Both the small and large intestine contain absorption sites for phenolic acids and several phenolic acids affect the expression and activity of enzymes involved in the production of inflammatory mediators of pathways thought to be important in the development of gut disorders including colon cancer (Russell & Duthie, 2011).

Phenolic and polyphenolic compounds constitute an important class of secondary plant metabolites that act as free radical scavengers and inhibitors of LDL cholesterol oxidation and DNA breakage. Thus, the role of food phenolics and polyphenolics in the prevention of cardiovascular disease and certain types of cancer is well recognised (Shahidi, 2004).

Proanthocyanidins have regulatory effects on signalling pathways, neuro-protection, and control of energy metabolism (Lee et al 2006).

Polyphenols exert their beneficial effects by influencing bacterial growth in the large intestine microbiota (Laparra & Sanz, 2010). There is increasing evidence that alterations in inflammatory pathways are a key step in the development of gut disorders including colon cancer (Terzić et al, 2010).

Consequently, one obvious molecular target for phenolic acids in maintaining gut health is cyclooxygenase 2 (COX-2) as this enzyme is strongly and rapidly induced in response to mediators of inflammation, growth factors, cytokines and endotoxins (Russell et al, 2008).

Phenolic compounds down-regulate the inflammatory response in inflamed intestinal epithelial cells by a pathway implicating largely a post-transcriptional regulatory mechanism (Sergent et al, 2010).

**Action**
- Scavenges free radicals and inhibits LDL cholesterol oxidation and DNA breakage (Shahidi, 2004).
- Exhibits LDL Cu2+-catalysed reaction (Nardini et al, 1995).
- Reduces the risk of the development of Alzheimer’s disease (Roth et al, 2000).
- Exhibits neuro-protection in Parkinson’s (Zbarsky et al, 2005).
- Inhibits Cox-2 protein and PGE-2 expression (Harris et al, 2006).
- Reduces inflammation, heart disease, and cancer (Middleton et al, 2000).
- Exhibits beneficial effects by influencing bacterial growth in the large intestine microbiota (Laparra & Sanz, 2010).

**Research**

**Chlorogenic acid**
Chlorogenic acid, a member of a family of naturally occurring organic compounds. These are esters of polyphenolic caffeic acid and cyclitol (-)-quinic acid.[1] It is an important biosynthetic intermediate.[2] It also is one of the phenols found in coffee, bamboo Phyllostachys edulis[3], as well as many other plants.[4] This compound, long known as an antioxidant, also slows the release of glucose into the bloodstream after a meal.

**Gut Microbiota & Phenolic Acids**
Many of the diverse species of bacteria that constitute the gut microbiome can perform reactions that transform complex plant phenolics such as anthocyanins, procyanidins, flavanones, flavonols, tannins and isoflavones into simple phenolic metabolites. Such phenolic acids may modulate the gut microbial population. Gallic acid and caffeic acid are reported to repress Clostridium and Bacteroides species (Lee et al, 2006).

Such phenolic–microbiota interactions are likely to influence the degradation and transformation pathways of more complex phenolic compounds. Despite such complexity, it is reasonable to assume that the colon is a rich source of potentially active phenolics, concentrations reaching the mm range for some molecules (Jenner et al, 2005). These may impact locally as well as systemically on gut health. The health benefits from phenolic consumption should be attributed to their bioactive metabolites and also to the modulation of the intestinal bacterial population (Selma et al 2009).

**Daidzein**
It has been established that intestinal bacterial metabolism of dietary compounds including flavonoids and isoflavonoids can alter their biological activities (Puupponen-Pimiä et al, 2004), which, in turn, could alter their potential to influence host health.

In vitro studies have shown that flavonoids, including equol and O-DMA, can inhibit enzymes involved in steroid hormone metabolism, such as aromatase, 5α-reductase, and 17β-hydroxysteroid dehydrogenase (Adlercreutz et al, 1993; Evans et al, 1995; Pelissero et al, 1996); therefore, some of the observed associations between equol/O-DMA and hormone levels and hormone-related factors might be due to their effect on the expression of enzymes involved in hormone metabolism.

It is possible that the daidzein-metabolizing bacteria could be involved in hormone metabolism. Studies of oral microflora in women in relation to the onset of puberty and during pregnancy suggest that changes in...
sex hormones during these times may result in alterations of the oral microbial environment (Jensen et al, 1994; Nakagawa et al, 1994; Muramatsu et al, 1994). This suggests that endogenous hormone levels could perhaps influence the intestinal bacteria. Among the women, equol excreters consumed a significantly higher percentage of energy as carbohydrate and greater amounts of plant protein and dietary fibre, both as soluble and insoluble fibre compared to non-excreters. Among women, dietary fibre or other components of a high-fibre diet may promote the growth and/or the activity of bacterial populations responsible for equal production in the colon (Lampe et al, 1998).

