Biosynthesis, Natural Sources, Dietary Intake, Pharmacokinetic Properties, and Biological Activities of Hydroxycinnamic Acids

Hesham R. El-Seedi,*,[†,§] Asmaa M. A. El-Said,[§] Shaden A. M. Khalifa,[#] Ulf Göransson,[†] Lars Bohlin,[†] Anna-Karin Borg-Karlson,[⊗] and Rob Verpoorte[⊥]

[†]Division of Pharmacognosy, Department of Medicinal Chemistry, Uppsala University, Biomedical Centre, Box 574, SE-75123 Uppsala, Sweden

[§]Department of Chemistry, Faculty of Science, El-Menoufia University, 32512 Shebin El-Kom, Egypt

[#]Department of Clinical Neuroscience, Karolinska Institutet (KI), Stockholm, Sweden

[⊗]Ecologial Chemistry Group, Department of Chemistry, School of Chemical Science and Technology, Stockholm, Sweden

[⊥]Section of Metabolomics, Institute of Biology, Leiden University, Box 9502, 2300 RA Leiden, The Netherlands

ABSTRACT: Hydroxycinnamic acids are the most widely distributed phenolic acids in plants. Broadly speaking, they can be defined as compounds derived from cinnamic acid. They are present at high concentrations in many food products, including fruits, vegetables, tea, cocoa, and wine. A diet rich in hydroxycinnamic acids is thought to be associated with beneficial health effects such as a reduced risk of cardiovascular disease. The impact of hydroxycinnamic acids on health depends on their intake and pharmacokinetic properties. This review discusses their chemistry, biosynthesis, natural sources, dietary intake, and pharmacokinetic properties.

KEYWORDS: *absorption, antioxidant, biosynthesis, dietary intake, hydroxycinnamic acids, phenolic acids, metabolism, natural sources*

■ INTRODUCTION

Hydroxycinnamic acids (HCAs) are one of the major classes of phenolic compounds found in nature.[1,2] They are secondary metabolites derived from phenylalanine and tyrosine, and they all have a C_6C_3 carbon skeleton with a double bond in the side chain that may have a cis or a trans configuration. Among the most common and well-known HCAs are cinnamic acid, o-coumaric acid, m-coumaric acid, p-coumaric acid, caffeic acid, ferulic acid, and sinapic acid (Figure 1). These species may

o-Coumaric acid, R1 = OH
m-Coumaric acid, R2 = OH
p-Coumaric acid, R3 = OH
Caffeic acid, R2 = R3 = OH
Ferulic acid, R2 = OCH3; R3= OH
Cinnamic acid, R1 = R2 = R3 = R4 = H
Sinapic acid, R2 = R4= OCH3; R3 = OH
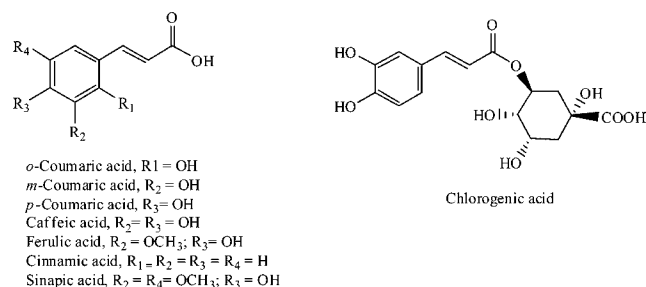
Chlorogenic acid

Figure 1. Chemical structures of common HCAs.

occur as the free carboxylic acids or as esters formed by condensation with hydroxylic acids such as quinic and tartaric acid, flavonoids, or carbohydrates. They also occur as amides, formed by the condensation of the parent acid with an amino acid or an amine. Chlorogenic acid, which is formed by the condensation of caffeic acid with quinic acid (5-O-caffeoylquinic acid), is probably the most abundant soluble hydroxycinnamic acid derivative (Figure 1); it occurs in a number of fruits and vegetables and also in coffee and tobacco.[3−7]

HCAs are widely distributed in the plant kingdom and have been found in most plant families,[8] including many species that are consumed as food or made into beverages, such as fruits, vegetables, and grains.[9−11] HCAs are also found in medicinal plants from both Western and Eastern cultures[12] and are used in both structural and chemical plant defense strategies. HCAs can occur freely or as components of plant polymers (cell wall).[13] During the past decade, HCAs received particular attention because they are the most abundant antioxidants in our diet and because of the increasing interest in the biological effects of antioxidants.[14,15] HCAs have been shown to have beneficial effects in various human diseases, particularly atherosclerosis and cancer.[10,16] Human colon cancer development is often characterized in an early stage by hyperproliferation of the epithelium, leading to the formation of adenomas.[17]

This is mainly a consequence of dysregulated cell cycle control and suppressed apoptosis. Protective effects against colon cancer development should consequently be associated with inhibition of cell proliferation and/or induction of the apoptotic pathway to delete cells carrying mutations and to maintain a normal cell population.[18]

Curly kale extracts have the ability to inhibit growth and induce apoptosis of different colon cancer cells in vitro. Further studies are required to clarify the link between results obtained in cell culture studies and the impact on human health before it can be determined that curly kale intake can affect colon cancer.[18] Very recently, research has been published providing

evidence that combinations of grape polyphenols at physiologically relevant concentrations may inhibit tumor growth.[19] Their presence in folk medicines and foodstuffs makes HCAs clinically important compounds. This review discusses their chemistry, biosynthesis, natural sources, biological activity, pharmacokinetic properties, and bioavailability and examines the HCA contents of various foodstuffs. We have compiled the data from various relevant studies focusing on well-documented sources of HCAs. The occurrence of specific HCAs in individual plant species is not discussed in detail.

Biosynthesis of HCAs. HCAs are produced by the same metabolic network that gives rise to lignins, coumarins, lignans, stilbenes, chalcones, anthocyanins, and flavonoids.[20] They are synthesized using the amino acid phenylalanine as a starting material, via the shikimate pathway. This pathway is a major biosynthetic route for both primary and secondary metabolism; it uses phosphoenolpyruvate and erythrose-4-phosphate as its starting materials and ultimately produces chorismate as illustrated in Figure 3.[21]

The shikimate pathway leads to the synthesis of aromatic amino acids such as phenylalanine and tyrosine.[22,23] HCAs are formed by the deamination of phenylalanine or tyrosine to yield the C_6C_3 unit that serves as the core structure of the phenylpropanoids; the deamination is catalyzed by the enzyme phenylalanine ammonia-lyase (PAL).

PAL has been purified and characterized from a number of plants. It is a large protein (240,000−330,000 kDa) composed of four subunits. PAL genes from several plants have been sequenced including parsley, beans, lemon, tobacco, rice, and wheat. Their organization and structure have been well established. The active site of PAL contains a dehydroalanine residue that participates in the elimination of ammonia. This residue is formed post-translationally by modification of a serine residue.[24,165] The induction of PAL expression is often observed as a component of plants' responses to invading microorganisms, indicating that phenylpropanoids may act as phytoalexins in certain plant species.[25,26] Every plant species that has been studied has been shown to contain several copies of the PAL genes, each with different expression patterns.

In plants, tyrosine ammonia-lyase (TAL) converts tyrosine into 4-hydroxycinnamic acid (also known as *p*-coumaric acid), which can subsequently be transformed into caffeic, ferulic, or sinapic acid. This pathway is responsible for the biosynthesis of a very large number of diverse secondary metabolites such as lignin[27] and lignin precursors including feruloyl CoA and *p*-coumaryl CoA.[28] These CoA derivatives are believed chemically to be the source of cell wall bound HCAs. Figures 2 and 3 summarize the various compound classes that are derived from HCAs.

These CoA derivatives serve multiple purposes. Notably, some HCAs are incorporated into the cell wall; it is believed that they are transported there as CoA conjugates. In addition, the formation of the thioester linkage between CoA and the cinnamate activates the carbonyl group of the HCA, facilitating various condensation and conjugation reactions that give rise to species such as flavonoids, which are among the most abundant plant natural products,[29,30] and stilbenoids.[23,24,31]

Natural Sources of HCAs. HCAs are ubiquitous in vascular plants; they have been found in all plants in which they have been sought. They occur in most tissues in a variety of forms. Free HCAs are rarely found[32] other than in processed foods that have undergone freezing, sterilization, or fermentation.[11] HCAs in foods typically occur as monomers, dimers, or polymers;
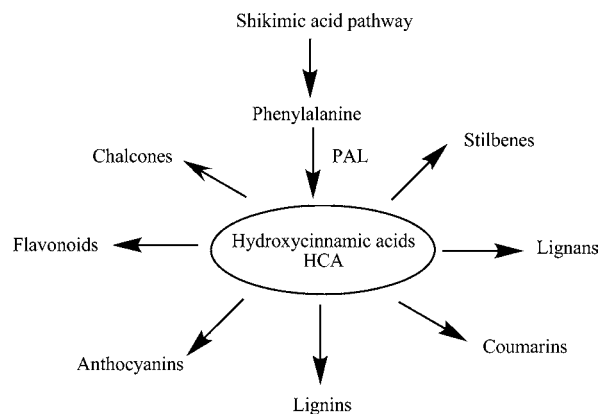

Figure 2. Scheme showing that hydroxycinnamic acids are central compounds in the polyphenol biosynthetic pathways in plants.

as esters formed by condensation with hydroxy acids, alcohols, or mono/disaccharides; or as amides formed by condensation with amines.

