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Review

The cardiovascular health benefits of apples: Whole fruit vs. isolated compounds

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ABSTRACT

Background: Apples are an important contributor to the intake of dietary components linked with cardiovascular disease (CVD) prevention. Apples have been shown to have beneficial effects on vascular function, blood pressure, lipids, inflammation and hyperglycaemia. The cardioprotective effects of apples, and other fruits, have been primarily ascribed to their high polyphenol content. There is emerging evidence that the bioavailability and bioefficacy of polyphenols is affected by the food matrix in which they are consumed.

Scope and approach: This review will discuss the differences in the consumption of apple as a whole food in comparison to the consumption of isolated key components, predominantly polyphenols and fibre. The bioavailability and absorption of major apple polyphenols, such as procyanidins, catechin, epicatechin, phloridzin, chlorogenic acid, and the quercetin glycosides, will be described. The methods by which apples may ameliorate risk factors for CVD will be discussed and results from key human intervention studies conferred. The list of studies described in this paper is exemplary and not exhaustive.

Key findings and conclusions: There are a number of factors influencing the bioavailability of polyphenols in an individual including colonic microbial composition, the dose consumed and the presence of other polyphenols and macronutrients within the food matrix. There is evidence of a synergistic relationship between the fibre and flavonoids found in a whole apple, which is likely mediated in part by the gut microbiota. Further human intervention studies investigating the effects of apples of cardiovascular risk factors, and the critical role of the gut microbiota, are warranted.

A diet low in fruit is one of the greatest contributors to total mortality, third only to high blood pressure and smoking (Ezzati & Riboli, 2013). Indeed, it is well recognized that a diet rich in fruit is associated with a lower risk of cardiovascular disease (CVD) events and mortality (Wang et al., 2014). Apples, the second highest consumed fruit due to widespread geographical and seasonal availability, are an important contributor to the intake of dietary components linked with CVD prevention. In observational studies, higher apple intakes are associated with lower risk of coronary heart disease and stroke (Larsson, Virtamo, & Wolk, 2013). Apples have been shown to have beneficial effects on vascular function and blood pressure (C. P. Bondonno et al., 2012), lipids (Jensen, Buch-Andersen, Ravn-Haren, & Dragsted, 2009), inflammation (Chun, Chung, Claycombe, & Song, 2008) and hyperglycaemia (Johnston, Clifford, & Morgan, 2002). The key components of apples having a cardioprotective effect are thought to include polyphenols and fibre. Currently research is focusing on individual compounds, rather than whole foods, with many studies concentrating on polyphenols present in apples. There is, however, emerging evidence that co-ingestion of polyphenols with other whole food components, such as fibre, can improve their bioavailability and bioefficacy. This leads us to question whether or not the same health benefits can be seen after consumption of apple products, such as apple juice, or isolated polyphenols.

This review will discuss the differences in the consumption of apple as a whole food in comparison to the consumption of

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isolated key components, in relation to bioavailability and health benefits.

1. Beneficial compounds in apples

Apples are made up of predominantly water (85%) and carbohydrates (14%), including fibre and sugar (primarily fructose) (US Department of Agriculture, 2015). Apples also contain vitamins (in particular vitamin C and vitamin E), minerals (mainly potassium) and polyphenols (Fig. 1). The principle apple products are apple juice (clear and cloudy) and fermented apple cider and vinegar. Cloudy apple juice has a lower sugar content and higher pectin and polyphenol content than clear apple juice but a much lower pectin and polyphenol content in comparison to whole apples (Ravn-Haren et al., 2013). Apple pomace, a by-product of apple juice production that is rich in fibre and polyphenols, has attracted attention due to the growing trend of industry to 'recycle' components of production which were previously seen as waste (Sudha, Baskaran, & Leelavathi, 2007). Dessert apples are favoured because of their taste, storability and nutritional properties while cider apples are generally small and, due to their high polyphenol content, very bitter.

1.1. Fibre

Apples contain approximately 2.21 g/100 g total fibre. Of that, 70% is insoluble fibre, including cellulose and hemicellulose, and 30% is soluble fibre, mainly pectin (Li, Andrews, & Pehrsson, 2002). Pectins are complex polysaccharides present in the cell wall of higher plants, which are not metabolized in the upper digestive tract in humans (Gulfi, Arrigoni, & Amadò, 2006). Beneficial health effects of pectin are attributed to its ability to lower cholesterol (Aprikian et al., 2003), slow down glucose absorption (Schwartz et al., 1988) and increase colonic short chain fatty acid (SCFAs) production (Andoh, Tsujikawa, & Fujiyama, 2003). Phenolic compounds can bind to macromolecules, such as cellulose and pectin, through covalent bonds via ether, ester and carbon-carbon bonds in the cell wall matrix, forming insoluble-bound phenolics (Shahidi & Yeo, 2016). Polyphenols such as procyanidins have a high affinity for pectin (Le Bourvellec, Guyot, & Renard, 2009).

1.2. Polyphenols

Polyphenol-rich foods are a major research focus due to epidemiological studies showing a correlation between high polyphenol intake and decreased CVD risk (Arts & Hollman, 2005; Knekt et al., 2002; Tresserra-Rimbau et al., 2014). Polyphenol compounds in food are predominantly found in their glycosylated form but conjugate-free compounds are also found and these are known as aglycones. The largest and most widely researched subclass of polyphenols is the flavonoids.

1.2.1. Polyphenol concentrations in apples

Currently there are two databases that report the flavonoid content of food: The United States Department of Agriculture (USDA) Database (US Department of Agriculture, 2004, 2007) and the more recently developed Phenol-Explorer database (Neveu et al., 2010). The USDA has a separate database for flavonoid content and for proanthocyanidin content of selected foods. The average flavonoid content of whole, raw apple (*Malus domestica*), as shown by these databases, is presented in Table 1. We have recently compared estimates of flavonoid intakes from over 1000 individuals using the Phenol Explorer and USDA databases (Ivey, Croft, Prince, & Hodgson, 2016). Our analysis showed overall good agreement, however there are several specific differences. The reasons for the differences are discussed in detail in the paper. Phenol-Explorer presents the content of all polyphenols, not just the flavonoids. The two databases contend with the complex structure of flavonoids as well as food content using different protocols. The USDA database reports flavonoid concentrations as levels of aglycone, which are obtained after hydrolysis of conjugates. These values may underestimate food flavonoid content as there is a potential for degradation of the flavonoid or incomplete hydrolysis of the conjugate (Ivey et al., 2016). The Phenol-Explorer database reports flavonoid concentrations as individual glycosides. There are also limitations for this method as glycosides could potentially escape detection due to low concentrations or lack of appropriate standards (Ivey et al., 2016). The level of agreement between the databases has been explored and a high correlation was found for total-flavonoids, flavanols, flavanones and anthocyanidins (Ivey et al., 2016). The total flavonoid content of apples can be calculated as 32 mg/100 g and 111 mg/100 g from the Phenol-Explorer and USDA databases respectively. The large difference in values is primarily due to the value for the proanthocyanidin polymer content of apples (85 mg/100 g), which is only reported in the USDA Database for the Proanthocyanidin Content of Selected Foods.

1.2.2. Polyphenol distribution in apples

Apple peel contains considerably more polyphenols than the flesh, attributed to the defensive role of the skin in protecting the fruit from harmful UV light and invading pathogens (Solovchenko

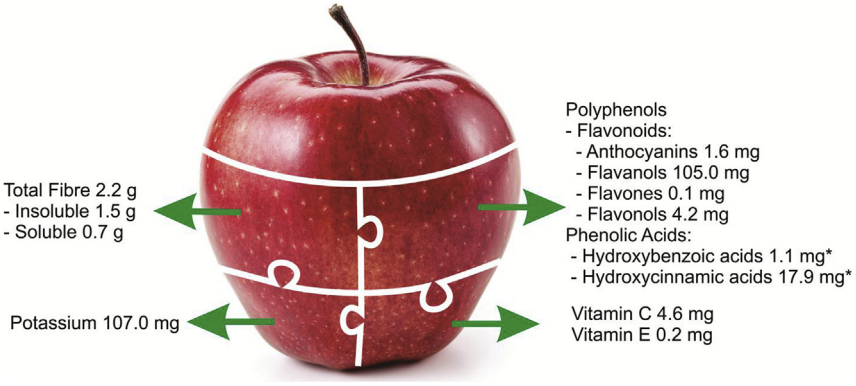

Fig. 1. Major classes of the potentially beneficial components of apples. This figure represents the average content of a whole, raw apple (*Malus domestica*).Values were obtained from the USDA Nutrient and Flavonoid Databases and *Phenol Explorer and are presented per 100 g fresh apple.

