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Non-Nutritive Bioactive Food Constituents of Plants: Bioavailability of Flavonoids

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Abstract: Flavonoids are polyphenols widely distributed in the plant kingdom, and are present in fruits and vegetables regularly consumed by humans.

In vitro metabolic studies of flavonoids in rat liver microsomes identified the 3', 4'-dihydroxylated derivatives as the major metabolic endpoint. However, *in vivo* in rats almost none of this metabolite and only minor amounts of the 4'-monohydroxylated derivative was produced. Flavonoids with the 4'-monohydroxylated structure were generally not metabolised and were excreted unchanged in urine in higher amounts than other flavonoids investigated.

It has for long been a controversy, whether flavonoids are absorbed as the intact glycoside or whether they have to be hydrolysed to the free aglycon prior to absorption. Recent data suggest that β-glucosidases and maybe also lactase phlorizin hydrolase (LPH) in the small intestine are capable of hydrolysing flavonoid glucosides and these compounds are thus taken up as the free aglycon and not as the intact glycosides.

LC-MS analyses of 12 dietary flavonoids in human urine showed that no flavonoid glycosides were excreted, and that the citrus flavanones and phloretin are excreted in higher amounts than the flavonols. Furthermore, total flavonoid excretion may be a useful biomarker for habitual fruit and vegetable consumption.

Key words: Metabolism, flavonoids, absorption, bioavailability, human, biomarker, fruits and vegetables

Occurrence and intake of flavonoids

Flavonoids constitute a large class of polyphenols that are widely distributed in the plant kingdom, and are thus present in fruits and vegetables regularly consumed by humans. They account for a variety of colours in flowers and fruits, from yellow to red and dark purple. The flavonoids are biosynthesised from phenylalanine (ring B) and three acetate units (ring A) giving the *chalcones* as the first identifiable intermediates (see Fig. 1) [1]. Ring closure of the chalcones give rise to the flavanones, that can be further oxidised or derivatised to *flavanonols, flavones* or *flavonols.* Reduction of the carbonyl group in the 4-position and subsequent removal of the hydroxyl group gives *catechins,* whereas oxidation of the C-ring affords the *anthocyanins.* Rearrangement of the flavonoid skeleton by an intramolecular 1,2-shift of the B-ring gives rise to the *isoflavonoids.* Substituents such as hydroxyl, methoxyl, and sugar moieties give rise to a multitude of different compounds, and more than 4000 different naturally occurring flavonoids have so far been described [2].

In plants, flavonoids occur primarily as glycosides with different sugar groups linked to one or more of the hydroxyl groups. They are mainly found in the outer parts of plants, such as leaves, flowers and fruits, whereas the content in stalks and roots usually is very limited.

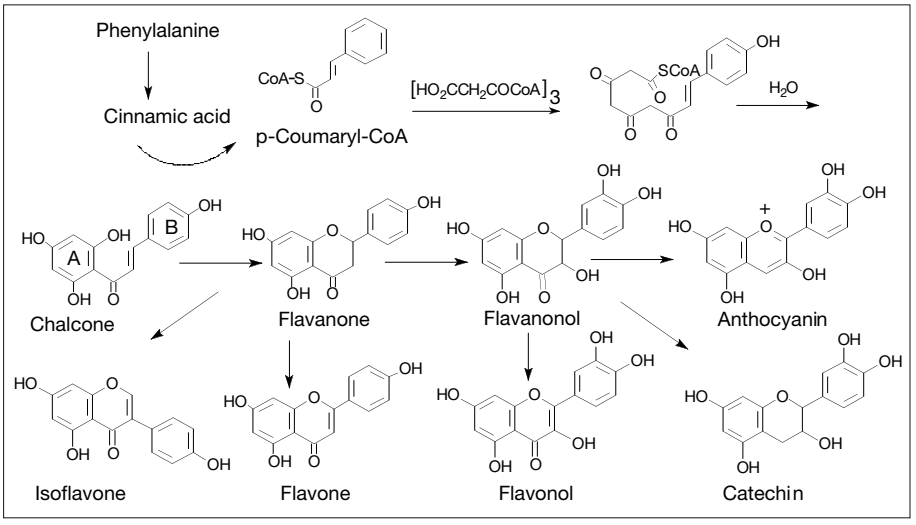

Figure 1: Biosynthesis pathways of common dietary flavonoids in plants.