**Ellagic Acid**

Ellagic Acid is metabolised by the microflora to yield urolithins A and B, which reduce DMH-induced large intestine tumours (Arroyo, 2008; Larrosa et al. 2006). The urolithins (Uro), inhibited PGE2 production. The mechanism of action implicated seems to be via the inhibition of activation of NF-kappaB and MAPK, down-regulation of COX-2 and mPGES-1 expressions (González-Sarriá et al, 2010). Ellagic acid, a polyphenolic compound widely distributed in fruits and nuts, protects against oxLDL-induced endothelial dysfunction by modulating the LOX-1-mediated signalling pathway (Lee et al, 2010).

**Procedures of the Nutrition Society**

A meeting of the Nutrition Society hosted by the Irish Section, University of Ulster, Coleraine.16–18 June 2010,

**Symposium on ‘Nutrition: getting the balance right in 2010’**

Session 3: Influences of food constituents on gut health

Plant secondary metabolites and gut health: the case for phenolic acids

Wendy Russella1 and Garry Duthiea1

Plant-based diets contain a plethora of secondary metabolites that may impact on health and disease prevention. Much attention has been focused on the potential bioactivity and nutritional relevance of several classes of phytochemicals such as flavonoids, carotenoids, phyto-oestrogens and glucosinolates. Less attention has been paid to simple phenolic acids that are widely found in fruit, vegetables, herbs, spices and beverages. Daily intakes may exceed 100mg. In addition, bacteria in the gut can perform reactions that transform more complex plant phenolics such as anthocyanins, procyanidins, flavanones, flavonols, tannins and isoflavones into simple phenolic metabolites. The colon is thus a rich source of potentially active phenolic acids that may impact both locally and systemically on gut health. Both the small and large intestine (colon) contain absorption sites for phenolic acids but low post-prandial concentrations in plasma indicate minimal absorption early in the gastrointestinal tract and/or rapid hepatic metabolism and excretion. Therefore, any bioactivity that contributes to gut health may predominantly occur in the colon. Several phenolic acids affect the expression and activity of enzymes involved in the production of inflammatory mediators of pathways thought to be important in the development of gut disorders including colon cancer. However, at present, we remain largely ignorant as to which of these compounds are beneficial to gut health. Until we can elucidate which pro-inflammatory and potentially carcinogetic changes in gene expression can be moderated by simple phenolic acids, it is not possible to recommend specific plant-based foods rich in particular phenolics to optimise gut health.

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Associations between human dietary patterns and risk of chronic disease are well documented. Numerous epidemiological and laboratory studies have suggested protective effects of a variety of nutritionally essential plant-based dietary components, such as fibre, antioxidant vitamins and trace elements. However, plants also contain more than 100,000 secondary metabolites ranging from structurally simple alkaloids to more complex phytoestrogens and polyphenolic molecules(1). These ‘phytochemicals’ are not recognised as essential dietary components as a lack is not associated with a specific deficiency condition. Nevertheless, many of these non-nutritive compounds exert biological activities in mammalian systems that may impact on health and disease risk. Indeed, some have been used therapeutically since ancient times and their molecular structures are the basis of many modern pharmaceuticals. As such, bioactive compounds are likely to be present in a wide range of plant-based foods and there is growing interest in their potential role in health and disease prevention in nutritionally relevant amounts. Several recent reviews discuss the potential importance to health of dietary phytochemicals (phyto-oestrogens, phytoflavonoids, etc.). To date, less attention has been focused on phenolic acids. These simple molecules are not only widespread in plant foods but are also the synthetic precursors and catabolic products of many more complex phytochemicals. There is growing interest in the possibility that some phenolic acids, such as salicylic acid, may prevent the development of gut disorders such as colon cancer. In this context, we consider whether phenolic acids of plant origin may benefit gut health.

What are plant secondary metabolites?

All living cells possess similar pathways for the synthesis of components such as sugars, amino acids, bases, carbohydrates, proteins, nucleotides and these are essential for primary metabolism. Plant secondary metabolites are derived from the products of primary metabolism but have a much more limited taxonomic distribution. Some are produced for appreciable reasons (e.g. defence, colourants and attractants), but for many their functions and benefits to the plant are essentially unknown. They can be broadly categorised according to their structure and biosynthetic pathways (Eq. 1). However, it should be appreciated that many secondary metabolites are derived by combining elements from all these biosynthetic routes.