Table 1
The polyphenol content of whole, raw apple (*Malus domestica*) according to Phenol-Explorer and the USDA Flavonoid and Proanthocyanidin databases.

Polyphenol Subclass	Phenol Explorer	mg/100 g	USDA Databases	mg/100 g
Anthocyanins	Cyanidin 3-*O*-arabinoside	0.06	Cyanidin	1.57
	Cyanidin 3-*O*-galactoside	0.81		
	Cyanidin 3-*O*-xyloside	0.06		
			Peonidin	0.02
	Total	0.93	Total	1.59
Dihydrochalcones	3-Hydroxyphloretin 2′-*O*-glucoside	0.11		
	Phloretin 2′-*O*-xylosyl-glucoside	2.58		
	Phloridzin	2.69		
	Total	5.38		
Flavanols	(+)-Catechin	1.22	(+)-Catechin	1.30
	(−)-Epicatechin	8.37	(−)-Epicatechin	7.53
			(−)-Epicatechin 3-gallate	0.01
			(−)-Epigallocatechin	0.26
			(−)-Epigallocatechin 3-gallate	0.19
	Procyanidin dimer B2	14.56	Proanthocyanidin dimer	10.99[a]
			Proanthocyanidin polymers	84.76[a]
	Total	24.15	Total	105.04
Flavones			Luteolin	0.12
			Total	0.12
Flavonols	Quercetin	0.13	Quercetin	4.01
	Quercetin 3-*O*-arabinoside	1.40		
	Quercetin 3-*O*-galactoside	2.36		
	Quercetin 3-*O*-glucoside	0.64		
	Quercetin 3-*O*-rhamnoside	1.33		
	Quercetin 3-*O*-rutinoside	0.22		
	Quercetin 3-*O*-xyloside	0.78		
			Kaempferol	0.14
	Total	6.86	Total	4.15
Hydroxybenzoic acids	Gentisic acid	0.22		
	Syringic acid	0.9		
	Total	1.12		
Hydroxycinnamic acids	4-Caffeoylquinic acid	0.54		
	4-*p*-Coumaroylquinic acid	2.25		
	5-Caffeoylquinic acid	13.37		
	5-*p*-Coumaroylquinic acid	1.05		
	Caffeic acid	0.33		
	Ferulic acid	0.07		
	p-Coumaric acid	0.27		
	Total	17.88		

Note: Phenol-Explorer presents the content of all polyphenols, whereas the USDA databases only provide data on flavonoid content.
[a] Values were obtained by averaging the values presented for all apple varieties, raw with skin.

& Schmitz-Eiberger, 2003). The polyphenols typically found in apple peel are flavonoids (such as procyanidins, catechin, epicatechin, phloridzin, and the quercetin glycosides), hydroxybenzoic acids and hydroxycinnamic acids (such as chlorogenic acid) (Escarpa & Gonzalez, 1998). These compounds are found in much lower concentrations in apple flesh except for chlorogenic acid, which tends to be higher in the flesh than in the peel. Quercetin glycosides are found almost exclusively in the apple peel. The quantities of these differ substantially between varieties and are affected by geographic region, growing season and storage. There is a significant increase in quercetin content in the skin of apples exposed to sunlight, however the levels of catechins, phloridzin and chlorogenic acid are largely independent of light exposure (Awad, Wagenmakers, & de Jager, 2001).

2. Bioavailability

2.1. Whole apple

The health effects of apples are influenced by the absorption, metabolism and distribution of bioactive compounds such as polyphenols. In 2004, Vrhovsek et al., quantified the polyphenol content of 8 western European apple cultivars and found that the total polyphenol content, determined with the Folin Ciocalteu assay, ranged from 66.2 mg–211.9 mg/100 g fresh weight (Vrhovsek, Rigo, Tonon, & Mattivi, 2004). However, apple polyphenol content may be grossly underestimated as a substantial proportion of polyphenols cannot be extracted with organic solvents and thus are not quantified in routine laboratory analysis (Saura-Calixto, 2012). This would affect estimation of polyphenol consumption in nutritional and epidemiological studies. Although they are not bioaccessible in the small intestine, through the action of gut microbiota, these polyphenols can be released in the large intestine (Saura-Calixto, 2012). These "non-extractable polyphenols" may significantly contribute to our polyphenol intake and may have a positive effect on gastrointestinal health, possibly mediated by the increased production of SCFAs (Saura-Calixto et al., 2010).

2.1.1. Absorption and metabolism

Apple polyphenols can be readily detected in human plasma and urine after apple consumption (Stracke et al., 2010). A study by Wruss et al., showed that plasma phenolic content, expressed as (+)-catechine equivalents, was highly variable (ranging from 1025 to 1749 mg/L) between individuals after the consumption of apple juice (Wruss et al., 2015). The authors suggest that sub-populations with different pharmacokinetics exist due to differences in the length of the small intestine, the gut microbiome or genetic factors. Interestingly, they also found lower polyphenol concentrations in the plasma and urine of the females when compared to the males.

The large degree of inter-individual variation that exists [approximately 64% (Wruss et al., 2015)], is predominantly attributed to variations in colonic microflora composition (Koutsos, Tuohy, & Lovegrove, 2015). These variations in individual metabolism have important implications in the potential health effects of polyphenols within the body and may explain the diverse findings of studies investigating the health benefits of apples and individual flavonoids. The uptake of polyphenols not only depends on the individual's gut micro-flora composition, but on genetic traits as well. For example, a human study by Egert et al., found that 150 mg/day quercetin supplementation for 6 weeks led to a significant decrease in BP (3.4 mmHg, $P < 0.01$) in overweight-obese carriers of the ApoE3 (Apolipoprotein E3) gene but not in carriers of the ApoE4 gene (Egert, Boesch-Saadatmandi, Wolffram, Rimbach, & Müller, 2010). Aside from an individual's gut microbiota and their genetic make-up, other factors influencing the bioavailability of polyphenols include the form in which the polyphenols are ingested, and the focus of this review: the food matrix in which they are found.

The bioavailability of polyphenols is the proportion digested, absorbed, and utilized in normal metabolism. This depends directly on the bioaccessibility; the amount of polyphenols released from the solid food matrix that may be able to pass through the intestinal barrier (Hedren, Diaz, & Svanberg, 2002). Fruits and vegetables naturally consist of hydrated cells with phenolic compounds contained in cell vacuoles and weakly linked to the cell wall (Parada & Aguilera, 2007). The mechanical action of mastication results in the rupture of some of these cells, allowing phenolic compounds and other nutrients to be released (Padayachee et al., 2012). The acidic environment of the stomach and the alkaline environment of the intestine can facilitate the release of other polyphenols linked more closely to the cell wall (Del Rio, Costa, Lean, & Crozier, 2010; Tagliazucchi, Verzelloni, Bertolini, & Conte, 2010).

