infarction and sudden cardiac death (Vita, 2005). Polyphenols may help to prevent the accumulation of atherosclerotic plaques and thus reduce CVD risk, for example by improving endothelial dysfunction, reducing blood pressure (BP) (López-Sepúlveda *et al.,* 2008) and improving plasma lipids (Annuzzi *et al.,* 2014). In the recent Primary Prevention of Cardiovascular Disease with a Mediterranean Diet (PREDIMED) observational study with a duration of 4.3 years and consisting of 7172 participants, a 46% reduction in CVD risk was observed in the highest versus the lowest quartile of polyphenol intake, with flavanols, lignans and hydroxybenzoic acids having the strongest inverse relationship to risk (Tresserra-Rimbau *et al.,* 2014). This was also supported in previous work by McCullough *et al.* (2012) in which total flavonoids, anthocyanidins, flavanols, flavones, flavonols, and proanthocyanidins were inversely associated with fatal CVD events.

Of the whole foods and beverages studied, moderate alcohol consumption has been shown to offer benefits in both those with CVD and also in healthy subjects, with the strongest association found for beverages rich in polyphenols such as wine and beer (Chiva-Blanch *et al.*, 2013). A more recent review by Estruch & Lamuela-Raventos (2014) highlighted this benefit, and further concluded that moderate wine consumption protects against the development and progression of atherosclerosis due to both its ethanol and polyphenol content. Chocolate consumption has been shown to reduce BP, myocardial infarct and risk of stroke (Buijsse *et al.,* 2010), and to increase flow mediated dilation (FMD) and reduced systolic (SBP) and diastolic blood pressure (DBP) (Hooper *et al.*, 2008). In a study by Hooper *et al.* (2008) soy protein isolates were also found to reduce DBP, soy and green tea were found to reduce low density lipoprotein (LDL) cholesterol and acute black tea consumption actually increased overall BP. Epidemiological studies on soy and CVD risk are limited, however a review by Zhang *et al.* (2003) found a strong inverse association between soy food intake and risk of coronary heart disease, and this association was strongest for myocardial infarct. Although black tea has was found to have no benefits for reducing CVD risk factors, green tea and its polyphenols have shown an inverse association with CVD (Wang *et al.,* 2011).

4.7 Green tea catechins, energy expenditure and weight maintenance

Research determining the effect of certain polyphenols in relation to weight maintenance is promising (Galleano *et al.,* 2012; Gonzalez-Castejon & Rodriguez-Casado, 2011), with a majority of studies assessing the effect of green tea and its polyphenols. A meta-analysis of 11 studies found that the polyphenols from green tea were beneficial for weight loss (Hursel *et al.,* 2009) and specifically the green tea catechins were thought to play a significant role in weight reduction (Rains *et al.,* 2011). Polyphenols may play a role in weight maintenance by altering the metabolic rate and promoting fat oxidation, and therefore by increasing the amount of energy expended postprandial. Diet induced thermogenesis (DIT) is the increase in metabolic rate after the ingestion of food (Rothwell & Stock, 1983), and results in 5 to 15 % of daily energy expenditure (EE) when on a mixed diet (Westerterp, 2004). Intervention studies have shown beneficial effects of green tea and its polyphenols for increasing fat oxidation (Boschmann & Thielecke, 2007; Murase *et al.,* 2002), EE (Auvichayapat *et al.,* 2007) and especially DIT (Harada *et al.,* 2005).

Catechins specifically have been shown to increase metabolic rate. Harada and others (2005) found that in healthy males who consumed a drink containing 592.9mg catechins, fat oxidation and DIT were significantly enhanced after 12 weeks. Brown adipose tissue has a high metabolic rate and although it is found in low amounts in human adults, stimulating this tissue may significantly increase EE (Trayhurn & Milner, 1989). Green tea has been shown to increase the respiration rate of brown adipose tissue *in vitro* (Dulloo *et al.,* 2000) and may enhance β-adrenoceptor activation and thus metabolic rate in this tissue in humans (Choo, 2003).   However results on green tea need to be interpreted with caution due to the thermogenic effect of caffeine in the tea (Dulloo *et al.*, 2000; Hursel *et al.,* 2011; Rumpler *et al.,* 2001). A recent meta-analysis investigated the effect of catechin rich teas and also caffeine on EE and fat oxidation (Hursel *et al.,* 2011). It was concluded that although both the catechin-caffeine mixtures and caffeine only supplementation increased EE, fat oxidation was only significantly increased after the catechin-caffeine mixtures. Another study by Dulloo *et al.* (2000) discovered that brown adipose tissue thermogenesis was stimulated by the green tea catechin-polyphenols and caffeine interaction and the thermogenic effect of green tea was greater than could be explained by the caffeine content alone. Therefore, these studies highlight the potential of polyphenols and caffeine to act synergistically as agents for increasing metabolic rate.

Other polyphenols have been shown to affect EE, yet studies are limited. A recent study found that the combination of EGCG and resveratrol increased both EE and DIT (Most *et al.,* 2014). In mouse models, quercetin was found to increase EE over a three week period (Stewart *et al.,* 2008), and in mice fed a high fat diet, supplementation with the coffee polyphenols caffeoyl quinic acids (CQA) increased EE as measured by indirect calorimetry (Murase *et al.,* 2011). Therefore, green tea and possibly other polyphenol compounds may increase EE and DIT partially mediated through brown adipose tissue, proving beneficial for obesity and its related disorders such as inflammation, CVD and type 2 diabetes.

5. Diabetes, glycaemia and polyphenols

5.1 Diabetes: background and characteristics

Last year the World Health Organisation (WHO) reported a total of 347 million people worldwide to have diabetes (WHO, 2013). In the UK there are an estimated 3.2 million cases of diagnosed diabetes and potentially 630,000 people who are currently undiagnosed (Diabetes UK, 2014). The disease is associated with an abnormal glucose metabolism in which glucose cannot enter the cells and therefore concentrations in the blood can reach pathological levels. There are two main forms of diabetes, type 1 and type 2 and the main characteristics of each disease are given in Table 5.1.

Table 5.1 Common characteristics of type 1 and type 2 diabetes

	Type 1 diabetes	Type 2 diabetes
Prevalence (% of cases)	10%	85-95 %
Onset	Acute and potentially fatal	Chronic, often undiagnosed for long periods of time
Age of onset	<40 yr, but can occur at any age	Predominantly 30+ yr, but can occur at any age
Symptoms	Excessive thirst, passing urine frequently, weight loss, fatigue, blurred vision and ketoacidosis	Excessive thirst, slow healing wounds, passing urine frequently, weight loss, fatigue, reoccurring thrush and blurred vision
Pathology	Destruction of pancreatic β-cells causing a lack of insulin production	Not enough insulin produced or insulin cannot function properly
Cause	Unknown, may be due to genetics, infection and/ or environmental factors	Genetics factor, increased risk caused by unhealthy lifestyle factors such as diet
Treatment	Daily insulin injections	Can be controlled through diet, yet may require medication and/ or insulin injections
Optimal blood glucose range	4-7 mmol/L pre meal <9 mmol/L post meal <10 mmol/L post meal for children	4-7 mmol/L pre meal <8.5 mmol/L post meal

Diabetes UK, 2014; yr, year.

Strict blood glucose control is important to prevent the further complications associated with diabetes, such as neuropathy, retinopathy and nephropathy (NICE, 2014). Disturbance in glucose metabolism can also increase one's risk of other chronic disease states such as insulin resistance, abnormal plasma lipid levels, hypertension and other risk factors of CVD. Diet plays a large role in the prevention of type 2 diabetes and the treatment of both type 1 and 2 diabetes and research has suggested the particular role of low GI foods for improving glycaemic control.

5.2 The glycaemic index

The concept of the GI of foods for managing type 1 diabetes and dyslipidaemia was brought about by Jenkins and others in 1981. The GI of a food is calculated by dividing the postprandial blood glucose area under the curve (AUC) of 50g of available carbohydrate (avCHO) from a test food by 50g of avCHO from a reference food (either white bread or glucose). The AUC is determined two to three hours postprandial and the resulting value is expressed as a percentage, with high GI foods thought to negatively exacerbate postprandial glycaemia (Pi-Sunyer, 2002). Routine GI tests are conducted in healthy subjects in the morning after a 10 - 12 hour overnight fast (FAO/ WHO, 1998).

Several factors can influence the GI of a food, including the ripeness, physical form, type and preparation of the food. For example, the GI of fruits will vary depending on whether they are consumed whole, as a purée or as a juice (Radulian *et al.,* 2009). The amylose to amylopectin ratio of the food may also affect GI, with the higher the amount of amylopectin the higher the resulting GI. Starch granules must be disrupted to allow for digestion and absorption of sugars and therefore processes such as cooking, grinding and the degree of chewing will alter the GI (Williamson, 2013). Foods are generally consumed as a meal instead of in isolation and the GI of mixed meals will depend on the amount of each macronutrient in the meal. The glycaemic load (GL) is also important to consider and is defined as the GI multiplied by the amount of avCHO consumed (Salmeron *et al.,* 1997). Foods such as watermelon which have a high GI value produce a low GL due to the low quantity consumed and the high water content of the fruit.

Published GI values may differ between studies as glucose was the initial reference food whereas now white bread is predominantly used (Jenkins *et al.,* 1988). The calculation of the AUC usually includes only the area above the curve (FAO/ WHO, 1998) which results in another conflict as some researchers do measure the entire glucose response, and therefore the GI may be higher when the area beneath baseline is included (Gannon & Nuttall, 1987). Also, the glucose from some foods such as those rich in dietary fibre and/ or low GI foods may take longer

than four hours to clear the blood stream and the entire glycaemic effect may not be accounted for (Brennan *et al.,* 2012). Although GI studies are designed to limit the amount of confounding factors, such as reducing physical activity, and caffeine and alcohol intake on the night before a test, several factors can still affect the postprandial GR to a test food. For example, it has been proposed that the foods in the hours and days prior to testing may significantly affect the 'next meal' blood glucose values (Robertson *et al.,* 2003); however other studies show no effect of a pre evening meal on next day glycaemia (Ning *et al.*, 2010).

5.3 Low GI diets and health

Although the postprandial response to food in healthy people may be highly efficient, this is not the case in various disease states and consuming food with a low GI may positively influence blood glucose levels and also other metabolic abnormalities. Glycosylated $HbA1_c$ is a precise marker of diabetes control and represents the average blood glucose concentration of plasma over the previous two to three months (Diabetes UK, 2014). In a recent Cochrane review assessing the effect of GI on risk factors of diabetes, hypo and hyperglycaemic episodes were fewer in the low versus high GI diet, with $HbA1_c$ also reduced in the low GI group (Thomas & Elliot, 2009). Low GI and low GL diets may also be beneficial for other associated disease risk factors, for example those following a low GI diet were found to lose significantly more weight, have a lower body mass and total fat mass and have fewer lipid abnormalities than those on control diets (Thomas *et al.,* 2007). A cross sectional study combining results from the Metabolic Syndrome and Cardiovascular Disease (CoDAM) Study and the Hoorn Study found an inverse association between GI and insulin sensitivity, and a positive association with a pathological lipid profile and chronic inflammation (Huaidong *et al.,* 2008).

Hyperglycaemia increases the production of ROS which can activate inflammatory cytokines, leading to an increase in inflammatory cell recruitment (Dickinson *et al.,* 2008). It was concluded from the Nurses' Health Study that the inflammatory marker CRP was positively associated with GI and GL, the relationship being stronger in overweight women (Bhupathiraju *et al.,* 2014). Obesity, diabetes and inflammation are linked largely by an increase in the release of these cytokines from adipocytes, which also have the potential to further affect glucose metabolism. For example, inflammatory mediators such as tumour neurosis factor (TNF) - α can increase tyrosine phosphorylation of the insulin receptor in adipose and muscle tissue, reducing glucose uptake into the muscle and cause resulting insulin resistance (Hotamisligil, 1999).

Therefore, high GI diets can influence blood glucose levels directly or indirectly through increasing the inflammatory response and increased inflammatory recruitment can potentially further worsen glycaemic control.

Diets rich in low GI carbohydrates may also improve plasma lipids as an attenuated, prolonged absorption of glucose into the blood can result in a reduction in plasma free fatty acids, and in time this can lead to an accelerated uptake of glucose into the cells (Dresner *et al*., 1999). A systematic review and meta-analysis on GI and markers of CVD found that the evidence from short term studies supported a low GI diet for reducing total and LDL cholesterol while having no effect on high density lipoprotein (HDL) cholesterol (Goff *et al*., 2013).

After the consumption of a high GI carbohydrate, the increase of glucose in the blood is followed by an exaggerated release of insulin, and resulting hypoglycaemia in the hours postprandial. The Glucostatic Theory proposed by Mayer in 1953 states that the frequent consumption of high GI foods can result in an increase in hunger due to these drastic changes in blood glucose and insulin levels. However, although short term studies support this hypothesis, more long term studies are need to confirm an association between glycaemia and satiety (Niwano *et al.,* 2008). Therefore, high GI foods may not only increase one's risk of developing diabetes by causing abnormal glucose and insulin levels, yet may also contribute to obesity by increasing the feelings of hunger (Arumugam *et al.,* 2008).

5.4 Diabetes and polyphenols: the evidence

To date, the most extensively studied polyphenols and polyphenol rich foods in the prevention of type 2 diabetes and the metabolic syndrome have been tea, both black and green for its high tannin and EGCG content, coffee for its phenolic acids, grapes and grape products such as wine for resveratrol and apples for their abundance of flavonoids (Williamson, 2013). Various herbs, berries, soy, vegetables and whole grain products have been recognised for their benefits for blood glucose control in humans. The assessment of dietary patterns in the development of type 2 diabetes has been reviewed. Huxley *et al.* (2009) found an inverse linear association between coffee, decaffeinated coffee and tea intake and risk of type 2 diabetes, while Carter *et al.* (2010) found green leafy vegetable consumption, but not fruit or vegetables, to be associated with a 14% reduction in risk of type 2 diabetes.

Overall flavonoid intake has been shown to reduce the incidence of type 2 diabetes, with the strongest association found for flavonols and to a lesser extent flavanols (Jacques *et al.,* 2013). Other epidemiological studies have looked at the effects of whole foods on diabetes risk factors,

particularly apples and black tea consumption has been linked to a lower incidence of type 2 diabetes (Song *et al*., 2005). The consumption of one plus apple a day resulted in a 28% reduction in diabetes compared to those consuming no apples, and four plus cups of black tea a day was also associated with a reduced risk. Vegetables are abundant in micronutrients, fibre and also polyphenols. In the Japanese Elderly Diabetes Intervention Trial, vegetable intake was compared against metabolic parameters in males with type 2 diabetes (Takahashi *et al.,* 2012). It was found that $HbA1_c$, triglycerides and waist circumference had an inverse association with vegetable consumption, specifically green vegetables were shown to have the most pronounced effect in reducing $HbA1_c$.

5.5 Summary of the evidence from animal and human studies

Studies in animals have shown the potential of polyphenol rich sources for reducing pathological markers of glycaemia, with results being strong for isolated polyphenols such as catechins from green tea (Sabu *et al,* 2002; Wu *et al.,* 2004), resveratrol (Ramadori *et al.,* 2008), quercetin (Kim *et al.*, 2011), isoflavones (Zimmermann *et al.,* 2012), anthocyanins (Seymour *et al.,* 2011) and proanthocyanidins (Young *et al.,* 2008). Randomised controlled trials in humans further support these animal studies, with the majority measuring longer term effects of polyphenol consumption on markers of glycaemia over a period of weeks and months.

Polyphenol rich sources such as cinnamon (Crawford, 2009; Lu *et al.,* 2012), resveratrol (Bhatt *et al.,* 2012) and green tea (Hsu *et al.,* 2011) have been shown to improve $HbA1_c$ in diabetic subjects, and therefore can improve glycaemic control over months. The reduction in fasting blood glucose is also important for improving diabetes control, as a high fasting level can result in an exaggerated postprandial response. Green tea extract has been shown to reduce fasting plasma glucose in both obese (Bogdanski *et al.,* 2012) and healthy subjects (Huang *et al.,* 2013), and black tea (Bahorun *et al.,* 2012), genistein (Squadrito *et al.,* 2013), quercetin (Lee *et al.,* 2011) and low amounts of soy isoflavones (Bahls *et al.,* 2011) have also been shown to reduce fasting glucose levels in long term intervention trials. Chocolate polyphenols may have a saturation effect in reducing glycaemia as Almoosawi and others (2010) found that a dose of 500mg of polyphenols from dark chocolate was equally effective at reducing fasting plasma glucose as 1000mg polyphenols in healthy overweight and obese subjects. At similar doses, the flavanols in dark chocolate have also been shown to increase insulin sensitivity in healthy lean subjects (Grassi *et al.,* 2005), and to reduce insulin resistance in women with type 2 diabetes when combined with 100mg of isoflavones (Curtis *et al*., 2012). Moderate red wine consumption

(approximately one glass a day) can reduce insulin resistance when controlling for the alcohol component, thus highlighting the beneficial effects of the wine polyphenols (Chiva-Blanch *et al.,* 2013), specifically resveratrol (Brasnyó *et al.,* 2011).

There is considerable variability and heterogeneity between different study designs, including the health status of the subjects, the form and dose of the polyphenol source, the combination with other nutrients and the length of the study, to name a few. The consumption of various fruits in relation to glycaemia is another area with extensive research, however trials are conflicting. For example, two studies found no difference in HbA1$_c$ (Christensen *et al.,* 2013) or insulin resistance (Wallace *et al.,* 2013) in those consuming little or no fruit compared to those with a higher intake. However, an improvement in overall glycaemic control was found in subjects with type 2 diabetes who were instructed to consume two pieces of fruit a day for three months (Hegde *et al.,* 2013). Although some studies show no effect of fruit on markers of glycaemia, the sugar content of fruit should be considered due to the adverse effects that may result from the addition of sugar rich fruit to the diet (Rock *et al.,* 2009).

Research has also been considering the role of polyphenols for improving postprandial glycaemia although again, results are conflicting. Wine and its components and various types of berries have been shown to reduce post-meal metabolic abnormalities including hyperglycaemia (Burton-Freeman, 2010). For example, 300mg of polyphenol rich grape seed extract was found to reduce postprandial GR in overweight people (Edirisinghe *et al.,* 2012), and 40g of sweetened dried cranberries low in sugar reduced the GR in subjects with type 2 diabetes (Wilson *et al.,* 2010). In healthy adults, although the consumption of an oatmeal drink with added bilberry showed no effect on postprandial GR, it was found to reduce the insulin demand for a given GR and therefore reduce acute IR (Granfeldt & Björck, 2011).

5.6 Carbohydrate digestion and absorption

Starch is a polysaccharide containing glucose molecules linked by α-1,4 and branched α-1,6 glycosidic bonds (Guzman-Maldonado & Paredes-Lopez, 1995). These chains are made up of either amylose or amylopectin, with amylopectin constituting most of the branched structure and different starch sources will contain different ratios of these molecules. Sucrose and lactose are the main disaccharides in the diet and are broken down into the monosaccharides glucose, and fructose and galactose, respectively (Williamson, 2013). The main enzyme for carbohydrate digestion is α-amylase which can only act on α-1,4 linkages and is secreted both by the salivary glands and by the pancreas (Woolnough *et al.,* 2008). α-Glucosidase enzymes are found in the intestinal border and are able to complete the digestion of starch chains by hydrolysing the

terminal α-1,4 glucose linkages to release free glucose. Maltase is also found in the intestine and can hydrolyse maltose into individual glucose molecules. Lactose is found in milk products and is digested by lactase, with only a small amount of lactase still active when people reach adulthood (Williamson, 2013). Polyphenols that are linked to sugars by glycosidic bonds can be broken down in the intestine by enzymes such as lactase and therefore most polyphenols contain a structural group that allows them to bind to glucose and the resulting glucose receptors (Day *et al.,* 2000b).

Glucose is hydrophilic and thus cannot cross the intestinal membrane unaided. Glucose transport is mediated by both sodium dependent and sodium independent transport (Scheepers *et al.,* 2004). Active transport takes place via sodium dependent glucose transporter 1 (SGLT1) in which two sodium ions bind to the transporter on the luminal side of the small intestine, initiating a conformation change to the transporter to allow glucose to bind. Another transformation then takes place permitting the sodium ion and glucose into the enterocyte. In the basolateral membrane, the facilitative transporter glucose transporter 2 (GLUT2) then causes a release of the glucose into the circulation. Glucose can also be absorbed through independent mechanisms by facilitated diffusion.

5.7 Abnormal glucose metabolism, insulin resistance and the molecular role of polyphenols

As glucose is taken up by GLUT2, a series of enzymatic reaction begin which result in an increase of the energy compound ATP (Hosokowa & Thorens, 2002). ATP-sensitive potassium channels are inactivated leading to depolarisation, and calcium flows into the cell through calcium channels allowing insulin to be released through exocytosis. Glucose transporters can be regulated at the transcriptional level and/ or by intracellular signalling (Williamson, 2013). In diabetes, the glucose transporters in the intestine become up regulated, as do certain enzymes such as lactase and sucrase resulting in an increase in glucose absorption.

The increase in postprandial plasma glucose causes a resulting increase in insulin secretion, which in turn binds to cells producing a series of signalling cascades that lead to glucose uptake. After the binding of insulin, phosphorylation of a tyrosine residue on insulin receptor substrate 1 (IRS1) activates phosphoinositide 3- (P13)-kinase, which in turn activates atypical protein kinase C (aPKC). The translocation of the GLUT4 vesicle from inside the cell is then relocated in the membrane, where glucose can enter into the cell (Williamson, 2013). In skeletal muscle, glucose is either stored as glycogen or is oxidized to produce energy (Huang & Czech, 2007). Insulin resistance is linked to an increase in the

phosphorylation of a serine residue which inhibits phosphorylation of the tyrosine on IRS1. There is a reduction in the downhill signal events resulting in a reduction of GLUT4 in the cell membrane and therefore an increase of glucose in the blood to abnormally high levels.

Chronic hyperglycaemia can lead to the destruction of pancreatic β-cells and therefore to the development of insulin resistance (Augustin *et al.,* 2002). As the pancreas overcompensates by producing more insulin, β-cells can lose efficiency leading to their malfunction. Insulin secretion by the pancreas is a potential target for flavonoids as these compounds have been shown to increase the efficiency of the insulin secretion pathways in those with insulin resistance (Birnbaum, 2001). There are various fates for those polyphenols that are absorbed in the intestine and enter the circulation. Such roles can include activating insulin receptors and facilitating glucose uptake into tissues (Hanhineva *et al.,* 2010; Martineau *et al.,* 2006), modulation of intracellular insulin signalling pathways (Cordero-Herrera *et al.,* 2013), an increase in insulin secretion (Zhang & Liu, 2011) and promotion of glucose release from the liver (Hanhineva *et al.,* 2010). However, when polyphenols reach the intestine they have potential to interact with carbohydrates and with digestive enzymes and local transporters, and therefore they may influence the rate and degree of glucose absorption into the blood. More specifically, polyphenols may show potential for reducing the GI of a carbohydrate rich source. The widely produced diabetic drug acarbose inhibits the digestive enzyme α-glucosidase but does not get absorbed into the body, and therefore works only at the intestinal level. Natural sources of glucosidase inhibitors such as polyphenols with actions similar to acarbose are currently being studied to act competitively with sugars to reduce carbohydrate absorption and to attenuate postprandial glycaemia (Scheen, 2003).

5.8 Polyphenols at the intestinal level

Polyphenol compounds can inhibit certain digestive enzymes either by binding to receptors on the enzymes and/ or by possibly denaturing these enzymes (Kuhnert *et al.,* 2011). Studies support the role of different polyphenols for inhibiting α-glucosidase activity and to a lesser extent α-amylase *in vitro* (McDougall, 2005). For example, extracts rich in polyphenols such as *Ficus species* (Olaokun *et al.,* 2013), Ginkgo *biloba* (Tanaka *et al.,* 2004) and grape seed extract (Yilmazer-Musa *et al.,* 2012), have been found to inhibit the activity of both α-amylase and α-glucosidase. Certain berries such as blackcurrant and rowanberry (Boath *et al.,* 2012), and also anthocyanins (Matsui *et al.,* 2001) and isoflavones (Tadera *et al.,* 2006) may be more potent at inhibiting α-glucosidase, whereas proanthocyanins (Hargrove *et al.,* 2011) and resveratrol (Maio *et al.,* 2013) have been shown to significantly inhibit α-amylase activity. Catechins from tea have been found to inhibit porcine pancreatic α-amylase

activity *in vitro*, epicatechin-gallate (ECG) and EGCG being the most potent inhibitors from the tea (Cheng *et al.,* 2013). Yilmazer-Musa *et al.* (2012) found that tea catechins showed greater inhibitory potential for α-glucosidase, and therefore different tea catechins and other polyphenols may inhibit digestive enzymes to varying degrees. Certain classes of polyphenols including flavonols and flavones influence digestive enzyme activity (Kim *et al*., 2000; Tadera *et al.,* 2006) and can also reduce the transport of glucose into the blood (Kwon *et al.,* 2007).