Fig. 1. Main biosynthetic routes in plants from which plant secondary metabolites are derived. Many secondary metabolites are derived by combining elements from all these biosynthetic routes. hv, energy from sunlight; TCA, tricarboxylic acid cycle.

**Nitrogen- and sulphur-containing compounds**

Alkaloids contain one or more amino acid-derived nitrogen atoms and are structurally the most diverse class of secondary metabolites. Their production is commonly associated with allelopathic effects in host plant defence and signalling. They have a wide range of pharmacological activities and a historical use as stimulants, medicines and toxins. For example, hemlock (Conium maculatum) was a mainstay of the poisoners’ repertoire as it contains coniine, an alkaloid that paralyses motor nerve neurones(2).

Many drugs in modern use contain alkaloids or their synthetic analogues. Examples are atropine, codeine, heroin,
morpine, cocaine and vinblastine. From a dietary perspective, alkaloids such as capsaicin are responsible for the fiery taste of chilli peppers and the purine alkaloid, caffeine, causes the stimulatory effects associated with drinking a cup of coffee(2).

Glucosinolates contain both S and N. Their function in plants is unclear, but a likely role is to discourage herbivory, as they produce enzymically hydrolysed toxic metabolites in damaged plants. These products also have the intermix of plants (e.g. mustard and horseradish). Broccoli and Brussels sprouts are rich dietary sources, but are not to everyone’s taste. Glucosinolates have limited biological activity but the glucose moiety is removed by myrosinase released from cell membranes by chewing or processing. The resulting aglycone can form compounds such as isothiocyanates and indoles. These have been extensively studied in relation to protection against carcinogenesis and immunostimulation(8–10).

Secondary metabolites of the acetate pathway
The acetate pathway produces a wide range of natural products including the fatty acids and polyketides. The formation of the polyketides from acetate results from carbonyl-CoA to malonyl-CoA and a series of Claisen condensation reactions. Plants contain predominantly unsaturated fatty acids with the dietary supplement γ-linolenic acid being derived from Borage (Borago officinalis) and Evening Primrose (Oenothera biennis). The growing poly-β-keto chain can be stabilised by cyclisation and further reaction. The resulting bioactive polyketides have a range of applications. These include use as stimulant laxatives, antibiotics and antifungals(11).

Secondary metabolites of the mevalonate pathway
Terpenoids, often referred to as terpenes or isoprenoids, are a structurally diverse group of hydrocarbons derived from the five-carbon precursors: isopentyl diphosphate. Over 25 000 terpenoids have been identified(6) and they are classified according to the degree of isoprene incorporation, i.e. hemiterpenes (C5), monoterpenes (C10), sesquiterpenes (C15), diterpenes (C20), sesterpenes (C25), triterpenes (C30) through to higher polymers such as rubber (>100). In addition to being biosynthesised as part of plants’ host defence, flowers emit terpenes to attract pollinating insects(12). Bioactive molecules derived from terpenoids include the herbal tranquilliser, valtrate, the principle component of valerian (Valeriana officinalis) and the anti-cancer drug, taxol, extracted originally from the Pacific Yew (Taxus brevifolia). The tetraterpenes (C5)8, collectively known as the carotenoids, play a major role in photosynthesis. They are highly coloured pigments. For example, lycopene imparts the characteristic red colour to tomatoes and β-carotene the orange colour in carrots. Higher modified terpenoids include the phytosterols, steroidal saponins such as diosgenin in Fenugreek (Trigonella foenum-graecum) and the cardioactive glycoside, digitoxin, from Foxglove (Digitalis purpurea). Additional major food sources include citrus fruits, apricots, soya, grapes, grains, spinach, kale and sweet potatoes. Numerous effects of terpenoids in mammalian cells have been reported including anti-tumour and antioxidative activity although whether such affects are achievable at nutritionally relevant intakes is not always clear. Several reviews consider the possible relevance of terpenoids to health and disease prevention(13–15).

Secondary metabolites derived from the shikimate pathway
Approximately 20% of carbon fixed by plants is channeled into the shikimate pathway. The plant metabolites produced by this primary pathway are an essential component of our diet. The shikimate pathway has been described as ‘a metabolic tree with many branches’(16) although chloroisomerase formation is generally considered as the major branch point. Beyond chloroisomerase the pathway provides a synthetic route to folate coenzymes, enterobactins, siderophores, lipid-soluble isoprenoid quinones and the three essential aromatic L-aminos, tryptophan, tyrosine and phenylalanine.

