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Chapter 16

POLYPHENOLS OF APPLES AND THEIR POTENTIAL HEALTH BENEFITS

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ABSTRACT

Based on the per capita consumption, apples are found to be one of the best sources of dietary polyphenols in the North American and European diet. The polyphenols found in apples are relatively effective antioxidants and demonstrated to have numerous of biological effects in prevention of various chronic diseases including cardiovascular disease, cancers and neurodegenerative diseases. Apples contain over 60 different phenolic compounds which mainly belong to flavonoids, cinnamic and benzoic acid derivatives. The apple flavonoids are mainly 3-hydroxy flavonoids which consist of anthocyanins, flavan-3-ols, procyanidins, and flavonols. Dihydrochalcones such as phloridzin are unique and predominant flavonoid precursors found in apples. The distribution and concentration of polyphenols vary greatly within the apple fruit as well as among apple cultivars. Apple peels have higher levels of polyphenols than flesh or core and also abundant in flavonoids such as quercetin glycosides and cyanidin galactoside. The flesh and core have relatively high concentrations of chlorogenic acid. There are many environmental factors that influence the accumulation of polyphenols in apples such as exposure to ultra-violet light, climate conditions and soil conditions such as nitrogen supply. While extensive research exists on the health benefits of apple polyphenols, this chapter focuses on the most recent literature regarding the apple polyphenols and their health benefits associated with cardiovascular disease, type II diabetes, and various cancers.

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INTRODUCTION

Apples are the most widely consumed fruit by Western populations [1] where 22% of fruit phenolics consumed in the United States are from apples [2]. Apple extracts and their constituent compounds are among the fruit bioactives most being experimented. The amount of bioactives in apples is greater than that of many other tree fruits such as peach and pear [3]. A serving of one medium apple [4] provides around 400 mg of total polyphenols expressed as gallic acid equivalents [5,6]. There are five major polyphenol groups found in apples: hydroxycinnamic acids, flavan-3-ols, flavonols, anthocyanins and dihydrochalcones [7-9]. Among them, flavonoids and phenolic acids are the most predominant. Apple peel contains three- to six-folds more flavonoids than flesh and hence peel extracts have greater antioxidant activities than flesh extracts [9].

In general, phenolic compounds of fruits have shown antioxidant and many physiological activities which in turn exert cardio protective properties *in vitro* [10,11] as well as in experimental animals [12-15]. There are also evidence that polyphenols of apples may also help to mitigate type II diabetes through various mechanisms including inhibition of starch digestive enzymes, α -amylase and α -glucosidase [16]. Anthocyanins, phenolics acids, flavonols and dihydrochalcones are also implicated for reducing the blood glucose level and increasing insulin sensitivity in experimental animals [17-20].

Furthermore, numerous *in vitro* and animal studies suggest that apple polyphenols can also modulate important cellular and molecular mechanisms leading to the prevention of carcinogenesis [21,22]. The mode of action of polyphenols seems to be multiple that involves the removal or trap of mutagens by antioxidant properties, detoxification of procarcinogens through enhanced xenobiotic metabolism, induction of apoptosis and antiproliferative effect. While extensive research exists on the health benefits of apple polyphenolics, this chapter focuses on the most recent literature regarding the apple polyphenolics, their bioavailability, antioxidant activity and health benefits associated with cardiovascular disease (CVD), type II diabetes, and cancers.

MAJOR POLYPHENOLICS PRESENT IN APPLES

A) Flavan-3-Ols: Catechin and Epicatechin

Catechin and epicatechin (Figure 1) are two monomeric flavan-3-ols that are found in higher concentrations in apple peel than in flesh [1]. In immature apples, about one half of the flavan-3-ols are monomeric and among monomers, concentration of epicatechin is higher than catechin [23]. The antioxidant activity of epicatechin measured by ferric reducing antioxidant power (FRAP) is greater than catechin and is considered as one of the most important polyphenolic compounds in apples [9]. A study demonstrated that the concentration of epicatechin in apple extracts had the strongest correlation with the extract's ability to inhibit peroxy radical-induced oxidation of methyl linolenate [24]. Both catechin and epicatechin are absorbed by small intestinal epithelial cells. Sulfation is the most common epicatechin metabolism pathway in plasma [25] and the majority of catechin detected in plasma or urine is methylated or present in the form of glucuronide conjugates [26].

B) Procyanidins or Condensed Tannins

Procyanidins are dimers, oligomers and/or polymers of flavan-3-ol monomer units. A study showed that the major source of procyanidins in the American diet was apples and accounted for the major flavonoid ingested in the Western diet [27]. Concentration of total procyanidins was the greatest among total polyphenols where 60% of apple peel and 56% of apple flesh polyphenols represented procyanidins [9].

Therefore, procyanidins can exert a greater effect on the total antioxidant activity of apples. Procyanidin content is significantly higher in unripe apples than in ripe apples [28]. Most common procyanidin dimers in apples are procyanidin B1 and B2 and among trimers procyanidin C1 is common [29]. The main structural difference in procyanidin B1 (Figure 1) and B2 dimmers is that procyanidin B1 has an epicatechin molecule linked to a catechin molecule and procyanidin B2 has an epicatechin molecule linked to another epicatechin molecule. This interflavan linkage is found between the C4 carbon in the C ring of the upper unit and C8 carbon in the A ring of the lower unit.

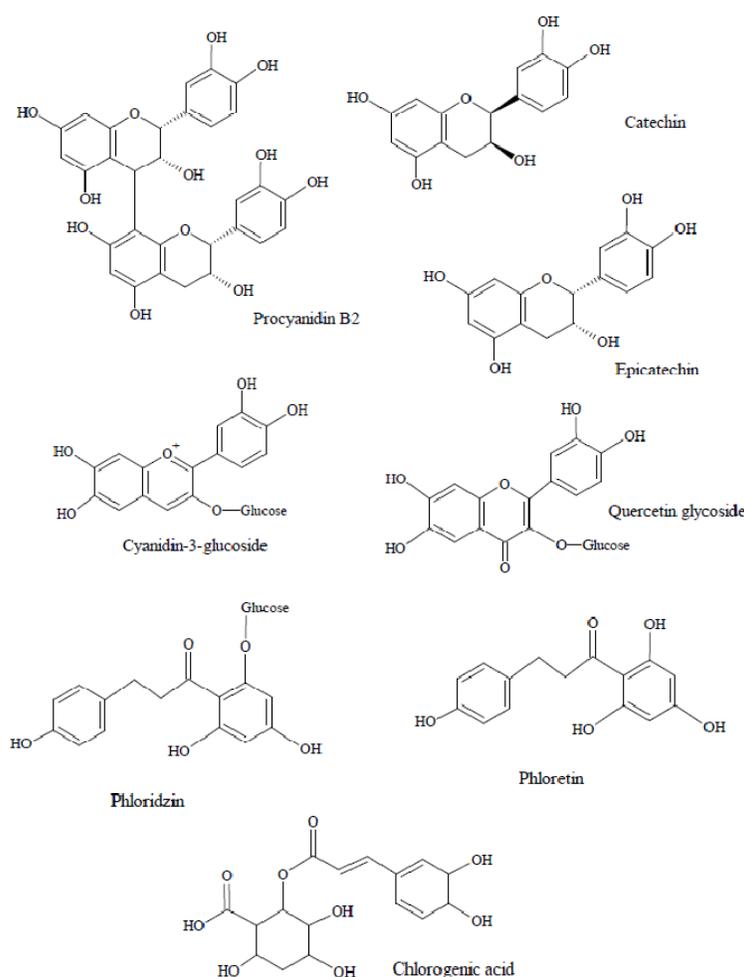


Figure 1. Chemical structures of selected major polyphenols found in apple.

The antioxidant capacity of procyanidins depends on their oligomeric chain length and the type of reactive oxygen species with which they react [30]. The procyanidin dimers have shown two times more antioxidant activity than the monomer units and procyanidin B2 had a greater antioxidant activity than the procyanidin B1 in *in vitro* studies [9]. It is difficult to estimate the oligomeric procyanidin content as they have a wide range of structures and molecular weights [7].

However, there is experimental evidence that oligomeric procyanidins have a stronger antioxidant activity compared to the monomer units [30]. It was reported that one half of the procyanidins in immature apples consisted of monomers, dimers and trimers where the other half consisted of procyanidins with higher degree of polymerization [28].

C) Anthocyanins

Anthocyanins are located exclusively in the peel [7] and are responsible for the red color of the apple peel. Apple anthocyanins are a mixture of two different cyanidin-3-*O*-glycosides of which cyanidin-3-*O*-galactoside is more common than cyanidin-3-*O*-glucoside (Figure 1). The quantity of these two compounds ranged from 10–551 mg/kg dry weight among different apple cultivars [7]. Apart from the two major cyanidins, 3-arabinoside and 3-xyloside are present in minor amount in certain red cultivars [23]. The major anthocyanin in the apple peel of most of the apple genotypes is cyanidin-3-*O*-galactoside [23,24,31]. Recently, high concentrations of cyanidin-3-*O*-galactoside were reported in the flesh of red-fleshed and crab apple species [32,33]. Anthocyanins have a greater antioxidant activity in terms of FRAP assay but as they account for less than 1% of the total polyphenols, this may explain why their concentration do not correlate with the antioxidant activity of apple bioactives [9].

D) Flavonols: Quercetin Glycosides

Flavonols, especially quercetins are exclusively located in the apple peel [1,34,35]. These compounds are often associated with different sugar moieties like galactose, glucose, rhamnose, arabinose and xylose [7]. Flavonols are in low concentrations and therefore is considered as a minor group of polyphenols in apples [7]. Although in low concentrations, quercetin has shown a strong bioactivity and has been studied extensively. A study showed that quercetins represent 1–11% of total apple polyphenols [23]. They are present mostly in their conjugated forms and apples contain traces of free quercetin and a mixture of six different quercetin glycosides (Figure 1): 3-galactoside, 3-arabinoside, 3-rhamnoside, 3-xyloside, 3-glucoside and 3-rutinoside [23,24]. Quercetin is recognized as a free radical scavenger as well as a radical chelator of transition metal ions [36]. The nature of the sugar residue in quercetin glycosides influences the extent of *in vivo* absorption [37].