Only some of the flavonoids are found in considerable amounts in the human diet, and the intake of flavonoids varies between countries and cultures (Table I): The *flavanones* are present mainly in citrus fruits, predominated by naringin (the glycoside of naringenin), responsible for the bitter taste of grapefruit, and hesperidin (the glycoside of hesperetin) found in oranges. Since the intake of orange juice is extensive in many Western countries e.g. Denmark and Finland, intake levels of especially hesperetin are very high [3, 4].

The flavanonols, also called dihydroflavonols, are only found in trace amounts in plants, and are therefore not important constituents of the human diet [5]. The contribution of *flavones* to the human intake is generally limited, however some spices and herbs contain high amounts of flavones. Parsley for instance contain large amounts of apigenin [6], and the highly methylated flavone, tangeretin is found in the peel of citrus fruits, and can thus occur in juices made from whole fruits [5].

The *flavonols* are one of the major groups of flavonoids present in the human diet. Quercetin is the most abundant flavonol, found in large amounts in onions, kale, apples, wine, and tea. Kaempferol, found in broccoli, kale and tea, and myricetin, found in tea and wine, are also major flavonols found in the human diet [7, 8].

The *isoflavones* are only present in legumes, especially in soy. The European intake of isoflavones is very low, but in Asia and especially in Japan, where soy products, such as tofu, are consumed in large amounts the intake is considerable, and as seen in Table I, it exceeds the intake of all other flavonoids.

Table I: Occurrence and estimated human dietary intake of flavonoids

Flavonoid subgroup	Major dietary sources	Major flavonoids	Estimated intake (mg/day)			
			Denmark[a]	Holland[c]	Finland[e]	Japan[f]
Flavanones	Citrus fruits, orange and grapefruit juice	Naringenin Hesperetin	7.1 9.3	n.d.	8.3 28.3	n.d.
Flavones	Parsley, celery, red pepper, spices	Apigenin Luteolin	1–2	2	n.d.	0.3
Flavonols	Onions, kale, broccoli, apples, berries, tea, wine	Quercetin Kaempferol Myricetin	8.6 3.4 1.5	17 4 1	7 2.2 1.1	16.4
Isoflavones	Soybeans, legumes	Genistein Daidzein	< 1[b]	n.d.	n.d.	47.2
Total flavonoid			~ 31.5	24	46.9	63.9
Catechins	Tea	epigallocatechin	45[b]	50[d]	8.3	~ 40
Anthocyanins	Berries, red wine	cyanidin	6–60[b]	n.d.	n.d.	n.d.

[a] Data obtained from Justesen *et al* [4, 44]. [b] Data are obtained from Dragsted *et al*, 1997, and are based on Danish consumption levels. [c] [45] [d] [46]. [e] [3]. [f] Data obtained from Arai *et al,* 2000 [47]. n.d. = no data available.

Catechins are quantitatively a quite large group within the human diet. They either occur as free catechins or derivatised with gallic acid, and are found mainly in green and black tea, chocolate and wine [9, 10]. In countries with a high intake of tea, such as Japan (mainly green tea) and the United Kingdom (black tea) the daily intake of catechins is substantial.

Anthocyanins are strongly coloured compounds responsible for the red, purple and blue colours found in fruits, berries and red wine. As seen in Table I, the median intake of anthocyanins may be extensive, in particular for regular consumers of black currant juice, red wine, berries, and grapes.

The estimated average daily intake of flavonoids including catechins and anthocyanins is above 50 mg for all countries presented in Table I and most likely the real intake is higher than 100 mg/day if data on all flavonoid subgroups were available. The daily intake of other dietary antioxidants such as vitamin C (80 mg/day), vitamin E (8.5 mg/day) and β-carotene (1.9 mg/day) [11] is comparable to or lower than the intake of the flavonoids, so these compounds certainly constitute an important part of the dietary intake of antioxidants.

Metabolism of flavonoids by cytochrome P450

Although an extensive number of studies have reported effects of flavonoids on enzymatic, biological and physiological processes, only very few researchers have attempted to determine the actual compound/metabolite responsible for the observed effects. It has been generally assumed, that the biological activities originate from the flavonoids investigated, although they may have been biotransformed into one or more structurally quite different compound.