As well as performing a significant role in the provision of primary metabolites, the shikimate pathway has a major function in higher plants in the production of secondary metabolites(16). The switch from primary to secondary metabolism occurs with the E2-elimination of ammonia from the amino acid, L-phenylalanine, to form cinnamic acid, the first metabolite of the phenylpropanoid pathway. The C6C3 structure of cinnamic acid is an essential building block for the largest range of natural products suggested as potentially beneficial for human health. Such compounds are characterised by having at least one aromatic ring with one or more hydroxy groups and range from complex structures with high molecular weight such as the plant polymer lignin to simple compounds. Such compounds include acting as antioxidants and signalling molecules, providing skeletal structure, aiding pollination and protecting against microbial infection, herbivorous grazing and excessive UV light(17).

Phenolic acids
Broadly speaking, simple phenolic acids in plants are derived from an ortho oxygenation and subsequent methylation substitution of cinnamic acid. This gives rise to the most common parent hydroxyxycinnamic acids, namely: p-coumaric acid, caffeic acid, ferulic acid and sinapic acid (Fig. 2). These are often considered as intermediates of lignin biosynthesis(18). However, they are also the important building blocks of many other natural products and are often found as specific esters and dehydrodimers (e.g. chlorogenic acid, truxillic and truxinic acid)(19,20).

Fig. 2.
Parent structures of the predominant phenolic acids found in plants: (a) Hydroxybenzoic acid and (b) hydroxycinnamic acid. Substitution of the aromatic ring provides the common hydroxybenzoic acids; salicylic acid, protocatechuic acid, gallic acid, syringic acid, vanillic acid and gentisic acid. Examples of hydroxyxycinnamic acids are p-coumaric acid, ferulic acid and sinapic acid. They are found in plants in their free form, as dimers and conjugated to other plant components such as simple sugars, organic acids and plant polymers. The C6C1-substituted hydroxybenzoic acids can be formed directly from intermediates early in the shikimate pathway. However, in plants they are more frequently formed by degradation of C6C3 cinnamic acid derivatives. Commonly found examples are 4-hydroxybenzoic acid, protocatechuic acid, vanillic acid and syringic acid (Fig. 2). Less abundant are hydroxyphenylacetic acids (the C6C2 derivatives). Generally, they are observed to have an aromatic ring substituted as observed for the hydroxybenzoic acids, except that the direct route to their biosynthesis is unclear. Phenolic acids can be found in plants not only in their free form but also conjugated (predominantly by esterification) to a variety of molecules including simple sugars, organic acids and plant polymers.

Dietary sources of phenolic acids
Food composition databases for macro- and micronutrients provide essential information for research on the health effects of nutrients, nutritional surveillance, clinical dietetic practice and food formulation and processing. However, analogous compositional information on potentially bioactive phytochemicals, including
phenolic acids, in foods is generally lacking. Several databases of some plant secondary metabolites in commonly consumed foods are under construction(21–23), but generally there is a marked disparity in the literature even for similar food items. This can be ascribed, in part, to differences in the analytical methodology employed between studies. Many employ redox colorimetric assays that show little specificity(24). Others measure only those compounds that are easily extracted into solvents. This is likely to result in a maldistribution content as many, in particular the hydroxycinnamic acids, are esterified to insoluble plant fibres. In addition, the phytochemical content of primary food products is also influenced by numerous other factors including plant varieties, seasonality, growing conditions, storage and cooking(25–27). Thus, estimating dietary intakes of phenolic acids becomes distinctly problematical and highlights the importance of regular quantitative analysis of food products used during human dietary interventions. With these caveats, copious literature of which a selection is cited(28–40) indicates that phenolic acids are ubiquitously distributed throughout plant primary products. Rich sources of hydroxycinnamic acids such as ferulic, sinapic and caffeic acids include legumes, cocoa, fruits, oils, herbs, spices, nuts, vegetables and cereals. In addition, beverages such as coffee, beer and wine are important dietary sources. Some foods are particularly rich in particular hydroxycinnamates. For example, coffee has been reported to contain 1-13 mg caffeic acid/100 ml and green olives have 83 mg sinapic acid/100 g. The hydroxybenzoic acids generally are found in lower concentrations in plant-based foods compared with hydroxycinnamic acids. Significant dietary sources include beverages (fruit juice, tea, beer, wine and spirits), berries, herbs and spices. Up to 14 mg vanillic acid/100 g basil has been reported. Walnuts are rich in syringic acid (57-45 mg/100 g) and 100 g tea can contain up to 10-35 mg gallic acid. Soft fruits appear to be particularly rich dietary sources of both free and esterified hydroxybenzoic and hydroxycinnamic acids compared with other commonly consumed fruit(41) (Fig. 3).