Polyphenols can also influence postprandial glycaemia at the intestinal level by binding to glucose receptors and inhibiting glucose transport into the body via SGLT1 and GLUT2. Galloylated catechins such as ECG and EGCG can inhibit SGLT1 transport activity, and can reduce glucose absorption under sodium dependent and to a lesser extent sodium free conditions (Kobayashi *et al.,* 2000; Johnston *et al.,* 2005). Phenolic acids and tannins found in the fruits strawberries and apples have been shown to reduce activity of GLUT2 and to a lesser extent SLGT1 (Manzano & Williamson, 2010), and anthocyanin rich berry extract (Alzaid *et al.,* 2013) and a mixture of herbs and spices containing cafferic acid and *p*-coumaric acid (Farrell *et al.,* 2013) have also been shown to inhibit both GLUT2 and SGLT1 activity.

Finally, certain polyphenols may also act to reduce glucose absorption by binding to starch chains therefore reducing carbohydrate breakdown (Beta *et al.,* 2001; De Castro Palomino Siller, 2007). High molecular weight proanthocyanidins specifically can interact with amylose, thereby reducing starch digestibility and increasing resistant starch, with polymeric and oligomeric molecules thought to be better than monomeric (Barros *et al.,* 2012).

5.9 Conclusion

Therefore in summary, the literature shows a strong correlation between polyphenol consumption in overweight and/ or disease populations and a reduction in risk factors of diabetes. However, the effect on healthy people is not as convincing. Polyphenols have shown potential for reducing acute GR and have shown great promise *in vitro* for reducing starch digestibility and glucose absorption into the blood after a carbohydrate rich source. To date there are no systematic reviews assessing the effect of different polyphenol rich sources on carbohydrate digestion and resulting glycaemic parameters, in healthy adults.

6. The impact of polyphenols on acute postprandial glycaemia: a systematic review

6.1 Introduction

From 1996 to 2013, the number of people diagnosed with diabetes in the UK has increased from 1.4 million to 2.9 million (Diabetes UK, 2013). This rate is predicted to continue to increase and by 2025 it is estimated that five million people will have diabetes. Most chronic disease associated with obesity and type 2 diabetes is treatable and, more importantly, preventable by maintaining a healthy lifestyle of which nutrition plays a key role. A healthy diet which is low in saturated fat and sugar yet rich in fruits, vegetables and dietary fibre, has frequently been shown beneficial in type 2 diabetes (Diabetes UK, 2013). Certain food compounds and food combinations are being reviewed with the intention to manage diseases associated with poor diet quality and overeating. For example, foods that can reduce blood glucose levels and/ or improve overall metabolic parameters are attractive for improving glycaemic control.

Polyphenol rich foods are thought to carry various health promoting properties including their effects on glycaemic regulation. To date, few systematic reviews have examined the effect of polyphenol rich sources and type 2 diabetes risk (Bhatti *et al.,* 2013; Shojaii *et al.,* 2011) and those that have been completed show conflicting results. For example, herb and herbal products are rich in polyphenols and have been extensively studied for their effects on blood glucose levels (Shojaii *et al.,* 2011). In a review of 38 randomised controlled trials investigating the effect of herbs on various parameters of glycaemic control, 26 of the herbs produced benefits for glycaemia, yet the evidence was inconclusive as to what components of the herbs were the contributing factors. Another review by Bhatti *et al.* (2013) found that tea, especially green tea was associated with a reduced risk of stroke and diabetes, with improved levels of blood glucose, abdominal obesity, cholesterol and blood pressure. Coffee intake showed an inverse dose-dependent association with diabetes, and also with CVD deaths and all-cause mortality.

Epidemiological studies are also conflicting as to whether polyphenol rich foods are associated with a reduced risk of type 2 diabetes (Mursu *et al.,* 2014; Nanri *et al.,* 2010). In a study by Mursu *et al.* (2014), 2,332 non-diabetic men between the ages of 42 - 60 years were followed for 19.3 years. Fruit, berry and vegetable intake and incidence of type 2 diabetes were assessed at follow-up, and fruit and vegetables, especially berries were found to reduce the risk of developing type 2 diabetes. In another study in 25,872 and 33,919 Japanese men and women, respectively, aged between 45 - 75 years and without diabetes, soy and isoflavone intake was determined (Nanri *et al.,* 2010). Although soy

products and isoflavones were not significantly associated with type 2 diabetes in men and women of normal weight, in overweight women a higher soy intake was associated with a lower risk of developing the disease.

Randomised controlled trials performed over weeks and months have shown beneficial effects of polyphenols in reducing fasting blood glucose and for longer term markers of hyperglycaemia such as HbA1$_c$ (El-Sayed, 2011; Jayesh-Kumar *et al.,* 2012; Squadrito *et al.,* 2013). However, the immediate effect of polyphenol sources on postprandial glycaemia and insulinaemia is also of interest. Recent trials looking at postprandial GR have shown promise for polyphenols in reducing blood glucose levels when consumed with a carbohydrate source, such as foods high in starch (Balisteiro *et al.,* 2010; Clegg *et al.,* 2011; Coe *et al.,* 2013; Rosen *et al.,* 2011; Törrönen *et al.,* 2013) and/ or sucrose (Bryans *et al.,* 2007; Gruendel *et al.,* 2007; Johnston *et al.,* 2002; Johnston *et al.,* 2003; Ochiai *et al.,* 2014; Törrönen *et al.,* 2010; Törrönen *et al.,* 2012a; Törrönen *et al.,* 2012b). Polyphenols may alter the postprandial GR in numerous ways, for example by increasing the overall glycaemic profile (GP). The GP was a term proposed by Rosen *et al.* (2009) with a high value representing a facilitated postprandial GR with a lower peak and a reduction in late stage hypoglycaemia. Therefore, any food or beverage that can prolong carbohydrate digestion thus reducing the rate of glucose absorption into the blood will have a high GP and a favorable effect on glycaemic parameters.

The mechanisms behind blood glucose and insulin reduction have been more extensively studied by *in vitro* methods. The molecular structure of specific polyphenols allows them to interfere with starch digestion at the intestinal level and therefore they can reduce and/ or prolong glucose absorption into the blood (Chai *et al.,* 2013). Polyphenols have been shown to inhibit digestive enzymes thus preventing enzyme attack on starch and sucrose chains, reducing the amount of free glucose released (Kwon *et al.,* 2008; Sapwarobol *et al.,* 2012). Polyphenols can also reduce glucose transport into the blood via the inhibition of specific glucose transporters in the intestinal lumen (Alzaid *et al.,* 2013).

However, there are issues when considering the combination of polyphenol and carbohydrate rich foods in an aim to reduce markers of postprandial glycaemia. For example, the optimal doses required to produce significant health benefits, the easy degradation of polyphenol compounds by such factors as light and heat, and the adverse effects of high polyphenol consumption, are all factors that need to be considered (Landete, 2012; Skibola & Smith, 2000). Polyphenols can be consumed alongside other foods or can be used as a functional ingredient by being added into foods, and therefore the form in which polyphenols are ingested may have different effects on glycaemia (Forester *et al.,* 2012; Liu *et al.,* 2011). Also, the effect of polyphenols on carbohydrate digestion may be influenced by factors such as the disease status of the person and/ or other metabolic parameters.

Considering the results from the variety of studies which have been performed to date, elucidation of the role of polyphenols in carbohydrate digestion is necessary in order to develop food products and/ or meal combinations for improving the GP in both healthy subjects and for people with type 2 diabetes. The aim of this systematic review was to assess studies which determined the acute, postprandial GR and IR after the consumption of a polyphenol rich source in combination with carbohydrate.

6.2 Methods

6.2.1 Data extraction

The databases Medline, CINHAL and Web of Knowledge were searched for studies in English language between 1970 and 2014 comprising of all human participants. A combination of MeSH search terms were used (flavan* or flavon* or isoflav* or EGCG or catechin or epicatechin or anthocyani* or cyanidin or procyani* or tannin or polypheno* or berry or fruit or resveratrol or stilbene or extract or phytochemical AND blood glucose or diabet* or glyc?mic response or glyc?mic index or insulin or glyc?mia or glucose tolerance or insulin sensitivity AND starch or sucrose or sugar or glucose or maltose or carbohydrate or amylose or starch digesti* AND {Medline and Web of Science only} human or subject or volunteer or participant or adult).

6.2.2 Inclusion of studies

Two independent investigators reviewed studies using a systematic hierarchy of exclusion criteria as shown in Figure 6.1. Papers were excluded based on titles if there was no mention of polyphenols or a potential polyphenol rich source, no mention of any type of metabolic outcomes (lipaemia, glycaemia etc.), cell, *in vitro* or animal mentioned in title, review or epidemiological study apparent from title or obvious from title that there was no starch or sugar source. A total of 873 abstracts were included for review. Of these, inclusion criteria of papers for further analysis were randomised controlled trials in all adult humans, an outcome of acute postprandial GR and/ or IR and abstracts with insufficient data. Papers were excluded if exercise was included as part of the intervention, there was no control group, the polyphenol source was debatable or not confirmed, there was >30 min between polyphenol intake and carbohydrate intake, GI studies, whole meals were used instead of a plain carbohydrate source, fasting blood glucose or long term measures of glycaemia/ insulinaemia and abstracts with insufficient data if no full paper

was available. Sixty eight full papers were put forward for further review based on the same guidelines for exclusion of abstracts. Further

exclusion criteria when reviewing full papers included no starch source or not an appropriate starch source and no mention or quantifying of

polyphenols in paper (methods; unless foods or beverages well known to be rich in polyphenols). A total of 13 papers fit all criteria for the review

and extracted data are shown in Table 6.1.

Figure 6.1 Flow chart of methodology used for identifying studies included in the systematic review.
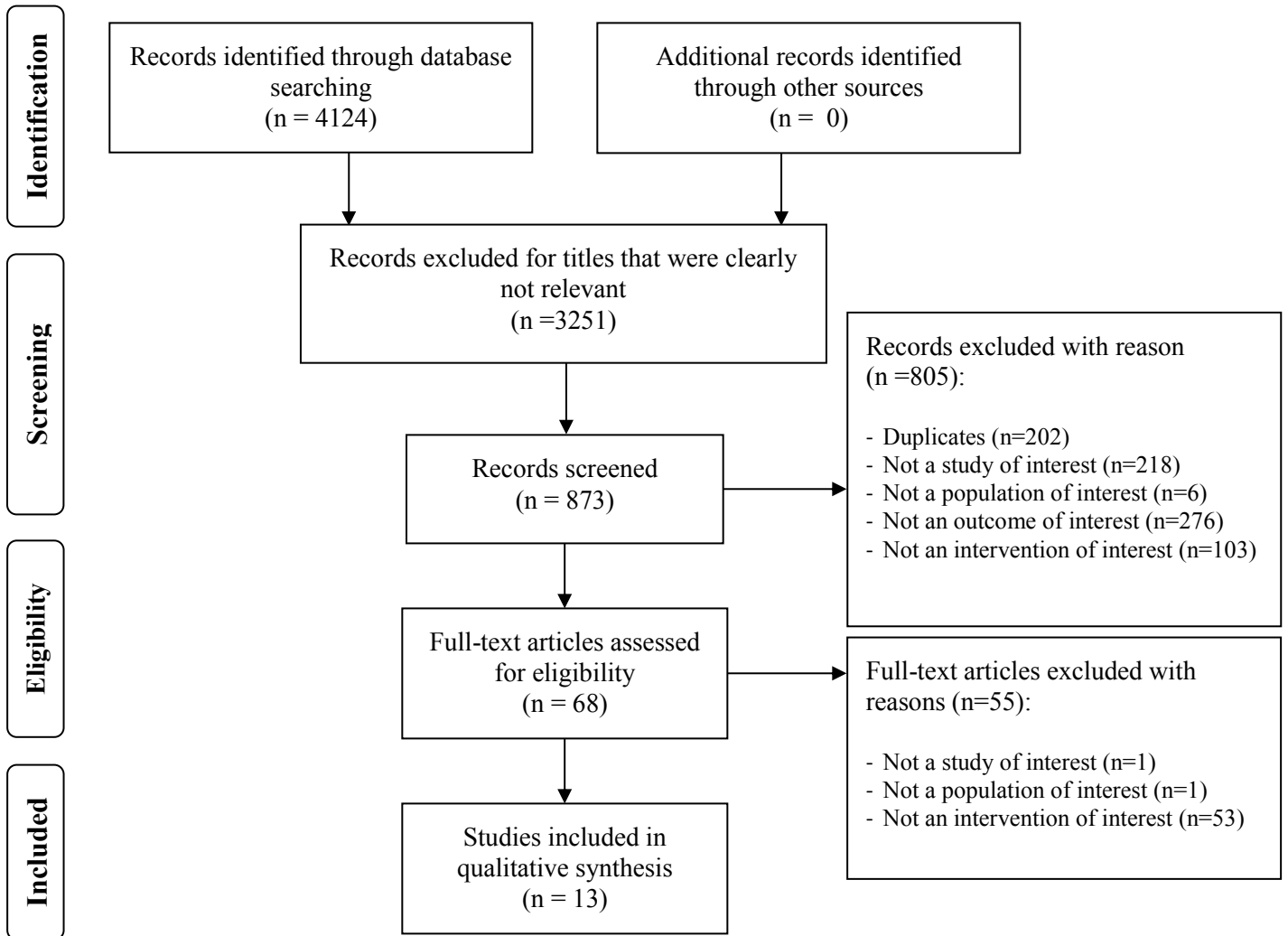
Table 6.1 Characteristics of 13 studies included for review

Reference	Subject characteristics	Intervention	Control	Polyphenol source and dose
Balisteiro *et al.* 2013	23 healthy adults (17 female) 29±6 y, 23.7±2.9 kg/m^2	White bread (25g avCHO) and 300mL clarified aracá juice	White bread (25g avCHO) and 300mL water	Aracá juice containing 1.48g proanthocyanidins TQE and 546mg total phenolics CE/ 300mL juice
Bryans *et al.* 2007	16 healthy adults (12 female) 35.5±1.5 y, 23.8±.7 kg/m^2	1g instant black tea w/ 75g glucose in 250mL water	75g glucose w/ .052g caffeine in 250mL water (+ con) 75g glucose in 250mL water (con)	Black tea containing 39 mg/g flavanol-3-ol, 21 mg/g theaflavins and 350 mg/g total polyphenols
Clegg *et al.* 2011	12 healthy adults (9 female) 33±13 y	Pancakes w/ 100g raspberries (50g in/ 50g additional) and 200mLwater Pancakes w/ 100g blueberries (50/ 50g) and 200mL water	Pancakes w/ 2.65g glucose/ 2.88g fructose and 200mL water Pancakes w/ 4.88g glucose/ 4.95g fructose and 200 mL water	100g raspberries or blueberries
Coe *et al.* 2013	9 healthy adult females 25.3±4.8 y, 22.3±2.6 kg/m^2	18.5g baobab fruit extract in 250mL water and 123g white bread 37g baobab fruit in 250mL water and 144g white bread	132g white bread and 250mL water	Baobab fruit extract containing 28.85±.47mg GAE/g total polyphenols
Gruendel *et al.* 2007	20 healthy adults (12 female) 29.4±2.6 y, 23±.5 kg/m^2	5, 10 or 20 g carob fibre w/ 50g glucose in 200mL water	50g glucose in 200mL water	Carob containing 2.8g/100g total polyphenols including gallic acid, gallotannins and flavonol glycosides
Johnston *et al.* 2002	9 healthy adults (5 female) 24±3.2 y	Clear apple juice 400mL (total of 25g glucose/ 30.7g fructose) Cloudy apple juice 400mL (total of 25g glucose/ 30.7g fructose)	Total of 25g glucose/ 30.7g fructose in 400mL water	Clear apple juice w/ phloridzin 5.7-11.9μg/mL and chlorogenic acid 35.1-68.6μg/mL Cloudy apple juice with phloridzin 4.1-27.3μg/mL and chlorogenic acid 79.1-255.6μg/mL Low amounts of phloretin xyloglucoside and catechin
Johnston *et al.* 2003	9 healthy adults (5 female) 26±3.2 y	400 mL caffeinated coffee w/ 25g glucose 400 mL decaffeinated coffee w/ 25g glucose	25g glucose in 400mL water	Coffee containing 2.5mmol/L cholorogenic acid

Reference	Results	Evidence direction	Quality score	Level of significance	Outcomes
Balisteiro *et al.* 2013	Aracà juice reduced maximum GR and total GR-AUC vs con	+	+	*p*<0.05	GR
Bryans *et al.* 2007	1. Tea reduced 120min GR vs con and caffeine 2. Tea increased 90min IR vs con and caffeine 3. Tea increased 150min IR vs caffeine 4. Tea reduced 30[a] and 120min[b] IR vs caffeine	+	+	1. *p*<0.01 2. *p*<0.01 3. *p*<0.05 4. *p*<0.01[a], *p*<0.05[b]	GR and IR
Clegg *et al.* 2011	No difference	-	ø	*p*<0.05	GR
Coe *et al.* 2013	37g baobab fruit reduced 60 and 120min GR-AUC vs con 18.5g baobab fruit reduced 120 and 180min GR-AUC vs con	+	+	*p*<0.05	GR
Gruendel *et al.* 2007	5 and 10g carob increased total GR vs con 5 and 10g carob increased total IR vs con	-	ø	*p*<0.001	GR and IR
Johnston *et al.* 2002	1. Clear apple juice reduced 15[a] and 30min[b] GR vs con 2. Cloudy apple juice reduced 15min GR vs con 3. Cloudy apple juice increased 45[a] and 60min[b] GR vs con 4. Clear[a] and cloudy[b] juice reduced GR-AUC 0-30min vs con 5. Cloudy reduced GR-AUC 30-90min vs con 6. Clear and cloudy juice reduced IR-AUC 0-90min vs con	+	+	1. *p*<0.0001[a], *p*<0.05[b] 2. *p*<0.001 3. *p*<0.05[a], *p*<0.005[b] 4. *p*<0.01[a], *p*<0.005[b] 5. *p*<0.01 6. *p*<0.05	GR and IR
Johnston *et al.* 2003	Caffeine coffee increased 0-30min GR-AUC vs non-caffeine and con Caffeine coffee increased 0-30min IR-AUC vs non-caffeine	-	+	*p*<0.05	GR and IR

avCHO, available carbohydrate; TQE, tannin equivalents; CE, catechin equivalents; GAE, gallic acid equivalents; GR, glycaemic response; AUC, area under the curve; IR, insulin response; GP, glycaemic profile; con, control.

Reference	Subject characteristics	Intervention	Control	Polyphenol source and dose
Ochiai *et al.* 2014	14 healthy adult males, 36.2±7.8 y, 22.7±1.8 kg/m^2, non-smokers	75g glucose w/ coffee polyphenols in 225mL water	75g glucose in 225mL water	Chlorogenic acid 600mg
Rosen *et al.* 2011	14 healthy adults (7 female) 23.6±.5 y, 22±.5 kg/m^2	Whole grain rye breads (50g avCHO): *Amilo, Nikita, D. Zlote, H. Loire, Rekrut* and all w/ 250mL tap water	White wheat bread (50g avCHO) and 250mL water	Various
Törrönen *et al.* 2010	12 healthy adults (11 female) 54.2 ±15.1 y, 25.4±2.9 kg/m^2	150g berry purée and sucrose 35g (natural sugar content 4.5g/p glucose and 5.1g/p fructose) in 120mL tap water	Sucrose 35g and 4.5g glucose/5.1g fructose in 250mL water	37.5g total blackcurrants, bilberries, cranberries and strawberries
Törrönen *et al.* 2012a	20 healthy adult females, 57±10 y, 24.6±2.4 kg/m^2	1. 150g berry purée and sucrose 35g in 150mL water	Sucrose 35g in 300mL water	150g blackcurrants or lingonberries
		2. 150g fresh berry nectars and sucrose 35g in 300mL water		
Törrönen *et al.* 2012b	12 healthy adults (10 female) 58±11 y, 24.3±2.2 kg/m^2	150g berry purée w/ sucrose 35g (natural sugar content 4.4g/p glucose and 4.7g/p fructose) in 120mL tap water	Sucrose 35g and 4.4g glucose/4.7g fructose in 250mL tap water	37.5g blackcurrants, bilberries, cranberries and strawberries
Törrönen *et al.* 2013	1. 15 healthy adult females, 48±14 y, 24.4±2.7 kg/m^2	1. Wheat bread and 150g whole berry purée and 200mL water	1. Wheat bread and 50g cucumber and 300mL water	1. 150g strawberries, bilberries or lingonberries
	2. 13 healthy adult females, 50±12 y, 24.2±3.2 kg/m^2	2. Wheat bread and 150g whole berry purée and 200 mL water	2. Wheat bread and 50g cucumber and 300mL water	2. 150g raspberries, cloudberries or chokeberries
	3. 20 healthy adult females, 57±12 y, 24.2±2 kg/m^2	3. Wheat bread or rye bread and a mix of berries and water	3. Wheat or rye bread and 50g cucumber and 300mL water	3. 150g total strawberries, bilberries, cranberries and blackcurrants
		(all w/out crust; 50g avCHO)	(all w/out crust; 50g avCHO)	

Reference	Results	Evidence direction	Quality score	Level of significance	Outcomes
Ochiai *et al*. 2014	No difference	-	ø	*p*<0.05	GR and IR
Rosen *et al*. 2011	*Amilo* reduced IR-AUC 0-60min vs con, *D Zlote* and *Nikita*	+	+	*p*<0.05	GR and IR
	Amilo and *Rekrut* reduced IR 60-120min vs con and *H Loire*				
	Rekrut reduced IR 120-180min vs *H Loire*				
	Amilo reduced peak IR vs all rye breads except *Rekrut*				
	Rekrut reduced GR-AUC 60-120min vs con				
	Correlation between total polyphenols and GR 0-60min				
Törrönen *et al*. 2010	1. Berries reduced 15[a] and 30min[b] GR vs con	+	ø	1. *p*<0.05[a], *p*<0.01[b]	GR
	2. Berries increased 150min GR vs con			2. *p*<0.05	
	3. Berries reduced maximum GR vs con			3. *p*=0.002	
	4. Berries (non-sig) reduced total GR-AUC vs con			4. *p*=0.29	
Törrönen *et al*. 2012a	*Purée*	+	ø	*Purée*	GR and IR
	1. Berries reduced GR 15min vs con			1. *p*<0.01	
	2. Blackcurrants reduced GR 30min vs con			2. *p*<0.05	
	3. Berries increased GR 60 and 90 min vs con			3. *p*<0.001	
	4. Lingonberries reduced GR120min vs con			4. *p*<0.001	
	5. Blackcurrants reduced maximum GR vs con			5. *p*=0.022	
	6. Berries increased GP vs con			6. *p*<0.001	
	7. Berries reduced IR 15min vs con			7. *p*<0.001	
	8. Blackcurrants[a] and lingonberries[b] reduced IR 30min vs con			8. *p<0.001[a]*, *p*<0.05[b]	
	9. Blackcurrants[a] and lingonberries[b] increased IR 60min vs con			9. *p*<0.001[a], *p*<0.01[b]	
	10. Berries increased IR 90min vs con			10. *p*<0.001	
	11. Blackcurrants[a] and lingonberries[b] increased IR120min vs con			11. *p*<0.05[a], *p*<0.001[b]	
	12. Blackcurrants[a] and lingonberries[b] reduced maximum IR vs con			12. *p*=0.052[a], *p*=0.03[b]	
	13. Blackcurrants[a] and lingonberries[b] increased IR-AUC vs con			13. *p* =0.002[a], *p*=0.004[b]	
	Nectars			*Nectars*	
	1. Blackcurrants reduced GR 0-45min vs con			1. *p*<0.05	
	2. Blackcurrants increased GR 90min vs con			2. *p<0.05*	
	3. Blackcurrants reduced maximum GR vs con			3. *p*<0.001	
	4. Blackcurrants reduced GR-AUC vs con			4. *p*=0.03	
	5. Lingonberries increased GR 60-120min vs con			5. *p*<0.05	
	6. Berries increased GP vs con			6. *p*<0.001	

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	7. Blackcurrants reduced IR 30min vs con			7. $p<0.05$	
	8. Blackcurrants increased IR 60 and 90min vs con			8. $p<0.01$	
	9. Lingonberries increased IR 90 min vs con			9. $p<0.001$	
	10. Blackcurrants[a] and lingonberries[b] increased IR-AUC vs con			10. $p=0.03$[a], $p=0.036$[b]	
Törrönen et al. 2012b	1. Berries reduced capillary[a] and venous[b] GR 15min vs con	+	ø	1. $p=0.021$[a], $p<0.007$[b]	GR and IR
	2. Berries reduced IR 15min vs con			2. $p=0.028$	
	3. Berries increased capillary[a] and venous[b] GR 90min vs con			3. $p=0.028$[a], $p=0.021$	
	4. Berries reduced maximum capillary[a] and venous[b] GR			4. $p<0.009$[a], $p<0.011$[b]	
	5. Berries reduced maximum IR			5. $p<0.005$	
	6. Berries improved capillary[a] and venous[b] GP vs con			6. $p<0.001$[a], $p=0.003$[b]	
	7. Berries increased IR 120min vs con			7. $p=0.042$	
Törrönen et al. 2013	1. Strawberries reduced maximum IR vs wheat con	+	ø	1. $p<0.05$	GR and IR
	2. Strawberries reduced IR-AUC 0-60min vs wheat con			2. $p<0.01$	
	3. Strawberries increased GP vs wheat con			3. $p<0.05$	
	4. Bilberries and lingonberries reduced IR-AUC 0-60 vs wheat con			4. $p<0.05$	
	5. Bilberries and lingonberries reduced IR-AUC 0-30min vs wheat con			5. $p<0.01$	
	6. Chokeberries reduced IR-AUC 0-60 vs wheat con			6. $p<0.05$	
	7. Chokeberries reduced IR-AUC 0-30min vs wheat con			7. $p<0.001$	
	8. Berry mix reduced GR-AUC 0-30min vs wheat control			8. $p<0.001$	
	9. Berry mix increased GP vs wheat con			9. $p=0.005$	
	10. Berry mix reduced IR 15 and 30min vs wheat con			10. $p<0.05$	
	11. Berry mix increased IR 120min vs wheat con			11. $p<0.05$	
	12. Berry mix reduced maximum IR vs wheat con			12. $p=0.001$	
	13. Berry mix reduced IR-AUC 0-120[a], 0-60[b] and 0-30min[b] vs wheat con			13. $p=0.041$[a], $p<0.001$[b]	
	14. Berry mix reduced GR-AUC 0-30min vs rye con			14. $p=0.026$	
	15. Berry mix increased GP vs rye con			15. $p=0.05$	
	16. Berry mix reduced IR 15 and 30min vs rye con			16. $p<0.05$	
	17. Berry mix increased 120min vs rye con			17. $p<0.05$	
	18. Berry mix reduced maximum IR vs rye con			18. $p<0.001$	
	19. Berry mix reduced IR-AUC 0-120[a], 0-60[b] and 0-30min[b] vs rye con			19. $p=0.03$[a], $p<0.001$[b]	

6.2.3 Quality assessment

Quality of the 13 final papers was assessed by the quality criteria checklist: primary research from the Academy of Nutrition and Dietetics (2014). Papers were allocated a quality rating of negative, neutral or positive based on a series of questions including subject characteristics, study bias, detail of intervention, clearly defined outcome measures and statistical analysis.