2.1.2. Effect of the apple matrix

The interaction of polyphenols with other macromolecules in the wholefood matrix can have a significant impact on the fate and function of these polyphenols in the body. These macromolecules include lipids, carbohydrates and proteins, and their impact on polyphenol activity has been recently reviewed by Jakobek et al. (Jakobek, 2015). The association between polyphenols and carbohydrates, in particular fibre, is very relevant to the health benefits of apples. Concurrent carbohydrate consumption, has been shown to significantly increase the uptake of polyphenols (Schramm et al., 2003; A.; Serra et al., 2010). Polyphenols can bind to dietary fibres which 'capture' them within a matrix, restrict the absorption of the polyphenols in the small intestine and allow them to be transported to the colon (Palafox-Carlos, Ayala-Zavala, & González-Aguilar, 2011). These form part of the non-extractable polyphenols discussed previously. It appears as though the interaction of polyphenol-rich foods with the gut microbiota is synergistic; the microorganisms in the large intestine can release the polyphenols from the fibre matrix and break them down into phenolic acids while the polyphenols are capable of stimulating the growth of beneficial bacterial species and inhibiting the growth of pathogenic species (Hervert-Hernandez & Goñi, 2011). Aprikian et al. described that co-ingestion of apple pectin and a polyphenol-rich apple concentrate was more effective on large-intestinal fermentation and lipid metabolism than separate ingestion, signifying a synergic effect of fibre and polyphenols in apple (Aprikian et al., 2003). While apples have only minor levels of protein and lipids, the possible interactions with proteins and lipids present in foods consumed concurrently with apple may be important. There is evidence that interactions between lipids and polyphenols may decrease the fat absorption process, a positive health effect (Jakobek, 2015). Additionally, both lipids and proteins could act as carriers of polyphenols through the gastrointestinal tract, protecting them from oxidation and degradation (Jakobek, 2015).

2.2. Pure polyphenolic compounds

Although the beneficial effects of apples on our health have been largely attributed to polyphenols in general, this review focuses mainly on the specific compounds, or groups of compounds, found in relatively high concentrations in apples. These include the flavanols (namely epicatechins and procyanidins), the dihydrochalcones (phloretin and phloridzin), the flavonol quercetin and the phenolic acids (5-caffeoylquinic acid and 4-*p*-coumaroylquinic acid) as shown in Table 1. Overall, quercetin glucosides are the most efficiently absorbed polyphenols in apples, followed by epicatechin and quercetin-rutinoside (rutin), while the hydroxycinnamic acids and procyanidins are poorly absorbed (Manach, Williamson, Morand, Scalbert, & Rémésy, 2005). An important question to ask is whether these compounds would be more or less beneficial to our health in isolation, where we could potentially consume them at a much higher dose than is found naturally occurring in plants. To determine this, it is crucial that we understand how each bioactive compound is absorbed, and whether this absorption is influenced by other components of the apple matrix. All polyphenols are influenced by pH, enzymatic activity and the microbiota of the digestive tract prior to absorption, as is discussed individually in more detail below.

2.3. Dihydrochalcones (phloretin and phloridzin)

2.3.1. Absorption and metabolism

The phloretin 2′-*O*-glucoside, phloridzin, is thought to be unique to apples and apple products. It has been shown, following apple juice consumption, that phloridzin is cleaved to phloretin by LPH (Scalbert & Williamson, 2000) and that only phloretin, phloretin 2′-*O*-xyloglucoside and phloretin 2′-*O*-glucuronide reach the colon (Kahle et al., 2007). Evidence suggests that most of the phloretin is absorbed in the small intestine as concentrations in plasma reached T_{max} of 0.6 h after ingestion of apple cider containing 46 μmol of phloretin-*O*-glycosides (Marks, Mullen, Borges, & Crozier, 2009). Studies have shown that phloridzin is transported by sodium-dependent glucose transporters (SGLT-1) in the small intestine and that this is followed by deglycosylation and glucuronidation in the intestinal epithelium or liver (Kahle et al., 2007).

2.3.2. Effect of the apple matrix

In a study investigating the bioavailability of phloretin and phloridzin in rats (Crespy et al., 2001), phloretin appeared more rapidly in plasma after the administration of phloretin vs phloridzin. Further studies are necessary to evaluate the matrix effect of apple on phloretin and phloridzin bioavailability.

2.4. Flavanols (epicatechins and procyanidin)

2.4.1. Absorption and metabolism

Unlike most other flavonoids, flavanols exist in plants mostly as aglycones, rather than glycoside conjugates (Crozier, Clifford, & Del Rio, 2012). They range from the simple monomers to the oligomeric and polymeric proanthocyanidins. Flavanol monomers are partially absorbed in the small intestine after which they are conjugated to form sulfate, glucuronide and methylated metabolites before entering the blood stream (Crozier, Jaganath, & Clifford, 2009). Flavanols oligomers, with a degree of polymerization >3, are not absorbed in the small intestine and thus travel to the large intestine where they are cleaved by the colonic microflora, releasing

aglycones which can then undergo ring fission leading to the production of phenolic acids (Crozier, 2013; Crozier et al., 2012). Accordingly, epicatechin peaks in the plasma around 2 h after ingestion. The main epicatechin metabolites; (−)-epicatechin-3′-glucuronide (II), (−)-epicatechin-3′-sulfate (III), and 3′-methyl-(−)-epicatechin-5-sulfate (IV), reach their peak plasma concentration 1–3 h after intake (Actis-Goretta et al., 2012; Ottaviani, Momma, Kuhnle, Keen, & Schroeter, 2012) and are mostly eliminated around 8 h (Hollands et al., 2013).

The results of numerous studies suggest that oligomeric and polymeric procyanidins are not absorbed in the small intestine due to their large molecular weight, polarity and high affinity for pectin (Le Bourvellec, Bouchet, & Renard, 2005). Rather, they reach the large intestine unaltered, where they interact with colonic microflora to generate a diverse range of phenolic acids (Espín, García-Conesa, & Tomás-Barberán, 2007; Kahle et al., 2007; Manach et al., 2005; Wiese et al., 2015). It has been suggested that absorption of procyanidins may not be a requirement for bioactivity and they may provide health benefits via modification of the gut microbiota and by exerting effects similar to that of prebiotics (Ou & Gu, 2014).

2.4.2. Effect of the apple matrix

To date, only one study has directly compared the bioavailability of epicatechin from a flavonoid extract and an apple matrix in humans (Hollands et al., 2013). When an epicatechin-rich apple extract was given rather than apple puree, maximum plasma concentration and time to peak for epicatechin was higher after the extract compared to the puree. Possible reasons for the reduced bioavailability of epicatechin in the presence of the whole apple matrix include the binding of epicatechin to apple fibre or entrapment within cells which may reduce bioaccessibility, and increased viscosity of the bolus due to the apple pectin. The apple puree had a very high pectin content of 17%, whereas the pectin content of a fresh apple is much lower. Other explanations given were reduced rates of enterocyte epicatechin uptake due to competition with other polyphenols for enterocyte phase-2 metabolising enzymes, and increased rates of apical efflux of conjugates thereby reducing absorption. Indeed, when pure epicatechin and pure quercetin were co-administered in rats, a decrease in plasma concentration was observed (Silberberg, Morand, Manach, Scalbert, & Remesy, 2005). In a human study with radiolabelled epicatechin it was found that 82% of the epicatechin, measured as total radioactivity, was absorbed (Ottaviani et al., 2016). The largest part seemed to be absorbed in the colon as metabolites/catabolites, indicating that the gut microbiota played a key role. Another important finding in this study is that there is a substantial degree of variation in the metabolism of epicatechin between species. Care should be taken when extrapolating the findings from mice and rat models to humans.

Although in the case of epicatechin it may appear as though concurrent ingestion of fibre reduces its bioavailability (Hollands et al., 2013), it is plausible that the absorption of epicatechin is only diminished in the small intestine, and that the association of epicatechin with fibre allows it to reach the colon where it can have beneficial effects on the gut microflora and can be degraded into phenolic acids which were not quantified in the plasma. In fact, a study by Schramm et al., found that concurrent carbohydrate consumption (in the form of white bread) significantly increased the uptake of flavanols (Schramm et al., 2003). The discrepancy in the findings of the two studies may be due to the quantity of insoluble fibre ingested as white bread only contains around 0.5% insoluble fibre (Li et al., 2002), or it may be due to carbohydrate content as flavanol absorption was enhanced when co-administered with sugar. Further randomized, crossover studies directly comparing bioavailability of pure epicatechin and epicatechin within a food matrix in humans are needed to account for the large inter-individual variability.