Fig. 3. Comparison of cinnamic (C6C3) and benzoic (C6C1) acids in commonly consumed and locally produced Scottish soft fruits compared with supermarket-purchased imported fruit. Values are specified on a wet-weight basis in mg/100 g, which corresponds to approximately one fruit portion and are given as mean (sd) (n = 3). Adapted from Russell et al.(41).

Consequently, daily intakes of phenolic acids are likely to be in the milligram range and comparable with many essential micronutrients. For example, estimated mean intakes (mg/d) of some phenolic acids in Finnish adults are: caffeic, 417; ferulic, 129, gallic, 33; p-coumeric, 16; sinapic, 11(42). Estimated intakes in a Bavarian population of protoxocatechic acid, vanillic acid and syringic acid are 1-69, 4-17 and 4-48 mg/d, respectively(43). However, such values may be an underestimate as the composition databases used to calculate daily intakes may not fully consider conjugated forms of the phenolic acids(40).

Gut microbiota as sources of phenolic acids

Many of the diverse species of bacteria that constitute the gut microbiome can perform reactions that transform complex plant phenolics such as anthocyanins, procyanidins, flavanones, flavonols, tannins and isoflavones into simple phenolic metabolites. Several Bacteroides, Streptococcus and Clostridium species have been observed in culture to metabolise quercetin, kaempferol, naringenin, diadzein and catechins(45–47). Phenolic acids have also been shown to be transformed by the gut microbiota to metabolites some of which retain the phenolic acid structure. For example, once ferulic acid is released from plant cell wall components by the gut microbiota it can then be further metabolised under going hydroxylation of the α,β-unsaturated bond, demethylation and selective dehydroxylation at C4 to form a plethora of related phenolic metabolites(48). However, the gut microbiome varies greatly between individuals and so, in vivo, it is difficult to fully ascertain the parent compounds from which phenolic metabolites are derived. In addition, such phenolic acids may modulate the gut microbial population. Gallic acid and caffeic acid are reported to repress Clostridium and Bacteroides species(49). As yet, such phenolic–microbiota interactions are not well understood but it is likely that they may influence the degradation and transformation pathways of more complex phenolic compounds. Despite such complexity, it is reasonable to assume that the colon is a rich source of potentially active phenolic acids, concentrations reaching the mm range for some molecules(50). These may impact locally as well as systemically on gut health.

Potential biological activity of phenolic acids

There is increasing evidence that alterations in inflammatory pathways are a key step in the development of gut disorders including colon cancer(51). Consequently, one obvious molecular target for phenolic acids in maintaining gut health is cyclo-oxygenase 2 (COX-2) as this enzyme is strongly and rapidly induced in inflammatory in this model system, possibly by affecting those signalling pathways leading to the upregulation of COX-2 (Fig. 4). Studies using cell and animal models show the effects of phenolic acids on both the expression and activity of enzymes involved in the production of inflammatory mediators(53–62). For example, COX-2 expression and activity are reduced by p-coumaric acid in dextran/sodium sulphate (DSS)-induced inflammation in a rodent model(57) and caffeic acid suppresses the expression of IL-17 and ameliorates DSS-induced colitis in mice(56). Clearly, these compounds have anti-inflammatory properties in model systems but results have to be interpreted with caution in a nutritional context. In such studies doses often markedly exceed that which may be achievable from the diet. Whether analogous effects occur at dietary relevant concentrations is often unclear. Although some observational studies(63,64) suggest inverse associations between the consumption of phenolic-rich diets and inflammatory markers, in general, the impact of dietary phenolic acids on gut health in human subjects has not yet been assessed in adequately powered and controlled dietary intervention trials.
Many potentially beneficial effects of phenolic acids interpolated from in vitro studies may not be of nutritional relevance unless phenolic acids gain access in vivo to appropriate cellular sites such as the colonocytes lining the gut. Systemic and colonic bioavailability of phenolic acids is not well understood and the complexity of absorption and hepatic and microbial metabolism make the correlation of dietary intake with physiological effects distinctly problematical. The exact mechanisms for the absorption of phenolic acids are not clear, but new data involve H+ and Na+ transport systems regarding hydroxycinnamic acids and hydroxycinnamic acids (65, 66). A recent review (67) concludes that the small intestine and colon can both be absorption sites for phenolic acids, with conjugated forms mainly present in the diet generally having a low bioavailability compared with the aglycones. Limited data indicate that relative bioavailability of some hydroxycinnamic acids is chlorogenic<caffeic<ferulic<p-coumaric (67).