Investigations on *in vitro* metabolism of flavonoids have so far been limited. The synthetic flavonoids, α- and β-naphthoflavone, well known inducers and inhibitors of monooxygenase activities, have been shown to be extensively hydroxylated by cytochrome P450 [12]. The polymethoxylated flavone, tangeretin, has been shown to be demethylated, although the structures of the metabolites were not elucidated [13]. The isozyme mainly involved in the metabolism of these compounds was found to be CYP1A1, but CYP2B and CYP3A isozymes might also be involved in the metabolism of tangeretin. The first systematic investigation of the structural requirements for metabolism of flavonoid aglycons by the cytochrome P450 enzyme system was provided only a few years ago in a study, where 16 different flavonoids were incubated with rat liver microsomes [14]. It was shown, that the flavonoids naringenin, hesperetin, chrysin, apigenin, tangeretin, kaempferol, galangin, and tamarixetin all were extensively metabolised by Aroclor induced rat liver microsomes and to a minor extent by uninduced microsomes. All metabolites were isolated and their structures elucidated by LC-MS and ^{1}H NMR, and the identity of the metabolites was consistent with a general metabolic pathway leading to the corresponding 3',4'-dihydroxylated flavonoids either by hydroxylation or demethylation. Examples of the hydroxylation of galangin and kaempferol are show in Figure 2. No metabolites were detected from eriodictyol, taxifolin, luteolin, quercetin, myricetin, fisetin, morin or isorhamnetin. Structural requirements for microsomal hydroxylation appeared to be a single or no hydroxyl group on the B-ring of the flavan nucleus. The presence of two or more hydroxyl groups on the B-ring seemed to abolish further hydroxylation. Furthermore, the results indicated that demethylation only occurs in the B-ring, when the methoxyl group is positioned at the 4'-position as in tamarixetin, and not in the 3'-position as in isorhamnetin. The CYP1A isozymes were found to be the main enzymes involved in flavonoid hydroxylation, whereas other cytochrome P450 isozymes seemed to be involved in flavonoid demethylation [14]. These present findings using rat liver microsomes were later verified in mouse and human liver microsomal preparations, where identical metabolic patterns was observed [15] (Breinholt, unpublished observations).

Most of the research on *in vivo* metabolism and disposition of flavonoids in experimental animals were performed in the sixties and the seventies. These investigations have been thoroughly reviewed by Griffiths in 1982, Hackett in 1986 and latest by Hollman and Katan in 1997 [16–18]. Except for the ring cleavage products (see later), the only metabolites identified from flavonoid aglycons in rodents have been: isorhamnetin and tamarixetin (3'- or 4'-methoxyquercetin) from administration of quercetin [19], 4'-hydroxy- and 3',4'-dihydroxy-flavone from flavone, apigenin (3'-hydroxychrysin) from chrysin, and eriodictyol (3'-hydroxynaringenin) from naringenin [18]. Although several additional flavonoid aglycons have been investigated in rodents [16], their metabolites may have escaped identification due to insufficient analytical methods, and the overall picture of the structural requirements for biotransformation and bioavailability is still not complete.

In order to evaluate the relevance of the identified metabolic pathways *in vitro,* the metabolism of different flavonoids was also investigated *in vivo* in rats. Urine samples were collected during 24 h from rats dosed with naringenin, chrysin, apigenin, tangeretin, genistein or quercetin as previously described [20]. The urine was en-