6.3 Results

6.3.1 General characteristics

A total of 13 papers fulfilled all inclusion criteria, totalling 218 adults (165 female and 53 male) with a mean age of 39.08 ± 7.78 years. All studies were cross-over trials in which subjects acted as their own control. Search criteria included all types of human adults however the final papers consisted of healthy adults only. Body mass index (BMI) was not available for three of the studies (Clegg *et al.,* 2011; Johnston *et al.,* 2002; Johnston *et al.,* 2003), and therefore mean BMI for the remaining 10 studies was 23.72 ± 2.03 kg/m^2.

Papers consisted of research conducted in Japan (Ochiai *et al.,* 2014), the United Kingdom (Bryans *et al.,* 2007; Clegg *et al.,* 2011; Coe *et al.,* 2013; Johnston *et al.,* 2002; Johnston *et al.,* 2003), Finland (Törrönen *et al.,* 2010; Törrönen *et al.,* 2012a; Törrönen *et al.,* 2012b; Törrönen *et al.,* 2013), Sweden (Rosen *et al.,* 2011), Germany (Gruendel *et al.,* 2007) and Brazil (Balisteiro *et al.,* 2013). Two studies had no reference to funding bodies (Gruendel *et al.,* 2007; Johnston *et al.,* 2002), and there was no conflict of interest for six of the studies (Clegg *et al.,* 2011; Johnston *et al.,* 2003; Ochiai *et al.,* 2014; Törrönen *et al.,* 2010; Törrönen *et al.,* 2012a; Törrönen *et al.,* 2012b) and no answer on conflict for the other seven. All but one study declared subject randomisation to test meals (Balisteiro *et al.,* 2013). Five studies used HPLC methods to assess individual polyphenols (Balisteiro *et al.,* 2013; Bryans *et al.,* 2007; Johnston *et al.,* 2002; Johnston *et al.,* 2003; Ochiai *et al.,* 2014) and three used the FCR method to assess total polyphenol content (Balisteiro *et al.,* 2013; Bryans *et al.,* 2007; Coe *et al.,* 2013). Wash out periods between control and interventions ranged from one day to one week, although four studies did not report the wash out period (Bryans *et al.,* 2007; Coe *et al.,* 2013; Johnston *et al.,* 2002; Johnston *et al.,* 2003).

6.3.2 Paper quality and outcomes

Papers were assigned a quality rating with six papers found to be positive (Balisteiro *et al.,* 2013; Bryans *et al.,* 2007; Coe *et al.,* 2013; Johnston *et al.,* 2002; Johnston *et al.,* 2003; Rosen *et al.,* 2011;) and seven neutral (Clegg *et al.,* 2011; Gruendel *et al.,* 2007; Ochiai *et al.,* 2014, Törrönen *et al.,* 2010; Törrönen *et al.,* 2012a; Törrönen *et al.,* 2012b; Törrönen *et al.,* 2013). No studies received a negative rating. Nine of the 13 studies had evidence in the positive direction (Balisteiro *et al.,* 2013; Bryans *et al.,* 2007; Coe *et al.,* 2013; Johnston *et al.,* 2002; Rosen *et al.,* 2011; Törrönen *et al.,* 2010; Törrönen *et al.,* 2012a; Törrönen *et al.,* 2012b; Törrönen *et al.,* 2013) and four in the negative direction when considering the effect of polyphenols on carbohydrate digestion and resulting postprandial glycaemia and/ or insulinaemia. Eleven of the studies showed significant results in either direction, with only two studies showing non-significance (Clegg *et al.,* 2011; Ochiai *et al.,* 2014).

All studies measured GR as either the primary or secondary outcome, and postprandial IR was also measured in nine of these (Bryans *et al.,* 2007; Johnston *et al.,* 2002; Johnston *et al.,* 2003; Ochiai *et al.,* 2014; Rosen *et al.,* 2011; Törrönen *et al.,* 2012a; Törrönen *et al.,* 2012b; Törrönen *et al.,* 2013). Only one study had GR and IR as the secondary outcome (Ochiai *et al.,* 2014, the primary outcome was endothelial function). Postprandial GR and IR were measured for between two to three hours after the initial consumption of the test food in all studies. Apart from one study which only measured GR and IR at baseline, one and two hours postprandially (Ochiai *et al.,* 2014), all other studies took measurements at baseline, and every 15 min for the first hour, then every 30 min for the remaining one to two hours.

6.3.3 Polyphenols as solutions

Two studies in this review used coffee and/ or its polyphenols. Both studies found no effect of coffee polyphenols (especially chlorogenic acids) on GR or IR when consumed with 25g (Johnston *et al.,* 2003) or 75g (Ochiai *et al.,* 2014) of glucose. In one of the studies (Ochiai *et al.,* 2014), GR and IR were only measured at baseline and at one and two hours postprandial and therefore these measurements may not have been frequent enough to show an effect. Caffeine was controlled for in both studies. Two different studies determined the effect of fruit juice consumed with a carbohydrate source on markers of glycaemia (Balisteiro *et al.,* 2013; Johnston *et al.,* 2002). In one study, 400 mL of clear or cloudy apple juice were assessed for their effects on GR and IR, the cloudy juice being richer in polyphenols (Johnston *et al.,* 2002). Fructose and glucose were added into the control water to match the sugar content of all juices. Early phase GR was reduced in both apple juices, with this reduction being greater in the polyphenol rich cloudy apple juice. In the second study, 300 mL of aracà juice was found to reduce maximal GR and total AUC-GR to white bread, however IR was not measured (Balisteiro *et al.,* 2013). Both the control and intervention were matched with 25g avCHO

(approximately 50g in weight) of white bread, however the juice provided additional carbohydrate in the form of sugars compared to the water control (Balisteiro *et al.,* 2013).

Only one study determined the effect of black tea on both GR and IR and it was found that at a dose of 1g, black tea significantly reduced 120 min GR, with variable effects on IR (Bryans *et al.,* 2007). Two studies in this review investigated the effect of polyphenol rich extracts in aqueous solution on GR. Baobab fruit extract at two doses of 18.5g and 37g was made up in solution with both doses found to have beneficial effects on mid-to-late phase GR when consumed with white bread (Coe *et al.,* 2013). However, carob pulp fibre at 5 and 10 g was shown to increase GR and also IR when consumed with 50g glucose in solution (Gruendel *et al.,* 2007). The avCHO in the baobab fruit drinks was matched between meals by reducing the bread content in the intervention meals (Coe *et al.,* 2013) whereas avCHO was not matched when consuming the carob pulp and therefore the higher the carob dose in solution, the greater the avCHO content of the drink (Gruendel *et al.,* 2007).

6.3.4 Food sources of polyphenols

Five studies in this review assessed the effects of different berry combinations on GR and IR. Törrönen and colleagues (Törrönen *et al.,* 2010; Törrönen *et al.,* 2012a; Törrönen *et al.,* 2012b; Törrönen *et al.,* 2013) performed four of these studies. Berries were found to reduce both the early phase and the maximal increase in GR to sucrose (Törrönen *et al.,* 2010). Berries in the form of purées and nectars both reduced the early phase yet increased the late phase GR and IR, with an overall increase in the GP when consumed with sucrose (Törrönen *et al.,* 2012a). Similar results were found in a later study by the same group (Törrönen *et al.,* 2012b) which found berries consumed with sucrose to again reduce early phase GR and IR, increase the late phase response and improve overall GP. The effect of berries on white wheat bread and rye bread was determined in three smaller studies (Törrönen *et al.,* 2013). Results found berries to reduce early to mid-phase GR and IR and slightly increase late phase IR to both breads. The only other study to date on berries consumed with a starch source and resulting effects on GR determined the effect of consuming berries in combination with pancakes, yet found no effect on GR (Clegg *et al.,* 2011).

Three of the studies by Törrönen (2010; 2012a; 2012b) assessed the effect of berries in combination with sucrose, of which two were matched for avCHO. Therefore one study provided additional sugars in the intervention yet still had favorable effects on glycaemia (Törrönen *et al.,* 2012a). Although lingonberries did not show beneficial effects on GR or IR, the intervention consisted of additional avCHO compared to the control solution and therefore lingonberries did not show negative effects on GR (Törrönen *et al.,* 2012a). When the carbohydrate source was

bread or pancakes consumed with berries, both studies matched avCHO for the starch source, however in one of the studies cucumber was consumed alongside the bread in the control group (Törrönen *et al.,* 2013). Therefore less avCHO was consumed in the control, yet favorable effects were still seen on both GR and IR for the berry intervention meal.   Due to the distinct sensory and physical properties of berries subject blinding to the intervention in all studies was not possible. It is important for the nutrients and other compounds between the meals to be as similar as possible to reduce confounding factors that may influence metabolism. Törrönen *et al*. (2013) used control meals containing less avCHO in the form of cucumber, and therefore meals were not closely matched for some compounds such as micronutrients. This adds some variability into the study and the reliability of the results may be altered.

Another study measured different rye breads for their polyphenol contents and all breads were found to be a rich source of a variety of compounds. Polyphenol rich rye breads were shown to significantly reduce the IR, especially the Amilo and Rekrut breads (Rosen *et al.,* 2011). Amilo significantly reduced early phase IR, Amilo and Rekrut reduced mid-phase IR and Rekrut reduced late phase IR and mid-phase GR, with all breads matched for avCHO.

6.3.5 Adverse effects

Adverse effects of consuming polyphenols and carbohydrate in combination were seen in some of the studies. Carob pulp at low doses increased the GR and IR compared to the control (Ochiai *et al.,* 2014). Cloudy apple juice increased the GR at certain time points versus the control, however overall GR was improved (Johnston *et al.,* 2002). All studies by Törrönen *et al.* (2010; 2012a; 2012b; 2013) found berries to increase the late phase GR and/ or IR, despite reducing both in the early stages after consumption. There was an increase not only in the IR at specific time points after tea consumption compared to the control, yet the 3g dose of black tea induced vomiting and palpitations, and therefore these data were excluded from the results (Bryans *et al.,* 2007).

6.4 Discussion and Conclusions

Polyphenol rich foods and beverages are well known for their potential health benefits, including their role in improving glycaemic control and in managing obesity. They show potential for reducing the postprandial GR and therefore for preventing the secondary disease measures associated

with elevated blood glucose levels. This review assessed the effect of polyphenol consumption with carbohydrates, and the resulting effect on two to three hour postprandial glycaemia and insulinaemia. Of the six studies that had a positive quality rating, five showed favorable effects on GR and/ or IR. Polyphenol sources in combination with sucrose, glucose or bread overall were found to reduce the early phase GR and IR (0-60 min), and prolong the rate of glucose absorption into the blood, thereby prolonging and sustaining insulin secretion.

6.4.1 Polyphenols as solutions

Coffee

Coffee consumption has been shown to have a protective effect against developing type 2 diabetes. In a prospective study of 28,812 postmenopausal women, coffee intake and especially decaffeinated coffee had a negative correlation with risk of type 2 diabetes (Pereira *et al.*, 2006). Coffee is rich in phenolic compounds such as the chlorogenic acids, with an average cup providing around 1-1.25 mmol of total CQA L^{-1} (20-657 mg CQA; Clifford, 1999). Chlorogenic acid and its components caffeic acid and quinic acid which are also found in coffee, have been shown to inhibit digestive enzymes such as porcine pancreas α-amylase (Narita & Inouye, 2009).

Two studies in this review investigated the effects of coffee on GR and IR, yet both studies found no effect of coffee and its polyphenols on either measure when consumed with glucose in solution (Ochiai *et al.*, 2014). These results may be partially due to the polyphenols in foods and beverages having synergistic effects with other components and therefore extracting these compounds and adding them into another medium such as was done with the isolated coffee polyphenols (Ochiai *et al.*, 2014), may reduce their bioactivity (Bastianetto *et al.*, 2000; Betts *et al.*, 2011).

Fruit juice

Sugar sweetened beverages can have adverse effects on blood glucose levels, however fruit juices rich in polyphenols show conflicting results. In a recent systematic review the consumption of soft drinks, 100% fruit juice and vegetable juice were assessed for effects on diabetes risk (Eshak *et al.*, 2013). Soft drink consumption was associated with an increased risk of type 2 diabetes, yet fruit juice and vegetable juices showed no association. In the current review, polyphenol rich fruit juice was shown to reduce the GR when matched for sugar content (Johnston *et al.*, 2002)

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and reduced both the GR and IR when consumed with white bread (Balisteiro *et al.,* 2013). Fruit juices are rich in polyphenols, specifically apple juice was found to be rich in phloridzin and chlorogenic acid and therefore these phenolics may responsible for the reduction in GR and IR (Johnston *et al.,* 2002).   The effect of polyphenols on digestive enzymes and/ or on overall carbohydrate digestion will differ depending on if sucrose or starch is the carbohydrate consumed however, studies on juices found favorable effects on GR regardless of whether the juice was consumed with simple sugars or with starch in the form of white bread.

Black tea

Tea polyphenols have been shown to inhibit intestinal glucose transport (Shimizu *et al.,* 2000) and increase insulin secretion *in vitro* (Hii & Howell, 1985). Uchida *et al.* (2013) found different varieties of black teas to inhibit α-glucosidase activity, and total polyphenol content of the teas was positively related to inhibitory activity. One study in this review assessed the effect of 1.5 cups black tea containing 350 mg/ g total polyphenols and 75g glucose, against a caffeine control and standard control (Bryans *et al.,* 2007). This study found a reduction in late stage glycaemia after tea consumption. The effects on late but not early phase GR and IR could be due to the delay in absorption of certain tea polyphenols (Leenen *et al.,* 2000).

Polyphenol extracts in solution

The polyphenol rich baobab fruit extract made up in solution, showed benefits for reducing GR when consumed alongside starch, yet carob pulp in solution exacerbated GR and IR when consumed with sucrose (Coe *et al.,* 2013, Gruendel *et al.,* 2007). Plant extracts have been shown to inhibit α-glucosidase, with certain extracts showing more potential for inhibiting α-amylase, and therefore extracts may be more efficient at reducing GR when consumed with a starch source compared to simple sugars (Bhat *et al.,* 2011). Extracts from fruits, and also different tea and coffee drinks will vary in composition depending on extraction and preparation techniques. Both extracts in this review were crude and therefore contained other nutrients such as fibre, vitamins and minerals, all of which could have influenced postprandial blood glucose levels. Fibre can delay digestion and therefore reduce GR (Marlett *et al.,* 2002), however both the insoluble and soluble fibre content were moderate in the solution drinks, and therefore only the higher doses of extracts consumed probably contained enough fibre to influence GR.

Carob pulp produced a greater GR than the control which may be partially due to the extra carbohydrate in the test meals versus the control. Some of the studies in this review matched avCHO between the control and intervention meals, either by reducing the amount of the carbohydrate source when adding the polyphenol source, or by the addition of sucrose, glucose or fructose to the control. However, other studies did not match the avCHO of the meals and therefore the intervention meals in these studies contained a higher amount of carbohydrate than the controls. In the latter, any reduction in GR or IR will be of greater importance considering the greater amount of avCHO in the intervention meals. In contrast to 5 and 10 g, 20g of carob extract did not produce a difference in GR compared to the control. Also, baobab fruit extract was consumed at doses of 18.5g and 37g in solution, and therefore it may be that the consumption of a larger dose of polyphenol rich extract is needed in order to have beneficial effects on GR.

6.4.2 Polyphenols consumed as whole foods

Berries

Berries are a well-known rich source of a variety of health promoting compounds including polyphenols. In the study by Clegg *et al.* (2011), no effect on GR was found when berries were consumed with pancakes. Compared to sucrose, glucose and white bread which are considered high GI foods, pancakes may contain a lower GI value and thus induce a lower postprandial GR and resulting IR. Berry addition to a carbohydrate source such as pancakes may therefore show no further improvement in the degree of degradation. Also, only 100g of berries were used whereas in the studies by Törrönen *et al.* (2010, 2012a, 2012b, 2013) 150g were consumed, and therefore a higher dose may be required to show effects on GR. Furthermore, IR was not measured in this study and therefore even though no effects were seen on GR, improvements in insulin sensitivity may have been found.

The four studies in this review by Törrönen and others (2010, 2012a, 2012b, 2013) showed positive results on GR and IR. When berries were consumed with sucrose, the GR in all studies was consistently shown to be reduced (Törrönen *et al.,* 2010; Törrönen *et al.,* 2012a; Törrönen *et al.,* 2012b). When consumed with a starch source the reduction in IR was more prevalent, with the reduction in GR still significant yet less apparent (Törrönen *et al.,* 2013). This may be due to the different structure of glucose versus bread, with the berries affecting the overall digestion and absorption to different degrees. Different types of berries will vary in their polyphenol contents as well as in their ability to inhibit digestive

enzymes. α-Glucosidase is the main enzyme for digesting sucrose while α-amylase acts on starch chains. Polyphenol rich extracts of blackcurrants, blueberries and strawberries have been shown to inhibit α-glucosidase *in vitro* (Cheplick *et al.,* 2007; Cheplick *et al.,* 2010; da Silva Pinto *et al.,* 2008; da Silva Pinto *et al.,* 2010; Johnston *et al.,* 2011; McDougall *et al.,* 2005). α-Amylase is thought to be inhibited by a variety of fruit polyphenols (Grussu *et al.,* 2011; McDougall *et al.,* 2005), yet raspberry extracts have been shown to be good inhibitors of α-glucosidase but not α-amylase (Zhang *et al.,* 2010). The intestinal transporters SGLT1 and facilitated sodium-independent GLUT2 have also been shown to be inhibited by a variety of the polyphenols found in berries (Cermak *et al.,* 2004, Johnston *et al.,* 2005; Manzano *et al.,* 2010; Song *et al.,* 2002; Welsch *et al.,* 1989). Berries inhibit α-glucosidase mainly due to their anthocyanin and proanthocyanidin contents (Hanhineva *et al.,* 2010). Anthocyanins are high in bilberries and blackcurrants as represented by their dark color (McDougall *et al.,* 2005) and the anthocyanin cyanidin-3-rutinoside is abundant in blackcurrants yet not in lingonberries (Slimestad *et al.,* 2002), and has been found to inhibit α-glucosidase activity *in vitro* (Adisakwattana *et al.,* 2011). This may be why lingonberries did not show beneficial effects on GR when consumed with sucrose (Törrönen *et al.,* 2012a).

The physical form of different berry combinations will affect resulting GR and IR. All berry meals assessed in this review were consumed in semi-solid form, either as whole berry purées or nectars, and therefore they may have delayed gastric emptying compared to the control beverage (Dikeman & Fahey, 2006). Whole berries will contain additional components including fibre, and vitamins and minerals and although juices and nectars will not contain fibre, they will also contain micronutrients and other compounds. In one study by Törrönen *et al.* (2013), GR and IR were only measured for two hours postprandial when bread was used as the carbohydrate source, however the fibre content of the breads may have prolonged the GR past this time period. Raspberries and cloudberries were found to have the highest fibre content in this study yet these berries had no effect on GR or IR, and therefore a further hour of testing may have provided more information about the GR to the breads. At equivalent avCHO profiles, the berry purée contained more soluble fibre compared to the nectar meals, and the nectar meals contained more than the control, however both test meals reduced GR and IR (Törrönen *et al.,* 2012a). Therefore it seems that the polyphenols and not solely the fibre content of berries may be responsible for the improvement in GR and IR. Overall all berry sources in this review were shown to be beneficial for reducing GR to a certain extent without showing any potential negative effects on glycaemia. This review highlights the effect of whole polyphenol sources such as berries for not only reducing overall GR and IR but for also reducing the degree of variability in these parameters compared to liquid sources of polyphenols.

Rye breads

Previous studies have shown that soluble fibre reduces carbohydrate absorption rates and also reduces IR after a meal (Del Toma *et al.,* 1988; Tabatabai & Li, 2000) whereas insoluble fibre can increase insulin sensitivity by altering the patterns of insulin secretion (Weivkert *et al.,* 2006). Rye breads in general contain a high amount of fibre, especially soluble fibre and studies have shown rye products to reduce IR without necessarily reducing the GR postprandial (Rosen *et al.,* 2009; Rosen *et al.,* 2011). Also, rye bread has a harder, less porous crumb which is thought to contribute to its lower IR compared to white bread (Juntunen *et al.,* 2003).

In one study in this review, different rye breads with endogenous polyphenols were compared against a white bread low in polyphenols, for effects on GR and IR. Amilo rye contained the highest amount of insoluble fibre and Rekrut rye contained the greatest content of soluble fibre, with both types of fibre showing a negative correlation with IR and GR (Rosen *et al.,* 2011). This study was the only one to have the polyphenol source and the starch source in the same medium (rye bread). Amilo had the greatest content of caffeic acid and H. Loire rye was high in other phenolic acids and a negative correlation was found between early phase GR and polyphenol content. Therefore in rye bread, factors such as the structure of the starch granules, the fibre content and total polyphenols all may be beneficial for both GR and IR compared white bread. Results from this study show that some rye breads provide more benefits on GR and IR than others, and this is at least in part due to the polyphenol content of the breads.

6.4.3 Further limitations

Limitations of the studies in this review include an absence of *in vitro* analysis to determine mechanisms by which polyphenols reduce carbohydrate digestion. When whole foods, beverages or extracts were used as the polyphenol source, or when no polyphenol analysis was performed, it is unclear if the polyphenols or other components were responsible for the reduction in glycaemia. Also, there are a wide range of polyphenol classes and structures, with the most abundant polyphenols in foods and beverages not necessarily being those that are most bioactive. All studies were performed in healthy participants and therefore the role of polyphenols on GR and IR in diabetic subjects may show different results. Foods are generally consumed in combination and not in isolation, and therefore the confounding effects of other food components may influence carbohydrate digestion and resulting GR and IR. For example, black tea can be consumed with milk and this may influence the ability

of polyphenols to act on carbohydrates (Ryan & Petit, 2010). This review includes variable sources of polyphenols and also a varied source of carbohydrates, with the range of doses used differing between studies.

6.4.4 Conclusions

Studies assessing the effect of polyphenols on carbohydrate digestion and resulting GR are limited and results are conflicting due to the heterogeneity between studies. Overall, polyphenol addition to a carbohydrate rich food reduces early phase glycaemia and prolongs glucose absorption into the blood. The degree to which polyphenols influence GR and IR depends on the source of polyphenols, the source of carbohydrates, and other factors such as the dose used, the medium in which products are consumed and the composition of the polyphenols used. Due to the lack of studies in this area, it is inconclusive as to what types of polyphenol sources have the most potential for lowering blood glucose, and at what dosage this effect is optimal. The observed pattern of glycaemic reduction from the papers in this review makes it apparent that polyphenols may work at the intestinal level to reduce carbohydrate breakdown and glucose absorption.

Nutrition plays a key role in the risk reduction and management of diabetes. Polyphenols are found abundantly in foods and are an easy addition to the diet. This systematic review shows that there is potential for the postprandial GR and resulting IR to a food or meal to be reduced with the addition of polyphenols at doses easily obtained in the diet. Consuming polyphenol rich sources in the form of beverages, foods or extracts may therefore be a strategy in diabetes management and obesity prevention.

6.5 Publication

Coe S & Ryan L (2014) The impact of polyphenols on acute postprandial glycaemia: a systematic review, *Diabetes* (Submitted).