In general, hydroxybenzoic acids appear to be more readily absorbed than hydroxycinnamic acids. Only the benzoic acids were detected in plasma of human volunteers following consumption of strawberries rich in both types of phenolic acids, 26% being recovered in the urine within 5 h of consumption (68) (Fig. 5). These observations strongly suggest that the bulk of the cinnamic acids escape early absorption in the gastrointestinal tract. They appear to be destined for the colon and metabolised by the microbiota. Moreover post-prandial concentrations of phenolic acids in plasma are low (<1 µm) indicative of low levels of absorption and/or rapid hepatic metabolism and excretion (68). It is likely that any bioactivity that contributes to gut health may predominantly be located in the lower gastro intestinal tract.

**Fig. 5.** Concentration (ng/cm3) of phenolic acids recovered in plasma after consumption of strawberries rich in hydroxycinnamnic and hydroxybenzoic acids. Data given as mean (sd) (n 4). Adapted from Russell et al. (68).

**Salicylic acid and colon cancer prevention**

In 1763, the Reverend Edward Stone informed the Royal Society that willow bark contained substances that effectively relieved 'ague' (malarial fever) (69). The anti-inflammatory component was eventually identified as salicylic acid. In Victorian times, large doses were routinely used to treat fever, pain and inflammation but had the unfortunate side effect of causing ulceration of the stomach (70). In order to partially address this, an acetylated form was produced (aspirin) at the end of the 19th century. Since then, aspirin has remained the most commonly prescribed drug for relieving pain, inflammatory symptoms and fever (71). More recently, evidence has been accumulating with regard to the fact that regular intake of aspirin inhibits the incidence of, progression, and death due to colorectal cancer. These benefits transcend study designs, and cohort characteristics (e.g. age, gender, nationality, risk factors) (72). Aspirin is deacetylated following consumption. The acetyl group directly and irreversibly binds to the PG H2 synthases, inhibiting the production of pro-inflammatory and potentially carcinogenic eicosanoids, contributing to this activity by competitive inhibition of arachidonic acid metabolism and/or some of the other postulated mechanisms of action (72). This has led to the intriguing suggestion that the recognised effects of consuming fruit and vegetables on lowering risk of colon cancer may be due in part to salicylates in plant-based foods (73). However, it is unclear as to whether sufficient salicylic acid can be obtained from dietary sources to exert disease preventative activity. Estimates of daily intake vary widely ranging from 0.4 to 200 mg/d (74). Using a recently constructed food composition database (74) describing median salicylate values for twenty-seven different types of fruit, twenty-one vegetables, twenty-eight herbs, spices and condiments, two soups, and eleven beverages, estimated median dietary intakes of a Scottish population were 4-4 and 3.2 mg/d for males and females, respectively. Major dietary sources of salicylates were alcoholic beverages (22%), herbs and spices (17%), fruit (16%), non-alcoholic beverages including fruit juice (13%), tomato-based sauces (12%) and vegetables (9%). Intuitively, such salicylate intakes appear insufficient to exert preventative effects and indeed could be negated by the substantial proportion of salicylic acid derived from beverages containing alcohol, a recognised pro-carcinogen. However, serum and urinary salicylate concentrations of vegetarians are higher than omnivores and overlap with individuals who regularly take low-dose aspirin (75) suggesting substantial absorption of salicylates from ingested plant-based foods. Moreover, populations that incorporate substantial amounts of salicylate-rich spices in foods may have markedly higher daily intakes of salicylates. Indeed, it has been suggested that the low incidence of colorectal cancer among Indian dietary salicylates throughout life from spice consumption (76). However, plant products found to contain salicylic acid are generally found to be rich in other phenolic acids and the contribution of these compounds to the protective effect should not be overlooked.

**Future perspectives**

Our gut is exposed to a plethora of simple phenolic acids and related metabolites arising directly from the food we consume and via the microbial degradation of more complex dietary phenolic compounds. Many of these metabolites have potential activities in model systems, which could potentially contribute to gut health. However, at present we remain largely ignorant as to which of these compounds are beneficial (or indeed detrimental). Moreover, understanding of their mechanisms of action in vivo and how they interact with the gut microbiota is likely to help in the prevention of conditions such as inflammatory bowel disease and colon cancer. Future research directions are likely to expand on current metagenomic and metabolomic approaches (77–79) to elucidate which pro-inflammatory and potentially carcinogenic changes in gene expression can be moderated by simple phenolic acids. In addition, the development of good methods for monitoring total bacterial communities and their metabolic activity in response to phenolic acids is essential. At present, there is arguably insufficient information to allow the recommendation of specific plant-based foods rich in particular phenolics to optimise gut health. However, in view of the preventative effects of acetylsalicylic acid on the development of colon cancer, more studies on the nutritional efficacy of phenolic acid-rich foods appear warranted.