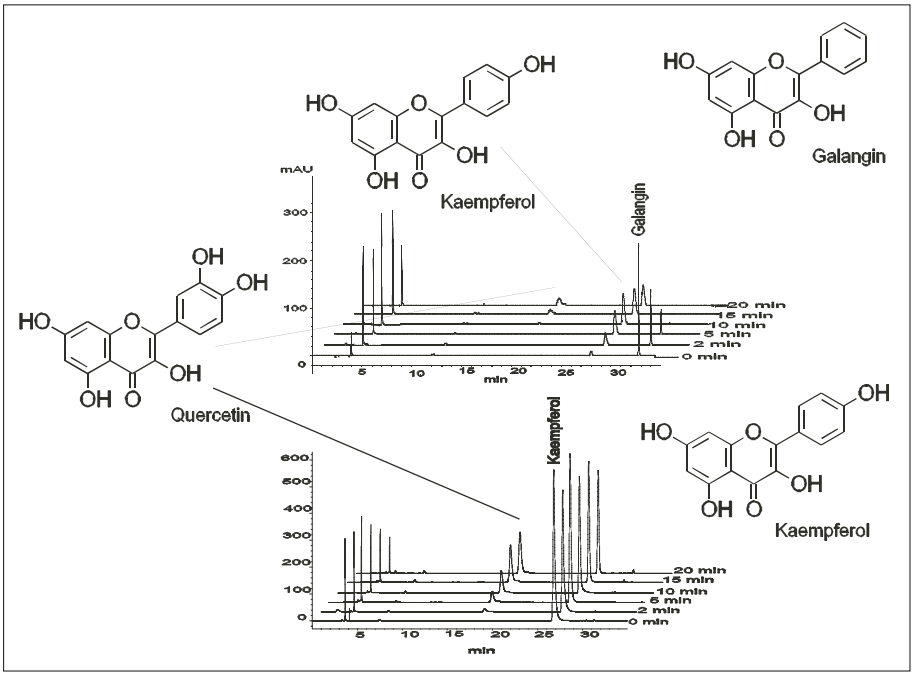

Figure 2: Examples of rat microsomal metabolism. Galangin is converted to kaempferol and quercetin by stepwise hydroxylation. The hydroxylation of kaempferol to quercetin confirms the endpoint of the *in vitro* metabolic pathway as the 3',4'-dihydroxylated derivative.

zymatically hydrolysed to release the flavonoid aglycons from glucuronic acid or sulphate conjugates. By means of LC-MS and proton NMR the presence of four previously reported metabolites were verified and quantitatively determined, along with the identification and quantification of ten metabolites of tangeretin which had not earlier been described *in vivo* [20]. The metabolites were either demethylated, hydroxylated or methylated derivatives of the parent flavonoids. The changes were found primarily to occur in the B-ring of the compounds investigated as also observed in the *in vitro* metabolic studies.

The amounts of flavonoids and metabolites with intact flavonoid structure excreted in urine were found to be highly dependent on the structure of the compound investigated. Parent compounds having a free 4'-OH group in the B-ring, were excreted with unchanged flavan nucleus in urine in larger amounts than the other compounds investigated, and the major metabolic products identified also contained a 4'-OH moiety, regardless of the remaining configuration of the flavonoid molecule (see Fig. 3). From these data it could be speculated that there is a systemic preference for the 4'-monohydroxylated structure of the flavonoids regardless of the configuration of the remaining part of the molecule.

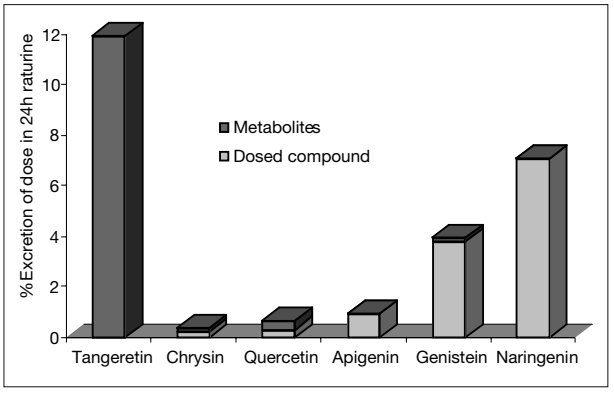

Figure 3: In vivo metabolism of dietary flavonoids. The bars show the amount of parent compound (B-ring structure shown above each bar) and of metabolites excreted in 24 h urine. The majority of the tangeretin metabolites (> 70%) had a free 4'hydroxyl group [20]. The metabolite of chrysin was identified as apigenin, whereas no metabolites of apigenin was observed, from quercetin, isorhamnetin and tamarixetin was identified, from genistein, small amounts of dehydrogenistein was found, and from naringenin minor amounts of eriodictyol could be identified [11].