2.5. Flavonols (quercetin)

2.5.1. Absorption and metabolism

The absorption of quercetin depends on the form in which it is ingested (glycosylated or aglycone) and the food matrix in which it is found (Donovan, Manach, Faulks, & Kroon, 2006). Absorption of glucosides in humans involves a crucial deglycosylation step that is mediated by glucosidase activity of human saliva (Walle, Browning, Steed, Reed, & Walle, 2005), or by lactase phlorizin hydrolase (LPH), or cystolic β-glucosidase (CBG) during passage across the gut wall (Németh et al., 2003). The resulting quercetin aglycone can then be absorbed in the small intestine via diffusion (Walgren, Walle, & Walle, 1998). Flavonoid glycosides that are not substrates for deglycosylation pathways in the small intestine (such as quercetin-3-*O*-rutinoside and quercetin-3-*O*-rhamnoside), pass into the colon where they can be deglycosylated by microfloral rhamnosidases and β-glucosidases that are present in the colon (Donovan et al., 2006). Absorption in the large intestine is less efficient due to the smaller exchange area and lower density of transporters in the colon yielding a lower bioavailability and longer time to peak for quercetin rhamnoglucosides when compared to quercetin aglycone or quercetin glucosides. Additionally, catabolism of polyphenols such as quercetin by the gut microbiota leads to a reduction in the absorbance of the parent compound. Following absorption, quercetin undergoes very efficient conjugation (sulphation, methylation or glucuronidation), meaning no aglycones or quercetin glycosides are found in the plasma (Mullen, Edwards, & Crozier, 2006). The main plasma quercetin metabolites are quercetin-3'-sulphate, quercetin-3-glucuronide, isorhamnetin-3-glucuronide, quercetin diglucuronide and quercetin glucuronide sulphate. Quercetin metabolites are eliminated slowly with reported half-lives ranging from 11 to 28 h (Manach et al., 2005; Scalbert & Williamson, 2000).

One of the most bioavailable forms of quercetin, quercetin-3-*O*-glucoside, peaks in the plasma within 60 min (Olthof, Hollman, Buijsman, van Amelsvoort, & Katan, 2003) and is absorbed in a dose-dependent manner (Bondonno, Bondonno et al., 2016). Quercetin aglycone is not absorbed as readily as quercetin glucosides, possibly because the intestinal mucus prohibits the absorption of lipophilic substances such as the quercetin aglycone (Bondonno, Bondonno, Hodgson, Ward, & Croft, 2015). As discussed above, quercetin-3-*O*-rutinoside is absorbed in the large intestine and hence has a relatively low bioavailability (Erlund et al., 2000).

2.5.2. Effect of the apple matrix

There is increasing evidence that co-ingestion of quercetin with other flavonoids and food components can influence its bioavailability. In contrast to the results with flavanols, consumption of apple pectin has been shown to enhance the absorption of quercetin. This has been shown with chronic pectin consumption in rats, where quercetin, but not quercetin-3-*O*-rutinoside, absorption was enhanced (Nishijima, Iwai, Saito, Takida, & Matsue, 2009). As there was no effect on the time to peak of plasma quercetin, the authors postulated that this effect was likely to be the result of biological improvements in the absorptive capacity of the small intestine induced by the pectin rather than physico-chemical interactions with quercetin. To further explore this effect, quercetin aglycone and pectin were co-administered to human subjects (Nishijima, Takida, Saito, Ikeda, & Iwai, 2015). It was found that the simultaneous ingestion of pectin improved absorption of quercetin and this effect was dependent on the dose and degree of pectin methylation. The dose of pectin in this study was comparable to the

level of pectin in an apple (Ferretti, Turco, & Bacchetti, 2014). High-methoxy pectin, the predominant form of pectin found in the cell walls of fruits and vegetables, was thought to enhance the absorption of quercetin through its high-viscosity which can influence the solubility and transit time of quercetin in the gastrointestinal tract (Nishijima et al., 2015). A decrease in the transit time of the bolus through the small intestine allows an increase in absorption of quercetin (Eastwood & Morris, 1992). Fibre is not the only component of apples that needs to be taken into consideration; as mentioned previously, there is evidence that co-administration of quercetin with other flavonoids lowers its absorption (Silberberg et al., 2005).

The effect of the apple food matrix on the bioavailability of quercetin was directly investigated in a randomised crossover trial (Petersen et al., 2016). Equal increases in quercetin and total flavonol plasma concentrations, after administration of vacuum impregnated apple chips containing apple peel extract, apple peel extract capsules or freeze-dried apple peel were found in comparison to the reference (quercetin dehydrate capsule). Due to the large inter-individual variability of responses, further studies with a greater number of participants may be necessary for statistically significant differences to be revealed. In addition, it is possible that freezing and freeze drying results in decreases in total polyphenol content and antioxidant activity in apples (Dalmau, Bornhorst, Eim, Rosselló, & Simal, 2017). Further studies are necessary to compare the bioavailability and bioactivity of quercetin administered as a pure compound as opposed to within the whole food matrix in which it is typically consumed.

2.6. Phenolic acids (5-caffeoylquinic acid and 4-p-coumaroylquinic acid)

2.6.1. Absorption and metabolism

The hydroxycinnamic acids, 4-*p*-coumaroylquinic acid (p-CA) and 5-caffeoylquinic acid, also known as chlorogenic acid (CGA), are the most abundant phenolic acids in apples (Phenol Explorer Database).

p-CA exists in apples at low concentrations in its free, unconjugated, form and at high concentrations in conjugated form (Pei, Ou, Huang, & Ou, 2016). In conjugated form, p-CA can be esterified with alcohols, amines, monosaccharides, polysaccharides or lignin. The bioavailability of p-CA has been reviewed by Pei et al. (Pei et al., 2016); briefly, free p-CA is quickly absorbed in the small intestine and is excreted in the urine. A large percentage of absorbed p-CA remains in the free form; around 50% of the free form was found in the plasma 60 min after oral administration. The conjugated forms of p-CA are absorbed much slower in the small intestine, and a high portion of these reach the colon. Like other polyphenols, p-CA can undergo further conjugation with glucuronide and sulphate after absorption. It has been postulated that p-CA conjugates that reach the colon may have local beneficial effects such as inhibition and promotion of particular microorganisms and they may be metabolised by the microbiota and these metabolites absorbed where they can exert beneficial effects towards the host (Pei et al., 2016). Approximately 30% of CGA is absorbed in the small intestine (Olthof et al., 2003) after which it is rapidly conjugated (Erk et al., 2012). Absorption can be increased by a decrease in transit time of the CGA through the gastrointestinal tract. However, the majority of CGA reaches the colon intact, where it is hydrolysed and transformed by the gut microbiota (Renouf et al., 2010). These microbial metabolites are then absorbed and can be further modified by human enzymes, yielding a large variety of end products in the plasma (Gonthier et al., 2006). While the metabolic fate of CGA is dependent on the individual's gut microbiota composition, it has been shown that all of the transformation pathways lead to the production of 3-(3-hydroxyphenyl)-propanoic acid, which in some individuals can be further metabolised to hydroxyphenyl-ethanol and/or phenylacetic acid (Tomas-Barberan et al., 2014).

2.6.2. Effect of the apple matrix

The bioavailability of phenolic acids has not received as much attention as that of flavonoids, despite them being the main phenol compounds consumed (Lafay & Gil-Izquierdo, 2008). Significant amounts of phenolic acids bind to cell walls, possibly restricting bioavailability in the small intestine (Padayachee et al., 2012). Covalent bonds can be formed between phenolic acids and polysaccharides, meaning phenolic acids can be transported to the large intestine where they undergo fermentation and metabolism by resident bacteria. Apples contain approximately 78 mg/100 g hydrolysable phenolics (Pérez-Jiménez, Díaz-Rubio, & Saura-Calixto, 2013), forming part of the non-extractable polyphenols discussed above. The effect of other apple components on the bioaccessibility of phenolic acids requires further exploration.