7. Polyphenol bioaccessibility and sugar reducing capacity of black, green and white teas.

7.1 Summary

Tea (*Camellia sinensis*) is a widely consumed beverage and recognised for its potential enhancing effect on human health due to its rich polyphenol content. While a number of studies have investigated the quantity and type of polyphenols present in different tea samples, no study has reported the potential effect of digestive enzymes on the availability of tea polyphenols for human absorption or the subsequent impact on glycaemic response (GR). The objectives of the present study were to assess the total polyphenol content of different teas; to assess the bioaccessibility of polyphenols in whole and bagged teas and to determine the effect of black, white and green tea infusions on sugar release. All of the teas were a significant source of polyphenols (10-116 mg Gallic acid equivalents {GAE}/ g). There was an overall increase in the release of polyphenols from both the bagged and the whole teas following *in vitro* digestion. Bagged green tea significantly ($p < 0.05$) reduced rapidly digestible starch (RDS) from white bread samples compared to control and black and white bagged teas. The present study confirms that tea is a rich source of polyphenols and highlights the potential benefits it may have on modulating GR in humans.

7.2 Introduction

One of the most widely consumed beverages throughout the world is tea produced from the tea plant (*Camellia sinensis*). Tea for consumption is classified according to the methods used in its production. Geographical consumption patterns of the different teas vary greatly with green tea consumed mainly in Asia and the Middle East and black tea consumed mostly in western countries. Tea has been found to be a rich source of polyphenols and antioxidants (Lakenbrink *et al.,* 2000). This together with evidence from epidemiological studies (Khan & Mukhtar, 2007) and high consumption rates worldwide has led to growing interest in tea as a product that may significantly contribute to human health.

The polyphenol profile of the different teas is affected by their different methods of production. Black tea is produced by wilting, crushing and partial oxidation and consequently is rich in theaflavins and thearubigins (Rusak *et al.,* 2008). In green tea production oxidation is minimised resulting in catechins being dominant (Cabrera *et al.,* 2006), particularly epigallocatechin-gallate (EGCG). White tea undergoes the least processing and is produced from young leaves and buds resulting in high levels of EGCG (Santana-Rio *et al.,* 2001), though generally lower than the levels found in green tea.

Reduced risk of coronary heart disease (Mukamal *et al.,* 2007), stroke incidence (Keli *et al.,* 1995), chronic inflammation (Sharma & Rao, 2009) and cancer incidence (Kuzuhara *et al.,* 2008) are associated with black and green tea consumption. Recent studies have focused on the impact of tea and tea polyphenols on blood glucose and insulin sensitivity. Black tea has been shown to decrease plasma glucose and enhance insulin concentrations post consumption in comparison to a control and a caffeine drink (Bryan *et al.,* 2007). Aldughpassi and Wolever (2009) showed that 250 mL of black tea with test meals actually increased overall mean peak blood glucose compared to water though a reduction in the standard error might indicate the ability of tea compounds to improve the precision of the GR. A study of green tea catechins in insulin resistant induced obese rats suggested that they may impact glucose control through several pathways (Yan *et al.,* 2012).   It was hypothesised that all teas would be rich sources of bioaccessible polyphenols, and that they would reduce the degradation of carbohydrate from white bread throughout an *in vitro* digestion.

The objectives of this study were as follows:

1. To determine the total polyphenol content of different commercial teas.
2. To determine the bioaccessibility of polyphenols from whole and bagged teas after *in vitro* digestion.
3. To determine the effect of black, white and green tea infusions on sugar release from bread after an *in vitro* digestion model.

7.3 Materials and Methods

7.3.1 Chemicals

All chemicals and reagents were of analytical grade and were purchased from Sigma-Aldrich (Poole, UK). The different teas were sourced directly from JING Tea Ltd as whole teas or purchased in bag form from a local Tesco supermarket. Teas included black teas: Jing Assam Breakfast, Jing Earl Grey, Jing Ceylon, Jing Darjeeling 2nd Flush; green teas: Organic Jade Sword, Organic Dragon Well, Jasmine Pearls, Kagoshima Sencha; white tea: Jasmine Silver Needle; herbal teas: Flowering Osmanthus, Flowering Jasmine & Lily, Moroccan Mint, Jun Shan Silver Needle; oolong teas: Tieguanyin, Yellow Gold Oolong, Taiwan Ali Shan Oolong; and three bagged teas (Tesco supermarket): Clipper Black Tea, Clipper Green Tea, and Clipper White Tea.

7.3.2 Study protocol

Samples from 19 brands of commercially available tea were chosen. The weight of each whole tea used was approximately 3g except for the 'Flowering Osmanthus' and 'Jasmine & Lily' teas where an entire bulb was used (the weight of each bulb was recorded).   For teas in bagged format, one tea bag (approximately 2.5g) was used. All tea samples were prepared using a standard protocol. Each tea was infused in 200 mL of boiling water (90 ^{o}C unless otherwise specified on the manufacturer's instructions) for three minutes and then stirred six times before the tea was removed. The resultant sample was then left at room temperature to cool for an additional 17 min before testing commenced. All tests were carried out on a minimum of three separate occasions and samples were analysed in triplicate for each test.

7.3.3 Folin-Ciocalteu (FCR) analysis

An aliquot (200 µL) of each tea sample was added to 1.5 mL of freshly prepared FCR reagent (1:10 v/v with water; Sharma & Gujral, 2010). The mixture was allowed to equilibrate for 5 min and then mixed with 1.5 mL of 60 g/L sodium carbonate solution. After incubation in a dark air-tight space at room temperature for 90 min, the absorbance of the mixture was read at 725 nm using the respective solvent as blank. The results were expressed as mg of GAE per gram of tea weight.

7.3.4 Bioaccessibility of tea polyphenols

Samples from 19 brands of commercially available tea were analysed using an *in vitro* digestion model adapted from Ryan *et al.* (2008). A total of 4 mL of each 200 mL tea infusion was added to an amber vial and made up to a volume of 15 mL with saline. A 1 mL baseline aliquot was taken

from each sample. The samples were acidified to pH 2 by the addition of 1 mL of a porcine (gastric muscosa) pepsin preparation (250 units/ mg solid; 0.04g pepsin in 1 mL 0.1 M HCl) and then incubated at 37 °C in a shaking water bath at 95 rpm for 1 hour. Gastric aliquots were taken. The pH was increased to 5.3 with 0.9 M sodium bicarbonate, followed by the addition of 200 µL of the bile salts glycodeoxycholate (0.04g in 1 mL saline), taurodeoxycholate (0.025g in 1 mL saline) and taurocholate (0.04g in 1 mL saline), and 100 µL of pancreatin from porcine pancreas (4 × USP specifications; 0.04 g in 500 µL saline) to each sample. The pH was adjusted to 7.4 using 1 M NaOH and overlaid with nitrogen. The samples were then incubated in a shaking water bath for two hours at 37 °C. Duodenal aliquots were taken and samples were frozen until analysis. Bioaccessibility was calculated by determining the percentage change realtive to baseline polyphenol (mg GAE/ g) values, throughout the gastric and duodenal stage of simulated digestion.

7.3.5 Measurement of sugar release

The effect of one black tea, one green tea and one white tea sample from the Clipper® brand on the inhibition of starch breakdown was determined with bread used as the starch source. This was achieved by subjecting samples of tea combined with bread to an *in vitro* digestion procedure and measuring the resultant reducing sugars released. Specifically, the RDS was measured which takes place at 20 min into the duodenal stage of digestion when the rate of sugar release is most pronounced. The RDS is therefore an indicator of the glycaemic index (GI) of a food (Englyst *et al*., 1996).

7.3.6 Bread Preparation

White bread dough was made to a recipe of 190g warm tap water, 1 tbsp virgin olive oil, 1 tsp salt, 1 tbsp sugar, 1 tbsp dried milk powder, 350g strong white flour (Hovis super strong premium white bread flour) and 1 ½ tsp of yeast. The dough was then baked in a Russell Hobbs bread maker (model no:18036, Manchester, UK) for a total of 3 hours and 20 min. All bread samples were baked the evening prior, sealed at room temperature in plastic containers overnight and tested the following morning.

7.3.7 In vitro Digestion

An *in vitro* digestion procedure was used to test the tea samples. This consisted of a simulated gastric digestion phase followed by an ileal digestion phase with timed sampling at the end of the gastric phase and during the ileal phase (Mishra *et al.,* 2008). Samples of the bread were prepared by weighing 2.5g samples and placing each into 60 mL specimen pots. Samples were finely chopped with a large kitchen knife into small uniform pieces (millimetres in diameter) to reflect the 'chewing process'. The pots were inserted into an aluminium heating block and covered with an insulating sheet in readiness for testing. A volume of 30 mL of each tea infusion (1 tea bag/ infusion) was added to its own individual bread sample. A 250 µL baseline sample was extracted for each sample at t = 0 min and added to a test tube in a ratio of 1:4 in ethanol. This was followed by the addition to each sample of 0.1 mL 10% porcine α-amylase (Type VI-B, ≥10 units/mg solid), 0.8 mL 1 M HCl and 1 mL 10% porcine pepsin (800-2500 U/mL) protein solution in 0.05 M HCl, to each. The resultant mixture was stirred slowly at 130 rpm every 15 sec for 30 min at 37 °C to complete the gastric digestion phase, and then gastric aliquots were taken. The ileal phase was initiated by the addition of 2 mL 1 M $NaHCO_3$ and 5 mL 0.2 M Na maleate buffer (pH 6) to each sample, and the volume was increased to 55 mL with distilled (dH_2O). In quick succession, 0.1 mL of amyloglucosidase (≥300 U/mL, aqueous solution) and 1 mL of 2% porcine pancreatin solution (8 x USP; in maleate buffer, pH 6), were added to each sample. Samples were then incubated for 120 min with constant slow mixing, and aliquots were taken at 20, 60 and 120 min during ileal digestion. The tubes were centrifuged (1000 x *g*, 2 min) in a Biofuge Primo Centrifuge (Heraeus Instruments, Kendro Laboratory Products, Germany) and an aliquot of the supernatant was removed for analysis of reducing sugars.

7.3.8 Analysis of reducing sugars released during digestion
Sugar released from the bread during digestion was measured by a colorimetric method adapted from Englyst and Hudson (1987) designed to measure monosaccharides after an amyloglucosidase secondary digestion to complete depolymerisation of starch fragments. A total of 0.05 mL of 10 mg/ mL glucose standard or sample from the *in vitro* digestion was added to 0.25 mL of enzyme solution A (1% amyloglucosidase {Megazyme, 3260 U/mL} in acetate buffer, pH 5.2). Each sample was incubated for 10 min at 25 °C and then 0.75 mL of 3,5-Dinitrosalicyclic acid (DNS) mixture (0.5 mg/ mL glucose: 4 M NaOH: DNS reagent mixed in ratio 1:1:5) was added. The resultant sample was then heated for 15 min at 95 °C in a water bath. Following this, 3 mL of water was added to each sample which was then left to cool for 20 min in a cold water bath. Absorbance was read at 530 nm on a Shimadzu UV-1201 spectrophotometer (Shimadzu Corporation, Australia) and sugar release was measured

in mg per g of bread sample. Slowly digestible starch (SDS) was extrapolated by subtracting the RDS measurement at 20 min from the reducing sugars measurement at 120 min during ileal digestion (Mishra & Monro, 2009).

7.3.9 Statistical analysis

All experiments were carried out in triplicate and each had a minimum of three replicates for each tea. The data are presented as means (± SEM) and comparisons between samples were carried out by an one way analysis of variance (ANOVA) and Tukey's multiple comparison test Statistical Package for Social Science (SPSS, version 17; SPSS Inc., Chicago Ill). A probability of 5% or less was considered statistically significant.

7.4 Results

7.4.1 Polyphenol content

Table 7.1 illustrates that all teas were a significant source of polyphenols. Of the whole teas, 'Kagoshima Sencha' brand[®] had a significantly higher polyphenol content ($p < 0.05$) compared to the other commercial whole teas analysed. Of the bagged varieties, green tea infusion had a significantly higher polyphenol content than both white and black teas, as measured by FCR ($p < 0.05$).

Table 7.1 Polyphenol content (expressed as gallic acid equivalents {G.A.E.} per gram and per serving {3g in 200 mL water})

Tea	G.A.E. (mg/ g Tea)	G.A.E. (mg/ serving)
Black teas		
Jing Assam Breakfast[1]	48.6	145.9
Jing Earl Grey[1]	62.2	186.7
Jing Ceylon[1]	58.7	176.0
Jing Darjeeling 2nd Flush[1]	47.3	141.8
Green teas		
Organic Jade Sword[1]	42.6	127.9
Organic Dragon Well[1]	54.9	164.6
Jasmine Pearls[1]	23.3	69.9
Kagoshima Sencha[1]	95.3[*]	285.8
White tea		
Jasmine Silver Needle[1]	20.4	61.1
Herbal teas		
Flowering Osmanthus[1]	10.3	75.2
Flowering Jasmine & Lily[1]	13.8	96.3
Moroccan Mint[1]	48.7	146.2
Jun Shan Silver Needle[1]	38.6	115.7
Oolong teas		
Tieguanyin[1]	28.5	85.5
Yellow Gold Oolong[1]	23.5	70.5

Taiwan Ali Shan Oolong[1]	20.1	60.3
Clipper Black Tea[2]	87.9	263.7
Clipper Green Tea[2]	115.5**	346.5
Clipper White Tea[2]	102.8	308.4

[1]Whole teas, [2]Bagged teas. *$p<0.05$ = significantly greater than all other whole tea samples. **$p<0.05$ = significantly greater than all other bagged tea samples. Values represent means of three independent experiments.

7.4.2 Polyphenol bioaccessibility

Table 7.2 illustrates that the polyphenol content of all tea infusions was enhanced following the gastric digestion phase. This enhancement continued into the duodenal phase, although some tea polyphenols became less bioaccessible relative to the gastric phase.

Table 7.2 % Bioaccessibility of the polyphenol content after the Gastric and Duodenal phases of digestion

Tea	Gastric (%)	Duodenal (%)
Black teas		
Jing Assam Breakfast[1]	140.7	121.2
Jing Earl Grey[1]	121.0	127.7
Jing Ceylon[1]	124.6	127.4
Jing Darjeeling 2nd Flush[1]	133.5	142.4
Green teas		
Organic Jade Sword[1]	133.2	131.0
Organic Dragon Well[1]	123.2	128.1
Jasmine Pearls[1]	172.2	204.0
Kagoshima Sencha[1]	143.7	134.7
White tea		
Jasmine Silver Needle[1]	204.1	233.6
Herbal teas		
Flowering Osmanthus[1]	176.8	189.3
Flowering Jasmine & Lily[1]	160.5	174.6
Moroccan Mint[1]	128.0	124.3
Jun Shan Silver Needle[1]	153.9	147.1
Oolong teas		
Tieguanyin[1]	161.7	185.2

Yellow Gold Oolong[1]	194.4	233.5
Taiwan Ali Shan Oolong[1]	207.9	231.1
Clipper Black Tea[2a]	136.6	126.6
Clipper Green Tea[2b]	132.0	125.3
Clipper White Tea[2c]	165.1	176.6

[1]Whole teas, [2]Bagged teas.

7.4.3 Sugar release

At 20 min into the duodenal phase of digestion, green tea significantly suppressed RDS release of white bread to 253.83 mg/ g bread sample when compared to white tea and the control bread ($p < 0.05$; Figure 7.1). Black tea showed no significant effect on sugar release at this time point. In all teas, there was a non-significant trend to increase SDS release compared to the control.
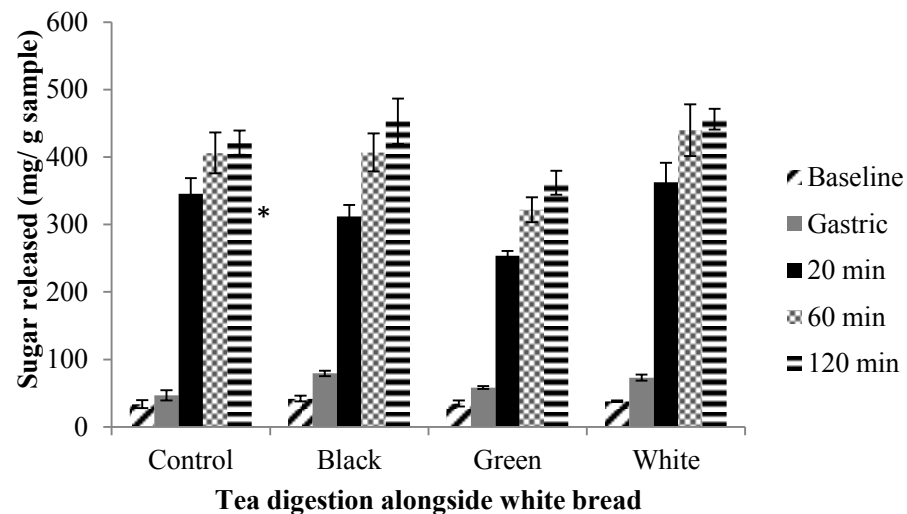
Figure 7.1 Sugar release from bread samples, when digested alongside either no tea (control), black, green or white tea.

*$p<0.05$= significant reduction in sugar release compared to control white bread. Values reported as mg sugar released/ g bread sample. Each patterned bar represents different stages of digestion, with 20, 60 and 120 min = intestinal stages of digestion. Values represent mean ± standard error of the mean (SEM) of three independent experiments.

7.5 Discussion

7.5.1 Polyphenol content

Of the bagged teas, the black tea had the lowest polyphenol content. The different methods of processing and production impact the polyphenol content of the resultant teas. Total black tea polyphenols decrease during fermentation, and the longer tea is subjected to processing, the lower the polyphenol content (Astill *et al.,* 2001). Turkman *et al.* (2006) found that black tea polyphenol content as measured by FCR reached a maximum of 131.9 mg GAE/ g tea extract compared to the 87.9 GAE/ g in the current study indicating that the polyphenol content of the same tea can vary widely.

The green tea infusion was shown to have more overall reducing power than both black tea and white tea. The production process used in black tea results in the formation of theaflavins. Theaflavins in black tea are dimers of catechins and contain more hydroxyl groups in their structure. In green tea, catechins remain dominant. This could in part explain the differences in reducing potential between black tea and green tea (Astill *et al.,* 2001).

There has been very little research to date on the polyphenol content and health effects of white tea. Rusak *et al.* (2008) found that green tea was a richer source of polyphenols than white tea and the current study supports this. They also found that the concentration of catechins was significantly higher in green tea leaves than white tea leaves.

Ryan and Carolan (2011) found that green teas varied in their polyphenol content, ranging from 250 - 750 mg GAE/ tea bag. They also found that both the structure of the tea bag and the infusion time influenced the polyphenol content of the teas. FCR values in the current study were slightly lower than those found by Ryan and Carolan (2011), averaging 115.5 mg GAE/ g tea bag. Rusak *et al.* (2008) found that extraction of catechins from green tea was affected by the form of tea used, with extraction from loose green tea leaves being more effective than from refined bagged tea leaves. However, the form of the tea did not affect white tea catechins. Greater tea bag size results in tea solids diffusing into

solution faster because of the larger surface area available in which the contents can diffuse (Astill *et al.,* 2001). The material of the tea bag can also have an impact on polyphenol diffusion. In the current study, tea bag infusion time and stirring/ squeezing of the tea bags were kept constant for all three teas. Tea bags were different weights, the black tea bag weighing more than both white tea and green tea. Weights were however corrected for upon calculations.

Of the whole teas analysed, 'Kagoshima Sencha' had a significantly higher polyphenol content compared to the other teas. This was the only whole tea that had a higher polyphenol content than any of the bagged teas. Whole teas tend to be more compacted and have undergone much less processing than bagged teas. Unpublished data from our laboratory indicate that the grinding of leaves to form the bagged teas has the effect of releasing polyphenols and this may in part explain the higher polyphenol content in the bagged teas. Overall, all teas were shown to be good sources of polyphenols.

7.5.2 Polyphenol bioaccessibility

The *in vitro* digestion model enables the measurement of polyphenols potentially available for absorption after the gastric and duodenal phases of digestion. Bioaccessibility refers to the proportion of polyphenols which are presented to the brush border for absorption after digestion and gives some indication as to their potential bioavailability *in vivo*. In the current study, polyphenol bioaccessibility increased in all teas from baseline to gastric phases suggesting that the polyphenols in these teas may become more available in humans after consumption. The bioaccessibility increase from baseline to gastric phase was similar for both bagged and whole teas. The compact and relatively unprocessed nature of the whole teas resulted in a lower polyphenol content at baseline. However, the bioaccessibility increases suggest that the digestive enzymes can further release polyphenols from the tea infusion. Green *et al*. (2007) looked at the effect of *in vitro* digestion specifically on catechins in green tea and found that catechins had less than 20% recovery after *in vitro* digestion. However, the current study is the first to report and compare the bioaccessibility of total polyphenols from green, white and black tea infusions. In the current study, with the exception of white bagged tea, the bioaccessibility decreased slightly from gastric to duodenal phases in bagged teas. However, at the duodenal stage values still remained above baseline for all tea varieties. This decrease may be due to the increase in pH during the duodenal phase. Rusak *et al*. (2008) showed that the form of tea, either loose or bagged did not affect white tea catechin stability. Therefore, compounds in white tea may be more stable, and thus less susceptible to degradation compared to those in the other teas.

7.5.3 Starch digestion and sugar release

Green tea was the only tea shown to significantly reduce sugar release from white bread. This is a promising finding in that green tea may reduce the RDS of starch rich foods such as bread. However, tea polyphenols have been shown to reduce starch retrogradation. Wu *et al.* (2009) found that increased levels of added purified polyphenols (50% EGCG) resulted in decreased retrogradation for resistant starch in rice. From these results it was predicted that the hydroxyl radical of tea polyphenols combined with rice starch to form hydrogen bonds, preventing the re-association of the starch chains.

In the current study, white tea had no significant effect on sugar release, although there was a slight increase compared to the control bread. Certain polyphenols or other compounds in white tea may be responsible for interfering with the natural chemical bonds in the bread, therefore rendering the starch more susceptible to degradation. White tea polyphenols were shown to be the most stable throughout digestion, and therefore they may have a greater impact on starch than those polyphenols which are degraded more readily.

Different teas contain different polyphenols, and therefore it is plausible that each tea may have a different effect on sugar release. Teas were used at low concentrations in this study, i.e. one tea bag per infusion. Therefore, green tea at low concentrations reduces sugar release from starch samples, whereas white tea at a low dose seems to have the opposite effect. Teas were made as infusions and then added to bread samples at the baseline phase of digestion, whereas other studies have baked tea into breads or looked at the effect of purified tea polyphenol rich extracts. Also, because bread was chopped to fine particle size before *in vitro* digestion, the starch digestibility may have been increased compared to if bread was not chopped and thus contained larger particle sizes (Edwards *et al.,* 2014). Therefore, different study designs may account for variability in study results. However, what can be seen is that different types of tea do have an effect on starch breakdown and sugar release from breads.

Unpublished data from our laboratory found that GR increased over 180 min following the consumption of white bread with added black tea extract in comparison to eating white bread alone. A more pronounced effect was also seen when a tea infusion was consumed alongside the white bread and the time taken for this combination to exert its peak GR was prolonged compared to either the control bread or the bread with tea extract added. However, Koh *et al.* (2010) found black tea to reduce starch digestion, yet found no effect with green or oolong tea. In the current study, each tea was presented as an infusion alongside bread during digestion and the black tea infusion had no significant effect on sugar release

from bread. The form in which the black tea is used, the preparation method and the concentration used, may all be factors to account for the variation in results between studies.

Deshpande and Salunke (1982) found that in isolation tannic acid and catechins decreased *in vitro* digestibility of various types of starch sources. Also, Bjork and Nyman (1987) found that phytic acid and tannic acid reduced starch hydrolysis in the digestive tract. However, isolated polyphenols may have a different effect on starch digestion compared to phenolics in combination. For example, green tea is one of the richest sources of phenolic compounds and includes catechins such as EGCG, procyanidins and quercetin (Neveu *et al.,* 2010). Therefore, a combination of the phenolics in tea may have synergistic effects, either enhancing or reducing the degree of starch breakdown and sugar release compared to isolated catechins or tannins (Conte *et al.*, 2003; Morre & Morre, 2006). The reason for the reduction in sugar release seen in the current study may be because of the structural bonding of the green tea polyphenols with starch molecules. Both black tea and green tea contain conjugate forms of catechins, and these compounds may interfere with the way in which starch is broken down. Therefore, future research could look into accessing the polyphenol profile of the different teas to determine which polyphenols have an effect on reducing starch breakdown.

Tea polyphenols may have an inhibitory effect on digestive enzymes such as α-amylase and α-glucosidase. Black tea and to a lesser extent green tea were shown to inhibit α-amylase in human saliva and removal of tea tannins resulted in loss of the inhibitory activity (Zhang & Kashket, 1998). Hara and Honda (1990) found that both catechins and theaflavins inhibited salivary α-amylase and whilst, various teas differed in the extent of inhibitory activity, black tea showed consistently greater inhibitory activity. Kwon *et al.* (2008) found that black tea and white tea showed almost a 40% inhibition of α-amylase, with green tea showing only a 30% inhibition. They also found black and white tea to have a higher α-glucosidase inhibitory activity than green and oolong teas. At higher concentrations of polyphenols, {50 μg GAE/ mL, 100 μg GAE/ mL and 200 μg GAE/ mL} α-glucosidase inhibition was increased in all teas. Although enzyme inhibition was not tested in the current study, a future area of study is to investigate the effect of tea polyphenols on digestive enzymes.