Bioavailability of flavonoids – a controversial subject

Until Hollman *et al* in 1995 suggested that the intact flavonoid glycosides are absorbable [21], it was generally

accepted, that the flavonoid aglycons had to be liberated from the glycosides in the large intestine, prior to absorption [10]. It was thought, that the hydrophilic nature of the glycosides precludes absorption in the small intestine, and that the flavonoid β-glycosides resist intestinal hydrolysis. Consequently, flavonoid glycosides would pass unaltered into the large intestine, where they can be hydrolysed by micro-organisms to the free aglycon and sugar moiety (see Fig. 4) [22]. This was supported by the findings, that incubations of flavonoid glycosides with intestinal micro flora resulted in the release of the free aglycons, and that in germ free rats the flavonoid glycosides were excreted unchanged with faeces [17]. It was later shown, that human intestinal bacteria also are capable of hydrolysing flavonoid glycosides to the free aglycons and sugar moieties [23]. As seen in Figure 4, the micro-organisms in the intestine are also capable of degrading the flavonoid aglycons by ring fission of the C-ring, resulting in a variety of phenolic acids (e.g. phenylpropionic and phenylacetic acid derivatives) and phloroglucinol (from the A-ring) [17]. These phenolic acids can thus be absorbed form the gut, and further metabolised by either hydroxylation or methylation reactions followed by Phase II conjugations.

The intact flavonoid aglycons are also absorbed from the large intestine and enter the systemic circulation. The liver is the main organ for metabolism, and as described above, flavonoids can be hydrolysed or methylated by the cytochrome (CYP) 450 enzyme system [14, 17, 20], methylated by catechol-O-methyl transferase (COM) [24] and conjugated to glucuronic acid and sulphate esters by the Phase II enzymes as seen in Fig. 5.

Hollman *et al.* stated in 1995, that intact quercetin glycosides were absorbed in healthy ileostomy patients [21]. The use of ileostomy patients lacking the large intestine, should exclude bacterial hydrolysis and degradation of the flavonoid glycosides. After ingestion of fried onions providing 89 mg quercetin (as quercetin glucosides), excretion of quercetin in the ileostomy effluent and in urine was determined. Acid hydrolysis of the samples was used prior to analysis to liberate the quercetin aglycon from conjugates, hereby facilitating the determination. Thus, neither the excreted amounts nor the plasma levels of intact quercetin glycosides were determined. The absorption was defined as the oral intake minus the amount excreted in the ileostomy effluent, which gave absorption of 52% quercetin glycosides from onions, whereas only 0.5% of

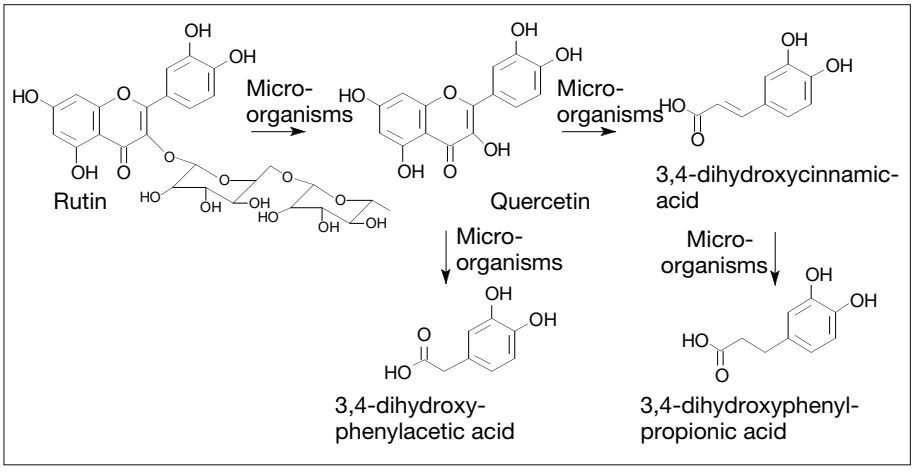

Figure 4: Microbial hydrolysis of flavonoid glycosides and degradation of flavonoid aglycons in the colon. Rutin is shown as an example.