2.7. Summary of bioavailability

The variance in health benefits of apple as a whole food in comparison to isolated key polyphenols may be due to differences in bioavailability between the two forms. There are a number of factors that influence how well an individual can absorb and metabolise polyphenols, including their microbial composition, the dose consumed and the presence of other polyphenols and macronutrients within the food matrix (Hollands et al., 2013). The co-ingestion of polyphenols with fibre may decrease their absorption in the small intestine, but increase the quantity that reaches the large intestine. Here the polyphenols can be degraded to phenolic acids and both the fibre and the polyphenols can improve the microbial composition of the colon. If whole food components such as fibre do indeed affect absorption, similar health benefits may not be achieved by the pure compound supplements, which are commercially available. If however, absorption of the pure compound is higher and bioactivity is not reduced, dietary supplements may be an easy way of increasing flavonoid consumption. Unfortunately many studies give freeze-dried apple powder or apple juices, which have different macronutrient profiles to whole apples that affect the absorption of polyphenols. More studies that directly compare the bioavailability of polyphenols given in isolation and as part of the apple matrix are needed.

3. Beneficial effects on cardiovascular health

3.1. Observational epidemiology

That a diet rich in fruit is protective against CVD is one of the most consistent relationships observed in observational lifestyle studies (Hung et al., 2004). An inverse association between apples in particular, and CVD mortality has been shown in early studies where participants in the highest tertile of intake had a relative risk of 0.87 (95% CI: 0.78–0.96) compared to those in the lowest tertile of intake (Mink et al., 2007). More recently, we have shown that higher apple intake is dose-dependently associated with lower risk of all-cause and cancer mortality with a hazard ratio of 0·89 (95% CI 0·81, 0·97) per sd (53 g/d) increase in apple intake (Hodgson et al., 2016), and lower odds of having severe abdominal aortic calcification in elderly women with an odds ratio of 0.76 (95% CI: 0.62–0.93) for each standard deviation (SD; 50 g/day) increase in apple intake (Bondonno, Lewis et al., 2016). The health benefits of a fruit-rich diet are primarily ascribed to their high flavonoid content. Knekt et al., showed a significant inverse association between

dietary flavonoid intake and total and coronary mortality in women (Knekt, Jarvinen, Reunanen, & Maatela, 1996). In this study, the relative risks for coronary mortality between the highest and lowest quartiles of apple intake for women and men were 0.57 (95% CI: 0.36–0.91) and 0.81 (95% CI: 0.61–1.09) respectively.

Due to difficulties in obtaining estimates of individual flavonoid consumption, few studies have examined the association of individual flavonoids with CVD. In the Finnish Mobile Clinic Health Examination Survey, high quercetin intake was associated with lower mortality from ischemic heart disease (Knekt et al., 2002), with apples and onions being the predominant source of quercetin. The relative risk (RR) between the highest and lowest quartiles of quercetin intake was 0.79 (95% CI: 0.63–0.99, p = 0.02). In this study, after adjustment for intake of vegetables and fruit other than apples, apple intake was inversely associated with occurrence of type-2 diabetes, thrombotic stroke, total mortality and ischemic heart disease mortality. In a prospective study of postmenopausal women from Iowa, a strong inverse association was seen between the intake of the flavonoids (+)-catechin and (−)-epicatechin and coronary heart disease death (Arts, Jacobs, Harnack, Gross, & Folsom, 2001). After multivariate adjustment, apple intake was significantly negatively associated with CHD with a relative risk from lowest to highest tertile of intake of 0.78 (95% CI: 0.62–0.98). In a recent prospective cohort study, epicatechin intake was inversely related to CHD mortality and was associated with 46% lower risk of CVD mortality in elderly men with prevalent CVD (Dower, Geleijnse, Hollman, Soedamah-Muthu, & Kromhout, 2016). In this study 28% of epicatechin intake came from apples.

Fibre has been shown to lower the risk of CVD and may be another component of apples contributing to positive effects on health. An increasing number of observational studies have reported a lower incidence of CHD and CVD in subjects who report consuming high amounts of fibre, as shown in a recent meta-analysis of 22 cohort studies (Threapleton et al., 2013). The pooled estimate for the risk ratio per 4 g/day increase in fibre from fruit was 0.92 (95% CI: 0.83–1.01) for CHD and 0.96 (95% CI: 0.93–1.00) for CVD.

3.2. Blood pressure and vascular function

Hypertension, a potentially modifiable risk factor for CVD, is a key outcome in many studies investigating the benefits of flavonoid-rich foods on cardiovascular health. Endothelial dysfunction is implicated in numerous cardiovascular pathologies including pre-hypertension, hypertension, atherosclerosis and stroke. A significant association has been observed between endothelial dysfunction, often caused by a decrease in the bioavailability and/or bioactivity of the vasodilator nitric oxide (NO), and increased risk of CVD (Halcox et al., 2002). In humans, ultrasonography of the brachial artery to measure NO flow-mediated dilatation (FMD) is the gold-standard method used to assess vascular endothelial function.

We have previously demonstrated that consumption of flavonoid-rich apples results in lower systolic blood pressure (−3.3 mm Hg, 95% CI − 4.9, −1.8 ($P < 0.001$)) and pulse pressure (−1.9 mm Hg, 95% CI − 3.2, −0.3 ($P = 0.02$)), in an acute, randomized, controlled, crossover study in 30 healthy men and women (Bondonno et al., 2012). We also saw an acute increase in NO and improved FMD of the brachial artery. In this study, the flavonoid-rich apple intervention was 120 g of apple flesh with 80 g of apple peel, providing a higher quercetin, (−)-epicatechin, and total flavonoid intake relative to the apple flesh only control.

In a double blinded crossover study by Auclair et al., 30 hypercholesterolemic men were asked to consume 2 bags of lyophilized polyphenol-rich or polyphenol-poor apples per day, for 4 weeks (Auclair et al., 2010). Phloretin excretion in the urine was used to assess compliance. They found no significant difference in FMD or BP between low polyphenol and high polyphenol apple powder. This study may be limited by the lack of analysis of the continuous FMD time-course curve, which was unavailable for some patients. Indeed, we have seen a significant improvement in FMD over the entire FMD time-course curve, but not when comparing the maximum change in FMD from baseline (unpublished data). Additionally, the authors discuss that the freeze-dried state of the apples may have influenced the bioavailability of the polyphenols.

To assess whether the acute changes in FMD and BP observed in our study described above were due to the flavonoid quercetin, we conducted a dose-response study with quercetin-3-*O*-glucoside in 15 healthy volunteers (Bondonno, Bondonno et al., 2016). We found no improvements in FMD or BP after any dose of Q3G ranging from 50 to 400 mg, despite seeing increases in quercetin metabolites in the plasma. Correspondingly, recent studies have only shown a decrease in systolic BP following quercetin supplementation in hypertensive individuals but not in pre-hypertensives or normotensives (Bondonno et al., 2015).

We have since conducted an acute and chronic study investigating the effects of high flavonoid apples with skin and low flavonoid apples (apple flesh only) on FMD and BP (unpublished). We saw significant improvements in FMD 1 h and 2 h after consumption of the high flavonoid apple intervention, in comparison to the low flavonoid apple intervention. If the beneficial effect on FMD is due to the high flavonoid content, it is most likely due to the flavonoids absorbed in the small intestine as effects were seen after just 1 h. In the same study, we saw similar improvements in FMD after 4 weeks of chronic apple ingestion. These improvements are unlikely to be due directly to the same flavonoid metabolites circulating in the plasma as they have a short half-life and would have been eliminated by the time the chronic FMD measurement was taken. In contrast to our first acute study, we saw no changes in BP. The results of our chronic study are in accordance with a 5 × 4 week dietary crossover study assessing the effects of whole apples (550 g/day), apple pomace (22 g/day), clear and cloudy apple juices (500 ml/day) on CVD risk factors in healthy volunteers, where no effect was seen on BP after any treatment (Ravn-Haren et al., 2013).

As the positive effects on FMD were seen after the flavonoid-rich apple with skin treatment, it is logical to consider whether or not the same effects can be seen after ingestion of those flavonoids alone. An acute intake of pure epicatechin (1 or 2 mg/kg body weight in one dose) was shown to increase FMD 2 h after ingestion in young healthy adults (Schroeter et al., 2006), however this was a limited pilot study as there were only 3 participants in each treatment group. In a recent chronic intervention study (−)-epicatechin (100 mg/day) improved FMD, however this did not reach significance (Dower et al., 2015). Additionally, two studies have shown that quercetin has neither an acute (Larson et al., 2012) nor chronic (Dower et al., 2015) effect on FMD.