Additionally, HCAs are often esterified or etherified to form the polymeric waxes that coat plants' external surfaces.[32] HCAs in the cell wall are generally covalently bound to species such as cellulose or proteins via ester linkages and are thus insoluble. Conversely, the cytoplasm typically contains soluble HCAs. Soluble HCAs such as simple esters are abundant in fruits and vegetables; insoluble HCA derivatives are more common in grains.[1,2,4,5,10] HCA-derived amides (formed by the condensation of cinnamic acids with amino acids or amines) are particularly common in coffee and cocoa.[10]

Insoluble HCAs that are covalently bound to other species can be released by alkaline or acidic hydrolysis[15,33] or by enzymatic treatment prior to extraction.[34−39] These components contribute to the mechanical strength of cell walls.[40,41] HCAs also play a regulatory role in plant growth and morphogenesis and in cells' responses to stress and pathogens.[40−42]

Fruits are a major dietary source of HCAs; they are abundant in fruits of almost every kind, including apples, various berries, plums, cherries, some citrus fruits, and peaches. In general, ripe fruits are bigger than their unripe counterparts and thus contain a greater total quantity of HCAs. However, fruits' HCA concentrations decrease as they ripen; unripe fruits thus have higher HCA concentrations.[43] Caffeic acid and coumaric acid are the most abundant HCAs in most fruits, accounting for between 75 and 100% of the total HCA content.[43,44] Fruits aside, foods such as cereals, carrots, eggplants, cabbage, and artichokes are also rich sources of HCAs.[45] By far the most abundant HCAs in cereals are ferulic acid, *p*-coumaric acid, and sinapic acid, with ferulic acid being the most common (every 100 g of wheat bran contains about 300 mg of ferulic acid).[38,46,47] The aleurone layer and the pericarp of wheat grain contain 98% of the total ferulic acid present in the seeds.[11] Sinapic acid is also more abundant in cereals than in other food plants.[38,46,47] The most important sources of sinapic acid are *Brassica* vegetables. Coumaric acid is most abundant in various berries such as strawberries (110 mg/100 g);[4] peanuts are also rich in this compound.[4]

Coffee is rich in chlorogenic acid, depending on the type of roast and the amount consumed.[10] Caffeic acid is also abundant in carrots and various berries.[4] Table 1 summarizes the levels of HCAs in various foodstuffs.
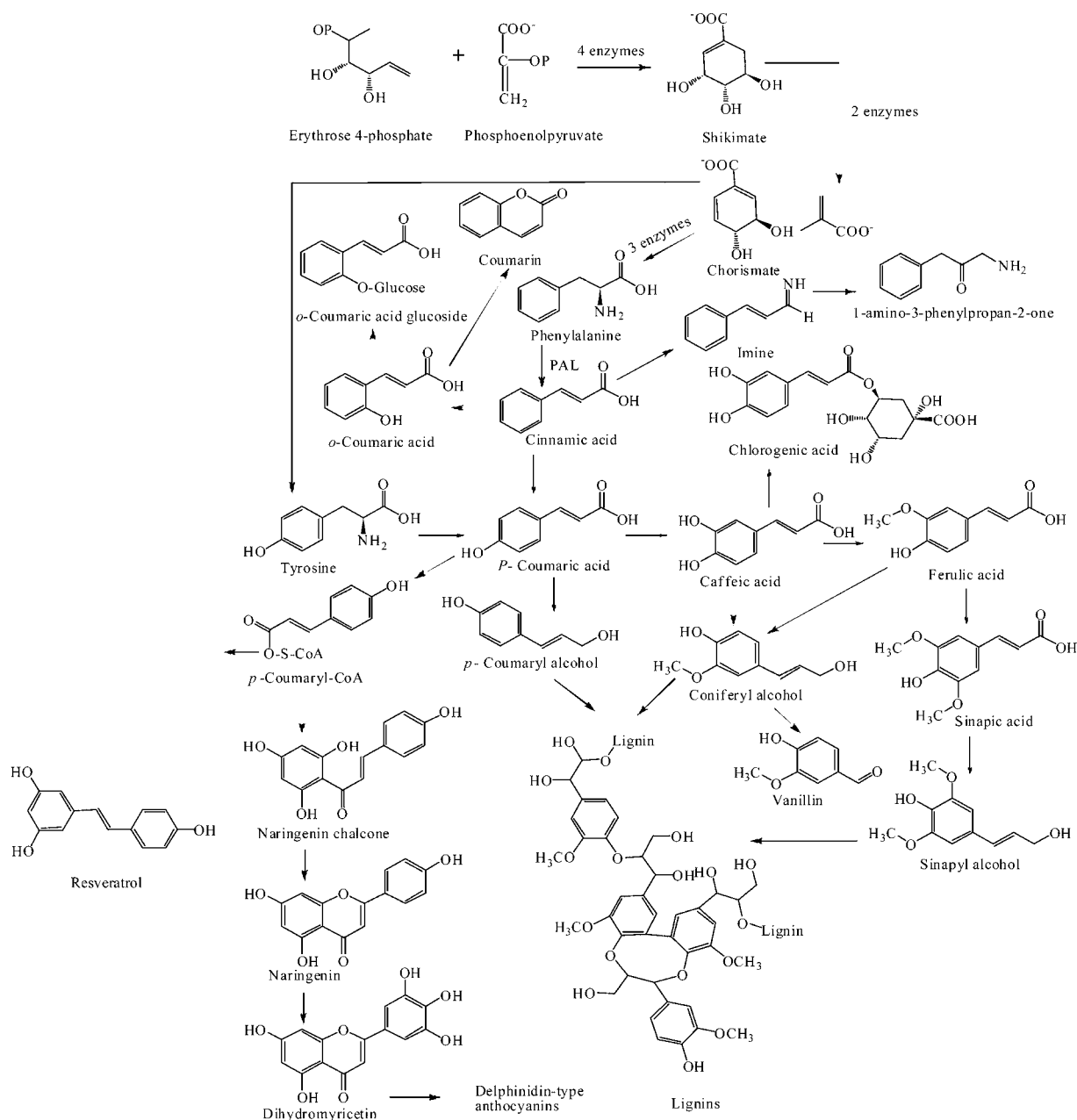
Figure 3. Biosynthesis of hydroxycinnamic acid derivatives in plants.[14,27,31]

Dietary Intake of HCAs. A growing number of epidemiological studies have provided evidence for the health benefits of a high dietary intake of HCAs.[51] Whereas the amount of HCAs consumed is likely to vary significantly from individual to individual, it has recently been estimated that the average person ingests a total of 211 mg of HCAs per day.[52] In another study the intake of caffeic acid alone was reported to be 206 mg/day.[53] The main dietary sources of HCAs are coffee (which is estimated to account for 92% of the caffeic acid consumed) and fruits and fruit juices combined (which are estimated to provide 59% of the p-coumaric acid),[53] although fruit juices are comparatively poor in HCAs, as shown in Tables 1 and 2. Other dietary sources of caffeic acid include apples, pears, berries, artichokes, and aubergines (eggplants).[10] HCAs have also been reported to occur in various wines.[54]

Ferulic acid is the most abundant HCA.[55] It is particularly abundant in cereal grains, which constitute its main dietary source. People who consume large quantities of cereal products often also consume significant levels (>100 mg/day) of ferulic acid.[14]

Tea is one of the most commonly consumed beverages throughout the world. Epidemiological studies have linked tea consumption to a reduced risk of suffering from many diseases as recently reported by Holst and Williamson.[56,57] These beneficial effects are attributed to the antioxidant properties of HCAs such as caffeic,[38] p-coumaric, and chlorogenic acid.[57]

Coffee contains high amounts of chlorogenic acid and other caffeoylquinic acid derivatives.[10] A single cup of coffee may contain 70−350 mg of chlorogenic acids; a heavy coffee drinker could ingest up to 2000 mg/day of chlorogenic acids and about 250−500 mg/day of caffeic acid,[10] whereas a non-coffee drinker might ingest <25 mg/day.[10] Because of its regular consumption in Western countries, coffee and related products are the most significant and constant sources of HCAs in the human