Finally, it should be noted that many studies evaluating the GR to foods contain the addition of tea or coffee in their test meals. Based on the current results and on previous studies, the polyphenols in tea may affect blood glucose, thus affecting the results of the test foods studied. It is important that when testing foods for different effects on disease parameters, the combination of ingredients is considered. A single food in isolation may have different effects on GR than that food with an additional tea beverage.

7.6 Conclusion

Tea is a commonly consumed beverage throughout the world, with different teas varying in their polyphenol profile and their total polyphenol content. This study was the first to look at the bioaccessibility of white tea, and it is hypothesised that tea polyphenols may show greater bioavailability in the body after digestion. Green tea reduced the amount of starch breakdown when consumed alongside bread, and therefore may show potential for reducing the amount of sugars available for absorption in the body. Therefore, teas are a rich source of accessible polyphenols and may show potential in reducing risk factors associated with certain metabolic disease states such as diabetes.

7.7 Publication

Coe S, Fraser A & Ryan L (2013) Polyphenol bioaccessibility and sugar reducing capacity of black, green, and white teas, *International Journal of Food Science*; available at http://www.hindawi.com/journals/ijfs/2013/238216/.

8. The polyphenol rich baobab fruit (*Adansonia digitata* L.) reduces starch digestion *in vitro*.

8.1 Summary

The baobab fruit (*Adansonia digitata* L.) is found throughout regions of Africa and is becoming increasingly recognised for its high micronutrient and polyphenol content. Fruits rich in polyphenols and antioxidants have shown benefits for metabolic parameters including their effect on reducing the postprandial GR. In the current study six extracts of baobab fruit were measured for their total antioxidant and polyphenol content and also for their bioaccessibility throughout *in vitro* digestion. The extracts included: five different extracts of baobab fruit donated from five different geographical locations in Africa and one commercially purchased baobab fruit extract (sample 6, Baobab super fruit powder, Min Vita, London, UK; Holland & Barrett, UK). The commercial sample of baobab fruit extract powder was then baked into white bread at different doses, to determine the optimal dose for reducing starch breakdown and sugar release. Results showed that all six extracts were good sources of bioaccessible antioxidants and polyphenols. Baobab fruit extract added to white bread at 1.88% was the optimal dose for significantly ($p < 0.05$) reducing RDS from white bread. Therefore, the polyphenol rich baobab fruit may be a potential functional ingredient for reducing the GI of starch rich foods.

8.2 Introduction

Africa is abundant in novel plant species known to be rich in health promoting compounds, many of which remain undiscovered or unused by western society (Lamien-Meda *et al.,* 2008). The baobab tree, *Adansonia digitata* L., is widely distributed throughout Sub - Saharan Africa and Western Madagascar and has many uses, including but not limited to its use in medicine, food and beverages (Diop *et al.,* 2006; Gebauer *et al.,* 2002). This fruit is of increasing nutritional interest because it may be a significant contributor to the daily intake of important nutrient and non-nutrient compounds (Nour *et al.,* 1980). Many studies have confirmed the baobab fruit pulp to rich in vitamins and minerals (De Caluwe *et al.,* 2010; Gebauer *et al.,* 2002; Osman, 2004; Oyelek *et al.,* 2012) and to contain a high amount of both soluble and insoluble dietary fibre (Arnold *et*

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al., 1985; De Caluwe *et al.,* 2010; Gebauer *et al.,* 2002; Magaia *et al.,* 2013; Osman, 2004; Oyelek *et al.,* 2012). This fruit is high in Vitamin C which contributes to its overall antioxidant capacity (Besco *et al.,* 2007; Blomhoff *et al.,* 2010; Gruenwald, 2009; Lamien-Meda *et al.,* 2008; Vertuani *et al.,* 2002), and is a good source of polyphenols, including certain flavonoids (Lamien-Meda *et al.,* 2008; Mulaudzi *et al.,* 2011) and tannins (Ghani & Agebejule, 1986).

As mentioned previously, polyphenols are thought to have a reducing effect on starch digestion in foods (Desphande & Salunke, 1982; Yoon *et al.,* 1983). Yoon *et al.* (1983) investigated the effect of phytic acid on starch digestibility *in vitro* and *in vivo*. When phytic acid was added to raw wheat starch, using an *in vitro* digestion model it was found that the addition of phytic acid directly after the salivary phase decreased sugar liberation. In addition, the higher the concentration of phytic acid added to a food in healthy people, the lower the GI of the food being consumed. Fruit extracts are rich in both polyphenols and fibre, with the baobab fruit extract powder containing around 30g of insoluble fibre and 30g of soluble fibre/ 100g extract. Foods rich in soluble fibre have been shown beneficial for altering the degree of starch breakdown in a food and for managing blood glucose levels (Cavallero *et al.,* 2002; Thondre & Henry, 2009). Therefore, polyphenols and/or the fibre in various fruit extracts such as the baobab fruit may impact starch digestion and resulting GR in humans. This study assessed the polyphenol and antioxidant composition of baobab fruit extract, and its overall potential for reducing starch breakdown from white bread *in vitro*. It was hypothesised that the baobab fruit would be a rich source of antioxidants and polyphenols and these compounds in addition to the fibre content of the extract would reduce the starch digestion from white bread.

The specific objectives of the present study were:
1. To analyse the antioxidant and polyphenol content, and the polyphenol bioaccessibility of six baobab extracts.
2. To test the commercially purchased extract for its effect on starch breakdown and resulting sugar release from white bread samples at different doses.

8.3 Methods and Materials

8.3.1 Chemicals

All chemicals and reagents were of analytical grade and were purchased from Sigma-Aldrich (Poole, UK). The extracts included: five different extracts of baobab fruit donated from five different geographical locations in Africa (samples 1-5) and one commercially purchased baobab fruit extract (sample 6, Baobab super fruit powder, Min Vita, London, UK; Holland & Barrett, UK). A total of six extracts were selected in order to test for variability in polyphenol content and bioaccessibility between extract locations. The commercially available extract was the only extract used for the remaining *in vitro* sugar release and contained the following ingredients per 100g: 170 kcal, <1 g total fat, 78g total carbohydrate, 1.8g protein, 30g soluble fibre, 30g insoluble fibre, 14g total sugars, 0.6mg Vitamin B1, 0.03mg Vitamin B2, 300mg Vitamin C, 2500mg potassium, 350mg calcium, 2mg iron, 148mg magnesium, 0.5mg sodium.

Figure 8.1 Commercial baobab (sample 6, Baobab super fruit powder, Min Vita, London, UK; Holland & Barrett, UK)

8.3.2 Study protocol

Each sample was weighed out at 500mg and added to a 50 mL solution of dH_20. All tests were carried out on a minimum of three separate occasions and samples were analysed in triplicate for each test.

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8.3.3 Ferric-ion reducing antioxidant power (FRAP)

Samples from six baobab extracts were analysed using the FRAP method adapted from Benzie and Strain (1996). The FRAP reagent was prepared from acetate buffer 300 mM, pH 3.6 (3.1g sodium acetate trihydrate and 16 mL glacial acetic acid made up to 1 L water), TPTZ 10 mM HCl (3.4 mL HCl made to final volume 1 L dH_2O) and ferric chloride $FeCl_3 * 6H_2O$ 20 mM (5.406 g/ L dH_2O) in a 10:1:1 ratio. The FRAP assay was performed by warming 1 mL of dH_2O to 37 °C before adding 25 µL of sample and 1 mL of reagent. The tubes were then placed in a water bath at 37 °C for 4 min. Absorbance was measured after the 4 min at 593 nm, with each standard and sample measured against the blank. The FRAP value was then calculated in µmol/ L against a standard of ferrous sulphate (1000 µM).

8.3.4 FCR

The polyphenol content of the six baobab extracts was analysed using the FCR method (Sharma & Gujral, 2010), as previously described in section 7.3.3.

8.3.5 Bioaccessibility of baobab polyphenols

Samples from six baobab fruit extracts were analysed using an *in vitro* digestion model adapted from Ryan *et al.* (2008) as previously described in section 7.3.4. Samples were frozen ay -20°C until analysis.

8.3.6 Measurement of sugar release

The effect of baobab fruit extract on the inhibition of starch breakdown was determined. Bread was used as the starch source. This was achieved by performing a dose-response using different concentrations of baobab fruit extract baked into bread at 1.25%, 1.88%, 2.50%, 3.13% and 3.75%, and then subjecting samples to an *in vitro* digestion procedure and measuring the resultant reducing sugars released.

8.3.7 Bread preparation

White bread dough was made to a recipe as in Table 8.1. The dough was then baked in a *Russell Hobbs* bread maker (model no: 18036, Manchester, UK) for a total of 3 hours and 20 min. When adding the baobab fruit extract powder (sample 6, Baobab super fruit powder, Min Vita,

London, UK) to the white breads, the flour and water content were altered in order to keep the overall weight of the loaf at 500g. Percentages were calculated based on the entire 500g loaf.

Table 8.1 Ingredients used to make white bread with added baobab [1]

Ingredients/ weights	Baobab % used per g bread (500g)	Baobab % used per g CHO [2]
190g warm tap water	1.25%	2.70%
1 tbsp virgin olive oil	1.88%	4.07%
1 tsp salt	2.50%	5.46%
1 tbsp sugar	3.13%	6.87%
1 tbsp dried milk powder	3.75%	8.30%
1 ½ tsp yeast		
350g strong white flour		

[1] Expressed as percentage per 500g loaf. Values also expressed as baobab percentage per g of available carbohydrate.
[2] avCHO= total available carbohydrate. Baobab extract was added into breads and water and flour content were altered in order to keep overall loaf weights at 500g after baking.

8.3.8 In vitro digestion

The *in vitro* digestion procedure by Mishra *et al*. (2008) was as previously described in section 7.3.7. Samples of the bread were prepared by weighing 2.5g and placing each into 60 mL specimen pots. A volume of 30 mL of dH_2O (compared to tea sample in previous section) was added to each bread sample.

8.3.9 Analysis of reducing sugars released during digestion

Sugar released from the bread during digestion was measured by a colorimetric method adapted from Englyst and Hudson (1987), as previously described in 7.3.8. Previous studies have shown that the *in vitro* analysis of RDS can be correlated to the GR *in vivo* (Englyst *et al.*, 1999).

8.3.10 Statistical analysis

The *in vitro* analysis experiments were carried out three times with a minimum of three replicates of each sample. Comparisons between samples were carried out by an ANOVA and Tukey's multiple comparison test, and statistical analysis was performed using SPSS, version 17. Results were expressed as the mean ± SEM and significance was set at $p < 0.05$.

8.4 Results

8.4.1 Antioxidant and polyphenol content

Baobab samples 3, 4 and 6 had a significantly ($p < 0.05$) greater antioxidant and polyphenol content than samples 1, 2 and 5, as measured by both FRAP and FCR (Table 8.2), respectively.

Table 8.2 FCR and FRAP values for baobab samples 1-6 at baseline, before *in vitro* digestion.

Baobab	FCR[1] (mg G.A.E./ g)[3]	FRAP[2] (µmol/ L)[3]
1	24.94 ± 0.32	1755.07 ± 28.3
2	21.85 ± 0.27	1666.79 ± 23.5
3	27.58 ± 0.20*	2093.76 ± 61.4*
4	26.95 ± 0.26*	2121.56 ± 44.8*
5	24.68 ± 0.33	1844.02 ± 43.7
6	28.85 ± 0.47*	2167.68 ± 52.9*

[1] FCR=Folin-Ciocalteu assay
[2] FRAP=Ferric reducing antioxidant power assay
[3] Values reported in µmol/ L relative to ferrous sulphate (1000 µM) and gallic acid equivalents (G.A.E.)/ g sample. Samples 3, 4 and 6 were significantly higher in polyphenols and antioxidant content than samples 1, 2 and 5; *$p < 0.05$. Values represent means ± SEM of three independent experiments.

8.4.2 Polyphenol bioaccessibility

Polyphenol content as measured by the FCR method significantly increased throughout digestion in all samples ($p < 0.05$; Table 8.3) compared to the baseline polyphenol content.

Table 8.3 Bioaccessibility of the polyphenol content after the Gastric and Duodenal phases of digestion, measured using the Folin-Ciocalteu assay.

Baobab	Gastric (%)	Duodenal (%)
1	270.8	330.6
2	293.0	361.2
3	222.5	274.5
4	239.7	292.0
5	252.2	299.4
6	223.9	269.1

All samples showed increased bioaccessibility of polyphenols at the gastric and duodenal phase compared to the baseline phase, $*p < 0.05$. Values expressed as percentage increase from baseline. Values are results from three independent experiments.

8.4.3 Sugar release

The addition of baobab to white bread at 1.88%, 3.13% and 3.75% significantly ($p < 0.05$) reduced sugar release compared to the control white bread at 20 min (RDS) and 60 min into intestinal digestion (Figure 8.2). There was no significant difference in the SDS calculated values between any samples.
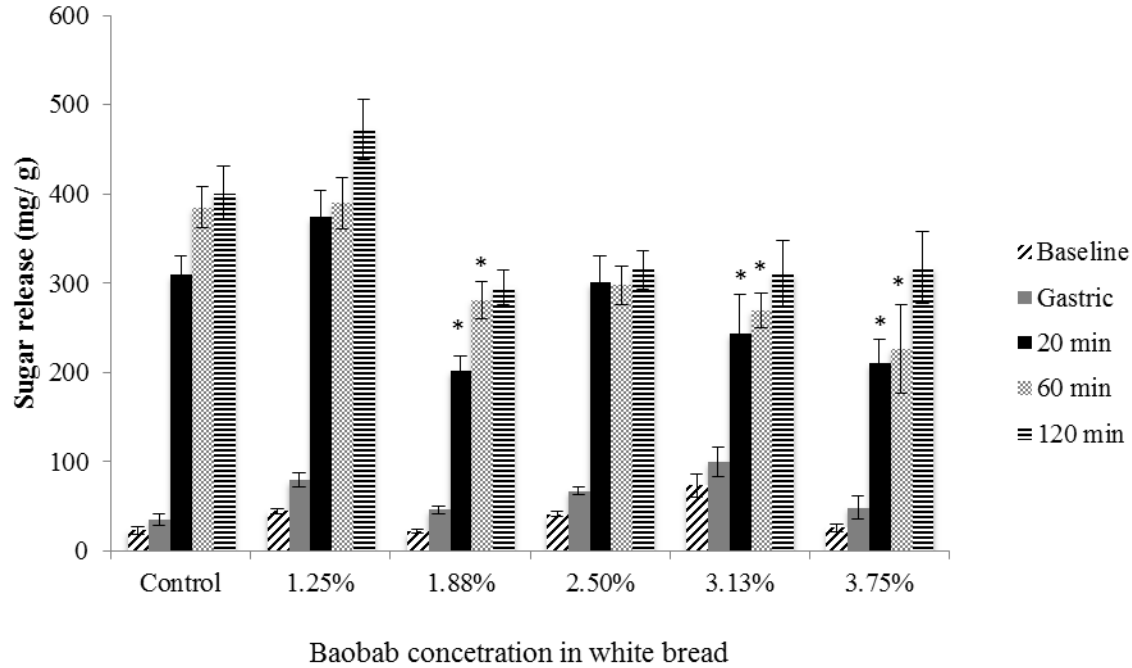
Figure 8.2 Sugar release in mg/ g bread sample for each bread containing different concentrations of baobab extract, expressed as percentages at each stage of digestion.

*$p<0.05$ denotes significant decrease in sugar release compared to control white bread (no added baobab extract). Percentages of baobab were calculated with baobab extract added into white bread to make a total loaf weight of 500g. Each patterned bar represents different stages of digestion, with 20, 60 and 120 min = intestinal stages of digestion. Values represent means ± SEM of three independent experiments.

8.5 Discussion

8.5.1 Polyphenol content and bioaccessibility

The antioxidant content of each sample was measured using FRAP and the high antioxidant potential was in agreement with previous studies, which used various methods of antioxidant detection (Besco *et al.,* 2007; Blomhoff *et al.,* 2010; Gruenwald, 2009; Lamien-Meda *et al.,* 2008;

Vertuani *et al.,* 2002). All baobab fruit extracts were shown to be good sources of polyphenols, with the commercial extract (sample 6) being the most concentrated polyphenol extract out of the total six extracts. The donated extracts (1-5) were kept in clear plastic bags, which may have been subjected to more air and light oxidation than the commercial extract which was in a solid dark container. Therefore, some of the polyphenols in the clear bags may have been destroyed before analysis. The antioxidant content of fruits and fruit extracts has been shown to be correlated with the amount of total polyphenols (Soon & Barlow, 2005; Tzulker *et al.,* 2007), and this was also found in the current study. It has previously been found that the bioaccessibility of phenolics increases throughout digestion (Ryan & Prescott, 2010; Wootton-Beard *et al.,* 2011). The bioaccessibility of polyphenols in the current study increased throughout the digestive process, with approximately a three-fold increase in polyphenol concentration between the baseline and duodenal phase.

8.5.2 Starch breakdown and sugar release from bread

In this study, there was a significant reduction in sugar release at 20 and 60 min into the duodenal phase of digestion in the 1.88% and also the higher concentrated baobab-white breads (excluding 2.5%), compared to control white breads. Although polyphenols have been shown to reduce sugar release from starch rich foods (Desphande & Salunke, 1982; Yoon *et al.,* 1983) other studies have shown an increase in starch digestibility with added polyphenols (Wu *et al.*, 2009). There are a few hypotheses that may account for the decrease observed in the baobab breads. Polymeric polyphenols are usually not absorbed to any significant extent, and thus they have potential to bind to starch molecules in foods, slowing the rate of starch breakdown (Gonthier *et al.,* 2003). Baobab is rich in compounds such as high molecular weight tannins (Ghani & Agbejule, 1986) which may be interfering with starch degradation. The polyphenols in the extract may be inhibiting digestive enzymes such as α-amylase and α-glucosidase, thereby preventing the breakdown of starch (McDougall & Stewart, 2005). However the exact polyphenol composition of the baobab fruit is currently unknown and further work is required to understand how these polyphenols may be exerting their effect.

The soluble fibre in the extract may also be contributing to a reduction in sugar release, as fibre has continuously been shown to reduce postprandial glycaemia (Wolever, 2006, Brennan, 2005; Kwong *et al.,* 2013; Slaughter *et al.,* 2002; Vosloo, 2005). In the current study, soluble fibre mostly in the form of pectin, from baobab may have formed a viscous solution reducing the rate of starch breakdown and sugar release into solution. Also, the polyphenols may also be major contributing factors to the reduced GR in carbohydrate-rich foods such as oats (Thondre *et al.,*

2011). Thondre *et al.* (2011) found oats to have a significant amount of polyphenols with a negative correlation between polyphenol content and RDS (-0.743). However, in this study there was no correlation between fibre content and the digestibility of the starch in oats and therefore it was the polyphenols of the oats contributing to the reduced sugar release. The reduction in sugar release after consumption of baobab fruit extract in the current study may therefore be partly due to the polyphenols in the extract.

It should be noted that only one parameter, sugar release, was measured in this study. Polyphenols do not only bind to molecules in foods, yet can act as unbound entities. Polysaccharides in the cell wall of plants have been found to form bonds with polyphenols (Le Bourvellec *et al.*, 2004). Charmorro *et al.* (2012) found that adding pectinase and tannase to grape seed extract and grape pomace increased not only the release of sugar from the polysaccharide rich cell walls, yet also increased release of polyphenols. Therefore, unbound polyphenols have the potential to be absorbed where they can modulate specific cellular processes in the body. In this study the vast majority of fates that polyphenols may have are not being considered.

Although the reduction of RDS is important, the resultant increase in SDS is another factor to consider, as it produces a gradual increase in postprandial blood glucose levels (Jenkins *et al.*, 1978). This is important because polyphenols can affect both the early and late stages of digestion. For example, berries have been shown to reduce concentrations of glucose in the blood in the early phase and increase concentrations in the later phase of digestion (Torronen *et al.*, 2010). It was concluded that the proanthocyanidins and other phenols in the berries may be reducing the rate of digestion and/ or absorption from the digestive tract, with the peak glucose increment also reduced. Therefore, even if polyphenol extracts cannot produce an overall reduction in total sugar release, they may still have to potential to reduce the rapid release of sugar. However, the method used by Englyst and Hudson (1987) may have limitations in regards to the amylolysis of starch material as it uses a first order kinetics model with a single digestibility constant. A new method developed by Butterworth *et al.* (2012) which involves two first order kinetic equations that differ in the digetiblity rate constant, takes into account the reduced rate of stach digestibility in the later stages of digestion and may therefore be a more accurate predictor of starch digestibility.

8.6 Conclusion

In conclusion, this is a novel study as no other study to date has looked at the effect of exogenously added baobab fruit extract on sugar release *in vitro*. The baobab fruit is a rich source of bioaccessible polyphenols and the current study shows the potential of baobab for reducing the GR to carbohydrate-rich foods. Future studies would need to assess enzyme inhibition to determine the effect of baobab fruit on amylase activity, and also the individual polyphenol content of the samples to determine the methods by which baobab reduces starch digestion and potentially the GR.

8.7 Publications

Coe S & Ryan L (2012) Polyphenol content and *in vitro* bioaccessibility of six baobab fruit extracts, *Proceedings of the Nutrition Society*, **71**, E26.

Coe S, Clegg M, Armengol M & Ryan L (2013) The polyphenol rich baobab fruit (*Adansonia digitata* L.) reduces starch digestion and glycemic response in humans, *Nutrition Research*, **33**, 888-896.

9. The effect of polyphenol rich extracts in isolation and in combination, on sugar release from various starch rich foods *in vitro*.

9.1 Summary

Foods rich in polyphenols have been shown to modify starch digestion from carbohydrate rich foods and lower resulting glycaemia. Several factors may influence the potential of polyphenols for reducing starch breakdown, such as the source under study and/ or the combination of polyphenolic compounds used. An *in vitro* dose-response analysis was performed to determine the optimal dose of three extracts, green tea extract (GTE), grape seed extract (GSE) and resveratrol (RES), for reducing RDS from white bread. These same doses were then measured in other starch foods, and were also tested in combination in white bread, and resulting RDS was measured. All extracts were found to reduce sugar release from white bread at optimal doses ($p < 0.05$). However, there was no significant effect on sugar release for any of the extracts when they were baked into flat bread or gluten free bread at the same doses used in white bread, nor when extracts were baked into white bread in combination. Therefore, this study highlights the potential of plant extracts for reducing starch breakdown from white bread, thus potentially reducing the GI of the bread.

9.2 Introduction

Polyphenols are well known for their health benefits including their potential in reducing risk factors of diabetes. Extracts from plant foods including Amla fruit extract (Akhtar *et al.,* 2011), whortleberry fruit extract (Kianbakht *et al.,* 2013), cinnamon extract (Lu *et al.,* 2012) and Sajabalssuk extract (Cho *et al.,* 2012) have been shown to improve blood glucose control in humans. Plant extracts contain a wide range of health compounds including polyphenols, which can be absorbed into the blood and exert their effects at the cellular level. Also current research is discovering the potential of certain metabolites for reducing postprandial glycaemia by inhibiting and/ or prolonging digestion in the intestinal tract when consumed with a carbohydrate rich source (Chai *et al.,* 2013; Sapwarobol *et al.,* 2012). For example, polyphenols have been shown to inhibit the digestive enzymes α-amylase and α-glucosidase thereby preventing enzyme attack on starch chains (Sapwarobol *et al.,* 2012) and have

been found to form complexes with amylose chains interfering with the amylose recrystallization and thus reducing their degradation (Chai *et al.,* 2013). White bread is a commonly consumed food with a high GI value, and is therefore able to induce a large increase in postprandial blood glucose and insulin levels. The addition of plant extracts to certain carbohydrate rich foods such as bread may reduce the GI, therefore reducing the resulting GR.

Some polyphenols and polyphenol rich extracts have shown more potential than others for improving various health parameters, specifically for diabetes control. For example, at a concentration of 22.4 mg/kg/day, resveratrol was found to normalise glycaemia and insulinaemia in mice fed a high-fat diet over a six month period (Baur *et al.,* 2006). Wu *et al.* (2004) found that green tea polyphenols increased insulin stimulated glucose uptake of adipocytes and therefore increased insulin sensitivity in rats. Forester *et al.* (2012) found that the administration of 100 mg/ kg body weight of EGCG to mice after the consumption of corn starch modulated amylase-mediated starch digestion and reduced postprandial blood glucose levels. Red and white wines have shown a positive correlation between total soluble phenolics and α-glucosidase inhibitory activity, with black, green, oolong and white tea were all shown to have high α-glucosidase inhibitory activities (Kwon *et al.,* 2008).

There are many foods and beverages rich in polyphenols, with two or more classes of polyphenols found in a single source. For example, grapes and red wine are rich in polyphenols including the anthocyanins, catechins and quercetin and the stilbene resveratrol (Neveu, 2010). As mentioned previously, green tea contains compounds such as catechins, EGCG, procyanidin and quercetin. Much research to date has focused on isolated polyphenols and their effect on health parameters. However, because foods are found to contain more than one polyphenol type, these naturally occurring polyphenols may have synergistic effects. For example, polyphenols have been found to synergistically benefit markers of cancer. Grape extract was found to work synergistically with decaffeinated green tea extract by inhibiting cancer cell growth and inhibiting tumor-associated nitric oxide and nitrogen dioxide (NOX) activity (Morre & Morre 2006). Based on previous studies, it was hypothesised that polyphenol rich extracts both in isolation and in combination, would reduce sugar release from various starch rich foods.