E) Hydroxycinnamic Acids

Apples contain a significant amount of hydroxycinnamic acids representing 4–18% of total polyphenols [23]. Hydroxycinnamic acids are the second major polyphenolic group in

apples and are mainly located in the flesh than the peel [7] and primarily contribute to the antioxidant activity of apple flesh [9]. The main compound under this group of apple polyphenols is chlorogenic acid (5'-caffeoyl quinic acid) (Figure 1) followed by *p*-coumaroyl quinic acid and traces of *p*-coumaric acid [23]. Chlorogenic acid is the major hydroxycinnamic acid and most importantly the most abundant phenolic acid in apple flesh of many apple cultivars [38]. Chlorogenic acid and *p*-coumaroyl quinic acid are the precursors of cider flavour when they are in low concentrations [7]. Chlorogenic acid is known as a strong alkyl peroxy radical scavenger [39] and this compound is absorbed in the small intestine with no structural changes [40].

F) Dihydrochalcones

Dihydrochalcones are mainly associated with glucose and xyloglucose [9] and accounts for two to six percent of total apple polyphenols [23]. Among this group, phloridzin (phloretin-2'-glucoside) is the predominant compound followed by phloretin-2'-xyloglucoside (Figure 1) in both apple peels and flesh [9,38]. Phloretin and 3-hydroxyphloridzin are two dihydrochalcones occasionally found in apples [7]. A major part of dihydrochalcones are located in the peel and seeds of apples [38,41]. Dihydrochalcones show a low antioxidant activity. In apple peel and flesh, dihydrochalcones accounted for 0.1% and less than 0.1% of calculated antioxidant activity respectively [9]. It confirmed that a catechol B-ring is vital for better antioxidant activity [42].

Although this group of polyphenols are found in low concentrations, they have been useful in distinguishing from other fruits and identifying apple cultivars due to their unique nature to apples as well as varied profile among cultivars [38]. However, in an aqueous emulsion system of omega-3 fatty acids, phloridzin is more effective than α -tocopherol [43].

DISTRIBUTION OF POLYPHENOLICS AMONG CULTIVARS

There are various cultivars of apples for specific purposes like cider making, cooking and dessert or fresh eating [44] and polyphenolic composition of these different apples cultivars can vary drastically (Table 1). It is desirable for cider apple cultivars to have more polyphenols in their cortex as well as the peel in order to develop the desired flavor, color and mouth feel where as for fresh eating or dessert cultivars, less polyphenolic contents are preferred as too much of the astringency would not appeal the consumers [44]. Therefore, different apple cultivars would essentially comprise of different polyphenolic profiles as well as the total polyphenolic content would vary [8,45,46].

Examples for cider apple cultivars are 'Avrolles', 'Guillevic', 'Rhode Island Greening'; dessert apple cultivars are 'Golden Delicious', 'Jonagold', 'Braeburn', 'Cortland', 'Empire' and cooking cultivars are 'Granny Smith', 'McIntosh', 'Rhode Island Greening'. Some of these cultivars are being utilized for more than one use. For example, cultivar 'Granny Smith' can be used as a culinary cultivar as well as a dessert apple cultivar.

Cider apple cultivars contain greater polyphenolic content with comparison to dessert apple cultivars [23,45,47,48]. The cider apple cultivars are classified according to their total polyphenolic content and total acidity of the juice where bitter cultivars contain more flavan-3-ols, hydroxycinnamic acids and/or dihydrochalcones in the pulp, peel and juice than the non-bitter cultivars [49]. For making of French cider, a typical apple cultivars are generally selected to get the unique taste. Bitter cider apple cultivars contained a higher amount of procyanidins in the flesh or cortex compared to sharp cultivars but the sharp cider cultivar 'Avrolles' was an exception with greater procyanidin content [50]. Cider apples contain procyanidins of polymerized at a higher degree. It was reported that, for cider cultivars, the average degree of procyanidin polymerization was 3 for methanol extractable procyanidins and around 11 for aqueous acetone extractable procyanidins [51]. Cultivars 'Guillevic' and 'Avrolles' contained procyanidins with a degree of polymerization as high as 40 to 50 [50]. Only rutin was present in cider apple cultivars when compared to dessert and cooking apples after analyzing four dessert cultivars, three cider cultivars and a cooking apple cultivar for their flavonol content [52].

Among numerous dessert apple cultivars, 'Golden Delicious' is studied extensively [23,48,53,54]. Dessert cultivars are known to have a lower polyphenolic content compared to cider cultivars and showed five folds higher antioxidant content compared to the later [47]. Similar to other apple cultivars, the dessert cultivars also contained procyanidins as their major class of polyphenolic compounds [48,55]. Depending on the cultivar and the tissue zone, the degree of procyanidin polymerization varied from 5.7 to 7.1. The three dessert cultivars studied: 'Golden Delicious', 'Granny Smith' and 'Braeburn' showed a similar flavan-3-ol monomeric profile and proportions in the procyanidins.

'Renetta' which is a traditional apple cultivar cultivated in France and Italy had the highest total polyphenol content among eight "old" and "new" dessert apple cultivars investigated [23]. The "old" apple cultivars were 'Golden Delicious', 'Red Delicious', 'Granny Smith', 'Morgenduft' and the studied "new" cultivars were 'Fuji', 'Braeburn' and 'Royal Gala'. The cultivars arranged in the increasing order of total polyphenol content were as followed: 'Fuji', 'Braeburn', 'Royal Gala', 'Golden Delicious', 'Morgenduft', 'Granny Smith', 'Red Delicious' and 'Renetta'. As reported by Vrhovsek and colleagues [23], 'Granny Smith' consisted of the highest amount of flavan-3-ols and similar results on this cultivar were reported [55]. 'Renetta' had the highest level of oligomeric procyanidins, flavonol glycosides and dihydrochalcones. Cultivar 'Fuji' had the highest concentration of hydroxycinnamates as a percentage of total polyphenols. Although chlorogenic acid is generally the major monomeric polyphenol in apples, epicatechin is higher in cultivar 'Red Delicious' and 'Granny Smith'. Similar contents of epicatechin and chlorogenic acid are found in cultivar 'Braeburn' [23,55]. Highest level of anthocyanins is found in cultivar 'Morgenduft' and 'Red Delicious'.

Among four different apple cultivars commonly used to prepare apple sauce: 'Rome Beauty', 'Idared', 'Cortland' and 'Golden Delicious', 'Cortland' had the lowest phenolics as well as the flavonoid content while 'Rome Beauty' had the highest [56]. 'Idared' cultivar had the greatest anthocyanin content among the four cultivars. Huber and Rupasinghe [24] investigated apple peel extracts prepared by 21 different apple genotypes. They reported that the selected crab/heritage genotypes had the highest total phenolics including the procyanidin and chlorogenic acid when compared to commercial and new breeding lines studied. According to their findings, the commercial lines had the highest flavonol concentrations and

lower concentrations of chlorogenic acid and procyanidins compared to the crab/heritage lines and new breeding lines. Among 36 old and 31 new apple cultivars investigated, old cultivars had a higher polyphenol content compared to new cultivars [7]. The old cultivars studied by the authors were the most widely cultivated in Poland and the mentioned new cultivars were becoming popular among Eastern and Western Europe consumers.

Six apple cultivars including 'Golden Delicious', 'Cortland', 'Monroe', 'Rhode Island Greening', 'Empire' and NY674 were studied for their total phenolic content and contribution to antioxidant capacity [48]. 'Rhode Island Greening' had the highest amount of total phenolics among all the cultivars tested. All the cultivars had quercetin galactoside as the major flavonol glycoside where as NY674 had quercetin rhamnoside as the most abundant. Cultivar 'Jonagold' consisted of the highest amount of flavonoids among four other cultivars namely 'Golden Delicious', 'Cox's Orange' and 'Elstar' [54]. The quercetin glycoside profiles of 'Jonagold' and 'Golden Delicious' were similar and therefore, could not be used to distinguish these two cultivars from each other. All the cultivars had epicatechin as the predominant compound in the catechin profile.

***IN VITRO* ANTIOXIDANT ACTIVITY OF APPLE EXTRACTS AND PHENOLICS**

Apple peel and the flesh are significant sources of polyphenols and the polyphenol composition is unique in both peel as well as flesh. For example, flavonol glycosides are exclusively located in the peel and hydroxycinnamic acids are mostly found in the flesh [7]. Therefore, the antioxidant activity of different apple extracts will essentially depend on which tissues of the apple fruit used. A study used different polyphenolic extracts prepared from apple juice, peel and pomace and investigated their antioxidant effectiveness [57]. One apple juice extract consisted mainly of hydroxycinnamic acid where chlorogenic acid was the most abundant and the other juice extract consisted mainly of procyanidins. The apple peel extract consisted mainly of quercetin glycosides while the pomace extract mainly consisted of oligosaccharides. The procyanidin-rich juice extract exhibited the highest antioxidant capacity followed by the chlorogenic acid-rich juice extract and the apple peel extract measured by both Trolox equivalent antioxidant capacity (TEAC) and oxygen radical antioxidant capacity (ORAC) assays. The apple pomace extract showed the lowest capacity. Among eight advanced cider apple breeding lines, a positive correlation between total phenolic content and total antioxidant capacity in both peel and flesh was observed [45].

Apple peel is a by-product of apple pie and sauce manufacturing and considered as a significant source of phytochemicals for value-added products since apple peel contains greater concentrations as well as unique phytochemicals not present in the flesh [35,56]. An estimated 2-3 million kg of apple peels are generated as a result of apple processing in Nova Scotia, Canada [58]. Apple peel contains approximately 46% of the total phenolics in apples [59], and the phenolic compounds quantified in the peel are: the proanthocyanidins (procyanidin B1 and B2), the flavan-3-ols (epicatechin and catechin), the flavonols (quercetin-3-*O*-galactoside, quercetin-3-*O*-rhamnoside, quercetin-3-*O*-glucoside, quercetin-3-*O*-rutinoside), the dihydrochalcone (phloretin-2-*O*-glucoside), the anthocyanin (cyanidin-3-*O*-galactoside) and the phenolic acid (chlorogenic acid) [24,35]. Cyanidin-3-*O*-galactoside was

found on average to be the most abundant (22%) in the apple peel extract and showed relatively high correlations with the antioxidant capacity measured by ORAC and FRAP assays and total phenolics concentration [24]. According to the authors, the concentration of chlorogenic acid correlated with the concentration of total phenolics but had lesser correlations with the antioxidant capacity measures than that of flavan-3-ols. Methanolic extracts of apple peels were effective inhibitors of peroxy radical-induced oxidation of methyl linolenate as all the extracts showed over 73% inhibition [24]. Interestingly, the apple peel extracts were found to possess strong lipid stabilizing ability of 73.8% to 97.2% inhibition of peroxy radical-mediated oxidation of methyl linolenate in an aqueous emulsion system [24].