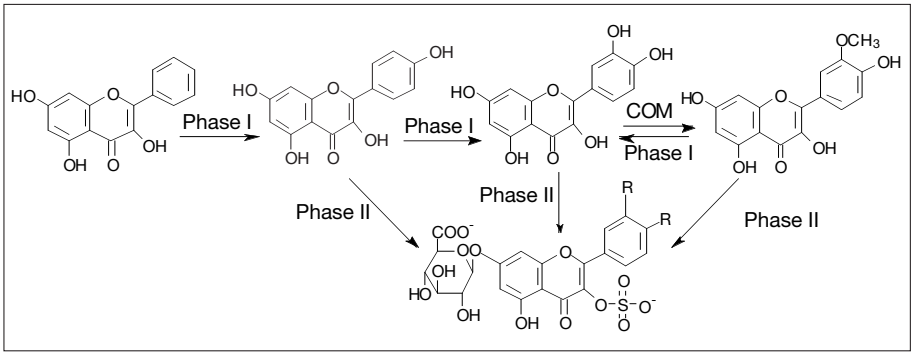

Figure 5: Metabolic pathways for systemic biotransformation of flavonoid aglycons. Major routes of metabolism are indicated with thick arrows.

the dose was detected in urine. The use of the ileostomy model has later been subject to debate, due to the possibility, that the micro flora normally present in the large intestine has settled in the lower part of the small intestine, resulting in hydrolysis of the glycosides and in ring cleavage of the flavonoid aglycons.

Since the study by Hollman *et al* (1995) in ileostomy patients, much effort has been done to demonstrate the absorption of intact glycosides. However, the majority of these studies have not been able, convincingly, to show the absorption of intact flavonoid glycosides in humans by determining them as such in plasma or urine. In a study on naringin from grapefruit juice by Fuhr and Kummert, 1995 [25], no naringin could be detected in urine; only the aglycon naringenin was determined. Also a study on diosmin, the 7-rutinoside of diosmetin (4'-OMe-Luteolin) failed to detect any diosmin in urine or plasma after oral administration of diosmin to healthy volunteers [26]. Only the aglycone diosmetin was detected as glucuronic and sulphate conjugates. Furthermore, no apiin (apigenin-7-apiosylglycoside), the major apigenin glycoside in parsley, could be detected in the urine after parsley consumption [27].

However, a few recent studies claim to have determined intact flavonoid glycosides in human plasma, although final verification of the identity of the compounds e.g. by mass spectrometry was not performed. Thus Paganga *et al* (1997) have published that they could detect flavonoid glycosides in high concentrations in plasma of normal unsupplemented humans by using a simple HPLC-UV analysis [28]. However, a comparison of the plasma levels reported in these subjects with those found in the onion supplementation studies would imply, that these subjects had consumed more than 0.5 kg of fried onions just prior to the plasma sampling to reach these high plasma concentrations. In addition, Aziz *et al* (1998) have reported that quercetin 4'-glucoside and isorhamnetin-4'-glucoside was determined in plasma after onion consumption. However, again no conclusive identification of the glucosides was performed.

In contrast to these studies, Walgren and co-workers has shown, that human intestinal caco-2 cell monolayers used as a model for human intestinal absorption was unable to absorb quercetin-mono or di-glucosides [29]. Furthermore, a recent study trying to redo Hollmans observations proved, that only free quercetin was left in the ileostomy fluid [30]. All attempts to determine quercetin glucosides in plasma failed, and only trace amounts of quercetin could be determined in plasma. The authors concluded, that since only 19.5–35.2% of quercetin was recovered in the ileostomy bags, 64.5–80.7% of the quercetin dose was absorbed as the free aglycone and not as the intact glycosides.

In another human study using onions as the quercetin source, Moon and colleagues were unable to determine quercetin glycosides in plasma by a very sensitive method using electrochemical detection, whereas quercetin was detectable, supporting the fact that no intact glycosides were absorbed [31].