The aims of this study were:

1. To perform a doses response analysis to determine the optimal dose of GTE, GSE and RES for reducing sugar release from high GI white bread.

2. To assess the sugar reducing capacity of optimal doses of isolated extracts in other starch systems such as gluten free bread, flat bread and English style pancakes.

3. To determine if polyphenol rich extracts show a synergistic effect in reducing sugar release from white bread.

9.3 Methods

9.3.1 Chemicals

All chemicals and reagents were of analytical grade and were purchased from Sigma-Aldrich (Poole, UK). The extracts included: GTE, GSE and RES (Biotivia®, UK). The polyphenol content and optimal dose of baobab fruit extract (BAO; Holland & Barrett, UK) for reducing sugar release from white bread was determined from previous results (Section 8). All bread ingredients were purchased from Tesco supermarket, UK. Extract compositions are given in Table 9.1.

Table 9.1 Contents, ingredients and bioactives in each extract tested

Extract	Contents
Green Tea Extract (Holland & Barrett, UK)	315mg green tea extract (*Camellia sinensis*, standardized to contain 15% polyphenols), maltodextrin, bulking agent (dicalcium phosphate), anti-caking agents (silicon dioxide, magnesium stearate)
Grape Seed Extract (Holland & Barrett, UK)	50mg grape seed extract (standardised to contain 50% proanthocyanidins, 25mg), Citrus bioflavonoids 500mg, (from 250mg of 2:1 concentrate), rice powder, lactose, maltodextrin, anti-caking agents (silicon dioxide, magnesium stearate, stearic acid)
Trans-Resveratrol (Biotivia®, UK)	Japanese knotweed (*polygonum cuspidatum*), dried rhizome extract (Biotivia®, UK) containing 50% trans-resveratrol, 2% emodin, hydroxypropyl methylcellulose, colour copper complex of chlorophyll and chlorophyllins

Contents based on per capsule amount.

9.3.2 Study protocol

All extracts were prepared using standard protocol. Extract samples were weighed: 250mg GSE, 100mg RES and 50mg GTE, and then added to 50 mL of dH_2O. Weights were determined by the level of detection in the FCR assay.

9.3.3 FRAP

Samples of three plant extracts were analysed using the FRAP method adapted from Benzie and Strain (1996), as previously described 8.3.3.

9.3.4 FCR

The polyphenol content of GTE, GSE and RES was analysed using the FCR method (Sharma & Gujral, 2010) as previously described in 7.3.3.

9.3.5 Bioaccessibility of plant extracts

Samples from three plant extracts were analysed using an *in vitro* digestion model adapted from Ryan *et al.* (2008) as previously described 7.3.4.

9.3.6 Measurement of sugar release

The effect of polyphenols on the inhibition of starch breakdown was determined. This was achieved by subjecting samples of bread with added polyphenol extracts to an *in vitro* digestion procedure, and measuring the resultant reducing sugars released at various phases throughout duodenal digestion.

9.3.7 Dose-response

A dose-response was performed using a range of extract concentrations to determine the lowest concentration of each extract that could significantly reduce RDS from white bread with percentages given based on a 500g loaf (Table 9.2). The optimal dose of BAO was previously determined in our lab (Coe *et al.,* 2013; Section 8).

Table 9.2 Doses of each extract (as a %) analysed for reducing sugar release from white bread.

GTE	GSE	RES	BAO[†]
0.2	0.2[*]	0.22[*]	1.25
0.4[*]	0.4	0.44	1.88[*]
0.6	0.6	0.66	2.50
0.8	0.8	0.88	3.13
1.0	1.0		3.75
1.2	1.2		
1.4	1.4		
1.6	1.6		
1.8			
2.0			
2.2			

*=optimal dosage for reducing sugar release ($p<0.05$).
Values represent the percentage (%) of the extract baked into a 500g loaf. GTE, green tea extract; GSE, grape seed extract; RES, resveratrol; BAO, baobab fruit extract. Sugar release was measured in mg/ g of bread sample.

[†]Baobab results found from previous results in our lab (Coe *et al.,* 2013).

9.3.8 Optimal doses added to other starch foods

The optimal doses of each extract found for reducing sugar release from white bread, was added at the same percentage (%) to both white flat bread and white gluten free bread. Resulting sugar release was then measured. Pancakes were also considered as a potential starch source, yet due to their low amount of sugar release measured *in vitro*, they were excluded from this study.

9.3.9 Bread preparation

White bread, flat bread and gluten free bread were made to recipe as in Table 9.3. Flat bread was made by mixing flour and polyphenol extracts before adding water. Dough was rolled flat and cooked in a large oiled frying pan for about 3 min, with occasional flipping. White bread and gluten free bread were made in a Russell Hobbs bread maker (model no:18036, Manchester, UK).

Table 9.3 Ingredients and amounts used to make each bread (based on the control for each bread).

White bread (500 g)	Flat bread (70.5 g)	Gluten free bread (750 g)
190 g warm water	100 g plain white flour	350 g warm water
1 tbsp virgin olive oil	75 ml cold water	1 ½ tbsp virgin olive oil
1 tsp salt		½ tsp salt
1 tbsp sugar		1 tbsp sugar
1 tbsp dried milk powder		400 g gluten free bread mix[1]
350 g strong white flour		1 tsp yeast
1 ½ tsp yeast		
2 g GTE (0.4%)	0.282 g GTE (0.4%)	3 g GTE (0.4%)
1 g GSE (0.2%)	0.141 g GSE (0.2%)	1.5 g GSE (0.2%)
1.1 g RES (0.22%)	0.155 g RES (0.22%)	1.65 g RES (0.22%)
9.375 g BAO (1.88%)	1.325 g BAO (1.88%)	14 g BAO (1.88%)

[1]=flour contents were altered when extracts were added into bread, in order to keep overall weights of breads (in grams) the same as control breads. This consisted of subtracting weight of extract from control flour weight to determine new flour weight. % based on optimal doses of extracts found for reducing

sugar release after being baked into white bread. Gluten free bread contained flour blend (rice, potato and tapioca), xanthan gum (Doves Farm brand). tbsp=tablespoon, tsp=teaspoon, g=grams, GTE=green tea extract, GSE=grape seed extract, RES=resveratrol and BAO=baobab fruit extract.

GTE, GSE, RES and BAO were added immediately before the baking process was initiated. When adding extracts to the breads, the flour content was altered in order to keep the overall weight of each bread the same as the control bread. This was achieved by subtracting the extract weight in grams from the flour weight in grams, to determine the new flour weight with added extract. All bread samples were baked the night before, sealed at room temperature in plastic containers overnight and tested the following morning.

9.3.10 Extracts in combination

The same optimal isolated extract doses for reducing sugar release were then combined in breads and sugar release was measured (with a 75% variation to increase the variety of extract combinations used). The type and amount of each extract used, as a percentage of total bread weight (500g), were as follows: bread 1=0.4 GTE/0.22 RES, 2=0.4 GTE/0.2 GSE, 3=0.4 GTE/1.88 BAO, 4=0.22 RES/1.88 BAO, 5=0.2 GSE/1.88 BAO, 6=0.4 GTE/0.2 GSE/1.88 BAO, 7=0.4 GTE/0.22 RES/1.88 BAO, 8=0.2 GTE/0.11 RES, 9=0.1 GTE/0.055 RES, 10=0.3 GTE/0.165 RES.

9.3.11 In vitro digestion

The *in vitro* digestion procedure (Mishra *et al.,* 2008) was as previously described in 8.3.8.

9.3.12 Analysis of sugar release

Sugar released from the bread during digestion was measured by a colorimetric method adapted from Englyst and Hudson (1987), as previously described in 7.3.8.

9.3.13 Statistical analysis

All *in vitro* experiments were carried out in triplicate and each had a minimum of two replicates for each sample. The data are presented as means ± SEM and comparisons between samples were carried out by an ANOVA and Tukey's multiple comparison test (SPSS, version 17; SPSS Inc., Chicago Ill). Statistical significance was set at $p < 0.05$.

9.4 Results

9.4.1 Antioxidant and polyphenol content, and bioaccessibility

The antioxidant content in μmol/ L ± SEM was as follows: GTE 2494.4 ± 77.0 μmol/ L, GSE 3361.0 ± 35.5 μmol/ L, and RES 3510.5 ± 882.3 μmol/ L. The polyphenol content in mg GAE per g of extract ± SEM was as follows: GTE 207.67 ± 2.84 mg/ g, GSE 67.95 ± 0.84 mg/ g and RES 161.96 ± 7.17 mg/ g. There was a trend for the polyphenol bioaccessibility of all extracts to increase following the gastric digestion phase ($p <$ 0.05; Figure 9.1); however the resveratrol extract had a non-significant increase. Values continued to increase ($p < 0.05$) into the duodenal phase.
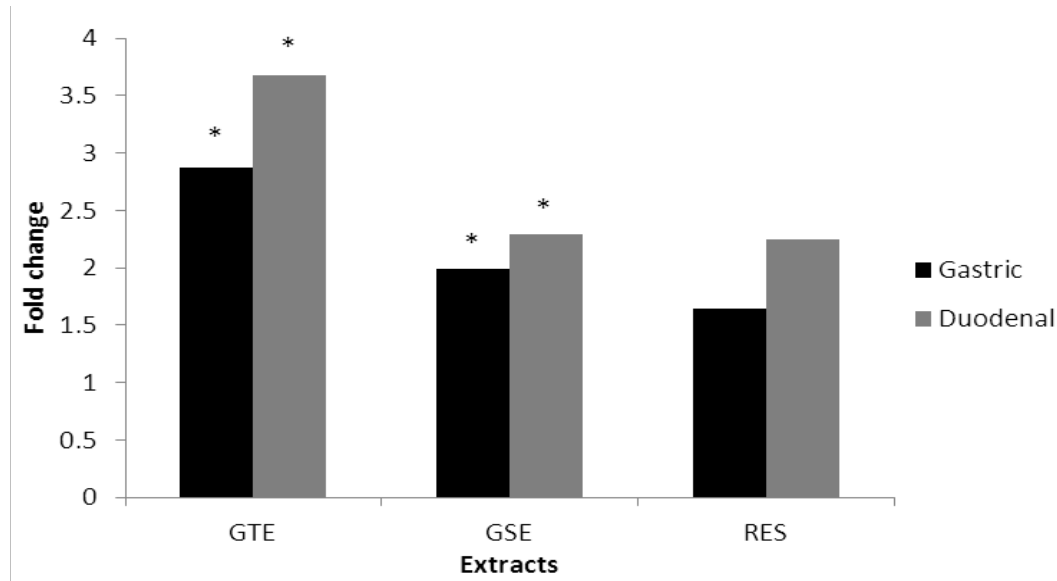
Figure 9.1 % Bioaccessibility of the polyphenol content after the Gastric and Duodenal phases of digestion.

*$p<0.05$ = significant increase compared to baseline values. GSE=grape seed extract, GTE=green tea extract, RES=resveratrol.

9.4.2 Analysis of sugar release: dose response in white bread

RDS sugar release was significantly reduced ($p < 0.05$) by the addition of GTE at 0.4%, GSE at 0.2% and RES at 0.22% to white bread compared to the CON (Figure 9.2, Table 9.4). These were the lowest doses to have a significant impact on RDS. There was a shift in the time of sugar release in the bread with all extracts, a greater majority taking place during the 120 min stage of ileal digestion.
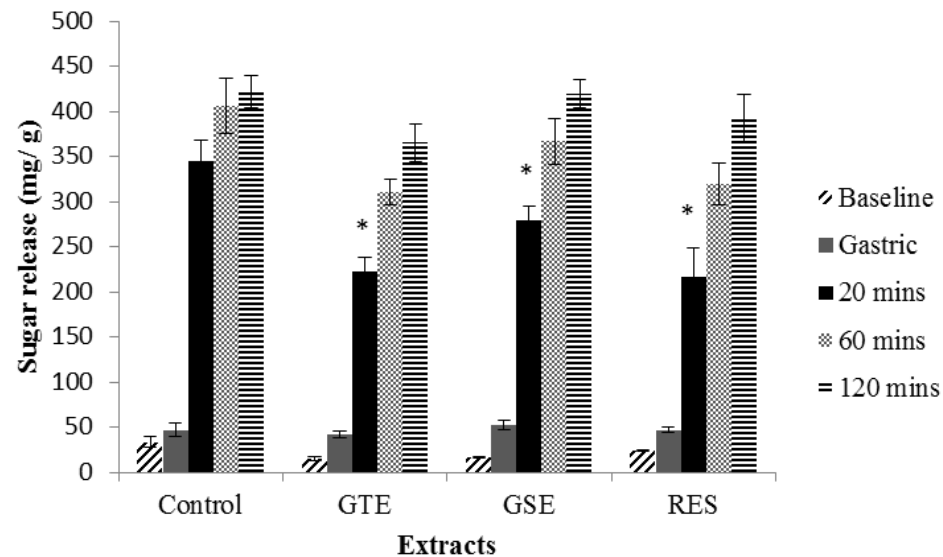
Figure 9.2 Sugar release (mg/ g sample) throughout digestion for each extract at optimal concentrations in white bread.

*$p<0.05$ denotes significant decrease in sugar release compared to control white bread (no added baobab extract). Percentages for each extract were calculated after the addition into white bread to make a total loaf weight of 500g. Each patterned bar represents different stages of digestion, with 20, 60 and 120 min = intestinal stages of digestion. GTE=green tea extract (0.4%), GSE=grape seed extract (0.2%), RES=resveratrol (0.22%). Values represent means of three independent experiments ± SEM.

9.4.3 Flat bread and gluten free bread

There was no reduction in RDS sugar release at any stage of digestion for any of the extracts, in either the flat bread or the gluten free bread, compared to the control (Table 9.4). White bread was shown to have a significantly greater sugar release than either the control flat bread or gluten free bread.

Table 9.4 Sugar release at 20 minutes into the duodenal phase (RDS) at optimal doses

	White bread	SD	Flat bread	SD	Gluten free bread	SD
Extracts						
Control	345.62	69.35	275.32**	61.43	256.84**	42.84
GTE	222.25*	41.19	252.33	37.97	247.78	80.93
GSE	279.47*	44.99	248.22	34.71	230.32	26.11
RES	216.07*	32.97	305.74	48.73	272.14	38.43
BAO	201.96*	39.58	277.08	29.46	266.85	46.79

*$p<0.05$=significant reduction in sugar release compared to the control. **$p<0.05$=significantly lower compared to white bread control. Values represent the optimal doses of each extract found for reducing sugar release from white bread and are means of three independent experiments ± standard deviation (SD).

9.4.4 Extracts in combination and resulting RDS

For combination breads 1 through 10, there was no significant difference between RDS or SDS values between breads or compared to the control bread (Figure 9.3). When comparing all single and combination extract breads to each other, combination bread 6 (0.4% GTE, 0.2% GSE and 1.88% baobab) had a greater ($p < 0.05$) RDS than isolated GTE, GSE and RES breads. Although bread 1 (0.4% GTE and 0.22% RES), 3 (0.4% GTE and 1.88% BAO) and 8 (0.2% GTE and 0.11% RES) showed a trend in reducing sugar release, no combination of polyphenols was better at reducing sugar release than any of the optimal doses of isolated polyphenols.
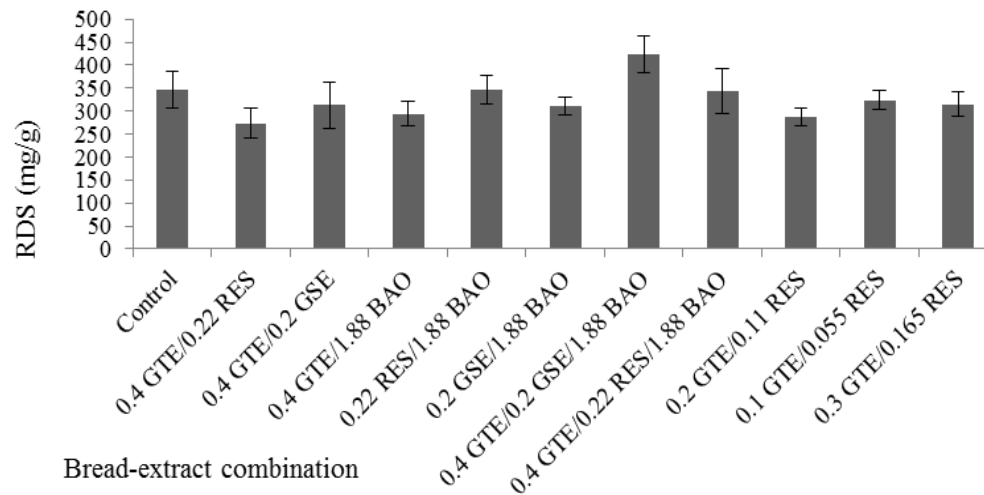
Figure 9.3 Rapidly digestible starch (RDS) sugar release from white bread with combinations of polyphenol extracts.

Doses were based on % of each extract added relative to 500g loaf and represent the optimal doses of each extract found for reducing sugar release from white bread. Values are means of three independent experiments ± SEM.

9.5 Discussion

9.5.1 Bioaccessible polyphenol rich extracts

The GTE and RES extract were rich sources of polyphenols, and although GSE was the least concentrated polyphenol extract it was still considered a rich source of polyphenols. All extracts had increased polyphenol bioaccessibility into the gastric and duodenal phase and therefore become potentially more available in the body throughout digestion.

9.5.2 Dose-response in white bread

Green and black tea extract have been found to reduce starch hydrolysis in wheat rice corn and potato, thought to be due to the interaction of polyphenols with starch structure (Guzar, 2012). In the same study by Guzar (2012), green tea extract was baked into sponge cake at 4%, 6% and 9%, yet only 6% and 9% were found to reduce in *vitro starch* digestibility and therefore different doses of extracts may show varying effects on GI and resulting GR. Results for GTE and also GSE and RES, showed this *in vitro* dose-response dependence in the current study.

An early study by Thompson *et al.* (1984) discovered that polyphenols were negatively correlated to the GR in both healthy and diabetic subjects. This was thought to be in part due to high-molecular weight tannins which have the ability to form complexes with macromolecules under certain digestive conditions (Horvath, 1981). Therefore, highly branched procyanidins found in GSE and GTE may reduce carbohydrate digestion by forming complexes with the starch chains, preventing their breakdown. Also, a high carbohydrate diet supplemented with both 100mg and 300mg of GSE was shown to reduce plasma glucose concentrations at 15 and 30 min after ingestion in healthy subjects, compared to a high carbohydrate control (Sapwarobol *et al.,* 2012). It was thought that the proanthocyanidins in GSE may be inhibiting α-amylase and α-glucosidase. The high stability of *Trans* - resveratrol may be the reason for the extract remaining intact and thus having a significant impact on reducing starch digestion (Trela & Waterhouse, 1996).

The polyphenol extracts were baked into bread at low doses, each loaf containing the same amount of polyphenols found in approximately two cups of green tea or a third of a small glass of red wine (Rothwell *et al.,* 2013). Therefore there is little risk of an overdose of polyphenols which would result in adverse effects in the body (Mennen *et al.,* 2005) and also little/ no discernible change to bread quality. Although RES decreased sugar release at all concentrations used in the current study, the change in bread properties at higher concentrations may decrease the sensory acceptability of the loaf.

9.5.3 Polyphenol rich extract addition to other starch foods

This study shows that freshly baked white bread has a high RDS value when measured *in vitro*, and therefore confirms the known high GI (approximately 100; Englyst *et al.,* 1999). Flat bread and gluten free bread were shown to have a less pronounced presence of RDS during analysis compared to the control white bread. Although polyphenol rich extracts have the potential to reduce the release of sugars from certain high GI starch rich foods such as white bread, flat bread and gluten free bread were estimated to only have a medium GI value and thus there may not have

been as much potential for polyphenols to further lower the GI of these carbohydrate rich breads. The food matrix can affect the starch digestibility from different sources, with factors such as the protein matrix, the compact structure of a food, the starch particle size and surface to starch ratio all able to influence disgestibility (Esther *et al.,* 2008). For example, compared to the flat bread, white and gluten free breads were baked instead of cooked, were porous and contained yeast compared to being compact. White bread and flat bread contained gluten protein and although gluten free breads can vary in starch digestibility, gluten itself does not seem to be a major contributor to the GI of a food (Packer *et al.,* 2000). English style pancakes were also considered as a potential starch source, yet due to their poor effect at reducing starch breakdown from white bread *in vitro* (containing a low/ medium GI), they were excluded from the study.

Polyphenols may reduce diabetes risk in ways other than by reducing starch breakdown in the gastrointestinal tract. For example, in streptozotocin-induced rats consuming a high-fat diet, GTE administered at 300 mg/ kg body weight for 30 days was shown to revert key enzymes of carbohydrate metabolism to near normal levels (Sundaram *et al.,* 2013), and grape seed procyanidins had an antihyperglycaemic effect which was thought to be due to insulinomimetic activity in cells by increasing glucose uptake (Pinent *et al.,* 2004). Therefore, these extracts may show beneficial effects on glycaemia *in vivo*, even if no beneficial results were found in the *in vitro* model used in the current study.

In this study, only three different bread systems were tested, and only one dose of each extract was used. Therefore, different doses used in other starch rich foods may show positive effects on sugar release. Liu *et al*. (2011) found that catechins reduced the GR in co-cooked normal and waxy maize starch, yet were shown to increase the GR and delay the blood glucose peak in high amylose maize starch. Amylose is less readily digested and absorbed into the blood than amylopectin and will generally produce a lower GR. This study highlights the potential of polyphenols to either have a sugar enhancing or reducing effect, depending on the starch source and thus the amylose/ amylopectin ratio.

9.5.4 Polyphenols in combination
Although polyphenol rich extracts in isolation were shown to reduce sugar release from high GI bread, in combination there was no reduction. Although isolated polyphenols have been shown beneficial to have effects on GR, other studies show different results. Intakes of single polyphenol compounds such as quercetin, kaempferol, myricetin, apigenin, and luteolin were found ineffective at reducing the risk of type 2 diabetes in The Women's Health Study (Song *et al.,* 2005).

Few *in vitro* and animal studies have shown synergistic effects of polyphenols for various disease risks (Bastianetto *et al.,* 2009; Betts *et al.,* 2011; Conte *et al.,* 2003). There is limited research on polyphenol extracts in combination for reducing risk factors of diabetes, and those that have been done are conflicting. Mackenzie *et al.* (2007) found no effect of combined tea supplements on reducing hyperglycaemia. Their study looked at the effect of a mixed green and black tea extract in subjects with type 2 diabetes, and found that $HbA1_c$ at three months was not significantly different between those taking tea supplements and those taking the control. Therefore, more work should explore the synergism that may take place between different classes of polyphenolic compounds for diabetes risk factors.

9.6 Conclusion

Many factors must be considered when using polyphenols as functional food additives. Although all extracts in isolation were shown to reduce starch breakdown and sugar release from white bread, these same doses were found to have no effect in other starch systems. Also, in combination there was no effect of any extract on sugar release. This is a unique study in that no other study to date has looked at determining the effect a combination of polyphenol extracts on sugar release from a starch rich food. Future work will look at increasing the dose of extract added into foods on sugar release, and also at the potential of polyphenols on sugar release from other medium to high GI foods.

9.7 Publications

Coe S & Ryan L (2013) Sugar release from various starch rich foods with added polyphenol extracts, *Proceedings of the Nutrition Society*, **72**, E201.

Coe S & Ryan L (2014) Investigation of polyphenol synergism for reducing sugar release from white bread, *Proceedings of the Nutrition Society* (Submitted).

Coe S & Ryan L (2014) White bread enriched with polyphenol extracts shows no effect on glycaemic response or satiety, yet may increase postprandial insulin economy in healthy subjects, *Nutrition Journal* (Submitted).

10. The polyphenol rich baobab fruit (*Adansonia digitata* L.) reduces the glycaemic response in humans.

10.1 Summary

Anecdotal evidence suggests that baobab may play a role in improving health, yet to date there is little research on the fruit. Based on previous results from our lab in which the baobab fruit extract reduced starch digestion *in vitro,* it was hypothesised that it would show potential for reducing the GR, and for increasing satiety and diet induced thermogenesis (DIT) in humans. Nine health females were recruited for a randomised cross over trial, in which baobab fruit extract was consumed in solution at both a low dose (LD, 18.5g) and a high dose (HD, 37g) in 250 mL of water along with white bread. Each meal provided a total of 50g available carbohydrate (avCHO), and postprandial GR, satiety and energy expenditure (EE) were measured. Results found that the baobab fruit extract at both LD and HD significantly ($p < 0.05$) reduced GR, although there was no significant effect on satiety or on DIT. Therefore, more studies need to look into the effect of baobab as a potential alternative therapy for improving blood glucose levels.