The antioxidant activity of apple polyphenols were compared with vitamin C and showed greater antioxidant activity except for chlorogenic acid [48]. Relative vitamin C equivalent antioxidant capacity was in the following order: quercetin > epicatechin > procyanidin B2 > phloretin > vitamin C > chlorogenic acid. The estimated contribution of these phenolics and vitamin C to the total antioxidant capacity of 100 g of fresh apples in the decreasing order was; quercetin > epicatechin > procyanidin B2 > vitamin C > phloretin > chlorogenic acid.

Quercetin has been studied extensively for its strong bioactivity. Quercetin is recognized as a free radical scavenger as well as a chelator of transition metal ions [36]. This compound possesses an antioxidant activity against copper-induced oxidation of plasma lipids even after absorption and metabolic conversion [14]. Quercetin glycosides were reported to be effective antioxidants against *in vitro* Cu²⁺- and 2,2'-azobis(2-amidino-propane) dihydrochloride (AAPH)-induced low density lipoprotein (LDL) [60] and polyunsaturated fatty acid [61] oxidation *in vitro*.

Apples can contain 80-128 mg of procyanidins per 100 g wet weight and due to its abundance, may contribute to more than 80% of the antioxidant capacity of apples, apple juice or extracts [6,9]. The antioxidant capacity of procyanidins depends on their oligomeric chain length and the type of reactive oxygen species with which they react [30]. The procyanidin dimers have shown two times more antioxidant activity than the monomer units and procyanidin B2 had a greater antioxidant activity than the procyanidin B1 in *in vitro* studies [9]. It is difficult to estimate the oligomeric procyanidin content in foods because they have a wide range of structures and molecular weights [7]. However, there is experimental evidence that oligomeric procyanidins have a stronger antioxidant activity compared to the monomer units and on average, around 80% of flavonols in apples are oligomeric procyanidins with a degree of polymerization three or higher [30]. Procyanidins have been shown to reduce the rate of hydroperoxide formation in isolated human plasma LDL through effective protection of α -tocopherol degradation and reduction of α -tocopheroxyl radical formation *in vitro* [62].

As anthocyanin compounds account for less than 1% of the total polyphenols of apples, this may explain why its concentration does not correlate well with the antioxidant activity of apple bioactives [9]. Anthocyanins have potent antioxidant and free radical scavenging activity. Protocatechuic acid is not found in high concentrations in fruits but it is a major metabolite of anthocyanin in humans [63]. This compound has shown to exert anti-inflammatory effects where an anti-monocyte adhesion effect as well as inhibition of intimal vascular cell adhesion molecule-1 (VCAM-1) and intercellular cell adhesion molecule-1 (ICAM-1) protein expression *in vitro* and *in vivo* has been observed [64].

The flavan-3-ols, epicatechin and catechin were shown to be more effective antioxidants than chlorogenic acid based on the β -carotene/linoleic acid model system, 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging antioxidant activity [65] and the 2,2'-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) radical scavenging antioxidant activity methods [66]. It was reported that epicatechin and procyanidin B2 were the most important compounds at individual levels to contribute to the antioxidant activity of apples [9]. It was also found that hydroxycinnamic acid exerted a significant antioxidant activity in the apple flesh rather than the peel due to its comparatively high content in flesh [9]. Dihydrochalcones had contributed lowest to the antioxidant activity in both apple peel [9] and flesh [9,42]. Even though with a low antioxidant activity, phloridzin was superior to vitamin C and E [42,48].

BIOAVAILABILITY OF APPLE POLYPHENOLS

There are many definitions to the term “bioavailability” and one of the most appropriate definitions by Visioli et al. [67] is “that a fraction of an ingested nutrient or compound that reaches the systemic circulation and the specific sites can exert its biological action.”

Therefore, it is imperative to understand the amount of a specific nutrient in food or dietary supplement as well as its bioavailability. Apples are a major contributor to the polyphenol intake in the Western diet. Bioavailability of apple polyphenols plays an important role when estimating their health benefits. Absorption and metabolism of apple polyphenols are summarized in the Figure 2.

Most of the ingested apple polyphenols were known to absorb in the small intestine [68]. It was believed that flavonoid glycosides were absorbed in the small intestine along with the sugar moiety intact but it is now debated where some believe these compounds do not reach the systemic circulation [69].

Absorption of some flavonoid glycosides can be very rapid where some can be very slow. Bioavailability of apple quercetin glycosides were 30% of that from onions [70].

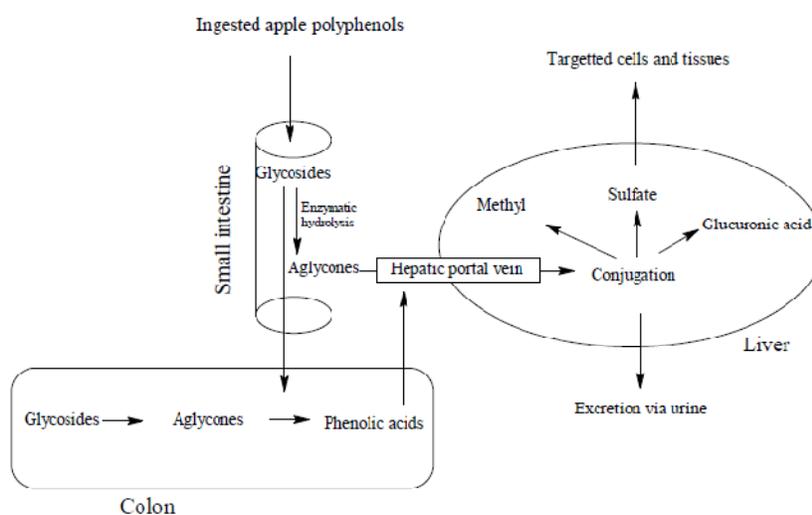


Figure 2. A simplified scheme of absorption and metabolism of polyphenols in mammals.

The associated sugar moiety had a greater influence on absorption where quercetin glucoside was absorbed 10 times faster and the concentration peaked 20 times greater in the plasma than quercetin rutinoid in humans [71]. It was suggested that the glucoside was absorbed in the small intestine where the rutinoid was absorbed in the colon after deglycosylation. The daily plasma level of quercetin was predicted to be substantially less than 1 μM [72]. Quercetin aglycone or its glucoside was generally not found in the plasma where its glucuronic acid, sulfate or methyl conjugates were exclusively present in the plasma [33,73]. These conjugates of quercetin are known to be less biologically active compared to the aglycone or the glucosides [73].

Catechins are rapidly absorbed and thus small intestine would be the site of probable absorption. The type of catechin monomer was not found to be a factor for absorption where dimerization reduced the bioavailability [72]. Catechins were found exclusively in the plasma as methyl, sulfate and glucuronic acid conjugates and generally had a shorter half life [73]. Epicatechins were mainly metabolized as sulfate conjugates and was not glucuronidated by neither liver, small intestine nor large intestine [1].

Although anthocyanins are rapidly absorbed, their bioavailability is the lowest compared to other flavonoids [72]. These compounds are not easily hydrolyzed to their aglycones [69]. Although glycosylated polyphenols needs to be hydrolyzed to its aglycone for intestinal or colonic absorption, anthocyanins could be absorbed and detected in the circulation with the aglycone or sugar moiety intact [74].

Absorption of phloretin and its glucoside phloridzin was suggested to be in the small intestine and phloretin was absorbed better than its glucoside [75]. After feeding a phloridzin diet to rats, no phloridzin was detected in the circulation, suggesting that the compound was hydrolyzed before absorption [76]. Phloridzin was believed to be hydrolyzed by the lactase phloridzin hydrolase enzyme and phloretin aglycone was taken up by the intestine [1]. This compound was also efficiently eliminated in urine and the major circulating metabolites were glucuronidated and/or sulfated derivatives of phloretin [76].

As summarized by Boyer and Liu [1], absorption of chlorogenic acid which was the major hydroxycinnamic acid was 33% and only traces were detected in urine. Major amounts of the chlorogenic acid reached the colon and were metabolized by the gut microflora in to hippuric acid and *m*-coumaric acid.

HEALTH BENEFITS OF APPLES

A) Cardiovascular Health

Most of the developed countries have undergone the “epidemiological transition” from infectious diseases to degenerative diseases like cardiovascular diseases (CVD) and cancer [77]. In the past history, the leading cause of death throughout the world was infectious diseases. From the midst of the 19th century to the midst of the 20th century, deaths from infectious diseases declined rapidly in most of the developed countries [78]. As Omran stated in his theory [77], now the developed countries have moved from the second stage which he called “the age of receding pandemics” to the third stage “age of degenerative and man-made diseases” where cancer and CVD become more prevalent. CVD along with diabetes

accounted for 32% of the total global deaths and contributed to 11% of the global burden of disability-adjusted life span in the year 2005 [79]. For example, the leading cause of hospitalization in Canada continues to be heart disease and stroke which account 16.9% of total hospitalizations and these diseases cost Canadian economy \$22.2 billion every year [80]. Therefore, CVD has become an increasing burden to the global economy.

CVD refers to the diseases related to the circulatory system. Under this category, rheumatic, hypertensive, ischemic, cerebrovascular and inflammatory heart diseases are included. Atherosclerosis is a degenerative process which involves accumulation of cholesterol in arterial walls, narrowing of arteries and formation of abnormal luminal surfaces in arteries and formation of atherosclerotic plaques [81,82]. With time, this degenerative process can become/lead to disease processes like CVD [82]. In addition to a person's genetic background, high blood pressure, diabetes, smoking and a diet high in cholesterol and lipids have been known to increase the risk of developing atherosclerosis [83], which is closely associated with dyslipidemia. According to the National Cholesterol Education Program [84] atherogenic dyslipidemia is defined as: triacylglyceride (TG) level ≥ 150 mg/dL; high density lipoprotein (HDL) cholesterol level ≤ 40 mg/dL; and presence of small LDL particles.