Although the positive effects of apple consumption on FMD that have been demonstrated by our research have been attributed to flavonoids, it is possible that the outcome may have been influenced by an increase in fibre intake. According to the USDA National Nutrient Database, apples with and without skin have 2.4 g and 1.3 g fibre per 100 g, respectively. Indeed, an acute improvement in FMD has been shown after a high fibre meal (Brock et al., 2006). Interestingly, microbial SCFAs, a product of the breakdown of dietary fibre in the colon, have been shown to lower blood pressure via endothelial G-protein coupled receptor 41 (Miyamoto et al., 2016).

Overall an improvement in vascular function has been shown, both acutely and chronically, after a flavonoid-rich apple

with skin treatment in comparison to low-flavonoid apple flesh. Improvements in FMD after administration of pure flavonoids has been shown mainly in acute studies with epicatechin (Schroeter et al., 2010). A decrease in systolic BP (−3.3 mm Hg) has been seen in healthy volunteers following consumption of a flavonoid-rich apple with skin treatment, however decreases in systolic BP following the administration of pure flavonoids have only been seen in hypertensive or pre-hypertensive populations.

3.3. Lipids

Elevated plasma cholesterol, a risk factor for CVD, may potentially be ameliorated by apples (Jensen et al., 2009). The potential for apples to reduce cholesterol levels has been investigated in numerous human, animal and *in vitro* studies and has recently been reviewed by Koutsos et al. (Koutsos et al., 2015). In a review on the effects of apples on cholesterol levels (Jensen et al., 2009), 9 human intervention studies were examined; in general the daily intake of approximately 3 apples resulted in a 5–8% decrease in total cholesterol, whereas the consumption of apple juice (375–720 ml) had no effect on plasma cholesterol levels and had an adverse effect on plasma triglyceride (TG) levels, possibly due to its high fructose content. Significantly lower levels of serum total cholesterol were found after 6 months of dried apple consumption in comparison to 6 months of dried plum, in postmenopausal women (Chai et al., 2012). In contrast some studies, including our as yet unpublished apple intervention study, concluded that increased apple consumption did not significantly improve plasma lipid profiles (including total cholesterol, LDL-c, HDL and TG) (Vafa et al., 2011).

Initially fibre, in particular pectin, was considered to be the key cholesterol-lowering component of apples. Apple pectin has been shown to decrease plasma cholesterol in humans; a meta-analysis determined that 1 g of pectin could decrease total cholesterol (TC) and LDL-cholesterol (LDL-c) by 0.070 and 0.055 mmol/L respectively (Brown, Rosner, Willett, & Sacks, 1999). In a dietary crossover study evaluating the effects of clear and cloudy apple juices, whole apples and apple pomace on plasma lipid levels, there was a trend for a decrease in TC and LDL-c in the apple treatment groups, with whole apples giving the largest decrease, when compared to the control group (Ravn-Haren et al., 2013). TC and LDL-c were increased with clear apple juice, which contains no pectin or cell-wall components, and these changes were inversely correlated with the calculated intake of pectin. HDL and TG were unaffected by the apple treatments. In a recent systematic review of the effect of pectin on cholesterol concentrations, there was a mean reduction in TC of 0.36 mmol/L (95% CI: −0.52 to −0.19 mmol/L, $p < 0.001$), although most of the studies were conducted in hypercholesterolemic populations (Mills & Mackerras, 2016). In human digestion, pectin can potentially reduce plasma lipid levels by binding to cholesterol in the gastrointestinal tract, although this appears to depend on the pectin source, degree of esterification and molecular weight (Brouns et al., 2012). Although the cholesterol lowering effect of pectin is well reported, the relatively low pectin content of apples suggests that there are other components of apples, such as polyphenols, which may have an effect.

In a study by Serra et al., rats fed a cholesterol-rich diet were randomised to receive one of three different apple varieties or nothing (A. T. Serra et al., 2012). Components in the apples that correlated with a decrease in lipids and oxidised LDL were catechin, epicatechin, procyanidin B1 and beta-carotene. In humans, 4 weeks supplementation of 1500 mg apple polyphenols decreased total cholesterol by 4.5% in 48 hypercholesterolemic men and women (Nagasako-akazome, Kanda, Ikeda, & Shimasaki, 2005). Whether the same effects could be replicated with isolated polyphenol compounds was investigated in several human intervention studies. In a randomized, double-blind, placebo-controlled, crossover trial neither (−)-epicatechin (100 mg/d) nor quercetin-3-glucoside (160 mg/d) for 4 weeks had any effect on plasma lipid profile (Dower et al., 2015). Overall most human studies, with both acute and chronic quercetin supplementation have not reported any significant changes in levels of plasma LDL or HDL cholesterol (Bondonno et al., 2015). The present theory is that the cholesterol-lowering property of apples is due to a synergistic effect between pectin and polyphenols, as they are more effective together than individually in reducing cholesterol (Aprikian et al., 2003; Auclair et al., 2010). The addition of fibre to clear apple juice may reduce adverse effects on blood lipids.

Potential mechanisms for lipid lowering effects of apples, such as the modification of lipid metabolism, have recently been described (Koutsos et al., 2015). There is evidence from animal studies that apples can increase the clearance of plasma cholesterol due to enhanced faecal excretion of bile acids (Osada et al., 2006). Additionally, gel-forming apple pectin fibres can bind to cholesterol, decreasing its absorption and increasing its excretion (Jensen et al., 2009). Apple phenolic compounds have been shown to upregulate lipoprotein lipase activity, thereby reducing cholesterol levels (Yao et al., 2014).

Overall, studies suggest that effects of whole apples on plasma cholesterol levels are due to a synergistic relationship between polyphenols and fibre. These outcomes have been mainly observed in hypercholesterolemic subjects. The exact mechanism remains to be elucidated and further human intervention studies are required to determine dose-related effects. While beneficial effects have been shown with fresh or dry apples and cloudy apple juice, clear apple juice has been associated with adverse effects possibly due to its low fibre and high fructose content. This highlights the importance of the matrix in which these bioactive components are consumed.

3.4. Antioxidant effects

Polyphenols have been shown to be excellent antioxidants *in vitro* (Cefarelli et al., 2006), and consequently the health benefits of polyphenol-rich food have been previously attributed to antioxidant activity. However, the free radical scavenging mechanisms of polyphenols are not replicable *in vivo* (Croft, 2016). It has been suggested that polyphenols should now be considered as 'bioactives' rather than antioxidants (Sies, 2010). Comparable results have been seen in studies exploring the protective effects of apples against CVD; despite the high antioxidant capacity of individual apple polyphenols, ingestion of large amounts of apples by humans does not appear to result in equivalent antioxidant effects (Lotito & Frei, 2004). Additionally, consumption of apples only increases plasma polyphenols to μmol concentrations at best (unpublished results), and the metabolism undergone by polyphenols after absorption is likely to diminish their antioxidant activity (Loke et al., 2008). The emerging theory of how nutritional antioxidants really work has been recently reviewed by Forman and colleagues (Forman, Davies, & Ursini, 2014). They propose that polyphenols, oxidised during their reaction with free radicals, activate nuclear factor erythroid 2-related factor 2 (Nrf2) which maintains or induces protective enzymes against oxidant damage. In support of this theory, an *in vitro* study demonstrated that pre-incubation of Caco-2/15 cells with dried apple peel polyphenols resulted in a significant increase in the expression of Nrf2 (Denis et al., 2013). Further human intervention studies are required to determine if this is one of the mechanisms behind the protective effects of

apples and whether this pathway is affected by other components of apples such as fibre.