Table 1. Total HCA Content (Free plus Bound) of Selected Vegetables, Fruits, Grains, and Beverages

source	contents[a] (mg/100 g)				ref	source	contents[a] (mg/100 g)				ref
	caffeic acid	ferulic acid	sinapic acid	p-coumaric acid			caffeic acid	ferulic acid	sinapic acid	p-coumaric acid	
potato cooked peel	40	9.4	0.51	0.66	5	grape, green	3.4	ND	ND	1.17	48
aubergine (eggplant)	21	0.57	ND[b]	0.19	5	grape, red	3.4	0.43	ND	3.8	48
avocado	0.42	1.1	0.97	0.81	47	plum, dark	23.4	1.47	0.14	2.1	48
button mushroom	0.06	ND	ND	ND	5	cherry	17.1	0.46	ND	5.1	48
carrot	26	1.5	ND	0.69	5	pear[c]	0.28	0.01	3.48	0.58	49
parsnip	1.8	2.2	0.2	0.34	5	orange[c]	0.36	0.30	2.88	2.08	49
radish	1.0	4.6	0.12	5.6	5	mandarin	6.6	9.24	1.51	0.88	48
turnip	0.2	0.23	0.84	0.09	5	grapefruit	5.5	11.6	0.99	1.35	48
cauliflower	0.38	0.35	1.8	1.2	5	kiwi	1.5	0.19	ND	0.25	5
broccoli	1.5	4.1	8.0	0.85	5	strawberry[c]	13.09	12.17	ND	110.76	49
Chinese cabbage	0.54	1.4	5.2	0.42	5	sea buckthorn[c]	0.96	1.52	ND	7.24	50
white cabbage	0.29	0.27	2.8	0.18	5	raspberry[c]	6.39	12.103	4.28	22.46	49
red cabbage	1.6	6.3	22	9.3	5	gooseberry	35.45	6.72	ND	50.48	5
pickled red cabbage	1.2	1.5	9.2	1.6	5	Saskatoon berry[c]	208.7	5.02	ND	8.21	50
garlic	ND	0.63	0.66	0.09	5	wild blueberry[c]	147	4.13	ND	3.93	50
onion	ND	0.08	0.10	0.21	5	cranberry[c]	2.3	0.6	0.52	1.4	48
green bean, fresh	0.46	1.2	ND	1.2	5	chokecherry[c]	645.5	4.3	ND	95.28	50
soybean	0.33	12	12	12	5	black currant[c]	53.72	12.09	3.73	59.13	49
peanut	2.4	8.7	14	103	5	buckwheat grits	8.5	1.2	2.1	1.5	4
swede (rutabaga)	0.07	0.06	0.79	0.05	5	corn flour	2.6	38	5.7	3.1	4
Jerusalem artichoke	21	0.55	ND	ND	5	millet grits	0.11	26	ND	1.8	4
pea, frozen	0.13	0.26	0.15	ND	5	rye bran	7.7	280	48	14	4
spinach, frozen	ND	7.4	ND	3.1	5	whole grain barley	0.42	86	13	6.4	4
pot-grown basil	23	1.5	ND	0.60	5	oat bran	0.54	33	9	1.2	4
iceberg lettuce	4.9	0.08	ND	0.09	5	wheat bran	3.8	300	20	9	4
pot-grown lettuce	20	0.33	ND	0.22	5	whole wheat flour	3.7	89	6.3	3.7	4
pot-grown lettuce/Lollo Rosso	49	1.4	ND	0.17	5	whole grain barley	0.17	25	0.11	0.4	4
						white wheat bread	ND	8.2	0.69	0.28	4
sweet pepper, green	1.3	0.37	0.18	2.2	5	apple cider	1.16	0.076	ND	0.39	48
sweet pepper, red	1.2	0.55	0.38	2.4	5	apple juice	3.6	0.10	ND	1.20	48
sweet pepper, yellow	1.2	0.53	0.32	1.9	5	beer	0.12	0.95	0.23	0.11	48
tomato	2	0.29	ND	1.0	5	black currant juice	2.6	0.78	0.78	3.2	48
tomato ketchup	2.8	0.29	0.09	0.69	5	black tea	1.42	0.16	ND	2.0	48
pot-grown parsley	ND	0.23	ND	5.8	5	coffee	87	9.1		1.27	48
zucchini	0.11	0.28	ND	0.32	5	green tea	1.34	ND	ND	1.0	44
peach	4.9	0.11	ND	0.52	48	orange juice	0.25	4.7	0.48	1.0	48
nectarine	4.9	0.14	ND	0.39	48	pear cider	0.106	ND	ND	ND	48
apple[c]	0.17	0.05	0.63	0.20	49	red wine	3.2	ND	ND	5.0	48
banana	0.20	5.4	ND	0.46	48	pasta	ND	12	1.7	0.36	4
grape[c]	0.04	ND	0.29	1.63	49						

[a]The contents were calculated by 100 g fresh edible part of foods. [b]ND, not detected. [c]The contents were calculated by 100 g dry matter.

Table 2. Dietary Sources of HCAs

Hydroxycinnamic acids (free + bound)	Dietary sources			Ref.
	Fruits	Vegetables	Beverages	
Caffeic acid	Blueberry, grapes, pear, cranberry, apple, orange, lemon, grapefruit, peach and cherry	Potato, lettuce, brassica, broccoli, spinach and cabbage	tea, coffee, cherry, orange juice and apple juice	(10, 63, 64, 65)
Chlorogenic acid				
Ferulic acid				
Neochlorogenic acid				
P-coumaric acid				
Caftaric acid			wine	

diet. It should be noted that consumption of coffee seems to be associated with a reduced risk of various cancers such as colorectal cancer.[58,59] Natella et al.[60] observed a significant 5.5% increase ($P < 0.05$) in plasma antioxidant activity in humans following a single intake of brewed coffee, further suggesting that coffee possesses antioxidant properties.[60] Depending on their normal consumption of coffee, bran, citrus fruits, and beer, an individual may ingest 500−1000 mg of HCAs/day.[10]

Red wine contains a complex mixture of various phenolic acids (caftaric acid, caffeic acid, and p-coumaric acid).[61] These compounds occur in red wine but are virtually absent from white wine because the skins, seeds, and stems are present during the fermentation of red wine but not during that of white wine. Red wine is one of the richest sources of polyphenols in the human diet. Highly tannic red wines can contain up to 3 g of total polyphenols per liter, and moderate red wine drinkers will consume polyphenols at levels well above the population average.[62]

Pharmacokinetic Properties of HCAs. *Absorption of HCAs.* The rates at which HCAs are absorbed from the gastrointestinal tract vary widely, depending on their structure.[66] Most studies[10,52] in this area have focused on caffeic acid, ferulic acid, and the main chlorogenic acid, 3-caffeoylquinic acid, as they are the most abundant HCAs in food. When HCAs and their derivatives are ingested, they are released from the matrix during and after mastication.[67] HCAs ingested in free form are rapidly absorbed from the stomach or the small intestine.[52]

For instance, caffeic acid has been shown to be absorbed from the stomach and from the small intestine after an in situ small intestine perfusion.[52,67,68] In an experiment in which caffeic acid was incubated in the stomach of a rat, the bulk of the added HCA was absorbed by the stomach and small intestine within 2 h,[69,70] and it was detected in the gastric mucosa, blood, bile, and urine. These results indicate that caffeic acid is subject to rapid gastric absorption within 1−2 h of consumption of the food that contained it.[52] On the other hand, both ferulic acid and p-coumaric acid showed >70% absorption after 25 min of incubation in the rat stomach and were detected in the gastric mucosa, blood, bile, and urine, suggesting they are subject to more rapid gastric absorption than cinnamic acid.[71] In situ and ex vivo absorption models suggest that ferulic acid and p-coumaric acid can be absorbed from the stomach,[71] jejunum,[72,73] ileum,[72] and colon of rats.[74]

Similarly, Yang et al.[75] showed that free ferulic acid was detectable in the plasma within 10 min of the ingestion of an oral dose of sodium ferulate in healthy subjects, indicating that free ferulic acid is also quickly absorbed in the human gastrointestinal tract. Overall, ferulic acid and p-coumaric acid have similar absorption efficiencies, which is higher than that for caffeic acid.[67,76−78]