LDL is the metabolite of very low density lipoprotein (VLDL); a TG and cholesterol carrier between liver and peripheral tissues and considered as one of the key mediators of atherosclerosis [85]. Long term elevated LDL levels and oxidative modifications of LDL are known to be leading causes for the formation of atherosclerotic plaques [85,86]. Oxidation of LDL in vessel walls leads to an inflammatory cascade that activates many atherogenic pathways [87]. Under dyslipidaemic conditions, the circulating LDL levels rise above normal level and the LDL not taken up by cells become more prone to oxidation. At a damaged arterial endothelium, native LDL can accumulate in the sub-endothelial space and can get oxidatively modified [88]. Circulating monocytes accumulate at the site of damage, infiltrate in to the sub-endothelial space and engulf the oxidized LDL. Generally, cellular uptake of LDL is controlled by a feedback inhibition mechanism of LDL receptors but accumulation of LDL within monocytes at the earliest pathological stage of atherosclerosis cannot be explained by this. These oxidatively damaged LDL (ox-LDL) are not recognized by LDL receptors and instead they are taken up by scavenger like receptors in monocytes. This uptake of ox-LDL is not regulated by the intracellular cholesterol content and facilitates ox-LDL and cholesterol build up in monocytes [87,89] converting them into macrophages. Macrophages rapidly accumulate lipids and cholesterol from highly ox-LDL resulting foam cells. Ox-LDL can stimulate the release of pro-inflammatory signals and induce expression of chemokines like monocyte chemoattractant protein-1 (MCP-1) which attract more and more monocytes to the site of endothelial damage [85]. It also can induce the expression of chemokines which are responsible for phagocytic and lymphocyte cell migration and activation. Ox-LDL also promotes the conversion of monocytes to macrophages and the macrophages express a cultivar of scavenger receptors which take up ox-LDL and convert themselves into foam cells. Macrophages are also known to secrete cytokines such as tumor necrosis factor alpha (TNF α) and interleukin-8 (IL-8) which stimulate endothelial cells to produce VCAM-1 [85]. VCAM-1 has been shown to bind blood monocytes facilitating the infiltration of monocytes into the endothelium. Due to this continuous process, more and more monocytes are converted to macrophages [85]. Accumulation and fusion of macrophages will lead to the formation of foam cells which in turn progresses to a fatty streak; the earliest visible lesion. This will cause further endothelial injury and evolve into a fibrous plaque [90] that can be

released in to the circulation. Such a ruptured thrombus can impede the blood flow to the heart tissue or brain causing a myocardial infarction or a stroke [82]. At a later stage of human atherosclerosis, atherosclerotic lesions acquire calcium salt deposits and become hard and increasingly complex [90,91]. These calcium deposits are found more frequently and in greater amounts in elderly individuals and in more advanced lesions [91]. This calcification process cannot be seen in rats, mice, hamsters or rabbits [92] and therefore, these animal models represent only the early stage of human atherosclerosis.

Effects on LDL, Lipid Oxidation and Atherosclerotic Plaque Formation

As mentioned before, LDL oxidation can lead to degenerative processes like atherosclerosis and there by CVD. Apple polyphenols have shown promising anti-atherosclerotic effects in different model systems. The total polyphenolic content of fresh apples ranges from 110-357 mg/100 g and 22% of fruit polyphenolics consumed in the United States were from apples [2]. These polyphenols mainly flavonoids and phenolic acids have shown to possess numerous health promoting effects including LDL oxidation inhibition through diverse mechanisms.

Apples can contain 80-128 mg of procyanidins per 100 g wet weight and they may contribute more than 80% of the antioxidant capacity of apples, apple juice or extracts [37]. Procyanidin content is significantly higher in unripe apples than in ripe apples [93]. These compounds have shown to reduce the rate of hydroperoxide formation in isolated human plasma LDL through effective protection of α -tocopherol degradation and reduction of α -tocopheroxyl radical formation *in vitro* [62]. A recent *in vitro* study suggested that procyanidins may attenuate the development of foam cell formation by reducing cholesterol accumulation and modulating the expression of key genes in the cholesterol flux and inflammation [94]. As a matter of fact, Chen and colleagues [10] revealed that there were multiple pathways such as regulating numerous genes and proteins involved in the cardio protection of procyanidin B2 dimmer against lipid-laden macrophages in cell culture studies (for details see Chen et al. [10]). As suggested by the authors, this dimmer may be capable of inducing the genes and proteins studied and exert effects by regulating numerous pathways and their complicated interactions [10]. Oligomeric procyanidins were also reported to show cardio protective effects through modulating the expression of genes associated with the key events of angiogenesis as well as exhibiting a less migratory phenotype of human endothelial cells [95].

Endothelial dysfunction is an early stage of atherosclerosis and is associated with lowered bioavailability of nitric oxide (NO). Endothelial nitric oxide is generated by NO synthase III in the caveole of endothelial cells [96]. In an *in vitro* model, marked loss of endothelial nitric oxide synthase due to oxidized LDL was effectively counteracted by (-)-epicatechin [97]. NO preserving action of (-)-epicatechin in vascular endothelial cells was also reported [98]. (-)-Epicatechin scavenged O_2^- and its *O*-methylated metabolite and prevented the generation of O_2^- via inhibition of endothelial NADPH oxidase activity in a human umbilical vein endothelial cell culture [98]. Catechins have shown to improve proper function of the vascular system through various mechanisms including antioxidant activity as well as reducing the development of fatty streak. In human intervention studies, catechins increased plasma antioxidant activity, decreased plasma lipid peroxides and malondialdehyde (MDA) concentration, increased plasma ascorbate concentration, decreased non-heme iron absorption and increased resistance of LDL to oxidation [73]. Plasma cholesterol content and average

aortic fatty streak accumulation (relative to the total area surveyed) was significantly reduced in hamsters receiving catechin as a treatment compared to the control [99]. These findings demonstrate the protective effects exerted by flavan-3-ols apart from their antioxidant and radical scavenging activity in the pathogenesis of atherosclerosis.

Quercetin has been studied extensively for its strong bioactivity. It is recognized as a free radical scavenger as well as a chelator of transition metal ions [36]. Quercetins are present mostly in their conjugated forms and apples contain a mixture of quercetin glycosides and traces of free quercetin [23,24]. Quercetin glycosides are reported to be effective antioxidants against Cu^{2+} - and AAPH-induced LDL [60] and polyunsaturated fatty acid [34,61] oxidation *in vitro*. Quercetin-3-glycosides accumulated in the aorta showed significantly lower thiobarbituric acid reactive substances and cholesterol ester hydroperoxides in rabbits fed a high cholesterol diet supplemented with quercetin-3-glycosides [36]. A recent study revealed that quercetin improved endothelial function by stimulating endothelium-dependant vasorelaxation *ex vivo* eventually leading to increase in NO bioavailability [100]. Quercetin, quercetin glycosides as well as a quercetin-rich apple peel extract showed a strong antioxidant activity against *in vitro* LDL oxidation (Thilakarathna and Rupasinghe, 2012, In Press). Quercetins were metabolized both in enterocytes and liver to methylated, glucurono- and sulfo-conjugated derivatives [33,101] and they possessed an antioxidant activity against Cu^{2+} -induced peroxidation of plasma lipids even after absorption and metabolic conversion [14]. Quercetin-3-glucuronic acid showed better LDL protection *in vitro* compared to quercetin-3'-sulfate metabolite (Thilakarathna and Rupasinghe, 2012, In Press). Apart from quercetin's antioxidant activity, they are also known to possess anti-atherosclerotic activity. Quercetin was reported to significantly reduce the aortic fatty streak area [99] and quercetin metabolites were known to specifically accumulate in the atherosclerotic plaques [102]. Quercetin may attenuate atherosclerosis in apo E-deficient (ApoE^{-/-}) gene-knockout mice by alleviating inflammation, improving NO bioavailability, and inducing heme oxygenase-1. Attenuation of lesion formation in ApoE^{-/-} knockout mice was associated with the expression of heme oxygenase-1 protein in aortic lesions [103].

Apple anthocyanins are a mixture of two different cyanidin-3-O-glycosides of which cyanidin-3-O-galactoside is more common than cyanidin-3-O-glucoside. The quantity of these two compounds ranges from 10–551 mg/kg dry weight among different apple cultivars [7]. Anthocyanins have potent antioxidant and free radical scavenging activity. Protocatechuic acid is not found in high concentrations in fruits but it is a major metabolite of anthocyanin in humans [63]. This compound has shown to exert anti-inflammatory effects where an anti-monocyte adhesion effect as well as inhibition of intimal VCAM-1 and ICAM-1 protein expression *in vitro* and *in vivo* is observed [64]. Adhesion of monocytes to the intima is considered as an early incident in the atherosclerosis development. Cyanidin-3-O-glucoside has also shown this effect where it inhibits VCAM-1 and ICAM-1 protein expressions in endothelial cells by down regulating the TNF- α -stimulated nuclear factor kappaB (NF- κ B) signal transduction pathway [5,6,104].

Cholesterol-Lowering Effects

Apart from inhibition of LDL oxidation and atherosclerotic plaque formation, apple extracts have shown to exhibit cardio-protection through cholesterol lowering effects in animal as well as clinical studies. There can be numerous mechanisms involved: decrease in

dietary cholesterol absorption, decrease or inhibition of de novo cholesterol synthesis, bile acid sequestration, cholesterol esterification, clearance, catabolism, etc.

Many studies have been conducted on cholesterol lowering effect of lyophilized apples [13,25], apple polyphenols extracts [25,105], apple fibres [106], apple juice [107] as well as major phenolic compounds in apples [93]. Apple polyphenol supplemented diet increased fecal bile excretion in rats [25]. These findings were repeatedly confirmed where fecal bile acid excretion increased two- to three-folds in hamsters [105] and a reduction of non-HDL was observed. As suggested, the later reduction was due to suppression of plasma cholesteryl ester transport protein (CETP) which mediated the transfer of cholesteryl esters from HDL to LDL or VLDL. Apple fibers and polyphenols reduced hepatic cholesterol in ApoE^{-/-} mice when administered at an equivalent intake level to humans; 1.6 g of polyphenols and 50 g of fiber per day [106]. Apple juice was effective in reducing hypercholesterolemic atherosclerosis by lowering the levels of serum total cholesterol, LDL and increasing the levels of serum HDL in a hypercholesterolemic rabbit study [107]. The apparent cholesterol absorption was markedly reduced while the bile acid digestive balance remained unchanged in rats fed a high-cholesterol diet accompanied with lyophilized apples [25]. The lipoprotein profiles of these rats were altered where a cholesterol reduction in the TG rich lipoprotein fraction and an increase in serum HDL fraction were observed. These results were confirmed in another study where 20% lyophilized apple supplementation lowered plasma total and LDL cholesterol levels as well as reduced TG accumulation in the heart and liver of obese hypercholesterolemic Zucker rats [13]. The diets contained 0.25% cholesterol and the rats had a greater excretion of bile acids in the feces compared to the control group with no supplementation.

Not only the whole apple or crude apple extracts, but also individual constituent phenolic compounds in apples have also shown cholesterol lowering ability. A study reported that oligomeric procyanidins could exert a hypocholesterolemic effect through enhancing the excretion of neutral steroids into the feces [93]. The authors argued that the hypocholesterolemic effect could be due to the inhibition of intestinal cholesterol absorption through reduction of the micellar solubility of cholesterol and hepatic catabolism of cholesterol [93]. Quercetin significantly reduced the serum total cholesterol and phospholipid levels in mice and reduced the activity and mRNA levels of enzymes involved in hepatic fatty acid synthesis [108].