3.5. Anti-inflammatory effects

Inflammation underlies a large variety of human diseases and there is evidence that polyphenols exert anti-inflammatory activities (Gonzalez et al., 2011). In an cross-sectional study of 8335 US adults, intake of apples was inversely associated with levels of C-reactive protein (CRP), a biomarker of chronic inflammation (Chun et al., 2008). Apple polyphenols, in particular procyanidins and phloretin, demonstrate anti-inflammatory activities *in vitro* and may function as transcription-based inhibitors of pro-inflammatory gene expression (Jung, Triebel, Anke, Richling, & Erkel, 2009). In a human intervention study, chronic (12 months) consumption of dried apple reduced CRP levels by 32%, however this did not reach statistical significance (Chai et al., 2012). Several other human intervention studies have shown no association between apple (Ravn-Haren et al., 2013), cloudy apple juice (Barth et al., 2012; Ravn-Haren et al., 2013) or apple pomace (Ravn-Haren et al., 2013) intake on markers of inflammation. In vitro, apple cultivars exhibiting high contents of procyanidins were the most potent at inhibiting nuclear factor-kappa B (NF-κB), a transcription factor implicated in the induction of pro-inflammatory enzymes (Andre et al., 2012).

Another important component of apples which may be, at least partially, responsible for its anti-inflammatory effect is fibre. In a meta-analysis of human intervention trials with increased consumption of dietary fibre, 6 out of 7 studies reported a significant decrease in CRP levels (North, Venter, & Jerling, 2009). As mentioned previously, dietary fibre increases the production of SCFAs in the colon, which have been shown to inhibit of NF-κB activation (Andoh et al., 2003). Additionally, prebiotic dietary fibre can reduce intestinal permeability and uptake of lipopolysaccharides (LPS), an endotoxin released by gram negative bacteria that elicits a strong immune response in humans (Koutsos et al., 2015). Whether the anti-inflammatory effects of apples are attributable to polyphenols, fibre, or a synergistic interaction between them is yet to be confirmed.

3.6. Effects on diabetes

Type 2 diabetes (T2D) can increase cardiovascular disease risk and incidence of T2D has grown worldwide (Shaw, Sicree, & Zimmet, 2010). Diet is a strong modifier of T2D risk; in particular a diet rich in fruits and vegetables is associated with a decreased risk (Carter, Gray, Troughton, Khunti, & Davies, 2010). Specifically, apples have been highlighted as an important dietary component with the potential to reduce T2D prevalence. In an observational study of 38, 018 women, the consumption of more than 1 apple per day was associated with a significant 28% reduced risk of T2D when compared to women who consumed no apples (Song, Manson, Buring, Sesso, & Liu, 2005). Johnston et al., showed that there were statistically significant delays in glucose absorption after acute consumption of clear and cloudy apple juice relative to the control (equimolar for total glucose) (Johnston et al., 2002). These effects were suggested to be due to phloridzin and other polyphenols. However, in a dietary crossover study evaluating the effects of long-term (4 weeks) consumption of clear and cloudy apple juices, whole apples and apple pomace on markers of glucose metabolism, no effect was seen after any of the apple treatments relative to the control. The authors discuss that the lack of observable effect may have been due to the chosen measuring time; blood samples were taken after 12 h of fasting (Ravn-Haren et al., 2013).

Evidence for the potential role of apples in ameliorating diabetes comes predominately from animal and cell-culture studies. Hyperglycaemia, a risk factor for diabetes, can be prevented through the inhibition of glucose uptake in the small intestine. It has been shown that apple polyphenols can influence glucose uptake in the small intestine by inhibiting the activity of glucose transporters (Manzano & Williamson, 2010). The main contributors to inhibition of glucose transport were quercetin-3-*O*-rhamnoside, phloridzin and 5-caffeoylquinic acid, in that order. Inhibition of GLUT2 by the apple extract was greater than the inhibition of SGLT1. Additionally, phloridzin inhibits SGLT1 (Rossetti, Smith, Shulman, Papachristou, & DeFronzo, 1987) and has been shown to reduce the postprandial glucose response in a diabetic animal model (Krook et al., 1997).

Another important apple component which may influence hyperglycaemia is pectin, which can slow glucose absorption by trapping carbohydrates (Furness, Cottrell, & Bravo, 2015). In a study of 12 non-insulin-dependent T2D patients, 4 week supplementation of 20 g apple pectin/day slowed gastric-emptying rate and improved glucose tolerance (Schwartz et al., 1988).

Evidence that certain subclasses of polyphenols can reduce the risk of T2D comes from an observational study of 2915 participants from the Framingham Offspring cohort which found that each 2.5-fold increase in flavonol intake was associated with a 26% lower incidence of T2D (Jacques et al., 2013). That flavonol intake may be associated with a decreased risk of diabetes is supported by several animal studies which show that quercetin lowers blood glucose levels and improves plasma insulin levels in a streptozotocin-induced diabetic mouse model (Kobori, Masumoto, Akimoto, & Takahashi, 2009). Conversely, in a randomized, double-blind, placebo-controlled, crossover trial, 4 weeks supplementation with quercetin-3-glucoside (160 mg/d) had no effect on plasma glucose, insulin, or insulin resistance, whereas (−)-epicatechin (100 mg/d) improved fasting plasma insulin (Δ insulin: −1.46 mU/L; $p = 0.03$) and insulin resistance (Δ homeostasis model assessment of insulin resistance: −0.38; $p = 0.04$) but had no effect on fasting plasma glucose (Dower et al., 2015). Conclusive evidence that apples can reduce the risk of T2D is still lacking, however there are indications that both apple polyphenols and pectin can reduce glucose absorption in the small intestine, preventing hyperglycaemia.

3.7. Effects on the gut microbiota

The two primary phyla found in the human colon are the *Firmicutes* and *Bacteroidetes* and the ratio between these is often used as a marker for intestinal health. Bacterial species involved in the production of SCFAs are considered as health supporting. As this area of research is still relatively new, there are very few studies that have investigated the effects of polyphenol-rich foods or individual polyphenols on the composition and health of the gut microbiome. As discussed previously, there appears to be a reciprocal relationship between polyphenol-rich foods and the gut microbiota (Hervert-Hernandez & Goñi, 2011). Dietary polyphenols have been shown to repress the growth of *Firmicutes* and enhance the growth of *Bacteroidetes* in the gut but this effect appears to be mediated by their biotransformation products, rather than the original plant polyphenols (Parkar, Trower, & Stevenson, 2013). Apple pomace can have beneficial effects on rat colonic health by increasing SCFAs production and decreasing caecal pH, thereby supporting the growth of beneficial microflora and inhibiting the growth of harmful microorganisms (Juśkiewicz et al., 2012). Few human studies have examined the effects of apples on gut microbiota: in a population of 23 healthy subjects, 4 weeks consumption of whole apple or apple pomace

Table 2
The cardioprotective effects of apples and apple polyphenols in human intervention studies.