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**References for article at end of document.**

**Gut Bacterial Metabolism of the Soy Isoflavone Daidzein: Exploring the Relevance to Human Health**

The indigenous intestinal microflora are involved in a variety of processes within the human body, and are important for maintaining host health. As such, individual differences in the ability to
harbor certain intestinal bacteria might be associated with interindividual differences in health and/or disease susceptibility. In the last decade there has been considerable interest in phytoestrogen intakes in relation to human health. Daidzein, an isoflavone phytoestrogen found in soy, is metabolized to equol and O-desmethylangolensin (O-DMA) by intestinal bacteria. The specific bacterium/bacteria responsible for equol and O-DMA production in humans have yet to be identified definitively, but in vitro and animal studies have suggested that equol and O-DMA are more biologically active than their precursor daidzein. Interestingly, substantial interindividual differences in daidzein metabolism exist; following soy or daidzein consumption, approximately 30%–50% of the human population produce equol, and approximately 80%–90% produce O-DMA. Observational and intervention studies in humans have suggested that the ability to produce equol and O-DMA may be associated with reduced risk of certain diseases including breast and prostate cancers.

The actual number of bacteria in the human colon is unknown, but it has been estimated that there are more than 400 species (Savage, 1977; Berg, 1996; Hooper et al, 2002). In some instances, as an alternative to physically isolating and identifying the bacteria, host phenotypes that result from the metabolic functions of certain bacteria can be used to indicate their presence in the intestines. For example, breath levels of methane indicate the presence of methanogenic bacteria (Bjorneklett & Jenssen, 1982). Similarly, breath levels of labeled carbon dioxide (produced by bacterial breakdown of administered [13C]labeled urea) indicate the presence of Helicobacter pylori (Savarino et al, 1999). Thus, the use of such phenotypes can provide information on gut bacterial populations without the need for laborious methods of bacterial identification, such as culture-based methods.

Although stable communities of intestinal bacteria exist within individuals (Zoetendal et al, 1998), substantial interindividual differences have been observed (Zoetendal et al, 1998; Hayashi et al, 2002). These differences may ultimately contribute to interindividual variation in health and/or disease susceptibility. In the last decade, there has been growing interest in soy and isoflavone intakes in relation to human health (Duncan et al, 2003).

Intestinal bacteria play an essential role in daidzein metabolism. Germ-free animals and young infants with underdeveloped gut microflora do not produce equol or O-DMA (Cruz et al, 1994; Rowland et al, 1999), and faecal incubation of daidzein with human faecal bacteria results in the production of equol and O-DMA (Atkinson et al, 2004). In cynomolgus monkeys (Blair et al, 2003), treatment with certain antibiotics causes marked reductions in plasma levels of equol, and some antibiotics inhibit the in vitro production of equol and O-DMA by human faecal bacteria (Atkinson et al, 2004). Equol and O-DMA are likely produced by different bacteria, and the bacteria involved also may differ between individuals; in vitro, faecal bacteria from some equol nonproducers can convert daidzein to O-DMA (but not equol) (Atkinson et al, 2004), and observational studies show that not all equol producers are O-DMA producers, and vice versa (Frankenfeld et al, 2004 Rowland et al, 2000; Frankenfeld et al, 2004). Furthermore, some antibiotics inhibit equol/O-DMA production by fecal bacteria from some individuals, but not others (Atkinson et al, 2004).

It has been established that intestinal bacterial metabolism of dietary compounds including flavonoids and isoflavonoids can alter their biological activities (Puupponen-Pimia et al, 2004), which, in turn, could alter their potential to influence host health. The metabolism of daidzein to equol and O-DMA in humans is of particular interest given that (i) substantial interindividual variation in equol and O-DMA production exists, and (ii) equol and O-DMA may be more biologically active than their precursor daidzein. To date, relatively few studies have been specifically designed to assess daidzein-metabolizing phenotypes in relation to human health.

In vitro studies have shown that isoflavones, including equol and O-DMA, can inhibit enzymes involved in steroid hormone metabolism, such as aromatase, 5(alpha)-reductase, and 17beta-hydroxysteroid dehydrogenase (Adlercreutz et al, 1993; Evans et al, 1995; Pelissero et al, 1996); therefore, some of the observed associations between equol/O-DMA and hormone levels and hormone-related factors might be due to their effect on the expression of enzymes involved in hormone metabolism. It is possible that the daidzein-metabolizing bacteria, or other bacteria associated with their presence, also could be involved in hormone metabolism. Studies of oral microflora in women in relation to the onset of puberty and during pregnancy suggest that changes in sex hormones during these times may result in alterations of the oral microbial environment (Jensen et al, 1994; Nakagawa et al, 1994; Muramatsu et al, 1994). This suggests that endogenous hormone levels could perhaps influence the intestinal bacteria and thus the manifestation of the daidzein-metabolizing phenotypes.