B) Type II Diabetes and Metabolic Syndrome

Type II diabetes is a chronic disease which results when the body cannot use insulin effectively. According to World Health Organization statistics, 346 million people worldwide have diabetes and deaths are projected to be doubled between 2005 and 2030 [109]. Metabolic syndrome is a cluster of atherosclerotic cardiovascular disease risk factors like insulin resistance, visceral obesity, hypertension, dyslipidaemia and proinflammatory state [110]. Obesity is related to the development of many degenerative diseases and has resulted due to the increase prevalence of metabolic syndrome characterized by visceral obesity, hypertension, dyslipidaemia and insulin resistance [111].

Obesity has led to the increasing prevalence of type II diabetes. As reviewed by Manabe [111], proinflammatory pathways can inhibit insulin signaling and chronic inflammation is

remarkably involved in developing type II diabetes. Insulin resistance and pancreatic β cell dysfunction are two pathological processes in type II diabetes and chronic inflammation was to be accountable for the development of both. Adipocytokines are biologically active molecules synthesized by the adipose tissue [112]. Inflammatory adipocytokines like TNF- α , IL-6, MCP-1 are expressed and upregulated [113,114] whereas adiponectin levels are down-regulated [115] under obese and type II diabetes conditions. The ongoing chronic inflammation in the adipose tissue is evident as the secretion of numerous proinflammatory cytokines and the increase in fatty acids release due to lipolysis [111]. Retinol binding protein 4 (RBP4) is another adipocytokine with a recent history and shows a close link with glucose uptake and insulin sensitivity [116]. It was found that when the expression of glucose transporter 4 (GLUT4) was reduced in adipocytes in obese or diabetes conditions, RBP4 expression and secretion to blood was increased [116]. This further caused impairment of insulin signaling in skeletal muscle and stimulated glucose production in the liver leading to high glucose concentration in the blood. Therefore, the authors stated that the regulation of adipocyte GLUT4-RBP4 system was strongly associated with type II diabetes involving in the metabolic syndrome. Apart from intercellular signaling molecules and inflammatory cytokines, recent investigations reported intracellular pathways like forkhead box O1 (FoxO1) regulating obesity-induced insulin resistance [117]. FoxO1 was known as a major transcriptional mediator of insulin signaling in cells like pancreatic β cells, adipocyte and hepatocyte and a negative regulator of insulin sensitivity [118].

Consumption of apples has shown to reduce the risk of developing type II diabetes [1]. It was reported that women who consumed at least an apple a day reduced the risk of type II diabetes by 28% than the women who did not. Postprandial hyperglycemia and hyperinsulinemia are risk factors for developing type II diabetes complications [119]. α -Glucosidase and α -amylase are two main enzymes targeted by different pharmacological drugs to suppress postprandial hyperglycemia. α -Glucosidase catalyzes the final step in the carbohydrate digestive process and α -amylase catalyzes the hydrolysis of internal α -1,4-glycosidic links in starch [16]. Pulp and peel extracts of ten different freshly harvested apple cultivars had high α -amylase inhibitory and α -glucosidase inhibitory activity, respectively [16].

Chlorogenic acid was found to exhibit a potential anti-obesity effect. The effects were mediated in high-fat diet induced-mice by altering plasma adipocytokine levels and body fat distribution, down-regulating fatty acid and cholesterol synthesis as well as up-regulating fatty acid oxidation and peroxisome proliferator-activated receptor (PPAR) α expression in the liver [18]. Chlorogenic acid reduced the body weight by 16% and epididymal adipose tissue weight by 46% in high-fat diet-induced-obese mice [18]. Plasma leptin and insulin levels positively correlated with the body weight and epididymal adipose tissue weight and the investigators observed a significant reduction in these mentioned hormone levels by chlorogenic acid supplementation [18]. A previous study reported similar findings where chlorogenic acid reduced plasma and hepatic lipids without interfering with the TG in the adipose tissue and improved glucose tolerance in (fa/fa) Zucker rats which was an established animal model for type II diabetes [120]. It was suggested that the chlorogenic acid treatment significantly improved the insulin sensitivity.

Thiazolidinediones are used as antidiabetic drugs and are synthetic PPAR ligands. These drugs are effective through adipocyte differentiation and activation of adipocyte genes [121]. Administration of these drugs has shown undesirable side effects like obesity and edema and

therefore selective PPAR γ modulators are much needed. Cyanidin-3-*O*-glucoside significantly upregulated GLUT4 and down-regulated RPB4 in the white adipose tissue of type II diabetic mice [122]. The action was accompanied by down-regulation of MCP-1 and TNF- α in the white adipose tissue and the treatment significantly reduced the blood glucose level and increased insulin sensitivity. Cyanidin-3-*O*-glucoside reduced blood glucose levels and improved insulin sensitivity in two diabetic mouse models and also showed beneficial effects against obesity-induced insulin resistance and hepatic steatosis in obese mice through inhibition of the JNK/FoxO1 pathway [17]. JNK is known as a mediator of TNF- α and hyperglycemia induced oxidative stress and abnormally activated in obesity and insulin resistance [123]. Stress activated JNK was found to directly increase FoxO1 activity by promoting import in the nucleus [124].

Quercetin improved adiponectin expression in white adipose tissue and increased the concentration of circulating adiponectin by a PPAR γ -independent mechanism in a rat model [19]. High circulating adiponectin levels are positively correlated with improved insulin sensitivity [125]. Quercetin aglycone was taken up by primary human adipocytes and was effective in attenuating insulin resistance and TNF- α mediated inflammation in the adipocytes [126]. Investigators suggested different direct and indirect mechanisms for this finding such as interfering with TNF- α receptor (TNFR) binding, suppressing TNF- α -TNFR signaling of altering the activity of proteins involved in glucose and lipid metabolism or inflammation.

Phloridzin is classified as an antidiabetic compound [75] due to its competitive inhibition of intestinal glucose uptake via sodium D-glucose cotransporter-1 (SGLT1) [127] as well as inhibition of renal glucose reabsorption [20]. Phloridzin treatment was known to counteract hyperglycemia and normalize plasma glucose levels in diabetic rat models by several mechanisms. The β -cell abnormalities were completely corrected [128] and the effects of insulin was normalized in liver and peripheral tissues like muscle and adipose tissue [129]. A mouse model with severe insulin resistance, hyperinsulinemia, dyslipidemia and hyperglycemia (MKR mice) improved circulating glucose levels after subcutaneously injecting phloridzin for two weeks [130]. The authors further stated that the circulating blood glucose reduction was not associated with any whole-body insulin sensitivity or glucose homeostasis and lipotoxicity affects the development and progression of type II diabetes rather than glucotoxicity in this mentioned mouse model.

C) Cancer

The polyphenol components from foods have been shown to prevent and reverse the carcinogenic process in a pleiotropic manner. Cancer chemoprevention refers to the use of an agent to block, inhibit or reverse the process of carcinogenesis through use of non-cytotoxic nutrients and/or pharmacological agents during the time period between tumor initiation and malignancy. High dietary intake of fruits and vegetables is consistently associated with a reduced risk of common human cancers.

Apples have been shown to contain several polyphenolics that are proven to be protective in cancer: these include flavonoids, phenolic acids and lignans. Numerous *in vitro* and animal model data suggest that apple polyphenols modulate important cellular and molecular mechanisms related to carcinogenesis [21,22]. It is a multistep process involving the removal or trap of mutagens by antioxidant properties, detoxification of procarcinogens through

enhanced xenobiotic metabolism, induction of apoptosis and antiproliferative effect. Epigenetic mechanisms and modulation of immune functions are recently the targets of chemoprevention.

Apple Consumption and Epidemiological Evidence for Cancer Prevention

Population-based studies, including case control and cohort studies, have suggested a link between apple consumption and reduction in the risk of developing cancer. A hospital-based, case-control study recently examined whether regular consumption of apples in humans had a beneficial effect on colorectal cancer risk [131]. The results revealed that the colorectal cancer risk was reduced by about 50% when consumed more than one apple a day. A cohort study of 35,159 Iowa women aged 55–69 years showed apple juice/cider consumption was associated with lower risk of development of non-Hodgkin's lymphoma (NHL) and follicular lymphoma [132]. In another hospital-based case-control study (2005 to 2008; University Hospital in Krakow, Poland) using 181 incident cases of colorectal cancer patients revealed that cancer was inversely correlated with daily number of consumed apple servings, but the most significant reductions of cancer was observed for an intake of one or more apple serving/s daily [133]. Association between the six main classes of phytochemicals in apple and the risk of colorectal cancer was examined using data from a national prospective case-control study in Scotland, including 1,456 incident cases and 1,456 population-based controls matched on age, sex, and residence area [134]. The reductions in colorectal cancer risk associated with the highest quartiles of intake (versus the lowest quartile) were 27% for flavonols, 32% for quercetin, 32% for catechin, 26% for epicatechin, and 22% for procyanidins. In a large and integrated network of case-control studies conducted, included over 6,000 participants from various regions in Italy, and examined the association between fresh apple intake and risk of cancer [135]. A larger cohort study of 10,054 men and women in different regions of Finland during 1966–1972 also found a significant inverse association of apple intake (as main source of flavonoids; of which 95% was quercetin) and lung cancer with a relative risk of 0.42 (95% confidence interval 0.23–0.76) [136,137].

Short Term Human Trials on Antioxidant Status and Oxidative Stress

Although *in vitro* and animal studies have indicated cancer preventive efficacy of apple products, extrapolation of the results to human situation is difficult. In humans, exposure to low doses of carcinogens and tumor promoters, genetic polymorphisms, variations in DNA methylation and epigenomic events may influence the response to carcinogens and protective agents. The proof of cancer preventive efficacy in humans requires very large and long-lasting controlled clinical trials [22]. Most of the reported short-term human trials focus on the modulation of antioxidant status and markers of oxidative stress by consumption of apple and apple juice. In a study by Ko et al. [138], antioxidant capacity was detected in serum of 10 healthy male volunteers 30 minutes after consumption of 150 mL of apple juice. Apple juice was compared with a variety of other fruit juices. Consumption of fruits or fruit juices reduced damage from oxidative stress which might be a consequence of the antioxidant activity of fruits in scavenging the reactive oxygen species generated in human plasma [138]. Similarly, in 12 healthy subjects, one serving of 1 L apple juice caused a significant increase in serum DPPH radical scavenging activity 1 hour after juice ingestion [139].