Study design	Treatment	Cohort	Significant Effects Observed in Treated Group/s	Endpoints with no observed effect	Reference
Randomised, controlled, cross over acute study	**Treatment:** 120 g of apple flesh with 80 g of apple skins **Control:** 200 g apple flesh only	**N** = 30 **Age:** 47.3 ± 13.6 y **Health status:** Healthy	↑ nitrite and NOx ↑ FMD ↓ SBP ↓ PP	DBP F_2-isoprostanes	(Bondonno et al., 2012)
Three-way, acute, single-blind, randomised, cross-over study	**Treatment:** 400 ml beverage consisting of either clear apple juice, cloudy apple juice **Control:** sugar-matched water	**N** = 9 **Age:** 24.0 ± 3.2 y **Health status:** Healthy	Delayed glucose absorption ↓ incremental glucose AUC ↓ plasma insulin over first 90 min	Plasma glucose	(Johnston et al., 2002)
Double-blind, randomized, chronic (4 weeks), crossover study	**Treatment:** 40 g of two lyophilized polyphenol-rich apples **Control:** 40 g of two lyophilized polyphenol-poor apples	**N** = 30 **Age:** 52.6 ± 5.5 y **Health status:** Hypercholesterolemic		FMD Lipids Glucose Plasma antioxidant capacity	(Auclair et al., 2010)
Randomised, single-blinded, chronic (4 weeks), crossover study	**Treatment:** whole apples (550 g/day), apple pomace (22 g/day), clear and cloudy apple juices (500 ml/day) **Control:** no supplement	**N** = 23 **Age:** 18–69 y **Health status:** Healthy	↑ LDL after clear juice compared to whole apple and apple pomace	HDL BP CRP Composition of the gut microbiota Markers of glucose metabolism	(Ravn-Haren et al., 2013)
Randomized, chronic (1 year), parallel study	**Treatment:** 75 g dried apple (about two medium-sized apples) **Control:** 100 g dried plum	**N** = 100 **Age:** 56.6 ± 4.4 y **Health status:** Healthy	↓ total cholesterol at 6 months ↓ weight ↓ LDL compared to baseline ↓ CRP compared to baseline ↓ lipid hydroperoxide	HDL TG	(Chai et al., 2012)
Randomized, controlled, chronic (8 weeks), parallel study	**Treatment:** 300 g apple per day **Control:** no supplement	**N** = 46 **Age:** 41.4 ± 4.0 y **Health status:** Hyperlipidemic and overweight		Plasma lipids Apo (B), Lp (a) LDL/HDL ratio.	(Vafa et al., 2011)
Randomized, double-blind, placebo-controlled, chronic (4 weeks), parallel study	**Treatment:** food tablets (Applephenon®) containing apple polyphenols (300, 600 or 1500 mg) three times per day **Control:** placebo	**N** = 46 **Age:** 40.8 ± 3.4 y **Health status:** Slightly elevated cholesterol	↓ LDL ↓ total cholesterol ↑ HDL in high dose	TG VLDL Blood sugar	(Nagasako-akazome et al., 2005)
Acute study	**Treatment:** 5 apples	**N** = 6 **Age:** 36.0 ± 3.0 y **Health status:** Healthy		Oxidation of plasma ascorbate, urate, α-tocopherol, and lipids	(Lotito & Frei, 2004)
Randomized, controlled, chronic (4 weeks), parallel study	**Treatment:** 750 ml/day cloudy apple juice **Control:** 750 mL/day isocaloric control beverage	**N** = 68 **Age:** 23–69 y **Health status:** overweight	↓ % body fat	Plasma lipids Waist circumference BMI	(Barth et al., 2012)
Chronic (2 weeks) study	**Treatment:** 2 apples per day	**N** = 8 **Age:** 2–60 y **Health status:** Healthy	↑ bifidobacteria ↓ lecithinase-positive clostridia ↓ Enterobacteriaceae ↓ fecal sulphide and ammonia	SCFA	(Shinohara et al., 2010)
Randomized, double-blind, placebo-controlled, acute, cross-over study	**Treatment:** Quercetin-3-*O*-glucoside (50, 100, 200 or 400 mg) **Control:** placebo	**N** = 15 **Age:** 60.8 ± 9.3 y **Health status:** Healthy	Linear dose-response ↑ in plasma Q metabolites ($p < 0.001$) 1 h post intervention	BP FMD Plasma NO	(Bondonno et al., 2015)
Acute study	**Treatment:** (−)-epicatechin at doses of 1 or 2 mg/kg of body weight (BW) dissolved in water	**N** = 3 **Age:** not available **Health status:** Healthy	↑ FMD ↑ PAT		(Schroeter et al., 2006) (Larson et al., 2012)

Double-blind, placebo-controlled, chronic (4 weeks), crossover study	**Treatment:** 1095 mg Quercetin aglycone **Control:** placebo	**N** = 5 healthy **Age:** 24 ± 3 y **N** = 12 stage-1 hypertensive **Age:** 41 ± 12 y	↓ SBP, DBP and MAP (in S1 hypertensives only)	plasma ACE activity ET-1 NO_2 FMD BP in normotensives	
Randomized, double-blind, placebo-controlled, chronic (4 weeks), cross-over study	**Treatment:** Quercetin-3-O-glucoside (160 mg/day) or (−)-epicatechin (100 mg/day) **Control:** placebo capsules	**N** = 37 **Age:** 66.4 ± 7.9 y **Health status:** Healthy (SBP: 125–160 mmHg)	↓ fasting plasma insulin (after epicatechin treatment) ↓ insulin resistance (after epicatechin treatment)	FMD BP Arterial stiffness Body weight Plasma glucose, insulin, and insulin resistance NO, Plasma lipids	(Dower et al., 2015)
Chronic (4 weeks) study	**Treatment:** apple pectin 20 g/day	**N** = 12 **Age:** 44.7 ± 7.9y **Health status:** Type 2 diabetes patients	↑ gastric emptying time ↓ incremental glucose AUC		(Schwartz et al., 1988)

ACE, angiotensin converting enzyme; AUC, area under the curve; APO, apolipoprotein; BP, blood pressure; CRP, C-reactive protein; DBP, diastolic blood pressure; FMD, flow-mediated dilatation; HDL, high density lipoprotein; LDL, low density lipoprotein; LP(a), lipoprotein a; NOx, nitrogen oxides; NO_2 nitrite; NO_3, nitrate; PAT, peripheral arterial tonometry; PP, pulse pressure; SBP, systolic blood pressure; TG, triglycerides.

resulted in a decrease in faecal pH but no change in microbial populations was detected (Ravn-Haren et al., 2013). In another small-scale intervention study (n = 8), 2 apples per day for 2 weeks resulted in a significant increase in *Bifidobacterium* and faecal acetic acid (Shinohara, Ohashi, Kawasumi, Terada, & Fujisawa, 2010).

Quercetin supplementation has been shown to counteract gut microbiota dysbiosis produced by a high-fat sucrose diet in rats (Etxeberria et al., 2015). In this study quercetin attenuated the *Firmicutes/Bacteroidetes* ratio and inhibited the growth of *Erysipelotrichaceae*, *Bacillus, Eubacterium cylindroides,* bacterial species associated with diet-induced obesity. Phloretin has been shown to act as an inhibitor of pathogenic *Escherichia coli* biofilm formation as well as an anti-inflammatory agent in inflammatory bowel diseases without damaging beneficial commensal *E. coli* biofilms (Lee et al., 2011). In a study investigating the potential health effects of apple-derived pectin in a rat model of diet-induced obesity, those rats receiving the high-fat diet treatment showed a decrease in *Bacteroidetes* phylum and an increase in *Firmicutes* phylum, and effect which was prevented by the addition of pectin to the diet (Jiang et al., 2016). Interestingly, pectins with a higher degree of methoxylation result in the greatest production of SCFAs (Gulfi et al., 2006).

4. Conclusion and future research

It is evident in observational studies that higher apple intake is associated with a lower risk of all-cause mortality, abdominal aortic calcification, coronary mortality, T2D, thrombotic stroke, and ischemic heart disease mortality. Beneficial effects on markers of cardiovascular health are less apparent in randomised controlled trials investigating whole apples, apple juice, apple pomace or apple polyphenol extracts (Table 2). While consumption of whole apples can improve vascular function, decrease systolic BP and reduce cholesterol levels, clear apple juice has been associated with adverse effects most likely due to its high fructose and low fibre content. As apple pomace is high in both polyphenols and fibre, this apple by-product could potentially be added to foods to increase nutritional value, however further research to validate this is required.

Individual compounds which may be “responsible” for the positive effects observed, in particular flavonoids, are a major research focus. But is it really one isolated compound or is it the unique combination of flavonoids and fibre in whole foods that is beneficial? There is evidence of a synergistic relationship between the fibre and flavonoids found in a whole apple, which is likely mediated in part by the gut microbiota. This underlines the importance of the matrix in which bioactive components, such as flavonoids, are consumed. Further research into the bioavailability of polyphenols when given in isolation and as part of a whole food matrix is needed. Additionally, more randomized controlled trials exploring potential health benefits of apples or apple products should be conducted in cohorts with risk factors for cardiovascular disease. The beneficial effects of apples may be more apparent in populations which have the potential for improvement. Many of the mechanisms believed to be behind the beneficial effects of apples are derived from *in vitro* and animal studies and remain to be established in humans. In particular, research to corroborate the emerging concept on how polyphenols actually act as antioxidants is required. Results from these studies could provide further incentive to breed apples for elite levels of polyphenol content, in both skin and flesh. If indeed it can be shown that “an apple a day keeps the doctor away”, this could prove to be a simple and economic way of reducing cardiovascular disease incidence world-wide.

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