References

References for: Gut Bacterial Metabolism of the Soy Isoflavone Daidzein: Exploring the Relevance to Human Health


Inhibition of DNA methylation by caffeic acid and chlorogenic acid, two common catechol-containing coffee polyphenols.


We studied the modulating effects of caffeic acid and chlorogenic acid (two common coffee polyphenols) on in vitro methylation of synthetic DNA substrates and also on the methylation status of the promoter region of a representative gene in two human cancer cell lines. Under conditions that were suitable for the in vitro enzymatic methylation of DNA and dietary catechols, we found that the presence of caffeic acid or chlorogenic acid inhibited in a concentration-dependent manner the DNA methylation catalyzed by prokaryotic M.SssI DNA methyltransferase (DNMT) and human DNMT1. The IC50 values of caffeic acid and chlorogenic acid were 3.0 and 0.75 microM, respectively, for the inhibition of M.SssI DNMT-mediated DNA methylation, and were...
2.3 and 0.9 microM, respectively; for the inhibition of human DNMT1-mediated DNA methylation. The maximal in vitro inhibition of DNA methylation was approximately 80% when the highest concentration (20 microM) of caffeic acid or chlorogenic acid was tested. Kinetic analyses showed that DNA methylation catalyzed by M.SssI DNMT or human DNMT1 followed the Michaelis-Menten curve patterns. The presence of caffeic acid or chlorogenic acid inhibited DNA methylation predominantly through a non-competitive mechanism, and this inhibition was largely due to the increased formation of S-adenosyl-L-homocysteine (SAH, a potent inhibitor of DNA methylation), resulting from the catechol-O-methyltransferase (COMT)-mediated O-methylation of these dietary catechols. Using cultured MCF-7 and MAD-MB-231 human breast cancer cells, we also demonstrated that treatment of these cells with caffeic acid or chlorogenic acid partially inhibited the methylation of the promoter region of the RARbeta gene. The findings of our present study provide a general mechanistic basis for the notion that a variety of dietary catechols can function as inhibitors of DNA methylation through increased formation of SAH during the COMT-mediated O-methylation of these dietary chemicals.

**Angiogenesis-inhibiting phytochemicals**

Medicinal herbs and their phytochemicals are potential novel leads for developing antiangiogenic drugs. This review aims to assess the current status of research with medicinal herbs and their phytochemicals for the development of antiangiogenic agents for cancer and other angiogenesis-related diseases including inflammation, diabetic retinopathy, endometriosis and obesity. Most studies reviewed have focused on vascular endothelial growth factor (VEGF)/vascular endothelial growth factor receptor 2 (VEGFR-2) signaling for endothelial response processes and have led to the identification of many potential antiangiogenic agents. Since human clinical trials with antiangiogenic modalities targeting VEGF/VEGFR-2 signaling have shown limited efficacy and occasional toxic side effects, screening strategies for herbal phytochemicals based on other signaling pathways important for cancer-endothelial and stromal crosstalks should be emphasized in the future.

<table>
<thead>
<tr>
<th>Compound classification</th>
<th>Compound</th>
<th>Scientific name</th>
<th>Crude Drugs</th>
<th>Efficacy</th>
<th>IC_{50}</th>
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<td>Flavonoid polyphenolics</td>
<td>Quercetin</td>
<td><em>Quercus</em></td>
<td>Rosae Fructus</td>
<td>100 µM</td>
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<td></td>
<td>Fisetin</td>
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<td></td>
<td>Apigenin</td>
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<td>Morin</td>
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<td></td>
<td>Epigallocatechin</td>
<td><em>Gallai</em></td>
<td><em>Thea</em></td>
<td>6.5–25 µM</td>
<td>Dona et al., 2003; Xu et al., 2006</td>
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<td></td>
<td>Isoflavone</td>
<td><em>Geronitin</em></td>
<td><em>Pueraria</em></td>
<td>10 µM</td>
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<td>Gallic acid</td>
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<td>Ellagic acid</td>
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<td>&lt;18 µM</td>
<td>Labroque et al., 2009</td>
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<td>1,3,5,7,8-pentahydroxy-</td>
<td><em>Pavonia</em></td>
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<td>4 µM</td>
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<td>Other non-flavonoid polyphenolics</td>
<td><em>Resveratrol</em></td>
<td><em>Veratrum</em></td>
<td>0.7 ± 0.1 µM</td>
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<td><em>Morus</em></td>
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<td>40 µM</td>
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<td>Curcumin</td>
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<td>Campesterol</td>
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