Table 1. Polyphenolic concentrations of selected apple cultivars

Cultivar	Use	Polyphenol Concentration	Remarks	Reference
‘Avrolles’	Cider	In cortex (mg/kg FW): traces of catechin and epicatechin; procyanidin 2424±250; chlorogenic acid 154±22; <i>p</i> -coumaroylquinic acid 104±11; phloretin xyloglucoside 55±14; phloridzin 25±4	Sharp cider variety. Has higher procyanidin content	[50]
‘Guillevic’	Cider	In cortex (mg/kg FW): traces of catechin and epicatechin; procyanidin 1066±170; chlorogenic acid 465±3; <i>p</i> -coumaroylquinic acid 134±1; phloretin xyloglucoside 19±0; phloridzin 19±0		[50]
‘Azpuru Garratza’ (Sweet-bitter)	Cider	Whole apple pulp (mg/kg FW): catechin 10±3; epicatechin 65±16; procyanidins 74±6; caffeoylquinic acid 158±49; <i>p</i> -coumaroylquinic acid 16.7±0.5; phloretin xyloglucoside 9±4; phloridzin 10.8±0.5; quercetin glucoside 0.21±0.03; quercetin rhamnoside 0.5±0.3	More flavan-3-ols, hydroxycinnamic acids and/or dihydrochalcones in the pulp, peel and juice than the non-bitter varieties	[49]
‘Palazio’ (sweet-Non-bitter)	Cider	Whole apple pulp (mg/kg FW): catechin 58.56±0.02; epicatechin 79±17; procyanidins 58±5; caffeoylquinic acid 373±2; <i>p</i> -coumaroylquinic acid 5±0.9; phloretin xyloglucoside 8±0.5; phloridzin 7.1±0.4; quercetin glucoside 0.55±0.06; quercetin rhamnoside 1.5±0.4		[49]
‘Dabinett’	Cider	Total flavonol in whole fruit 32.5±0.3 µg/g FW	Contains rutin as the major flavonol	[52]
‘Michelin’	Cider	Total flavonol in whole fruit 40.9±0.8 µg/g FW		[52]
‘Yarlington’	Cider	Total flavonol in whole fruit 41.9±2.2 µg/g FW		[52]
‘Renetta’	Cider	mg/100g FW: Total hydroxycinnamates 38.40±8.30; total flavanols 203.30±26.5; total anthocyanins nd; total dihydrochalcones 15.48±2.7; total flavonols 3.4±1.1	High polyphenol content. A traditional cider variety. High content of oligomeric procyanidins, flavonol glycosides and dihydrochalcones	[23]
‘Golden Delicious’	Fresh eating, Apple sauce, Baking	* mg/100g FW: Total hydroxycinnamates 10.69±1.9; total flavanols 70.8±8.7; total anthocyanins nd; total dihydrochalcones 2.79±0.5; total flavonols 7.14±2.0	Old variety. Quercetin glycoside profiles similar to that of ‘Jonagold’	[23]*; [54]; [55]

Cultivar	Use	Polyphenol Concentration	Remarks	Reference
'Granny Smith'	Fresh eating, Baking	* mg/100g FW: Total hydroxycinnamates 4.48±0.86; total flavanols 105.45±9.2; total anthocyanins nd; total dihydrochalcones 2.02±0.4; total flavonols 4.93±1.7	Old variety. Highest amount of flavanols among the studied varieties. Epicatechin is the major monomeric polyphenol	[23]*;[55]
'Braeburn'	Fresh eating	* mg/100g FW: Total hydroxycinnamates 6.45; total flavanols 58.92; total anthocyanins 0.52; total dihydrochalcones 2.28; total flavonols 8.32	New variety. Similar contents of epicatechin and chlorogenic acid	[23]*;[55]
'Red Delicious'	Fresh eating	mg/100g FW: Total hydroxycinnamates 9.87±1.2; total flavanols 112.44±17.0; total anthocyanins 2.52±1.25; total dihydrochalcones 4.36±0.9; total flavonols 5.86±3.0	Old variety. Epicatechin is the major monomeric polyphenol. Highest level of anthocyanins	[23]
'Morgenduft'	Fresh eating	mg/100g FW: Total hydroxycinnamates 18.89±3.2; total flavanols 99.64±5.7; total anthocyanins 3.67±2.8; total dihydrochalcones 2.47±0.5; total flavonols 5.43±1.6	Old variety. Highest level of anthocyanins	[23]
'Fuji'	Fresh eating	mg/100g FW: Total hydroxycinnamates 13.41±4.6; total flavanols 52.24±16.9; total anthocyanins 0.40±0.1; total dihydrochalcones 2.01±0.4; total flavonols 4.77±2.0	New variety. Highest concentration of hydroxycinnamates as a percentage of total polyphenols	[23]
'Rome Beauty'	Apple sauce, Baking	Peel: total phenolics 500.2±13.7 mg gallic acid equivalent/100g peel; total flavonoids 306.1±6.7 mg catechin equivalents/100g peel; total anthocyanins 2.1±0.2 cyanidin-3-glucoside equivalents/100g peel	Highest polyphenolic content among the studied cultivars	[148]
'Idared'	Apple sauce	Peel: total phenolics 588.9±83.2 mg gallic acid equivalent/100g peel; total flavonoids 303.2±41.5 mg catechin equivalents/100g peel; total anthocyanins 26.8±6.5 cyanidin-3-glucoside equivalents/100g peel	Highest anthocyanin content among studied	[148]

Table 1. (Continued)

Cultivar	Use	Polyphenol Concentration	Remarks	Reference
'Cortland'	Fresh eating, Apple sauce, Baking	Peel: total phenolics 388.5±82.4 mg gallic acid equivalent/100g peel; total flavonoids 167.4±20.2 mg catechin equivalents/100g peel; total anthocyanins 8.4±1.7 cyanidin-3-glucoside equivalents/100g peel	Lowest polyphenolic content among studied	[148]
Crab/heritage genotypes ('Dolgo')	Jelly	Total phenolics 642 mg/100g peel DW	Highest total phenolics including the procyanidin and chlorogenic acid compared to commercial and new breeding lines studied	[24]
'Rhode Island Greening'	Cider, Cooking	Whole apple: chlorogenic acid 14.28; epicatechin 19.16; phloridzin 2.08; phloretin xyloglucoside 5.88; procyanidin B2 21.68; quercetin galactoside 4.32; quercetin rhamnoside 4.08	Highest amount of total phenolics among all the cultivars tested	[48]
NY674	Cooking, Processing	Whole apple: chlorogenic acid 4.40; epicatechin 4.32; phloridzin 1.84; phloretin xyloglucoside 3.56; procyanidin B2 5.04; quercetin galactoside 1.92; quercetin rhamnoside 2.40	New variety. Rhamnoside was the most abundant flavonol glycoside	[48]
'Jonagold'	Fresh eating	Whole apple (mg/kg FW): total quercetin glycosides 98±16; total catechin 197±17; phloridzin 28±13; chlorogenic acid 201±15	Highest amount of flavonoids among studied cultivars. Quercetin glycoside profiles similar to that of Golden Delicious	[54]

*Values mentioned are from Vrhovsek et al. [23]. FW, fresh weight basis; DW, dry weight basis.

Table 2. Antioxidant and antiproliferative properties of apple extracts observed in various cancer cell lines

Source	Extraction solvent and concentration	Type of cancer cell line	EC50 (µg/L) (e) or dosage (d)	Key findings	Reference
Industrial apple waste	80% Methanol + 0.18 N HCl (extractable polyphenols)	Human HeLa, HepG2, and HT-29 cancer cells	1 mg/mL (d)	Significant antiproliferation efficacy	[145]
Cider Apple Juice	Ethanol	Caco-2 colon carcinoma cells	1-100 µg/mL (d)	Diminished DNA damage and ROS level	[57]
‘Royal Gala’ organic apples peel	ddH2O	Prostate (CWR:22Rv1,DU145); Breast (Mcf-7,Mcf-7:Her18, NCI-ADR)	0.5–2.5% (d)	Strong antiproliferative effects against cancer cells	[146]
Fermented apple juice extracts	6 and 24 h fermentation (with human fecal slurry)	Caco-2	1 mg/mL (d)	Decreased the ROS	[186]
‘Red Delicious’	80% Acetone phenolic extract	MCF-7 cell line and MDA-MB-231 cell line	15, 30, and 50 mg/mL apple extracts (d)	Inhibited cell in a dose-dependent manner	[147]
‘Bramley’ pomace after juice extraction	Ethyl acetate	HT29, HT115 CaCo-2	0.01% to 0.1% (d)	Protect against DNA damage, improve barrier function and inhibit invasion in dose dependent manner	[187]
‘Rome□Beauty’	Chilled 80% acetone	HepG2 human liver cancer cell	26.5 mg/mL (flesh+peel) (e) 12.4 mg/mL (peel) (e)	Apple peel extract inhibit the growth of HepG2 cells more than the whole apple extract	[148]
‘Red Delicious’	80% Acetone	Caco-2 and HepG2	50 mg/mL (d)	Strong inhibition of tumor-cell proliferation	[149]

ROS, reactive oxygen species; EC50, half maximal effective concentration.

Table 3. Anti-cancer effect of apple extracts in animal studies

Source	Preparation	Type of animals	Dosage	Key findings	Reference
Edible part of 'Red Delicious' cultivar	80% acetone	Female Sprague Dawley rats	Apple extracts at 3.3 (low), 10.0 (medium) and 20.0 g (high) of fresh apples/kg of body weight equivalent to human consumption of 1, 3, and 6 apples per day	Suppress mammary carcinogenesis, proliferative activity and induce apoptosis in mammary tumors	[153]
Modified apple polysaccharide	Mixed in diet	Male ICR mice	2.5, 5, and 10% diet	Anti-tumor attributed to its ability to induce apoptosis	[188]
Raw whole apple 'Shampion' and 'Poland'	Along with diet	DMH-treated male Fisher 344 rats	5 or 10 g apple/animal daily	Lowered the number of colon ACFs	[150]
Apple juice	Apple juice for 10 days	Male Sprague–Dawley rats	Adlibitum	Induction of antioxidant gene expression mainly by cloudy apple juice	[152]
Apple oligogalactan (AOG) from apple pectin	Basal diets mixed with AOG	Institute of Cancer Research mice	2.5%, 5% or 10% AOG in diet	AOG against inflammation and carcinogenesis from colitis	[189]
Apple polyphenol extract (APE)	Methanol containing 1% butylated hydroxytoluol (BHT)	Apc ^{Min/+} mice	60 µmol/L catechin/ animal	Reduction of the number and size of adenomas and polyps; prevention of DNA hypomethylation	[182]
Clear, cloudy apple juices, total polyphenolic fraction from mixed apple varieties	96% ethanol	Male Fischer 344 rats	Adlibitum	Cloudy juice had a higher cancer-preventive potential than the fractions	[155]; [156]
'Gala' apple extract	Distilled water	AP-1-luciferase reporter transgenic mice	Adlibitum	Blocks ROS-mediated AP-1-MAPK activation.	[157]

ROS, reactive oxygen species; ACF, aberrant crypt foci; DMH, dimethylhydrazine; AP-1, activator protein-1; MAPK, mitogen-activated protein kinases; Apc, Adenomatous polyposis coli.