10.2 Introduction

Epidemiological studies have shown that plant foods rich in polyphenols can reduce the risk of diabetes (Mursu *et al.,* 2014; Nanri *et al.,* 2010). They may also be beneficial for increasing both DIT and satiety (Bolton *et al.,* 1981; Gruendel *et al.,* 2006; Raben *et al.,* 1994; Tanko *et al.,* 2008; Torronen *et al.,* 2012). Bolton *et al.* (1981) found that whole fruit produced a stronger satiety rating than fruit juice, and it was found that the return of appetite was also delayed with whole fruit consumption. This increase in fullness was thought to be due at least in part to the fibre content of the whole fruit. However, studies looking at the effect of different foods and food compounds on the association between satiety and DIT show conflicting results. In a study looking at the polyphenol and fibre-rich carob pulp, satiety post consumption was increased compared to the control (Gruendel *et al.,* 2006) and the pulp was also found to increase postprandial EE by 42.3%. In contrast Raben *et al.* (1994) found a

reduction in DIT and an increase in the feeling of fullness in subjects after they consumed a high fibre meal when compared to a low fibre meal. Therefore, polyphenols are thought to play a significant role in the increase of EE (Harada *et al.,* 2005).

Following on from previous work in our lab (Section 8) focusing on the role of the baobab fruit in starch digestion *in vitro*, it was further hypothesised that when tested in humans baobab extract would reduce the GR postprandially. It was also predicted that the extract would increase the feeling of fullness and the amount of energy expended after consumption, and that these three test parameters may show an association.

Based on the results from previous published data from our lab, the aim of the present study was to determine the effect of a LD baobab drink and a HD baobab aqueous drink (sample 6, Baobab super fruit powder, Min Vita, London, UK) on postprandial GR, DIT and satiety in humans.

10.3 Methods

10.3.1 Subjects

The current study was a randomised, single-blind, repeated-measures design with volunteers fed three different test meals on three different days. Ten subjects were originally recruited based on the guidelines on measuring the GR by the Food and Agricultural Organization/World Health Organization (FAO/WHO) (1998) to take into account individual variations, with this subject number being the same as that used by Sanaka *et al.* (2007). However, one subject dropped out due to personal reasons, and therefore nine healthy female subjects (25.3±4.8 yr; height 1.66±0.05m; weight 61.2±8.0kg; body mass index 22.3±2.6 kg/m^2; body fat 25.4±7.7%; values are means ± standard deviation{SD}) were recruited for the study by means of advertisements and personal communications. Before inclusion in the study, potential participants were briefed on all aspects of the experiment and were given the opportunity to ask questions. This was followed by a health assessment, which included anthropometric measurements and a health questionnaire (giving details of food allergies/ intolerances, metabolic diseases, special dietary needs, and smoking habits). Those who fulfilled all the acceptable criteria (age 18-60 yr; body mass index <30 kg/m^2; blood pressure between 110 and 120/75 and 85 mm Hg; fasting blood glucose <6mmol/ L; not on prescription medication; no genetic or metabolic diseases) were included in the study. On the day before each test, subjects were asked to restrict their intake of alcohol and caffeine-containing drinks and to refrain from strenuous physical activity.

The study was conducted at the Functional Food Centre at Oxford Brookes University. All participants gave written informed consent before starting and the study was initiated after the approval by the Oxford Brookes University Research Ethics Committee according to the guidelines laid down in the Declaration of Helsinki. On each test day subjects arrived between 7 and 9 am on the morning after an overnight fasting (10 - 12 hour before testing time) and without undertaking any physical activity.

10.3.2 Test meal

The three test meals were a control (C), a LD solution of baobab fruit extract in 250 mL of water and a HD solution of baobab fruit extract in 250 mL of water (Baobab super fruit powder, Min Vita, London, UK). The control consisted of 132g of white bread (Sainsbury's White Sliced loaf, London, UK), and 250 mL still water. The LD consisted of 123g of white bread and 250 mL still water with 18.5g of baobab fruit extract added to make a drink (37% baobab per g avCHO) and the HD consisted of 114g of white bread and 250 mL still water with 37g of baobab fruit extract (74% baobab per g avCHO).

Each of the three meals contained 50g of avCHO which was calculated for each test meal using the procedure from FAO/ WHO (1998), according to the nutrition information available from the bread label and the baobab suppliers. Because previous controlled trials using baobab extract have not been performed, the doses of baobab used in this study reflect the amount and type of dietary fibre used in previous studies when measuring postprandial effects on GR (Gruendel *et al.,* 2007; Keogh *et al.,* 2007; Yan *et al.,* 2014).

10.3.3 Study design

Volunteers participated in a randomised, balanced, controlled crossover study where they consumed the C, LD and HD on separate days in a random order. On the day prior to testing, volunteers were asked to record their food intake and repeat it prior to subsequent tests.

10.3.4 Energy expenditure

On arrival in the laboratory, volunteers were asked to rest for 30 min in a supine position on a bed before baseline measurements of resting metabolic rate (RMR) were taken. RMR was determined in the morning between 7 and 9 am. RMR was measured at one-min intervals for 30 min

under the ventilated hood indirect calorimetry system (Deltatrac™ II Metabolic Monitor, Datex-Ohmeda Inc., Finland). The analyser was calibrated on each test day with standardised gases containing 5% CO_2 and 95% O_2.

DIT was determined after the breakfast meal for 15 min in the first hour and then every 30 min until 180 min (Reed & Hill, 1996). The first 5 min of every 15 min time period was discarded to allow for stabilisation within the Deltatrac hood and the average of the remaining 10 min was used. This time period was recommended to be appropriate to measure the DIT (Reed & Hill, 1996). DIT was calculated as the increase in EE per min above pre-meal values for 3 hour after meal intake. EE and fat oxidation were calculated using the equations of Lusk (1928).

10.3.5 Glycaemic response

The protocol used to measure the blood glucose response was adopted from that described by Brouns *et al.* (2005) and is in line with procedures recommended by FAO/ WHO (1998). Blood was obtained by finger prick using the Unistick three single-use lancing device (Owen Mumford, Woodstock, UK). Before a finger prick, subjects were encouraged to warm their hand to increase blood flow. To minimize plasma dilution, fingertips were not squeezed to extract blood but were instead gently massaged starting from the base of the hand moving towards the tips. The first two drops of expressed blood were discarded, and the next drop was used for testing.

Blood glucose was measured using the HemoCue 201+ Glucose analyser (HemoCue Ltd, Dronfield, UK). The HemoCue is a reliable method of blood glucose analysis (Stork *et al.,* 2005). The HemoCue was calibrated each morning before testing, using control solution and ensuring displayed results were in a specified range (located in the control solution box). Fasting blood samples were taken at −5 and 0 min, and the coeffienct of variation between the two readings was required to be less than 3% before testing could proceed. The test food was consumed immediately afterwards within 15 min at a comfortable pace. Further blood samples were then taken at 15, 30, 45, 60, 90, 120, 150 and 180 min after consuming the test meal.

The change in GR was calculated by computing the difference between the blood glucose concentration at a time point and mean baseline blood glucose concentration (based on two baseline values taken 5 min apart). Because it represented the relative increment in the GR at any time point compared with the baseline value, it was this change in GR that was used for all further analyses, including incremental area under the curve (IAUC) calculated using the trapezoidal rule (Brouns *et al.,* 2005; Wolever, 2006), blood glucose response curve construction and statistics.

10.3.6 Satiety

One hundred millimetre continuous line visual analogue scales (VAS) were utilised to measure subjective feelings of hunger, fullness, desire-to-eat and prospective food consumption. The volunteers provided VAS data at baseline (0 min) and at 30, 60, 90, 120, 150, and 180 min after the commencement of eating the test food, and after lunch. The specific questions asked were, 'How hungry do you feel?', 'How full do you feel?', 'How strong is your desire to eat?' and, 'How much food do you think you can eat?'

10.3.7 Statistical analysis

Statistical analysis was performed using SPSS (version 20.0; SPSS, Chicago, IL, USA) and data and figures were processed in Microsoft Excel spread sheet (2006, Reading, UK). The IAUC was determined for blood glucose, total DIT and fat oxidation using the trapezoidal rule for values above the baseline. The relative increment in the GR and EE at any time point compared with the baseline value was used to assess the differences at each time. The differences were assessed using a three-factor repeated-measures (RM) ANOVA with differences between the meal assessed using contrasts within the ANOVA. Significance was set at $p < 0.05$. Values are presented as means $\pm$ SD.

10.4 Results

10.4.1 Glycaemic response

The GR showed an initial rise in blood glucose following the test meal to peak at 45 min for the two baobab doses and at 60 min for the control. All three tests reached their nadir at 180 min (Figure 10.1). The GR-IAUC was significantly different between the three meals after 180 min, 120 min and 60 min ($p < 0.05$), with the C meal having the greatest GR (Table 10.1). In the first 60 min the HD had the lowest GR while over the entire 180 min LD attenuated the GR the most.

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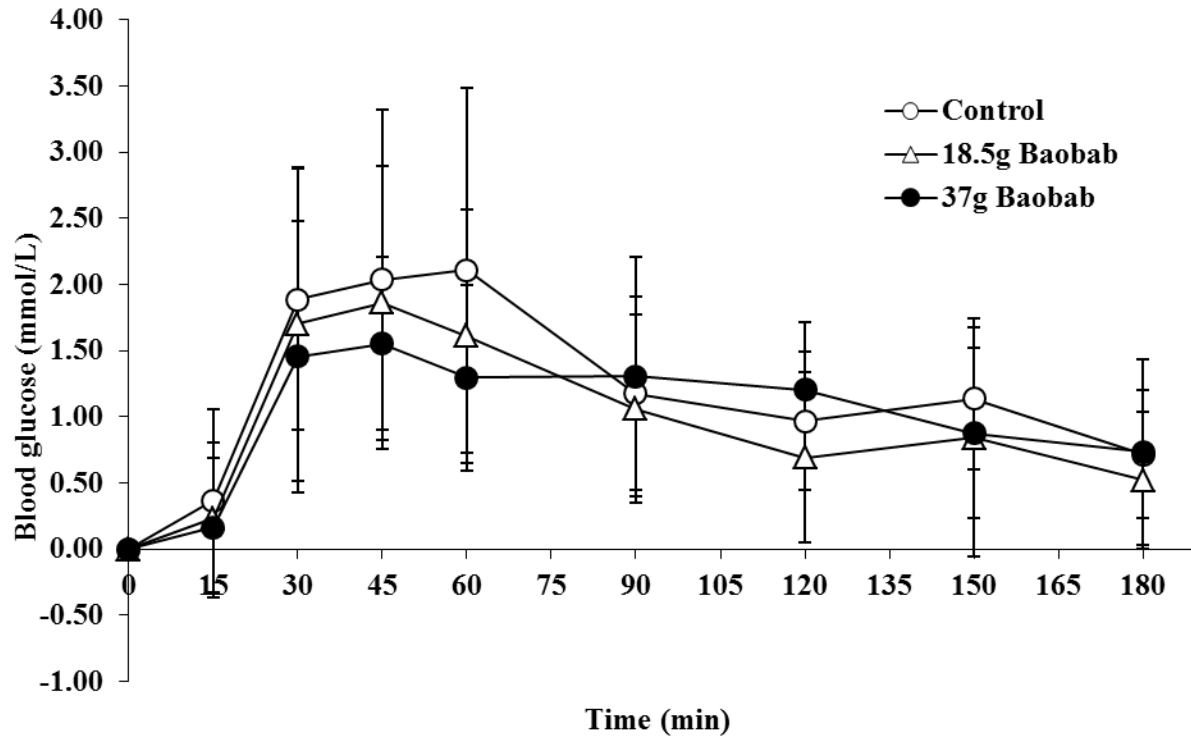
Figure 10.1 The change in blood glucose response from baseline.

Following the consumption of white bread in conjunction with 250 mL water (control), or 18.5g baobab or 37g baobab made up in solution with 250 mL of water, to equal 50 grams of available carbohydrate.
Finger prick blood samples were taken at 15 min intervals for the first hour, and then every 30 min for the last two hours, following the consumption of the test meal. Data are given as means ± SD (n=9).

Table 10.1 Glycaemic response area under the curve (AUC) after consuming three test meals.

GR (mmol/min/L)	Control	18.5g Baobab	37g Baobab
GR AUC 60	86.1±46.6	72.4±44.4	59.6±35.6*
GR AUC 120	175.3±80.4	142.6±76.7*	135.8±74.6*
GR AUC 180	237.4±104.9	188.1±114.4*	193.1±104.3

*$p < 0.05$ denotes significant decrease in glycaemic response (GR) compared to control at each time point (an ANOVA was performed followed by a Tukey's test). AUC at 60 min, 120 min and 180 min for each test meal to provide 50g of available carbohydrate. The three test meals consisted of the control white bread and 250 mL water, white bread and 18.5g baobab (low dose) made up in solution with 250 ml water and bread and 37g baobab (high dose) made up in solution with 250 mL water. Values represent mean ± SEM.

10.4.2 Energy Expenditure

There were no significant differences in resting EE between the three test days (Control; 0.880±0.087 kcal/min; 18.5g; 0.897±0.066 kcal/min; 37g; 0.872±0.084 kcal/min) with all three days having similar baseline measurements. EE increased postprandially following each of the three test meals. There were no significant differences in EE following the three meals with total DIT being similar for the three meals (C: 13.75±6.51 kcal; LD: 11.25±5.91 kcal; HD: 13.42±6.22 kcal).

10.4.3 Satiety

There was no significant difference between the visual analogue scores for any of the satiety parameters (Hunger: Control; 567±437; 18.5g; 550±411; 37g; 650±363 mm/min; Fullness: Control; 689±404; 18.5g; 750±424; 37g; 653±362 mm/min; Desire to eat: Control; 430±184; 18.5g; 578±413; 37g; 670±339 mm/min; Prospective consumption: Control; 409±452; 18.5g; 361±345; 37g; 567±328 mm/min).

10.5 Discussion

In the current study, the consumption of the LD and HD baobab fruit extract drinks significantly reduced the GR to white bread. What may be more important than lowering overall AUC may be preventing the large spikes in blood glucose 30-45 min after a meal (Ceriello, 1953). The HD baobab meal stabilised the blood glucose peaks more over the 3 hour period compared to the other two meals. Baobab is a rich source of dietary fibre, specifically soluble fibre, and the presence of fibre in a high carbohydrate food has been shown to blunt postprandial GR (Kwong *et al.,* 2012; Vosloo, 2005; Wolever, 2006). Soluble fibre specifically has been shown to be beneficial on postprandial glycaemia and on insulin levels in type 2 diabetic subjects (Flammang *et al.,* 2006). Baobab is also rich in flavonoids and Zhou *et al.* (2009) found that flavonoids extracted from lotus (*Nelumbo nuficera* Gaertn) reduced fasting blood glucose levels in diabetic mice. Therefore, both the fibre and the polyphenol content of the concentrated baobab extract may be contributing to the reduction in GR *in vivo*.

Satiety is dependent on many aspects of food intake, including the change in the GR, nutrient composition, hormonal changes and gastric emptying rates. In the current study, baobab fruit extract had no effect on satiety measures. The Glucostatic Theory states that the change in blood glucose levels between meals has an effect on hunger (Mayer, 1953). Therefore, any food that can stabilise blood glucose levels, such as seen in the HD meal, may reduce hunger and thus prove beneficial in weight management. Taking this into account, and also that the fibre content was increased in the HD baobab meal, it is of interest that there was no corresponding increase in satiety as shown in previous studies (Gruendel *et al.,* 2006; Raben *et al.,* 1994). In the current study, there were the limitations of a low subject number for measuring satiety (Stubbs *et al.,* 2000) and VAS were used as a measure of subjective hunger and they may not always reflect actual subsequent food intake (Ranawana & Henry, 2011). Therefore, an *ad libitum* test meal may have been a more accurate reflection of satiety, however given the desire to measure DIT and GR for 180 min postprandially this was not deemed possible as by 180 any differences in satiety would not be visible.

There was no significant difference between the control meal and the LD or HD meal on postprandial EE. Macronutrients have a hierarchy for increasing postprandial EE, alcohol and protein increasing DIT more than carbohydrate and fat (Westerterp, 2004). In the current study, avCHO was kept constant between groups and neither white bread nor baobab are major sources of protein/ and or fat, therefore macronutrient content between groups was not largely different. However, there was a large increase in the amount of fibre being consumed as baobab content increased. As mentioned, although fibre rich foods have previously been shown to reduce the GR and increase satiety, the findings on DIT are more controversial with both increases and decreases in DIT demonstrated (Gruendel *et al.,* 2006; Raben *et al.,* 1994). Polyphenols found in green tea, such as EGCG and other catechins have been shown to increase 24 hour EE (Dulloo *et al.,* 1999). However, Dulloo *et al.*

(1999) analysed polyphenols in isolation of other macronutrients, and therefore more studies need to be done on whole polyphenol rich extracts and total EE.

10.6 Conclusion

In conclusion, this is a novel study as no other study to date has looked at the effect of different doses of baobab fruit extract in aqueous solution on GR, satiety or DIT. The baobab fruit is a rich source of bioaccessible polyphenols and the current study shows the potential of the polyphenol rich extract for reducing the GR to carbohydrate-rich foods *in vivo*. This is in good correlation with the results from previous studies, and with results from the *in vitro* dose response digestion in our lab. However, there was no effect of baobab fruit on satiety or on DIT for either the LD or HD drink. Apart from the use of VAS and the low subject number, there are some other limitations to the methods used in this study. Future studies will need to determine the individual polyphenols present in the extracts to identify which polyphenols or group of polyphenols may be eliciting the effects on GR. To elucidate the role of polyphenols in the extract, the soluble (pectin) and insoluble fibre content would need to be controlled for as they may be potential confounders. Finally, further human studies on GR would be essential for determining the optimal dose of baobab fruit extract in reducing postprandial glycaemia.

10.7 Publication

Coe S, Clegg M, Armengol M & Ryan L (2013) The polyphenol rich baobab fruit (*Adansonia digitata* L.) reduces starch digestion and glycemic response in humans*, Nutrition Research*, **33**, 888-896.

11. White bread enriched with polyphenol extracts shows no effect on glycaemic response or satiety, yet may increase postprandial insulin economy in healthy subjects.

11.1 Summary

Carbohydrate rich foods that produce a slow, gradual rise in blood glucose levels have been shown to increase insulin sensitivity and postprandial satiety. Polyphenol rich plant sources have been shown to modify starch digestion from carbohydrate rich foods and lower resulting glycaemia. The aim of this study was to determine the effect of extracts rich in polyphenol compounds added to white bread, on GR, insulin response (IR) and satiety in healthy subjects. The two extracts at optimal doses with the greatest sugar reducing potential from the *in vitro* digestion were used in this study. On separate days thirteen volunteers (nine female and four male) consumed a control white bread, white bread with GTE (0.4%) and white bread with BAO (1.88%), and GR, IR and satiety were measured three hours postprandially. Although enriched breads did not reduce GR or hunger, white bread with added BAO significantly ($p < 0.05$) reduced the total (0-180 min) and segmental insulin AUC at 0-90, 0-120 and 0-150 min, and therefore reduced the amount of insulin needed for a given blood glucose response. This preliminary research suggests that there is potential for baobab fruit extract at low doses to be used as a functional additive to starch rich foods for improving parameters associated with insulin sensitivity.

11.2 Introduction

Foods that increase the postprandial GR have been associated with increased weight gain and therefore managing postprandial glucose excursions could be seen as a potential target for reducing the prevalence of metabolic syndrome and its associated disease. Because the compounds in various plant extracts can reduce the starch hydrolysis and postprandial GR, much interest has been focused on the role of certain extracts as potential functional food ingredients for improving health (Queen & Tollefsbol, 2010). As previously mentioned, postprandial blood glucose levels may influence feelings of satiety through glucoreceptors (Mayer, 1953), and IR is also thought to be highly associated with GR and satiety

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(Flint *et al.,* 2007). Therefore, polyphenols may exert not only glucose lowering effects by reducing starch digestion, but also may reduce postprandial IR and increase satiety. It was therefore hypothesised that the addition various plant extracts to high GI white bread would slow the rate of starch breakdown, and therefore reduce the postprandial GR and IR and increase satiety in healthy humans.

The aim of this study was to use the results from the dose-response in our previous study (Section 8 and 9) to identify the two polyphenol rich extracts with the greatest sugar reducing potential added to white bread, and to determine their effects on postprandial GR, IR and satiety in healthy adults.

11.3 Methods

11.3.1 Materials

All chemicals, extracts and ingredients were as previously described in sections 8.3.1 and 9.3.1.

11.3.2 Bread preparation

White bread was made in a Russell Hobbs bread maker as previously described. When adding extracts to the breads, the flour content was altered in order to keep the overall weight of each bread the same as the control bread. This was achieved by subtracting the extract weight in grams from the flour weight in grams, to determine the new flour weight with added extract. All bread samples were baked the night before, sealed at room temperature in plastic containers overnight and tested the following morning.

11.3.3 Subjects

Fifteen healthy volunteers were recruited for the study by means of advertisements, flyers and personal communications. Potential volunteers were briefed on all aspects of the study and were allowed to ask questions before giving written formal consent. Volunteers participated in a health assessment which included anthropometric measurements (height, weight, waist to hip ratio, Tanita BC-418MA segmental body composition analysis) and filled in a health questionnaire (asking about medications, vitamin and mineral supplement use, previous and present diseases, smoking habits and allergies). If volunteers fulfilled the acceptable criteria (BMI: <30 kg/m^2; age: 18–65 yr; fasting blood glucose: 4–6 mmol/ L;

not on prescription medication; non-smoking; no genetic or metabolic diseases), they were given a food frequency questionnaire (FFQ) to complete with regards to their average food intake over the previous three months, and they participated in a 24-hour dietary recall given by qualified personnel. The data for two volunteers was excluded based on failure to arrive at the second test visit. Therefore, a total of thirteen healthy normal or slightly overweight volunteers (nine female and four male; BMI: 19.4-29.7 kg/m2; 20-46 yr; 50.2-91.6kg mass) participated in the study. Each volunteer was asked to avoid the consumption of caffeine, alcohol, nicotine, marijuana or any other drugs and strenuous exercise on the evening prior to each trial, and to ensure that their food intake was as similar as possible for the 18 hour before all trials. Testing began each morning between 7.30 – 9.30 am after an overnight fast (10 - 12 hour).

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.

11.3.4 Test meals

The test meals consisted of 50g of avCHO from freshly baked control white bread (CON), white bread with added GTE (0.4% of loaf weight in g) or white bread with added BAO (1.88% of loaf weight in g), and 250 mL of still water. This equated to 103.61g CON bread, 104.16g GTE bread with a total of 96.33mg added polyphenols and 106.97g BAO bread with 61.24mg total added polyphenols. Volunteers were instructed to consume the test meals at a comfortable pace within 15 min. The extracts used in this part of the study were the optimal doses/ extracts for reducing starch digestion *in vitro* (specifically RDS) and resulting sugar release from white bread (Sections 8 and 9). GSE and RES were also found to reduce RDS yet to not as great an extent. Also, the colour change and sensory properties of RES made it unacceptable for human consumption.

11.3.5 Study design

This study was a single blind, randomised-controlled trial consisting of three meals each tested on one occasion in a random order on separate days, with at least one wash out day between each test. The volunteers were advised to keep their daily routines and diets similar throughout the testing period.

11.3.6 Glycaemic response

The protocol used to measure the blood glucose response was as previously described, adopted from that described by Brouns *et al.* (2005), reproduced by Coe *et al.* (2013; Section 10.3.5), and is in line with procedures recommended by the FAO/ WHO (1998). Blood samples were collected at baseline and 15, 30, 45, 60, 90, 120, 150 and 180 min postprandial.

11.3.7 Insulin response

As previously outlined in Ranawana and Henry (2011) and then in Ranawana *et al.* (2011), blood samples for insulin determination were obtained at the times mentioned above for GR. Using finger prick samples, 300 μL of capillary blood was collected at each time point into Microvette collection tubes treated with di Potassium EDTA (CB 300 K2E; Sarstedt Ltd, Leicester, UK) and immediately stored in crushed ice. Microvette tubes were centrifuged at 4000 rpm for 10 min (Centrifuge MC-6; Sarstedt Ltd), and 120 μL of the supernatant plasma was pipetted into eppendorf tubes and frozen at -40 °C. Upon analysis, samples were defrosted and insulin concentrations were determined by electrochemiluminescence immunoassay using a reliable automated analyser (Cobas E411; Roche Diagnostics, Burgess Hill, UK (Siahanidou *et al.,* 2011). The unit of measurement was μU/ mL.

The change in GR and IR from baseline was calculated by subtracting the mean baseline values from each time point. Therefore, the 'change in GR/ IR' was used for analyses including the incremental AUC, AUC graph constructions and statistics. The trapezoid rule was used to calculate AUC (without area beneath the baseline) by adding together the individual areas of the triangles and trapezoids for time periods up to the whole 180 min testing period (Brouns *et al.,* 2005; Wolever, 2006).

11.3.8 Satiety

One hundred millimetre continuous line VAS were utilised to measure subjective feelings of hunger, fullness, desire-to-eat and prospective food consumption. The volunteers provided VAS data at each time point mentioned previously, and AUC was calculated.

11.3.9 Statistical analysis

A minimum sample size of twelve was required based on a difference in mean GR-AUC of 10 mmol/ L, a mean SD of 5 with a power of 0.9 and an [alpha] of 0.05. This was also based on previous studies in our lab measuring GR (Clegg *et al.,* 2011). Therefore, a sample size of thirteen was used in the current study. Statistical analyses were conducted using the SPSS version 21 (SPSS Inc., Chicago, Illinois) with descriptive data presented as means.