The absorption of HCAs has also been studied in Caco-2 cell monolayers.[77−80] Konishi et al.[81,82] showed that HCAs may be transported across the intestinal epithelial cells by monocarboxylic acid transporters (MCTs); it has also been reported that MCTs may be responsible for the absorption of caffeic acid.[77,82] In rats, p-coumaric acid and ferulic acid have been shown to cross the cells of the small intestine via the monocarboxylic acid transporter (MCT).[77,82] It appears that the transepithelial flux of caffeic acid is lower than that of ferulic acid and p-coumaric acid[79] and that the transepithelial flux of chlorogenic acid is much lower than that of caffeic acid.[83] The mechanism of absorption of chlorogenic acid (as determined for Caco-2 cells in vitro) involves removal of the quinic moiety by a mucosa esterase and subsequent absorption of caffeic acid via the MCT.[77]

On the other hand, Poquet et al.[84] observed significant evidence for passive diffusion in a monolayer of cocultured Caco-2 and HT29-MTX cells; their findings are consistent with those of Adam et al.[76] Zhao et al.[71,85] suggested that a passive diffusion mechanism may be involved in the gastric absorption of

ferulic acid. Dupas et al.[86] also observed very limited but significant in vitro and in vivo absorption of chlorogenic acid by Caco-2 cells in humans and rats, respectively, suggesting that absorption of intact chlorogenic acids may occur.[67,83,86]

Other in vitro or ex vivo studies have suggested the existence of a H⁺-driven transport system for the uptake of cinnamic acid and structurally related substances such as ferulic acid and p-coumaric acid.[73,80,85,87] However, such transporters have not yet been identified. Wolffram et al.[73] showed the involvement of a Na⁺-dependent, carrier-mediated transport process in the uptake of cinnamic acid and ferulic acid across the brush border membrane of rat jejunum.[73]

Olthof et al.[69] showed that the esterification of caffeic acid with quinic acid, as in chlorogenic acid, markedly reduced its absorption, as is the case for other HCA esters. The absorption of chlorogenic acid in rats has been studied to determine where and in what form (free or bound) it is absorbed.[83] Most publications dealing with the absorption of chlorogenic acid in rats suggest that it is absorbed in two forms, with some absorption of the intact ester occurring through the stomach[68,80] and some being absorbed via the small intestine, where it may be hydrolyzed by the enterocytes before entering general circulation. This occurs in rats with an absorption percentage that represented 8% hydrolysis in the mucosa.[83] The chlorogenic acid that is not absorbed in the upper part of the gastrointestinal tract reaches the cecum. In rats, 8% of the ingested chlorogenic acid is absorbed via the mucosa and hydrolyzed.

Several workers have identified chlorogenic acid in the urine produced after the ingestion of coffee or pure chlorogenic acid, suggesting it is directly absorbed in the gut.[9,69,88−90] However, in many studies, it was not detected in the plasma or bile.[70,91−95] Chlorogenic acid may be hydrolyzed in the gut before absorption, as suggested by the presence of free caffeic acid in the intestinal effluent after the perfusion of chlorogenic acid and by the reported presence of tissue esterases in the upper intestinal mucosa.[96,97]

In rats fed 2.8 mmol of chlorogenic acid, the rate of absorption was 33 ± 17%, but only traces of the ingested acid were excreted in the urine. Thus, one-third of the total chlorogenic acid consumed by humans is absorbed in the small intestine. This implies that whereas some of the ingested chlorogenic acid will enter the blood, most will reach the colon.[69]

To date, there have been no reports on the bioavailability of sinapic acid in humans.[96] However, it has been shown that its conjugates are present in rat urine.[98] Moreover, it is sulfated in Caco-2 cells.[97]

Metabolism of HCAs. In both rats and human subjects, dietary plant HCAs can undergo metabolic modification in the gastrointestinal tract,[99−101,113] intestinal mucosa, intestinal microflora, liver, or kidney.[32,70,86−97] These modifications include dehydroxylation, demethylation, hydrogenation, O-methylation, sulfation, glucuronization, GSH conjugation, and/or glycination (Figure 4).[11,33,64,67,69,72,83,85,88,90,102−110]

Early studies suggested that the metabolic fate of ferulic acid was qualitatively identical to that of caffeic acid, for which a large number of metabolites have been reported (Figure 4).[111,112] For example, the same 3-hydroxy- and 3-methoxy-4-hydroxy derivatives of phenylpropionic acid, hydracrylic acid, and glycine conjugates such as dihydroferulic acid, vanillic acid, and vanilloylglycine were observed following the ingestion of caffeic acid or ferulic acid, in both human[91,103,113] and rat urine (Figure 4).[32,85,114]

In addition to unchanged caffeic acid, ferulic acid and other metabolites (such as sulfates and glucuronides) were detected
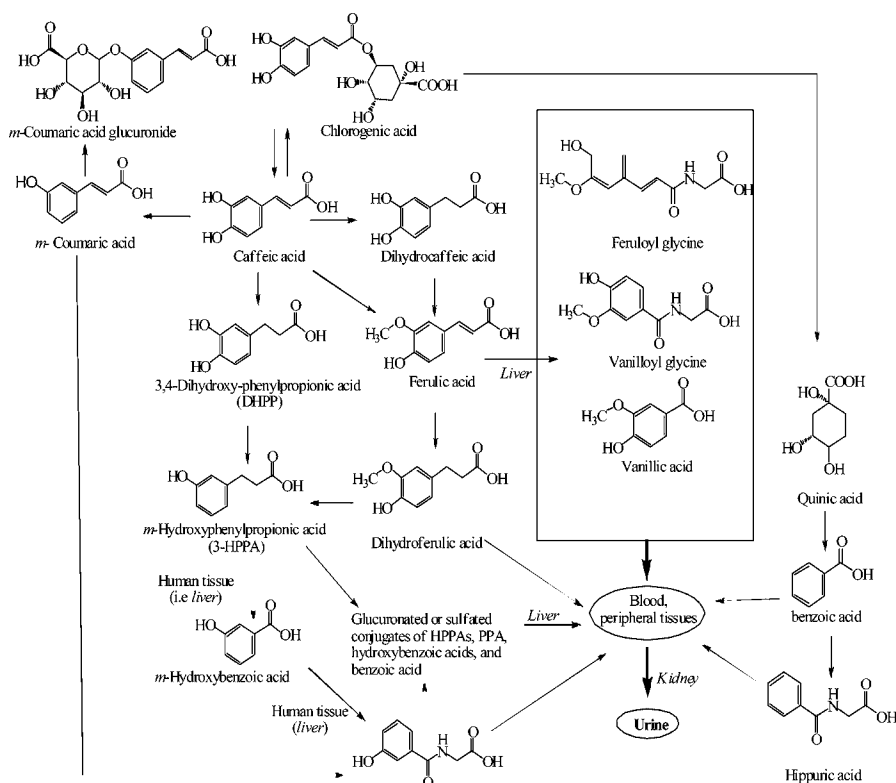
Figure 4. Metabolism of HCAs.[32,65,75,76,91–93,95,107,108,119,123–125]

in rats 2 h after the ingestion of 700 μmol/kg of pure caffeic acid.[70] These molecules were first detected 30 min after the start of the incubation, but reached their peak concentrations only after 2 h (Figure 4).[70,85,89,92,103,105,111,113,115,116]

Feeding studies in rats and humans demonstrated that ferulic acid undergoes metabolic conversion to a dehydroxylated derivative and the same hydroxymethoxy derivatives, which are typically then conjugated with sulfate and/or glucuronides in the liver.[71,103,107] The suggestion that 3-hydroxyphenylpropionic acid is a major urinary metabolite of ferulic acid in rats was supported by additional studies involving intraperitoneal administration.[114] Free caffeic acid was rapidly detected in plasma following the consumption of red wine, at concentrations varying from 0.084 to 0.3 μM (Table 3).[117,118] Similarly, sulfate and glucuronide[120] conjugates of ferulic acid were rapidly detected in the plasma following the consumption of tomatoes [92] or beer (Table 3).[119]

A study of human chlorogenic acid metabolism showed that the unabsorbed chlorogenic acid that reaches the colon is hydrolyzed to caffeic acid and quinic acid by the colonic microflora.[90] After dehydroxylation by the colonic microflora and absorption and further metabolism in the liver and kidney, it is converted to benzoic acid and conjugated to glycine to form hippuric acid. About half of the ingested chlorogenic acid is converted to urinary hippuric acid in this fashion.[90] Hippuric acid, 3-hydroxyphenylpropionic acid, and m-coumaric acid, thought to be derived from colonic microbial degradation of chlorogenic acid, have been detected in both the plasma and the urine of rats.[88] The presence of isoferulic acid was reported in the mesenteric plasma of rats perfused in situ with chlorogenic acid.[70,83] In a clinical trial featuring ileostomized patients, only 0.3% of the ingested chlorogenic acid was excreted without modification in the urine.[69]