Table 4. Anti-proliferative effect of various polyphenol constituents of apple

Polyphenolic Sub-class	Polyphenol compound	Type of cell line or animal model	EC50 (μ M)(e) or dosage (d)	Key findings	Reference
Flavonol	Quercetin	Caco-2 cells	9 μ M (e)	Most potent inhibitor of Cyp1A activity, a carcinogen activator	[166]
	Quercetin glucoside (Q3G)	MCF-7 cells	46.4 μ M Q3G and 10.8 μ M (combined with apple juice) (e)	Combination of apple juice and Q3G had a potent synergistic effect toward cell proliferation	[160]
	Kaempferol	Male Wistar rats	200 mg/kg (d)	Inhibited CYP1A mRNA, protein and catalytic activity	[162]
Flavan-3-ol	Catechin (apple extract)	Apc ^{Min/+} mice	8 μ mol/L equivalent (d)	Chemopreventive effect	[182]
	Epicatechin	Sheep seminal vesicles	2.2 μ g/mL (7.5 μ M) (IC50)	Anti-inflammatory potential-inhibited Cox-1 activity	[165]
Chalcones	Phloridzin	mouse mammary glands in culture	18.1 μ M (4.9 μ g/mL) (IC50)	Anti-inflammatory potential; inhibited Cox-1 activity	[165]
	Phloritin	HT-29 cells Rat mammary adenocarcinoma and Fischer bladder cell carcinoma cell lines	0-100 μ mol/L (d)	Restrain the tumor cell growth - inducing apoptosis, inhibition of glucose transmembrane	[179];[180]
Anthocyanins	Cyanidin	Human colon cancer cells HT29 and estrogen receptor-positive breast cancer cells MCF-7	0.025% to 0.5% (d)	Decreased cell proliferation	[181]
Condensed tannins	Proanthocyanidins B1 and Proanthocyanidins B2	Human vulva carcinoma cell line A431 and human colon carcinoma cell line HT29	235 \pm 68 μ M and 191 \pm 39 μ M, respectively (IC50)	Proanthocyanidins B1 possess growth / EGFR-inhibitory properties	[171]

EC50, half maximal effective concentration for cell line studies; Cyp1A, Cytochrome P450 1A; Q3G, Quercetin-3-O-glucoside; Cox-1, cyclooxygenase-1; EGFR, Epidermal Growth Factor Receptor; IC50, half maximal inhibitory concentration.

In a similar study, six healthy, non-smoking male volunteers consumed a homogenate obtained from 600 g unpeeled apples. Results indicated a significant inhibition of H₂O₂-induced micronuclei frequency in lymphocytes collected at 3 hours after apple consumption, compared with samples at 0 hour [140]. Mayer and others [141] used a high-throughput fluorescence screening method to measure antioxidative capacity in human serum where 47 healthy human volunteers consumed 1 kg of apples daily for four days, providing 2.7 g total phenolics/kg fresh apples. Apple consumption increased the antioxidant capacity in the aqueous phase, but not in the lipophilic phase of the serum 3 hours after the first apple consumption. The effect was only transient and did not increase with longer apple intake for four days [141]. Consumption of a blueberry/apple juice mixture (1 L daily, providing an extra 18 mg quercetin) for four weeks, significantly increased the plasma quercetin from 1.5 ng/mL plasma to 3.1 ng/mL plasma at the end of the study and thereby significantly increased the total antioxidant capacity of plasma of eight female volunteers [142]. In a follow up study with 168 subjects, who consumed a blueberry/apple juice mixture (1 L daily, providing an extra 97 mg quercetin) for four weeks showed increased plasma concentrations of quercetin and ascorbic acid, and thereby antioxidant capacity [143].

Apple Extracts and Cancer Prevention

In Vitro Studies Using Cell Lines

Several investigations used cultured colonic cells, both healthy and cancer-derived cell lines to examine the *in vitro* effects of apple extracts and polyphenols on cancer-related processes (Table 2). Oxidative stress, known to play a role in the pathogenesis of cancer, has been the focus of many new studies to determine the effectiveness of apple products in enhancing antioxidant capacity in the biological system. Cetkovic et al. [144] demonstrated strong free radical scavenging and antiproliferative activities of six different apple pomace extracts in cervix epithelioid carcinoma (HeLa) and colon adenocarcinoma (HT-29) human cancer cell lines. The potential of polyphenolic extracts prepared from apple juices (AE), apple pomace extraction juice (APE), and apple peel (AP) to protect against DNA oxidation damage was assessed in Caco-2 colon carcinoma cells [57]. Polyphenols (including oligomeric procyanidins) represented the major proportion of extract constituents (58–88%), consisting of oligomeric procyanidins and low molecular phenolics (flavan-3-ols, dihydrochalcones, hydroxycinnamic acids, and quercetin glycosides). All extracts exhibited distinct antioxidant capacities at 1 mg/mL extract concentrations. Quercetin-rich APE most effectively diminished DNA damage and reactive oxygen species (ROS) level after 24 h incubation (AE > APE), whereas the AEs were only moderately effective [57]. Similar to extractable polyphenols, non extractable polyphenols (Proanthocyanidins and prodelphinidins) demonstrated to have potent antiproliferation effect on human HeLa, HepG2, and HT-29 cancer cells at 1 mg/mL concentration [145]. Extract from organic ‘Royal Gala’ apple peel possessed strong antiproliferative effects in prostate and breast cancer cell lines [146]. The antiproliferative activities of ‘Red Delicious’ apple extracts on human breast cancer cells (MDAMB-231 and MCF-7) were demonstrated to be due to the modulation effects on cell cycle machinery, resulting from the induced G1 arrest with decreased expression of cyclin D1, Cdk4, and pRb proteins [147]. Wolfe et al. [148] demonstrated that apple peel extracts alone inhibited HepG2 cell proliferation significantly more than whole apples. For example, peel extracts of ‘Idared’ apples had an EC₅₀ of 13.6 mg/mL whereas the

whole apple had an EC50 of 125.1 mg/mL [148]. When Caco-2 colon cancer cells were treated with apple extracts, cell proliferation was inhibited in a dose-dependent manner reaching a maximum inhibition of 43% at a dose of 50 mg/mL. The same trend was seen in HepG2 liver cancer cells with maximal inhibition reaching 57% at a dose of 50 mg/mL [149].

Animal Studies Related to Cancer

Numerous animal studies investigating the effects of apple and its products in cancer or cancer related conditions have been reported (Table 3). Regular consumption of whole fresh apple has potential chemopreventive properties by significantly lowering the number of aberrant crypt focus and lower DNA damage in the rat colon [150]. Oxidative stress induced by free radicals causes DNA damage, which can subsequently lead to base mutation, single- and double-strand breaks, DNA cross-linking, and chromosomal breakage and rearrangement [151]. In male Sprague–Dawley rats, the clear and cloudy apple juice obtained from a mixture of different cider apple varieties, significantly increased expression of most antioxidant genes in the colon (GPX2, GSR, CAT, Nrf2) and liver (GPX1 and NQO1) [152]. Feeding rats with whole fresh apple had potential chemopreventive properties by affecting the occurrence of preneoplastic changes in the rat colon. Liu et al. [153] treated rats with a carcinogenic agent (7,12-dimethylbenzanthracene) to induce mammary tumors and then fed extracts of whole apples by gavage to the animals. Application of low, medium, and high doses of whole apple extracts, comparable to 3.3, 10, and 20 g of apples/kg of body weight, reduced the tumor incidence by 17, 39 ($p < 0.02$), and 44% ($p < 0.01$), respectively, which is comparable to human consumption of one (200 g/60 kg), three, and six apples per day. There was a concurrent and dose-dependent increase in expression of Bax, a proapoptotic protein and downward expression of Bcl-2, an antiapoptotic protein [154]. Barth et al. [155] used a well-established rat model of chemically induced colonic damage (using 1,2-dimethylhydrazine) to examine alterations associated with colon cancer and to test the effects of clear and cloudy apple juice. Both apple juice preparations, reduced DNA damage and hyperproliferation and lowered the number of large aberrant crypt foci in the distal colon [155]. It was found that cloudy apple juice fraction was more effective than an apple juice derived polyphenolic-rich extracts [156]. Oral administration of apple peel extracts decreased the number of non-malignant and malignant skin tumors in mouse induced by 12-O-tetradecanolyphorbol-13-acetate in 7,12-dimethylbenz(a)anthracene-initiated mouse skin [157]. It was the first evidence that an extract from fresh apple peel inhibited tumor promoter-induced carcinogenesis and associated cell signaling, and suggested that the chemopreventive effects of fresh apple might be through its antioxidant properties by blocking ROS-mediated activator protein-1-mitogen-activated protein kinase (AP-1-MAPK) activation [157].

Apple Polyphenols and Anticancer Properties

Cell Lines

Anti-proliferative, anti-cancer and anti-tumor properties based on experimental cell lines and animal of most of the polyphenol constituents of apples has been reported (Table 4). Quercetin and quercetin-3-*O*-D-glucopyranoside showed potent antiproliferative activities against HepG2 and MCF-7 cells, with EC50 values of 40.9±1.1 and 49.2±4.9 µM to HepG2 cells and 137.5±2.6 and 23.9±3.9 µM to MCF-7 cells, respectively [2,158]. Quercetin 3-*O*-

glucoside, a flavonol abundant in apple skin, protects SH-SY5Y cells against H₂O₂-induced oxidative stress by the up regulation of genes involved in cholesterol biosynthesis [159]. 'Red Delicious' apple extracts (in 80% ethanol) in combination with quercetin 3- β -d-glucoside had a potent synergistic effect toward MCF-7 cell proliferation [160]. NO is a free radical involved in the pathogenesis of cancer by increasing tumour vascularization and metastasis. At a concentration of 640 μ g/mL, polyphenols and flavonoids in methanolic apple extract showed protective effects against NO-induced proliferation of MCF-7 cells [161]. The initiation progress of carcinogenesis involves DNA oxidation damage that can occur with ROS leading to mispairing of DNA bases or DNA strand breaks. Colorectal cancer is due to persistent oxidative stress resulting in DNA damage and inhibition of tumor suppressor gene. Kaempferol, a flavonol in apple at the dose of 200 mg/kg lowered ROS and rejuvenated anti oxidant enzyme in rats with 1,2-dimethyl hydrazine-induced colorectal cancer [162]. The apple juice constituents, rutin, epicatechin and caffeic acid reduced oxidative DNA damage of Caco-2 (human colonic) cells, while chlorogenic acid efficiently decreased cellular ROS levels of HT29 (colon adenocarcinoma cells) and Caco-2 [163]. The aglycone quercetin and phloretin exhibited the highest preventive/antioxidant capacity in all assays.