In the studies on bioavailability of quercetin performed in 1995–1997 by Hollman and co-workers [21, 32, 33], it was observed that the amount of quercetin absorbed from quercetin *glucosides* was higher than that from the free aglycon and other glycosides with different sugars than glucose as the terminal sugar, such as e.g. rutin. Furthermore, quercetin was also more rapidly absorbed when given as quercetin glucosides, with peak plasma levels already between 30–60 min, whereas other quercetin glycosides from apple showed peak levels at 2.4 h and pure rutin at 9.3 h [33]. This was not in accordance with the general concept, that the glycosides have to pass unaltered all the way to the large intestine prior to hydrolysis and absorption, as this would have required a much longer transit time than the 30–60 minutes observed for the quercetin glucosides. Thus, the glucosides must be absorbed already in the small intestine. This was supported by the findings by Day *et al* in 1998 [34], demonstrating that both human and rat small intestine have β-glucosidase activities capable of hydrolysing flavonoid glucosides. Furthermore, studies by Gee *et al* using isolated preparations of rat small intestine investigated the possible involvement of the sodium dependent glucose transporter SGLT1 in the absorption of quercetin monoglucosides [35, 36]. These studies confirmed a more rapid absorption of the 3-or 4'-monoglucosides of quercetin compared to the 3,4'-diglucoside or quercetin itself. The authors conclude that there probably are two mechanisms for the transport of the quercetin monoglucosides i) SGLT1 transport the intact quercetin glucoside into the epithelial cells, where the glucose moiety are released by β-hydroxylases, and the free quercetin is then absorbed, or ii) the extra cellular enzyme LPH (lactase phlorizin hydrolase) that also was demonstrated to be able to hydrolyse quercetin glucosides [37], deglycosylate the quercetin glucoside, prior to the passive diffusion of the aglycon into the epithelial cells. Furthermore, the study verified that no intact quercetin glycosides were able to cross the intestinal epithelium, and only quercetin or quercetin glucuronides were determined after transfer. The scheme in Figure 6 shows the possible routes for deglycosylation and absorption of flavonoid glucosides as it is thought to occur in the small intestine. Flavonoids with other sugars attached than glucose, pass unaltered down to the large intestine, where the micro flora can cleave the glycosidic bond as reviewed by Kühnau in 1976 [10].

Very recently, both Hollman and Day have demonstrated the difficulties in distinguishing between the

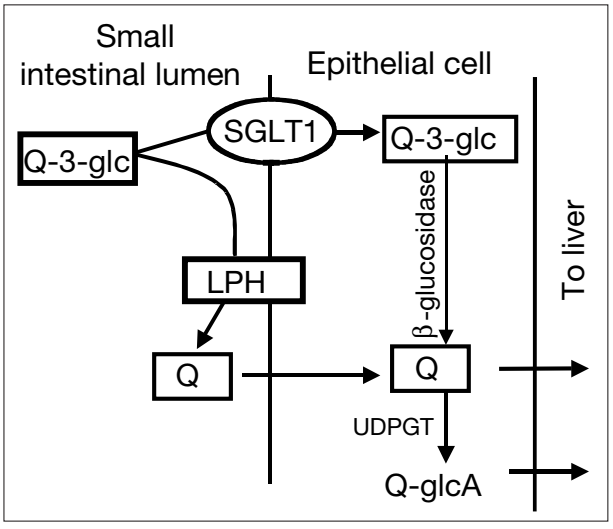

Figure 6: Scheme showing the routes of deglucosylation and absorption of flavonoid glucosides in the small intestine. Other flavonoid glycosides will pass unaltered down to the large intestine. SGLT1: Na+ dependent glucose transporter; LPH: lactase phlorizin hydrolase. Redrawn from Gee *et al,* 2000 [35] with permission.

flavonoid glycosides and glucuronides when performing chemical analyses of urine or plasma samples. Both types of conjugates have UV-absorption spectra that are almost identical with quercetin itself [38], and very similar retention times even with the most specific gradient systems. Using a very sensitive and specific HPLC method with coulometric detection and confirmation by LC-MS Hollman showed that only the glucuronides and not the glucosides are present in human plasma after consumption of pure quercetin-3-glucoside or quercetin-4'-glucoside [39].

Flavonoids as biomarkers

During the last few years, the number of human intervention studies with flavonoids has increased extensively. As seen in Table II, most of the studies employ large doses of flavonoids, some of them quite unrealistic in relation to the median daily intakes of these flavonoids in countries such as Denmark or Holland (see Table I). An important issue regarding the potential health effects of dietary