The amounts of caffeic acid and its methylated metabolite, ferulic acid, recovered in the urine after the administration of chlorogenic acid by gastric intubation were 100-fold lower than those observed after administration of caffeic acid.[70] Nardini et al.[94] reported an increase in the levels of conjugated caffeic acid in human plasma, reaching a maximum concentration 1 h after the ingestion of 200 mL of coffee. Chlorogenic acid has been observed in human urine and plasma after the ingestion of coffee, prunes, or the pure molecule.[9,69] Other studies have failed to detect chlorogenic acid in the plasma of both rats and humans after its ingestion as a pure compound or in coffee.[30,70,78,120–122] For example, Choudhury et al.[93] did not detect chlorogenic acid or any of its metabolites mainly in urine collected from rats 0–24 h after the ingestion of an acute dose. In contrast, a variety of conjugated metabolites, mainly glucuronides and/or sulfates of caffeic acid and ferulic acid, were identified in the plasma of rats after feeding with chlorogenic acid.[70]

Excretion of HCAs. The rapid and extensive metabolism of HCAs results in low plasma concentrations and quick elimination from circulation. HCA metabolites are excreted via one of two pathways: the biliary or the urinary route. Large, extensively conjugated metabolites can be excreted in the urine but are more likely to be eliminated in the bile; as such, they are returned to the gastrointestinal tract and may be (partially) reabsorbed. Small conjugates are preferentially excreted in the urine.[43]

The biliary pathway of HCA excretion may differ substantially in humans and in rats because, unlike rats, humans have a gall bladder. Also, intestinal bacteria possess β-glucuronidases that are able to release free aglycones from the conjugated metabolites secreted in the bile. Aglycones can be reabsorbed, resulting in enterohepatic cycling.[10] The biliary excretion of HCAs in the rat has previously been established by liver perfusion and

Table 3. Bioavailability of HCAs in Humans and Animals[a]

hydroxycinnamic acid	animal species	source	dose	t_{sample} (h) after last administration	$C_{determined}$ (mg/L)	24 h urinary excretion (% of intake)
Animal, Oral						
caffeic acid[132]	Wistar rats	purified	110 mg/day	**0.5**	99	13.7
caffeic acid[133]	Zealand white rabbits	purified	10 mg/kg	**0.5**	**1.08**	
caffeic acid[70]	Wistar rats	purified	125 mg/kg	**2**	**10.8**	
caffeic acid[88]	Wistar rats	purified	45 mg/day	12	*19.56*	40.9
caffeic acid[78]	Wistar rats	purified	1.8 mg/kg	**0.33**	**5.47**	
ferulic acid[76]	Wistar rats	purified	1.94 mg/day	12	ND	42.5
ferulic acid[76]	Wistar rats	purified	9.7 mg/day	12	0.213	51.8
ferulic acid[76]	Wistar rats	purified	48.55 mg/day	12	1.474	38.6
ferulic acid[76]	Wistar rats	whole wheat flour from duriac	50 mg/kg	12	ND	3.2
ferulic acid[124]	Sprague−Dawley rats	purified	5.15 mg/kg	**0.5**	**1.03**	43.4
chlorogenic acids[b 93]	Wistar rats	purified	50 mg/kg			ND
chlorogenic acid[70]	Wistar rats	purified	248 mg/kg	**0.5 ± 1**	0.28	
chlorogenic acids[88]	Wistar rats	purified	88.6 mg/day	12	40.14	58.8
p-coumaric acid[82]	SD rats	purified	1.64 mg/kg	**0.16**	22.8	
p-coumaric acid[82]	SD rats	10% propylene glycol	1.64 mg/kg bw	**10**	23.78	
Humans, Digestion						
caffeic acid[69]	humans	purified	500 mg			10.7
caffeic acid[115]	humans	red wine	0.06 mg		**0.014**	
caffeic acid[118]	humans	red wine	55 µg/kg bw	**1**	**0.015**	
caffeic acid[134]	humans	red wine (200 mL)	1.8 mg	**0.5 ± 1**	**0.01**	
caffeic acid[135]	humans	red wine (100 mL)	0.9 mg	**1**	**0.001**	
caffeic acid[135]	humans	red wine (200 mL)	1.8 mg	**1**	**0.003**	
caffeic acid[82]	humans	red wine (300 mL)	2.7 mg	**1**	**0.005**	
ferulic acid[92]	humans	tomatoes	30 mg			11 ± 25
ferulic acid[119]	humans	beer (4 L)	9.4 mg			21 ± 95
ferulic acid[85]	humans	breakfast cereals	260 mg	**1 ± 3**	**0.03 ± 0.04**	3.1
chlorogenic acid[69]	humans	purified	1 g			0.3
chlorogenic acids[42]	humans	coffee	149.7 × 6 cups = 898 mg[c]			5.9
chlorogenic acids[42]	humans	artichoke extract	102.9 × 3 times = 308.7 mg[c]		0.004 ± 0.015	
chlorogenic acids[136]	humans	artichoke extract	124 mg[c]			5.6
chlorogenic acid[79]	humans	coffee (200 mL)	96 mg	**1**	**0.19**	
chlorogenic acids[138]	humans	artichoke extract	107 mg[d]	**1 ± 6**	**0.023 ± 0.049**	4.7
chlorogenic acids[138]	humans	artichoke extract	153.8 mg[d]	**1 ± 6**	**0.034 ± 0.07**	4
chlorogenic acids[92]	humans	coffee	258.8 mg			9.4
chlorogenic acids[135]	humans	coffee	1170 mg	**1 ± 2.5**	**2.83**	
total hydroxycinnamic acids[82]	humans	apple cider (1.1 L)	15 mg	<2		

[a]Bold values correspond to t_{max} and C_{max}; C refers to maximum concentrations in plasma. Italic values denote the concentration of the aglycone + conjugated metabolites + microbial metabolites. ND, not detected; equiv, equivalents; bw, body weight. [b]Chlorogenic acid + glucuronide chlorogenic acid. [c]Equivalent 5-caffeoylquinic acid. [d]Equivalent caffeic acid.

intraperitoneal injection of caffeic acid.[105,112] As reported, the biliary excretion did not exceed 0.4% of the perfused dose of caffeic acid, suggesting that this route does not contribute extensively.[83] Absorbed chlorogenic acid is poorly excreted in the bile or in the gut lumen.

The urinary pathway of HCA excretion has been studied extensively in humans. Relative urinary excretion is currently used to estimate the minimal absorption rate,[85,89] although in cases when biliary HCA excretion is significant, this will result in an inaccurate estimate of the overall absorption.[116] The relative rates of urinary excretion of caffeic and ferulic acid and their metabolites range from 5.9 to 27%.[30,92,123] In rats fed 2.8 mmol of caffeic acid (505 mg), 11% of the ingested dose was excreted in the urine.[69]

Total urinary excretion (aglycone and metabolites) increased progressively, peaking 7 or 8 h after the ingestion of beer or tomatoes, respectively.[92] Free ferulic acid accounted for only 5−24% of the total quantity of metabolites.[71,107,124,125] Increased levels of ferulic acid, isoferulic acid, vanillic acid, dihydroferulic acid, hippuric acid, and 3-hydroxyhippuric acid were detected in urine samples from five male subjects after three ingestions of two cups of coffee at 4 h intervals.[113,126]

The fate of absorbed chlorogenic acid has been investigated in animal models. Relatively little is known about the bioavailability of chlorogenic acid as opposed to its excretion, because it and its metabolites are typically determined in the gut contents, plasma, and urine of rats fed diets supplemented

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with chlorogenic acid.[83] In such studies, the acid is observed in the plasma and urine within 1.5 h of the beginning of the meal.

BIOAVAILABILITY OF HCAS

Bioavailability relates to the uptake of the active compound, its metabolism, and excretion. The uptake is dependent on its availability from the matrix and the metabolism of the active compound in the gut.

The compounds' effects and pharmacokinetics are determined by their concentrations in the system. Their concentrations vary in time and are determined by their uptake, on the one hand, and metabolism and excretion, on the other (i.e., their pharmacodynamics). Bioavailability depends on several endogenous and exogenous factors; the exogenous factors seem to be related to the food matrix, size, and amount ingested, whereas the endogenous factors relate to the activity of the digestive enzymes, biliary/urinary excretion, biotransformations involving the gut microflora, the gastrointestinal epithelium, and the liver. Obviously, the physicochemical properties of the compound also affect its bioavailability.[52,127] There is significant variation in the amounts of HCA and HCA derivatives consumed by different individuals. It is therefore essential to understand the bioavailability and pharmacodynamics of the HCAs consumed as part of a normal diet to determine their effects on health.