Interestingly, prolonged exposure to polyphenolic apple juice extracts resulted in even greater antioxidant capacity for some compounds, suggesting that the stability of the compounds inversely correlated with their preventive effectiveness [163]. In a separate study, apple polyphenol extracts protected from H₂O₂-induced cytotoxicity in Caco-2 colon cancer cells, and prevented H₂O₂-mediated inhibition of gap-junctional intracellular communication that contributed to tumor promotion in B-F344 rat liver epithelial cells [164].

Inducing the activity of phase I enzymes of drug metabolism such as cytochrome P450 1A (Cyp1A) further increase the risk to produce ultimate carcinogens. Zessner et al. [165] identified the flavonoid quercetin as the most potent inhibitor of Cyp1A activity from apple juice extracts with an IC₅₀ value of 26 mM. In Caco-2 cells, apple juice extracts, quercetin, and phloretin effectively inhibited CYP1 mRNA, protein catalytic activity [166]. Among the phase II enzymes, glutathione S-transferases (GST) comprise one of the most efficient detoxifying enzyme systems in humans. Apple polyphenols induced GSTT2 (GST theta-2) expression in HT29 colon carcinoma cells by enhancing GSTT2 promoter activity [167].

Apple polyphenols induced gene expression of enzymes related to tumor suppression, cell cycle arrest, regulation of cell cycle, apoptosis signaling, stress and signal transduction and, in particular, detoxification enzymes systems [GST and UGT (Uridine 5'-diphosphoglucuronosyltransferase)] in LT97 adenoma cells [168,169].

It was also reported previously that ileostomy samples from volunteers who consumed apple juice induced GSTT2 in HT29 cells and protected the cells from genotoxic insult [168,169]. Fermentation of apple extracts by gut flora resulted in an increase of short chain fatty acids. Polyphenols except catechin and procyanidin derivatives, which has complex structures of these particular compounds, are susceptible to the action of the gut microflora enzymes and are degraded. Investigation in human colon cell Lines LT97 and HT29 showed fermented apple extracts were 3-fold less bioactive than the corresponding apple extracts, pointing to a loss of chemoprotective properties through fermentation [170].

Malignant transformation is associated with changes in cellular signaling cascades regulating cell growth and differentiation. The extracellular signal-regulated (ERK)/MAPK pathway represents one of the major intracellular signaling cascades in the control of cell proliferation. Activation of a respective cell surface receptor, such as the epidermal growth

factor receptor (EGFR), initiates an exchange of GDP versus GTP at the G-protein Ras. GTP loaded Ras recruits the serine/threonine kinase Raf-1 from the cytosol to the cell membrane, resulting in the activation of the kinase activity. Raf-1, an interface between cell surface receptors and nuclear transcription, is the entry point to the ERK/MAPK pathway. Effective inhibition of the upstream located EGFR resulted in a suppression of the subsequent MAPK cascade, leading to the inhibition of cell growth. Ethanolic polyphenol-rich extract of apple juice blend suppressed the subsequent MAPK cascade in human colon cancer cell line HT29. Apple juice constituents, the proanthocyanidins B1 and B2 as well as quercetin-3-glucoside and quercetin-3-galactoside were found to possess EGFR-inhibitory properties [171]. In another study, Kern et al. [172] showed the treatment with apple extract induced the onset of apoptosis in HT29 cells resulting from activation of caspase-3 (a cysteine-dependent aspartase that is a characteristic element of the apoptotic process), DNA fragmentation, and PARP (a common indicator of apoptosis) cleavage. Apple polyphenols were also reported to protect DNA damage and NF- κ B activation (causes inflammation) in human lung epithelial A549 cells [151].

Among the apple polyphenols, apple procyanidins specifically had a major effect on cell proliferation and induced apoptosis *in vitro*. Apple procyanidins altered intracellular signaling pathways, polyamine biosynthesis (small polycations that are essential for cell growth and differentiation) and triggered apoptosis in tumor cells [173]. The apple procyanidins increased mitochondrial membrane permeability and cytochrome *c* release from mitochondria, and activated caspase-3, -8 and -9 and TRAIL (TNF-related apoptosis-inducing ligand) within the tumor cells [174,175]. Induction of apoptosis by procyanidins isolated from apple was observed in human stomach cancer KATO III cells [176]. Apple procyanidins inhibited the growth of human metastatic colon carcinoma-derived SW620 by triggering apoptosis and alter signal transduction pathways [173]. In this study, procyanidins fraction (50 mg/mL) that contained 78% phenolic monomers inhibited SW620 cell growth (IC₅₀ = 45 mg/mL) compared to non-procyanidin fraction that contained 73% phenolic monomers and no procyanidins. In cancer therapy, the reactivation of hypermethylated tumor suppressor genes remains an attractive strategy. Polyphenols extracted from ‘Annurca’ apples, containing chlorogenic acid, catechin, and epicatechin as major components, were active in regulating apoptosis and cell viability in RKO and SW480 cells [177]. This apple polyphenol extracts lead to the reactivation of silenced tumor suppression genes (potent demethylating activity) by inhibiting DNA methyltransferase (DNMT) protein expression. The lack of toxicity in ‘Annurca’ apple extracts makes them excellent candidates for the chemoprevention [177].

Prostaglandins are endogenous mediators of inflammation and are formed from arachidonic acid by cyclooxygenase-1 (Cox-1) and the inducible form Cox-2, which is often elevated in tumor tissue. Excessive production of prostaglandins is a causative factor of cellular injury and may ultimately lead to carcinogenesis by inhibition of apoptosis (programmed cell death) as well as stimulation of cell proliferation, formation of new blood vessels (angiogenesis) and tumor invasiveness [178].

In a study to determine which apple constituents contribute to potential chemopreventive activities, (-)-epicatechin and phloridin showed the highest inhibition of Cox-1 activity which dose-dependently inhibited Cox-1 activity by 50% at a concentration of 2.2 μ g/mL (7.5 μ M) and 18.1 μ M (4.9 μ g/mL), respectively. Oligomeric procyanidins may also contribute to the anti-inflammatory potential [165].

Phloretin (0–100 $\mu\text{mol/L}$) inhibited HT-29 cell growth by inducing apoptosis, which could be mediated through changes in mitochondrial membrane permeability and activation of the caspase pathways [179]. Phloridzin and its aglycone, phloretin were proved to block glucose transport via the inhibition of glucose transmembrane transport in whole viable tumor cells lines: rat mammary adenocarcinoma and Fischer bladder cell carcinoma cell lines which restrain the tumor cell growth [180].

Anthocyanins are essentially located in apple peel and represent less than 1% of total polyphenols. In human colon cancer cells rat mammary adenocarcinoma and Fischer bladder cell carcinoma cell lines, the anthocyanin fraction of apple extract decreased cell proliferation but not in estrogen receptor-positive breast cancer cells MCF-7 cells [181]. Chlorogenic acid has very high alkyl peroxy radical ($\text{ROO}\bullet$) scavenging activity, which may also contribute to the protective effect of apples against cancer [1].

Animal Studies

An apple polyphenol extract (6.1 mmol/L catechin equivalents) was diluted 1:100 and treated in Apc(Min/+) mice (a model of familial adenomatous polyposis, FAP) with a daily dose of 8 $\mu\text{mol/L}$ catechin equivalent /kg body weight and found as an effective chemopreventive agent. FAP is characterized by the appearance of hundreds or thousands of adenomatous polyps in the colon. Investigators suggested the presence of monomeric polyphenols (catechin, epicatechin, chlorogenic acid, caffeine acid and rutin) and polymeric polyphenols (procyanidins) promoted the reduction of the polyp number in CRC Apc(Min/+) mice. DNA hypomethylation can cause genomic instability predisposing to DNA strand breakage and recombination. Chronic inflammation can increase the level of global hypomethylation, thus increase the susceptibility to cancer. An apple polyphenol extract had antiinflammatory properties, antioxidant activity and ability to reduce lipid peroxidation that represent as a plausible chemopreventive agent at high risk for colorectal neoplasia [182]. Another apple polyphenol extract inhibited proliferation and metastasis and significantly suppressed the serum lipid peroxide level [183].

In an *in vivo* study, colon carcinogenesis was induced in Wistar rats by azoxymethane injection for a week and treated with a fraction of apple procyanidin (0.01%) dissolved in drinking water. After 6 weeks of treatment, the colon of rats receiving procyanidins showed a significant reduction of the number of preneoplastic lesions when compared with control animals receiving water. The total number of hyperproliferative crypts and of aberrant crypt foci was reduced by 50% in rats receiving 0.01% apple procyanidins in their drinking water. The results showed that apple procyanidins alter intracellular signaling pathways, polyamine biosynthesis and trigger apoptosis in tumor cells. These compounds antagonized cancer promotion *in vivo*.

Catechin, at 0.5–4 mmol/kg diet, had no adverse health effects, and delayed tumor onset in a linear dose-dependent manner in a transgenic animal model of neurofibromatosis [184]. Chlorogenic acid inhibited 8-dehydroxy-deoxyguanosine formation in cellular DNA in a rat model following treatment with 4-nitroquinoline-1-oxide [185]. Animal and *in vitro* studies have demonstrated that apple polyphenols have high antioxidant activity, ability to inhibit cancer cell proliferation, decrease lipid oxidation, and potentially explain their role in reducing risk of various types of cancer.

CONCLUSION

The potential application of apple-derived polyphenolics as natural health products to prevent the pathology of certain chronic diseases of humans is well documented. Apple-based crude extracts and constituent polyphenolics have mainly been evaluated using *in vitro* research models, animal and human cell cultures and experimental animals. However, further research on the efficacy and safety as well as systematically designed human clinical trials are required before introducing apple polyphenolics-based natural health products for prevention of CVD, type II diabetes and various cancers.

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