A RM-ANOVA with Bonferroni correction was used after the data was tested for normality using the Shaprio-Wilk statistic. If data were skewed, a Friedman one-way ANOVA (non-parametric) was used to compare AUC for GR, IR and satiety between the three test meals. VAS data for each of the four satiety measures were analysed using a two-way RM-ANOVA with two within subject factors – time and meal. Pearson's correlation was used to compare GR, IR and satiety for each test meal. Statistical significance was set at $p < 0.05$.

11.4 Results

11.4.1 Glycaemic response

Data are presented as change in GR from baseline in Figure 11.1. The data were analysed by total and segmental AUC. There was no difference in postprandial total or segmental GR-AUC (Figure 11.2) between any of the tests. However, there was a non-significant trend for BAO and GTE to have a lower peak compared to CON.
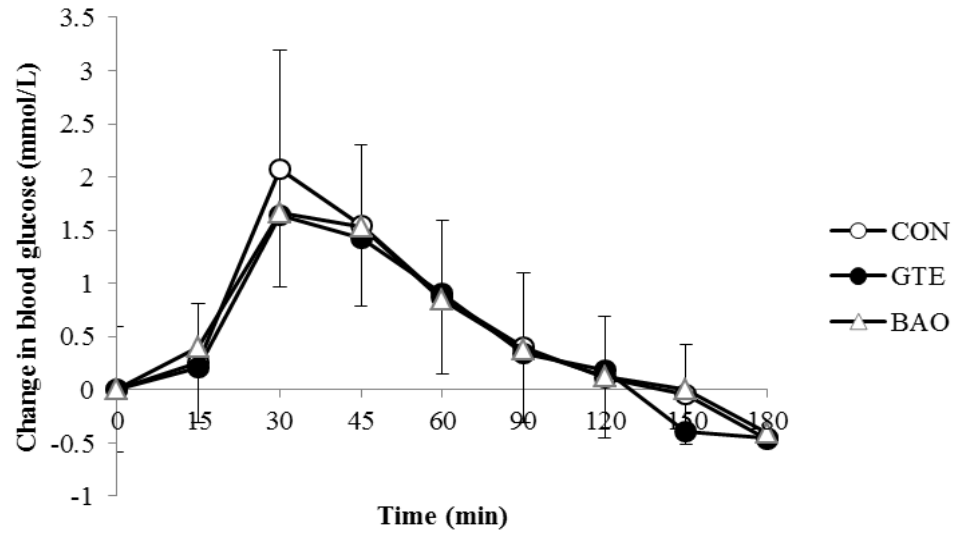
Figure 11.1 Change in blood glucose from baseline for 0-180 min (mmol/L).

50g available carbohydrate was consumed from a control white bread (CON; ○), white bread with 0.4% green tea extract (GTE; ●) and white bread with 1.88% baobab fruit extract (BAO; Δ). Values are means (n 13).
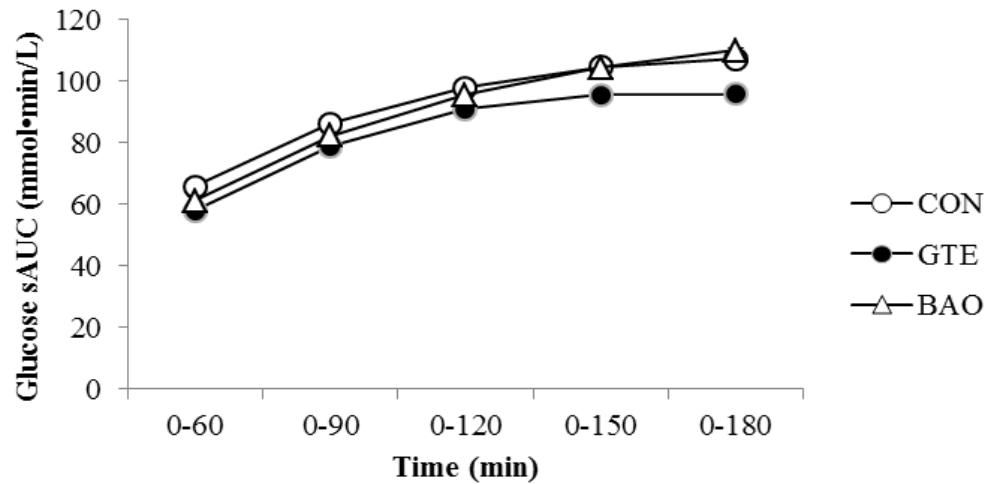
Figure 11.2 Segmental areas under the blood glucose response curve (sAUC) for 60 – 180 min.

50g available carbohydrate was consumed from a control white bread (CON; ○), white bread with 0.4% green tea extract (GTE; ●) and white bread with 1.88% baobab fruit extract (BAO; Δ). Values are means (*n* 13).

11.4.2 Insulin response

The change in IR from baseline is shown in Figure 11.3. BAO-enriched bread had a significantly ($p < 0.05$) lower total IR-AUC, a lower segmental AUC for 0- 90, 0-120 and 0-150 min and a lower incremental peak for IR compared to the CON and GTE breads (Figure 11.4). There was also a significant correlation between the GR and IR for the CON bread only ($p < 0.05$).
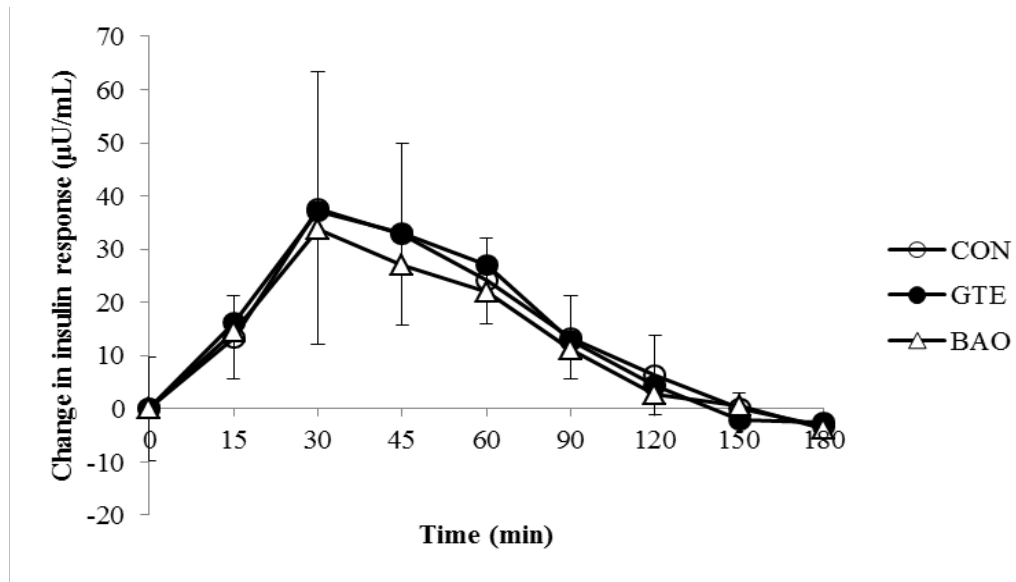
Figure 11.3 Change in insulin response from baseline 0-180 mins (μU/mL).

50g available carbohydrate was consumed from a control bread (CON; ○), white bread with 0.4% green tea extract (GTE; ●) and white bread with 1.88% baobab fruit extract (BAO; ∆). Values are means *(n* 13).
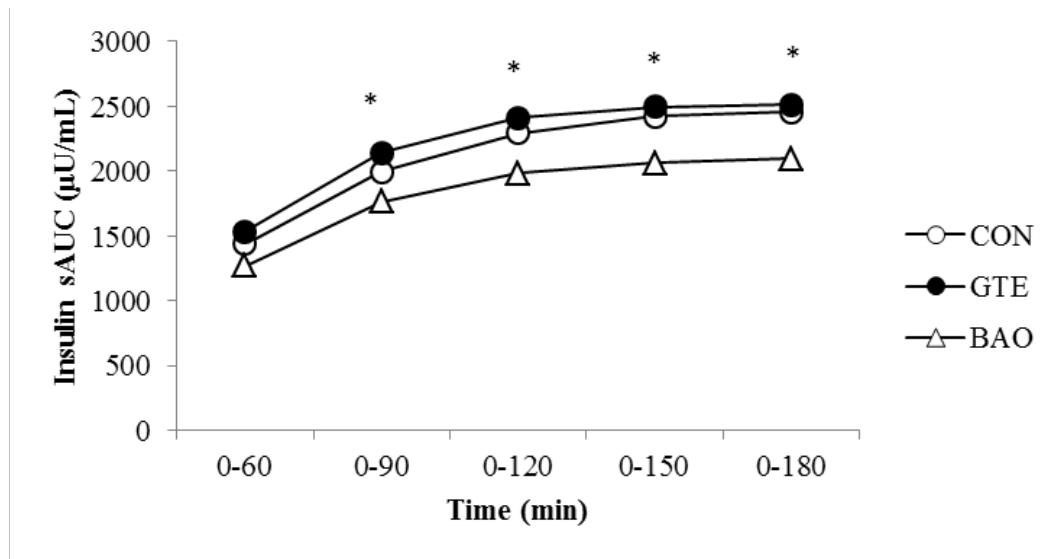
Figure 11.4 Segmental areas under the blood insulin response curve (sAUC) for 60 – 180 min.

*$p<0.05$ =significantly lower insulin response for BAO compared to CON and GTE. 50g available carbohydrate was consumed from a control bread (CON; ○), white bread with 0.4% green tea extract (GTE; ●) and white bread with 1.88% baobab fruit extract (BAO; Δ). Values are means *(n* 13).

11.4.3 Satiety

There was no difference in any of the four measures for satiety AUC (Table 11.1). No significant difference in pleasantness or tastiness of the three breads was found. There was a trend for BAO to non-significantly increase satiety and for the CON to show the lowest ratings on all satiety measures.

Table 11.1 Mean total area under the curve satiety scores using visual analogue scales.

	CON	SD	GTE	SD	BAO	SD
How hungry do you feel	360.40	312.43	372.90	267.93	445.05	301.15
How full do you feel	413.34	293.57	449.16	229.50	464.36	326.48
How strong is your desire to eat	361.07	340.36	433.82	260.95	450.15	331.11
How much food do you think you can eat	312.50	295.05	324.29	256.96	347.22	287.98

CON, control white bread; GTE, white bread with green tea extract; BAO, white bread with baobab fruit extract. Values reported in millimetres/minute ± SD (A two-way repeated measure ANOVA with two within subject factors – time and meal).

11.5 Discussion

The addition of GTE and BAO extracts to white bread had no effect on GR or satiety in healthy subjects, yet BAO was shown to reduce the postprandial IR compared to the CON and GTE breads.

The *in vitro* digestion model can be used to predict the digestibility of various foods and has shown good correlation with the human digestive response (Araya *et al.,* 2002; Monro & Mishra, 2010). Specifically, the RDS *in vitro* is thought to be highly correlated to the GI of starch rich foods in humans (Englyst *et al.,* 1996; Englyst *et al.,* 1999). Polyphenols were shown to be negatively correlated to GR and therefore may influence the rate and degree of starch digestibility (Thompson *et al.,* 1984).

Although low doses of all extracts tested were shown to reduce *in vitro* starch digestibility, in the current study these same doses of BAO and GTE did not reduce the GR in humans. It is impossible to mimic the human digestive system exactly as there are many different processes

taking place in the body that can influence the GR which may not be accounted for *in vitro*. A study by Eelderink *et al.* (2012) found no association between *in vivo* starch digestibility and GR.

A variety of factors may influence the effect of starch breakdown and resulting GR. For example, Clegg *et al.* (2011) found no effect of berries on GR or satiety when consumed with starch rich pancakes, yet Törrönen *et al.* (2012) found that berry consumption with sucrose improved the overall GR and IR. As previously mentioned, tea polyphenols were shown to increase GR from high-amylose maize starch, yet reduced the GR to normal and waxy maize starch (Liu *et al.,* 2011). The administration of EGCG (at a dose equivalent to 1.5 cups of green tea) alongside common maize starch in mice was found to reduce postprandial blood glucose levels and inhibit *in vitro* pancreatic α-amylase (Forester *et al.,* 2012). However, in the current study and also in the study by Liu *et al.* (2011), extracts were cooked into the starch source under investigation and were therefore subjected to baking. Whereas in the current study the objective was to develop potential functional foods, Forester *et al.* (2012) used a design similar to that of consuming a food and a beverage as separate entities. Various compounds in food undergo structural changes when subjected to heat, yet in the current study the overall polyphenol content of the breads was not measured after baking. However, the results from the *in vitro* part of the study found that extracts reduced sugar release from breads after being added to breads at low doses. Therefore, it was thought that certain compounds remained intact *in vitro* and therefore were predicted to exert their actions *in vivo*.

A meta-analysis by Liu *et al*. (2013) including 17 trials found that green tea consumption decreased fasting blood glucose and HbA1$_c$ and reduced fasting insulin concentrations. The monomeric and/ or oligomeric phenolic compounds in Kombucha green and black teas have been shown to inhibit starch hydrolysis via porcine pancreatic α-amylase (Kallel *et al.,* 2012). However, as shown by Guzar (2012) the dose of green tea added to bread was important for determining starch digestion. Therefore, the dose of GTE used in the current study was not optimal for reducing the GR in humans.

Baobab fruit extract has been shown to be rich source of phenolic compounds such as the tannins, hydrocyanic acid, oxalates and a variety of flavonoids (Ghani & Agbejule, 1986; Tanko *et al.,* 2008). This fruit has also been shown to have anti-diabetic activity, thought to be largely due to its high fibre and polyphenol content (Nour *et al.,* 1980). Apart from the study done by our lab (Coe *et al.,* 2013; Section 10), only one other study has looked at the glycaemia reducing effects of baobab. In a streptozocin-induced diabetes rat model, extract of baobab fruit reduced the GR compared to the control (Tanko *et al.,* 2008). Baobab fruit contains soluble fibre which has been shown to reduce postprandial GR and IR

compared to a low fibre meal (Keogh *et al.,* 2007), however the dose of extract used in this study only contributed 1.2g of total fibre to the bread and therefore this low amount was unlikely to contribute to the GR and/ or IR.

From our previous study, baobab fruit extract consumed at higher doses of 18.5g and 37g in liquid form was found to reduce the postprandial GR to white bread (Coe *et al.,* 2013; Section 10). This contrasts to the much lower (yet sufficient) dose of 9.375g added to the white bread both in the *in vitro* digestion in the same study, and also in the measurement of postprandial GR in the current study. Although baobab was consumed in different forms and doses between the studies, none of the previous literature can be used to explain the inconsistency between the results on starch digestion and GR when testing the same doses of extracts. Different food sources may be the result of inconsistency between methods, for example the *in vitro* starch digestion and the GR *in vivo* have been shown to correlate well when studying fibre rich foods (Regand *et al.,* 2011). However the association between the two methods when considering polyphenol-starch interactions is more controversial and relies on the structural properties of the polyphenols (Piparo *et al.,* 2008). Therefore, more studies need to look at the validity and reliability of the different digestion methods.

In healthy normal weight individuals, the GR and IR are highly associated after the consumption of a typical meal resulting in a rise in plasma glucose followed by an increase in insulin release to combat these glucose levels (Mckeown *et al.,* 2004). In the current study, GR and IR were found to be correlated for the CON only and not the GTE or BAO breads. Therefore, it can be concluded that the addition of plant extracts to bread altered the relationship between blood glucose levels and resulting insulin release. This has been shown in a study by Liese *et al.* (2005) who found no association between GI and glycaemic load (GL) with insulin sensitivity or fasting insulin levels.

In the current study, BAO yet not GTE was shown to reduce the postprandial IR. BAO reduced the segmental and total plasma insulin AUC despite having no effect on blood glucose levels, and therefore for the same glucose response a low concentration of BAO addition to bread increased the efficiency of the IR. Tannins and flavonoids, such as those found in baobab fruit (Ghani & Abejule, 1986; Tanko *et al.,* 2008) have been shown to increase insulin sensitivity both *in vitro* and in mice, yet studies in humans are limited (Pinent *et al.,* 2008). Those with type 2 diabetes are at risk of having high circulating levels of insulin in the blood, and therefore consuming foods that can increase insulin efficiency would prove beneficial for maintaining plasma glucose (Kahn, 2003). Törrönen *et al.* (2013) found that certain berry mixtures improved the IR without improving the GR to white bread. Therefore, white bread consumption with berries reduced the amount of insulin needed for a normal or slightly improved GR, with the IR to rye bread also becoming further reduced by berry addition.

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There were no differences in satiety measures between the breads which could be due to the lack of difference in GR between the tests. However, Flint *et al.* (2007) found insulin to be associated with short term satiety regulation, but GR showed no effect. In the current study, there was a non-significant trend for increased satiety in the BAO bread. Fibre and polyphenols have both been shown to increase satiety and therefore the reduction in IR in the BAO without its effect on satiety may have been counterbalanced by the slight increase in fibre, in polyphenols or both (Josic *et al.,* 2010; Slavin & Green, 2007). Sensory properties of the three breads in the current study were not affected by extract addition which could be due to the low extract doses used.

11.6 Conclusions

In conclusion, white bread enriched with GTE and BAO showed no effect on GR or satiety, yet BAO was shown to increase insulin economy. This is the first study to look at the effect of the addition of various extracts into white bread and the effects on GR, IR and satiety in humans. The reduction in IR after BAO may be due to the polyphenol association with carbohydrate digestion, or to the absorbed compounds from the extracts altering insulin action in the body. Relatively low doses of extracts were used in this study, and therefore the improvement in insulin parameters is of great significance. Future studies may look at C-peptide levels to determine if the the reduction in IR is due to a diminished pancreatic secretion of insulin rather than enhanced liver extraction. Although the dose was low, extracts were baked into bread and therefore may have directly interacted with starch chains requiring a lower amount to show a positive effect on glycaemic parameters. However, higher doses of extracts may be needed to see a reducing effect on GR. Due to the conflicting results from previous studies in our lab which found BAO to reduce starch digestion *in vitro* (Coe *et al.,* 2013) with no effect on GR in the current study, further research needs to determine the effects of different doses of BAO both in solid and liquid mediums, and the effect this has on metabolic parameters.

12. Conclusions and future prospects

It can be concluded from the literature that there is sufficient evidence to suggest the role of polyphenols for improving risk factors of metabolic diseases. Specifically, research is highlighting the importance of polyphenols for improving markers of glycaemia and for reducing the GI of carbohydrate rich sources. Foods with enhanced functional characteristics offer the potential to target populations that are at risk of chronic disease, such as obese individuals and the elderly, for whom attempts to change lifelong dietary habits may be challenging. Dietary interventions may be a simple and cost effective way to bring benefits for the health of the population and thereby improve overall quality of life.

A systematic review of the literature showed that various polyphenol rich sources positively influenced the postprandial GR when consumed with simple or complex carbohydrates. Overall, polyphenols in combination with sucrose, glucose or bread were found to reduce the early phase GR, prolong the rate of glucose absorption into the blood and to sustain insulin secretion. Berries specifically were found to improve the GP when consumed with both simple sugars and complex starches as found in the four studies by Törrönen's group (2010, 2012a, 2012b, 2013). Based on the pattern of change in GR, it was concluded that polyphenol compounds were acting at the intestinal level either by reducing carbohydrate breakdown and/ or by reducing glucose transport, and may therefore contribute to the reduction in GI. However due to the large heterogeneity between different study designs and with only 13 papers included in the review, a confident conclusion is difficult to draw.

The research component of this PhD included both *in vitro* and *in vivo* methods. Tea is a commonly consumed beverage throughout the world, and green, white and black teas were found to be abundant in bioaccessible phenolic compounds (study 1, Title: Polyphenol bioaccessibility and sugar reducing capacity of black, green and white teas). Green tea is a widely accepted source of antioxidants and polyphenol compounds and is well known for its contribution to health. One green tea bag reduced starch breakdown and sugar release when digested alongside white bread *in vitro* and therefore potential benefits were seen at amounts easily obtainable from the diet. Green tea extract was then baked into white bread and it was found to reduce starch digestion at specified doses, highlighting the potential of different forms and doses of green tea for reducing the GR when consumed with carbohydrate rich foods (study 3, Title: The effect of polyphenol rich extracts in isolation and in combination, on sugar release from various starch rich foods *in vitro*). Although green tea extract was shown to reduce starch digestion *in vitro*, no effect was seen on the GR *in vivo* when consumed at equivalent doses as found in study 5 (Title: White bread enriched with polyphenol extracts shows no effect on

glycaemic response or satiety, yet may increase postprandial insulin economy in healthy subjects). Thus caution should be taken when comparing results between *in vitro* and *in vivo* digestion methods.

   Grape seed and resveratrol are the health promoting components found in grapes and red wine, and in study 3 they were found to reduce starch digestion from white bread at specific doses *in vitro*. Although promising, more work would need to assess the glycaemia reducing potential of grape seed and resveratrol in human models before conclusions can be drawn.

   Few trials to date have investigated the role of baobab fruit in health and the extract has only recently been made available for consumer purchase in the UK. In solution, baobab at relatively high doses of 18.5g and 37g was found to improve the postprandial GR to white bread in healthy females (study 4, Title: The polyphenol rich baobab fruit {*Adansonia digitata L.*} reduces the glycaemic response in humans). In study 5, when baked into white bread at 1.88% of loaf weight, baobab fruit had no effect on GR, yet reduced the IR. Therefore the IR for a given GR was significantly improved and this may be of benefit for increasing insulin sensitivity in those with type 2 diabetes in whom circulating plasma levels of insulin are high. Due to the low doses used when baking baobab into white bread, the improvement in IR is of great importance. It can be concluded that polyphenol rich sources such as baobab fruit can improve markers of glycaemia and it may therefore be considered as a functional additive. Further studies would need to elucidate the longer term implications of this work.

   The polyphenol bioaccessilbity throughout *in vitro* digestion varied between sources, with the baobab extract, GTE, GSE and RES increasing into the gastric stage and further increasing into the duodenal stage. However, some teas tended to increase into the gastric stage and then begin to decline into the duodenal stage. This may have been a result of the form (bagged/ whole teas versus pure extracts), the concentration of polyphenols (lower in some teas compared to extracts) or the types of polyphenol present in the different sources. Different preparation methods may influence starch digestibility and resulting glycaemia and this PhD assessed various polyphenol preparation methods, including brewed tea (black, green, white and herbal varieties), extracts baked into bread (BAO, GTE, GSE and RES), and a raw extract made up as a cold drink (LD and D baobab extract). Also, different methods were used for starch sources, including baking (white and gluten free breads), cooking (flat bread) and the use of a commercial bread consumed alongside baobab drinks. Due to this and also the different doses used, it is therefore difficult to directly compare results between study designs.

   In an attempt to establish recommended intake values for different polyphenols, more rigorous randomised controlled trials are needed to determine optimal doses and in what forms these compounds are most beneficial for health. Adverse effects of consuming high levels of

polyphenols must be acknowledged and the inconsistencies between *in vitro*, animal and human models are a major limitation. Knowledge on bioavailability is limited and due to the poor stability of these compounds, adequately assessing the content in foods proves challenging. Methods for determining the polyphenol content of various sources have limitations which may further lead to inaccuracy when measuring polyphenol intakes in different population groups. Although the current research could be strengthened by including additional assays such as HPLC and enzyme inhibition, the focus was not on the specific mechanisms taking place between the extracts and starch digestion. It is assumed that certain polyphenols may inhibit enzyme action, and this would need to be assessed in future studies looking at the in depth mechanisms of polyphenols on the GR. Also, the polyphenol content of breads after baking was unknown and some of the compounds may have been degraded during the baking process. However, because effects were seen on both starch digestion and on glycaemic parameters, it is assumed that a substantial amount of polyphenols remained intact after baking.

This PhD adds relevant knowledge to the expanding area of research into polyphenols and health. With the growth in the market of functional foods and the increasing consumer interest in nutrition, modifying the diet is becoming increasingly acknowledged for its role in the management of obesity and its related comorbidities. The research into polyphenols is only beginning to reveal the potential these compounds have in contributing to the longevity of the population.

Acknowledgements

I would first and foremost like to thank my supervisor Dr Lisa Ryan, for her continuous support and guidance throughout my PhD. Dr Ryan has mentored me for the past six years, throughout both my undergraduate and postgraduate degree and has been one of my biggest support systems during this time. Dr Ryan has motivated me to excel in my work and to continuously publish throughout my PhD, and has not only been an excellent supervisor yet is someone I consider to be one of my closet friends.

Shirley Coe, my mother and best friend has supported me through both the achievements and the disappointments. Without my mother I don't believe I would have had the emotional strength to successfully complete my degree. During times of stress she has continuously been a positive role model and she has always believed in my success even when I had doubt in my own ability.

I would like to thank Dr Miriam Clegg, Ann Fraser, Dr Sangeetha Thondre and Dr Helen Lightowler who have provided support and advice over the previous three years. Dr Thondre assisted in training me in on various laboratory assays, as did Ms Sarah Warner, Dr Sarah Hillier and Ms Patricia Shaw.

Irene Cantero-Gonzalez and Mar Armengalo assisted in my research, collecting data and providing support in the laboratory.

My full degree and salary was funded by the USA Military Post 9-11GI bill funding scheme.

I would also like to thank Oxford Brookes University for providing support for me as a postgraduate student, and for providing me with the resources to complete my degree.

I would like to thank JING Tea Ltd for the generous gift of all the whole tea samples.

Finally, I would like to thank all the participants for their time in completing the studies.

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