In several studies, the bioavailability of ferulic acid has been examined by quantifying urinary excretion, albeit with variable results: the calculated bioavailabilities range from 0.4 to 98%, in part depending on the food source.[116,119,128] For instance, the bioavailability of ferulic acid in cereal products (particularly bran) seems to be low: 3% in humans[129] and 2.5−5% in rats;[76] that of corn bran is even lower, 0.4−0.5% in rats.[130] In other food matrices, its bioavailability was somewhat higher: 11−25% in tomato, [92] ∼28% in rye bread,[131] and 19−98% in beer.[119]

The overall bioavailability picture is quite complex, because foods contain complex mixtures of various free and conjugated HCAs, all of which differ in terms of bioavailability, metabolism, and excretion. It is important to keep the data presented in Table 3 in mind when reading the following paragraphs on the biological activity of the HCAs, as the low absorption of some of their derivatives may make it impossible to benefit from any beneficial health effects they may have when consumed in the diet.

BIOLOGICAL ACTIVITIES OF HCAS

HCAs are considered to be nutritionally important because of their antioxidative activities. Howevere, also a number of other activities have been reported such as antiallergic, antimicrobial, and immunomodulatory activities, which might explain the activity of some folk medicines.[2,41,132,139−143] In this section, we briefly discuss the various reported biological activities of the HCAs.

Antioxidant and Free Radical Scavenging Properties of HCAs. Oxidative stress is involved in aging and the pathology of many diseases including cancer, atherosclerosis, diabetes, and neurodegenerative, liver, and kidney diseases. Dietary antioxidants may afford protection against oxidative stress-related diseases. Antioxidants are defined as organic molecules that can protect the body's cells from damage caused by free radicals and reactive oxygen species.[14]

Substantial attention is presently focused on the potential health effects of HCAs, which have been described as chain-breaking antioxidants that probably act as radical scavengers.[163] This function is related to their hydrogen atom donating ability and their ability to stabilize the resulting phenoxyl radicals via the conjugated system comprising the arene and the alkenyl carboxylate side chain.[12,149−151]

Rice-Evans et al. have discussed the relationship between antioxidant activity of HCAs, as hydrogen donating free radical scavengers, and their chemical structures.[152]

Incorporation of a hydroxyl group into *p*-coumaric acid adjacent to that in the para position as in caffeic acid gives activity. Comparison of the cinnamates with the phenylacetic acid derivatives reveals that the activity is considerably lower than that of 3,4-dihydroxyphenylacetic acid. This is consistent with the electron-donating effects on the ring of the COOH−CH− CH versus COOH−CH$_2$− groups and the relationship with the number and position of hydroxyl groups in the ring. The monohydroxyl group in the cinnamic acids being more available as hydrogen donors than the monohydroxyl groups in the phenylacetic acid. On the other hand, dihydroxylation in the 3,4-position enhances the efficacy of the latter while decreasing that of the *p*-coumaric acid. In fact, the antioxidant activity of caffeic acid (3,4-dihydroxycinnamic acid) is almost the same as that of protocatechuic acid (3,4-dihydroxybenzoic acid).[152,158,164]

Furthermore, investigation of the antioxidant potential of phenolic acids in lipophilic systems consisting of accelerated autoxidation of methyl linoleate under conditions of intensive oxygenation at 110 °C for several hours has been undertaken.[153] Introduction of a second hydroxyl group in the ortho position caffeic or para position protocatechuic acid enhances the antioxidant activity[154] in lipid systems, making these phenolic acids more efficient than their respective monophenols, *p*-hydroxybenzoic acid and *p*-coumaric acid.

The results from these lipid studies also illustrated that the antioxidative efficiency of monophenols is increased substantially by one or two methoxy substitutions in positions ortho to the OH as in ferulic acid: sinapic acid is more protective than ferulic acid, which is better than *p*-coumaric acid, and syringic acid is more active than vanillic and *p*-hydroxybenzoic acids.[152−154] However, in the aqueous phase ferulic acid is 150% as efficient as caffeic and chlorogenic acids.[155]

The presence of the −CH═CH−COOH groups in cinnamic acid ensures greater H-donating ability and subsequent radical stabilization than the carboxylate group in benzoic acids. The reduction potentials of radicals derived from 3,4-dihydroxybenzoate decrease with the electron-donating power at C1; for example, dihydroxybenzoate radicals have a higher reduction potential than dihydroxycinnamate radicals.[156] Thus, caffeic, sinapic, ferulic, and *p*-coumaric acids were found to be more active than protocatechuic, syringic, vanillic acid and *p*-hydroxybenzoate, especially in lipid systems.[154] It may be that the −C═C−CO$_2$H linked to the phenyl ring plays a role in stabilizing the radical by resonance and hence, caffeic acid is more efficient than pyrocatechol.[152]

Recently, ferulic acid was studied in clinical practice as an antioxidant to neutralize hydroxyl radicals by means of the aromatic hydroxylation reaction. The hydroxyl radicals can be generated by ferric ions without any oxidizing agent. In view of the well-known damaging effect of poorly chelated iron in the human body, numerous natural products containing iron-binding agents can be essential in the maintenance of human health.[157]

Several authors have reported that HCAs protect low-density lipoprotein (LDL) against oxidative modifications

(see Figure 5).[143−145] It has been suggested that oxidized LDL plays a key role in the pathogenesis of atherosclerosis,
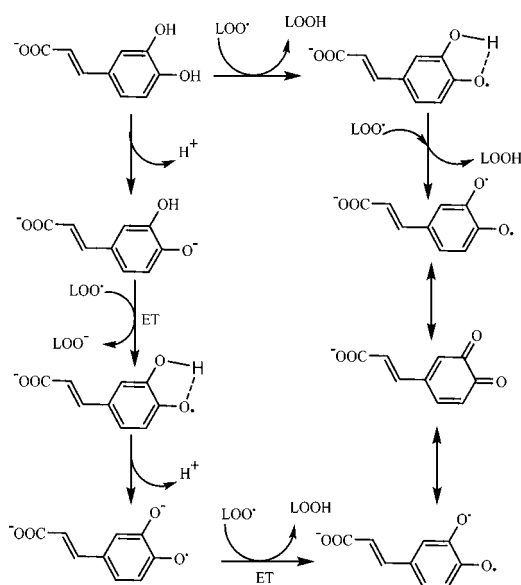

Figure 5. Mechanism of the inhibition of LDL peroxidation by caffeic acid. ET, electron transfer.

leading to the buildup of plaque in the arteries and consequently causing coronary diseases.[146,147] The ability of HCAs to inhibit the oxidation of human LDL was tested in vitro; the antioxidant activities of purified monomeric and dimeric hydroxycinnamates were investigated using an in vitro copper-catalyzed human LDL oxidation assay. The most abundant ferulic acid dehydrodimer found in rye inhibited LDL oxidation at 10−40 μM, and activity was found to decrease in the following order: caffeic acid > chlorogenic acid > sinapic acid > ferulic acid > p-coumaric acid.[42,145,146,148,149]

HCAs have been shown to inhibit the oxidation.[162] Only caffeic acid at 5 μM concentration gave complete protection of LDL from modification as could be seen from analysis of the formation of conjugated dienes and apo B-100 fragmentation.[23,38,141,173−178] Ferulic acid offered good radioprotection in vitro and in vivo conditions to DNA and enhanced the DNA repair process in the peripheral blood leucocytes of mice in vivo. Administration of ferulic acid (50 mg/kg body weight) to mice bearing fibrosarcoma tumors, 1 h prior to or immediately after radiation exposure (4 Gy), showed preferential radioprotection to normal cells,[61,179−184] and they appear to be suitable for the prevention of diseases associated with the process of lipid peroxidation,[182−184] such as cancer and cardiovascular diseases and for the treatment of inflammatory injuries.[159,160,185−189] A diet rich in natural antioxidants such as HCAs can thus significantly increase the reactive antioxidant potential of the organism[190−192] and thereby decrease the risk of free radical-related diseases. In addition to their uses as natural antioxidants in preventive medicine, HCAs have many industrial uses including as preservatives in foods and cosmetics and for preventing the degradation of rubber and gasoline.[193] Caffeic acid has been employed as a natural antioxidant for inhibiting oxidation of fish lipids present in different food matrices.[194]

It has been shown that HCAs can protect LDL against Oxidation of Cu^{2+} and 2,2′-azobis(2-amidinopropane) dihydrochloride (AAPH).[141] They can also act as scavengers of hypochlorite,[167] peroxynitrite,[168] and the 2,2-diphenyl-1-picrylhydrazyl radical (DPPH),[169] inhibit lipid peroxidation, and protect against LDL oxidation[149,170−172] as shown in Table 4.

Prevention of Cardiovascular Diseases by HCAs. Previous studies have shown that the hydroxycinnamic acids (p-coumaric acid, caffeic acid, ferulic acid) and their derivatives were effective in the treatment of hypercholesterolemia and type 2 diabetes.[198−200] Chlorogenic acid was reported to reduce the risk of cardiovascular disease by preventing the oxidation of LDL-cholesterol and total cholesterol.[140] Recently, Hsu et al.[201] reported that the IC_{50} value of chlorogenic acid in 3T3-L1 preadipocytes was 72.3 μM. This result indicates that the inhibition of preadipocyte population growth by chlorogenic acid might have further implication for its in vivo anti-obesity effects, which may provide an approach for treating obesity. Also, it was found that under normal dietary conditions, oral administration of chlorogenic acid (30 and 60 mg/kg/day) for 14 days reduced hepatic triglyceride levels.[202] Therefore, there is increasing interest in the antiobesity effects of chlorogenic acid in vivo. Cho et al.[203] compared the efficacy of chlorogenic acid and caffeic acid at reducing body fat in induced-obese mice fed a high-fat diet (37% calories from fat); the diet was supplemented with the appropriate HCA at a level of 0.2 g/kg diet. Caffeic acid and chlorogenic acid both caused significant body weight reductions (by approximately 8 and 16%, respectively) and reduced epididymal adipose tissue weight by approximately 22 and 46%, respectively. They also lowered triglyceride (in plasma, liver, and heart) and cholesterol (in plasma, adipose tissue, and heart) concentrations. These results suggest that caffeic acid and chlorogenic acid reduce body weight, lipid metabolism, and obesity-related hormone levels in high-fat-fed mice.[203] However, the levels at which these effects are induced are very high compared to the typical daily intake.

Yeh et al.[204] investigated the effects of dietary caffeic, ferulic acid, and coumaric acid to determine their possible roles in lowering plasma cholesterol levels. It was found that individuals whose diets were augmented with one of these compounds at a level of 0.2 g/kg diet exhibited reduced plasma cholesterol concentrations High-density lipoprotein (HDL). Further studies by this group suggested that the plasma lipid-lowering and antioxidative effects of caffeic acid, ferulic acid, and coumaric acid

Table 4. Antioxidant Capacity of HCAs in Different Model Systems

model system	caffeic acid	ferulic acid	p-coumaric acid	m-coumaric acid	ref
hydrogen peroxide scavenging activity ($\times 10^{-2}$ μM^{-1})	8.2	1.10	0.23	0.18	195
efficient quantity (mg L^{-1})	8	72	126		196
% inhibition in LDL oxidation at 10 μM	97.9	55.7	40.7		145
specific antioxidant activity in copper-mediated LDL oxidation (μM)	54	8.5	0.6		118
antioxidant activity (peroxyl radical scavenging) relative to Trolox	3.97	0.90	0.04		197
singlet oxygen quenching, given as rate constants kq ($\times 10^5$ M^{-1} s^{-1}) (kq)	40	20	6	7	177
radical (DPPH) scavenging activity (%)	49.6	17.3	7.0		143

supplements were very potent in high-cholesterol-fed rats. In fact, at a concentration of 5 mg/mL in vitro only caffeic acid completely protected LDL from modification (as judged by the formation of conjugated dienes and fragmentation of apo B-100) and also preserved α-tocopherol.[140]

Caffeic acid also had a more potent biological effect in terms of reducing plasma cholesterol and hepatic HMG-CoA reductase activity than either ferulic acid or coumaric acid. This is supported by the findings of Santos et al.,[205] who reported on the hypolipidemic effects of HCA administered via intraperitoneal injection at a dose of 5 mg/kg body weight. The intraperitoneal injection of caffeic acid at 30 mg/week for a period of 10 weeks has been shown to reduce plasma triglyceride, total cholesterol, and LDL-cholesterol levels in spontaneously hypertensive rats while increasing plasma HDL-cholesterol levels in normal Wistar rats.[206]

Prevention and Treatment of Cancer. The major carcinogenic agents are exogenous or metabolically generated reactive oxygen species (ROS) and electrophiles arising from the environment and from normal in vivo oxidative processes.[207] Enhanced ROS levels are involved in tumor initiation and promotion and may ultimately lead to carcinogenesis.[208] The antioxidant activity of the HCAs may thus play a role in cancer prevention.[209] It has recently been reported that HCAs have some effect when applied directly to cancer cell lines. For example, Jian et al.[210] studied the inhibitory effects of caffeic acid on the growth of the U937 tumor cell line by examining the cells' voltammetric behavior when exposed to doses >5 mg/L. Caffeic acid was shown to have a negative effect on cell health, suggesting that it may not be suitable for the treatment of tumor cells.

Several studies have shown caffeic acid to be an inducer of apoptosis in cancer cell lines and capable of causing tumor growth inhibition and regression in animals.[211,212] Notably, it has been reported to induce apoptosis in HL-60 cells and has novel and therapeutic effects on hepatocarcinoma cells.[211,213] It selectively inhibits matrix metalloproteinases (MMP)-2/9, can be used to treat HepG2 cells at a dose of 10 mg/L, and also protects WI-38 human lung fibroblast cells against H_2O_2 damage at 50 μM.[214]

Chlorogenic acid and other hydroxycinnamic compounds such as caffeic and ferulic acids showed inhibitory effects on 4-nitroquinoline-1-oxide (4-NQO)-induced tongue carcinogenesis in rats at concentrations of 250 mg/L (chlorogenic acid) and 500 mg/L (others).[215] Kasai et al.[216] suggested some mechanisms that may account for the cancer chemopreventive activity of chlorogenic acid and other naturally occurring phenolic compounds.

The effects of topically applied chlorogenic acid, caffeic acid, and ferulic acid on 12-O-tetradecanoylphorbol-13-acetate (TPA)-induced epidermal ornithine decarboxylase activity have been studied. The topical application of 10 μmol of chlorogenic acid, caffeic acid, or ferulic acid inhibited the induction of ornithine decarboxylase activity by 5 nmol of TPA by 25, 42, and 46%, respectively. Similar treatment of mice with 10 μmol of chlorogenic acid, caffeic acid, and ferulic acid together with 5 nmol of TPA inhibited the number of TPA-induced tumors per mouse by 60, 28, and 35%, respectively, and higher doses of the HCAs caused more pronounced inhibition of tumor promotion.[217]

Chlorogenic acid has also been reported to prevent the growth of different cancers in the large intestine, liver, and tongue in several animal models.[215,218−221] Chlorogenic acid has a chemopreventive effect on N-methyl-N-nitrosourea (MNU)-induced glandular stomach carcinogenesis when applied during the postinitiation phase at a concentration 400 mg/L.[222] Also, it is known to inhibit methylazoxymethanol acetate-induced colon and liver carcinogenesis in hamsters.[220] Moreover, chlorogenic acid has recently been shown to cause the regression of carcinogen-induced colonic aberrant crypt foci (ACF) in rat colons.[223]

Ferulic acid has also been shown to protect gastrointestinal organs such as the stomach, the intestines, and the colon, including the liver, against carcinogenesis. In rats that were treated with 4NQO (administered in their drinking water at dose of 20 mg/L for 5 weeks) and whose diet was then augmented with ferulic acid at a dose of 500 mg/L, the incidences of tongue carcinomas and preneoplastic lesions were significantly lower than in their counterparts who did not receive additional HCA. These results suggest that ferulic acid has some chemopreventive activity.[224]

Protection against Side Effects of Chemotherapy by HCAs. Cancer chemotherapy causes severe nausea, vomiting, and abdominal discomfort, which limits its administration.[225] Most anticancer agents slow gastric emptying.[226−228] Cisplatin is extensively used for the management of oncological disorders, particularly of the ovary, testis, bladder, head, and neck.[229] Although effective, it is associated with many adverse drug reactions such as renal damage, gastrointestinal dysfunction, auditory toxicity, and peripheral nerve toxicity.[230] Cisplatin's side effects in the gastrointestinal tract can be mitigated by treatment with metoclopramide[231] and antioxidants.[232]

The gastrokinetic activity of HCAs may be partly due to their interference with the production of nitric oxide (NO) production; NO plays a key role in the regulation of gastrointestinal motility by virtue of its smooth muscle relaxing and vasodilating activity.[220] Soliman and Mazzio[233] reported that chlorogenic acid and caffeic acid significantly inhibited NO production, probably via inhibition of NO synthase gene expression, at <300 mg/L. Further studies on the mechanism of action of HCAs on gastrointestinal motility may be helpful in determining their therapeutic value in gastrointestinal motor disorders.

The beneficial effects of the HCAs are also probably due in part to their stimulatory effects on the gastrointestinal tract and their antioxidant effects.[234] As antioxidants, they may prevent cisplatin-induced delay in gastric emptying in rats. These agents may therefore be useful in reducing cisplatin-induced emesis and in the treatment of gastrointestinal symptoms such as abdominal discomfort induced by cytotoxic agents.[235]

Antimicrobial Activity of HCAs. HCAs have been reported to possess antimicrobial activity.[236−240] Barber et al.[241] reported that HCAs were generally active against *Bacillus subtilis* subsp. *niger*, *Escherichia coli*, and *Pseudomonas syringe* (mean MIC = 3.0 mM), but were only weakly active against *Saccharomyces cerevisiae*, *Schizosaccharomyces pombe*, and *Sporobolomyces roseus* (MIC ≥ 8 mM). Ferulic acid was an exception, having a MIC of 4.0 mM against *S. cerevisiae*, as shown in (Table 5). On the other hand, Baranowski et al.[237] reported ferulic acid to be an effective antimicrobial against *S. cerevisiae*, causing complete inhibition at 0.25 mM. On the other hand, ferulic acid was less active against *S. roseus* at MIC = 4.0 mM compared to tolnaftate and eugenol as reference compounds, which have MIC at 0.5 and 1 mM, respectively (Table 5).

Some of the HCAs are also reported to inhibit *Listeria monocytogenes*, as shown in Table 5.[242−244] Because of their modest

Table 5. Minimum Inhibitory Concentrations (MIC) of the HCAs[a]

	minimum inhibitory concentration (mM)						
	yeast			bacteria			
compound	*S. cerevisiae*	*S. pombe*	*S. roseus*	*B. subtilis*	*E. coli*	*P. syringae*	*L. monocytogenes*[233]
caffeic	>8.0 (8)	>8.0 (25)	>8.0 (5)	4.0	8.0	4.0	0.16
ferulic	4.0	8.0 (8.0)	2.0	2.0	2.0	2.0	0.14
sinapic	>8.0 (51)	>8.0 (0)	>8.0 (15)	2.0	2.0	4.0	
p-coumaric	>8.0 (93)	8.0	8.0	2.0	2.0	2.0	0.13
chlorogenic							0.28
cinnamic							0.135
tolnaftate[a]	0.5	1.0	0.5				
eugenol[a]	2.0	2.0	1.0	4.0	4.0	4.0	

[a]Percentage inhibition at 8.0 mM is given in parentheses in cases where MIC > 8.0 mM.

antimicrobial properties and low toxicity, HCAs are broadly applicable as additives for food preservation.[245−247]

Antiosteoclast Activity of HCAs. Bone homeostasis is maintained by a delicate balance between osteoblastic bone formation and osteoblastic bone resorption. Pharmacological and nutritional factors are associated with reduced postmenopausal bone loss, as chemical compounds present in certain foods appear to modulate bone turnover.[248]

Recently, it has been found that HCAs and other related compounds have unique anabolic effects on bone components.[249,250] Lai and Yamaguchi[251] demonstrated that oral administration of 1, 2, or 5 mg/100 g of HCAs has an inhibitory effect on various bone-resorbing factor-induced osteoclast-like cell formations in mouse bone marrow cultures in vitro. Oral administration of HCAs (250 or 500 μg/100 g body weight) was found to cause a significant increase in calcium content, alkaline phosphatase activity, and DNA content in the femoral-diaphyseal (cortical bone) and -metaphyseal (trabecular bone) tissues of rats in vivo.[252]

This finding is in agreement with the observation that HCAs inhibit osteoclastic bone resorption in vitro.[251] HCAs did not affect the proliferation of bone marrow cells and were not toxic to the cells. Therefore, supplemental intake of dietary HCAs may have preventive effects on bone loss with increasing age.

Comments on the Reported Activities of the HCAs. Most of the reported activities are preventive and are observed only at relatively high concentrations (typically, an order of magnitude higher than those routinely consumed in the diet). At present, there are no drugs or botanical medicines having activity based on free HCAs. However, HCAs may be important in any synergistic effects associated with the medicinal plants used in herbal medicine. Epidemiological studies suggest that the consumption of fruits and vegetables as well as grains is associated with reduced risk of chronic diseases[253−255] such as heart disease, cancer, diabetes, and Alzheimer's disease.[253,254]

This conclusion is consistent with the results of previous human clinical trials examining the health benefits of single antioxidants, which have given inconsistent results.[256] Whereas many of the phytochemicals in plants have been identified, a large percentage remains unknown.[54] Different plants contain different mixtures of phytochemicals with different structures and thus offer different protective functions. Consequently, to obtain the strongest possible beneficial effects, a sufficiently large quantity and broad range of phytochemicals from a variety of sources (e.g., fruits, vegetables, and whole grain-based foods) should be consumed, as recommended in previous publications.[1,5,10,16,65] Clinical trials will be important to conclusively

demonstrate the activity of pure HCAs and/or HCA-rich medicinal plants.

Clinical Trials. There is evidence supporting the beneficial health effects of foods that contain HCAs. Epidemiological studies have suggested an inverse relationship between the consumption of HCA-rich foods such as fruits, tea, coffee, and wine[19,51,166,168] and diseases such as Alzheimer's disease and cancer.[257−262] Epidemiological studies usually involve the measurement of dietary intake or serum concentrations of the studied antioxidant. Human supplementation trials are clinical trials designed to test the hypothetical merits of antioxidant supplementation.[263] Although only a few clinical trials of HCAs have been conducted to date, their results suggest that HCAs do indeed possess anti-inflammatory and analgesic activities.[264]

Inconsistent results were obtained from clinical trials examining protective effects of dietary supplementation with pure antioxidant compounds against lung cancer in smokers, invasive cervical cancer, esophageal, gastric cancers, colorectal polyps, and coronary heart disease. This may be because the antioxidants examined in these trials act as components of a more complex system rather than as protective agents by themselves.[265,266]

The in vitro and in vivo antioxidant activities of a number of beverages derived from vegetables have been tested (beer, white and red wines, green and black teas, and coffee).[267−269] Coffee and tea are widely consumed beverages, but only the antioxidant effects of tea have been studied in vivo. The antioxidant capacity of plasma before and after supplementation with 200 mL of coffee (0, 1, and 2 h) was measured by the TRAP and crocin tests; an increase in plasma antioxidant capacity was detected, peaking at 7%. Coffee-related beverages significantly increased the quantity of uric acid in the plasma (5%). Uric acid and other molecules (probably phenolic compounds) are likely to be responsible for the increase in plasma antioxidant capacity after the consumption of coffee.[60]

In recent years, a number of in vivo studies have focused on the protective antioxidant-related effects of tea drinking in humans.[270] Green tea has been suggested to reduce hypertension, atherosclerosis, and thrombogenesis; several biological mechanisms for these effects have been proposed.[160,161]

In conclusion, HCAs occur as free monomers, as esters formed by condensation with hydroxy acids or mono/disaccharides, and as dimers or polymers. They also occur as amides (formed by condensation with amino acids and amines), particularly in coffee and cocoa. The presence of these compounds is found in a wide variety of food plants and beverages, often at high concentrations.

The present review provides information on the total HCA (free plus bound) content of a number of foods and summarizes the data on the bioavailability of dietary HCAs in both experimental animals and humans. Despite the importance of HCAs in the human diet, comparatively little is known about their bioavailability, absorption, metabolism, and transport.

The potential health benefits of HCA consumption depend on both their intake via food and their bioavailability. The free HCAs are better absorbed than conjugated HCAs. There is a shortage of information on the fate of these compounds in the gastrointestinal tract and on their absorption, metabolism, and excretion in humans; much more work needs to be done in this area.

The potential health benefits of HCAs are probably mostly related to their antioxidant capacity. Several studies have shown them to have potent antioxidant activities, and some animal studies have suggested they may have antiatherogenic and antidiabetic activity. The HCAs have also shown some potential as agents for the treatment of Alzheimer's disease. Their antioxidant activities are well documented, and their use as food preservatives may also be important for the prevention of some human disorders.

Clinical studies are needed to confirm the supposed therapeutic and preventive profiles of the HCAs. Such studies will determine how important HCAs are in the inverse relationship between the consumption of HCA-rich food and the incidence of cardiovascular disorders and diabetes observed in epidemiological studies.

■ AUTHOR INFORMATION

Corresponding Author

*Phone: +46-18-4714207. Fax: +46-18-509101. E-mail: hesham.el-seedi@fkog.uu.se.

Notes

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■ ABBREVIATIONS USED

HCAs, hydroxycinnamic acids; PAL, phenylalanine ammonia-lyase; TAL, tyrosine ammonia-lyase; MCTs, monocarboxylic acid transporters; LDL, low-density lipoprotein; ROS, reactive oxygen species; MMP, matrix metalloproteinase; HDL, High-density lipoprotein.

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