Table II: Human intervention studies on flavonoids

Flavonoid	Source	Flavonoid dose (mg)	No. of subjects	Cmax in plasma (μM)	Time to reach Cmax (h)	Urinary excretion (%, 24h)	Reference
Flavonols							
Quercetin glucosides	Onion	89	9	–	–	0.3 (13h)	[21]
Quercetin-rutinoside	Pure comp.	100	9	–	–	0.07 (13h)	[21]
Quercetin	Pure comp.	100	9	–	–	0.12 (13h)	[21]
Quercetin glucosides	Onion	68	9	0.74	< 0.7	1.39	[33]
Quercetin glycosides	Apple	98	9	0.3	2.5	0.44	[33]
Quercetin-3-O-rutinoside	Pure comp.	100	9	0.3	9.3	0.35	[33]
Quercetin glucosides	Onion	391.8	5	1.5	1.9	0.8	[48]
Quercetin glucosides	Apple/black currant juice	4.8, 6.4, 9.6[a]	5	–	–	0.47[b]	[49]
Quercetin-4'-O-glc	Pure comp.	144	9	3.5	< 0.5	–	[50]
Quercetin-3-O-rutinoside	Pure comp.	189	9	0.18	6	–	[50]
Quercetin-3-O-glc	Pure comp.	151	9	5.0	0.6	–	[51]
Quercetin-4'-O-glc	Pure comp.	154	9	4.5	0.45	–	[51]
Quercetin glucosides	Onion	100	12	7.6	0.68	4.4 (48h)	[52]
Quercetin-4'-O-glc	Pure comp.	100	12	7.0	0.70	3.3 (48h)	[52]
Quercetin glycosides	Buckwheat tea	200	12	2.11	4.32	0.8 (48h)	[52]
Quercetin-3-O-rutinoside	Pure comp.	200	12	1.1	6.98	0.7 (48h)	[52]
Flavones							
Chrysin	Pure comp.	200	7	6.11	1	1–7%	[53]
Apigenin glycosides	Parsley	50	14	–	–	0.58	[27]
Flavanones							
Naringenin-glycoside	Pure comp.	500	1	–	–	4.9	[54]
Hesperetin-glycoside	Pure comp.	500	1	–	–	3.0	[54]
Isoflavones							
Genistein	Soy milk	19	12	0.74	–	19.8	[55]
Daidzein	Soy milk	25	12	0.79	–	5.3	[55]

flavonoids in humans is to evaluate the effects of these compounds at realistic dose levels. However, besides the difficulties in distinguishing between different flavonoid conjugates as discussed above, merely the detection of the flavonoid aglycons in blood and urine samples (after hydrolyses of the conjugates) is often a very difficult analytical task, due to the low amounts present. The fact that flavonoids bind to plasma proteins and co-elute with a number of interfering compounds in urine further complicates these analyses. This often leads to the choice of large flavonoid doses in intervention studies, to ease the detection of the compounds in the biological samples, whereby the study looses some of its physiological relevance to normal dietary intakes.

In order to analyse low levels of flavonoids in human urine and plasma samples, a very selective and sensitive LC-MS assay was recently developed in our laboratory [40]. By this methodology we are able to analyse for 12 different dietary flavonoids simultaneously in urine from human subjects.

The methodology was applied on urine samples from 94 subjects on their habitual diet and after eating a diet either high or low in fruits, berries and vegetables for 6 weeks [41]. In the intervention period with controlled dietary intakes, we found highly significant differences between the urinary excretion of all flavonoid aglycons included in the assay on the high fruit and vegetable diet as compared to the low. None of the included flavonoid glycosides were detected. Furthermore, the amount of flavonols (quercetin, kaempferol, isorhamnetin and tamarixetin) excreted in urine was much lower than the amounts of the citrus flavanones (naringenin and hesperetin) and of the chalcone phloretin, found in high amounts in apples. However, the diet did not contain higher amounts of citrus fruits or apples than of other fruits, berries and vegetables, so it seems as if these flavonoids, phloretin and the citrus flavanones are excreted and thus presumably also absorbed to a higher extent than the flavonols.

The correlation between the habitual intake of fruits and vegetables as determined by three days of food registration, with the total excretion of flavonoids was 0.35, $p < 0.001$, which is similar to what has been found using plasma carotenoids as a marker of fruit and vegetable intake [42]. Again the excretion of the flavonols was much lower than of the other flavonoids included in the assay, and quercetin showed the poorest correlation with the habitual intake of fruits and vegetables. Thus, this study suggests, that other flavonoids from the diet may be more important to our health than the flavonols, and that it is quite unlikely that the beneficial effects of fruits and vegetables is due to quercetin, given this poor correlation. Other studies are however, necessary to confirm these observations, before definitive conclusions can be drawn.

Conclusion

The present knowledge indicate, that although the *in vitro* metabolism of flavonoids by CYP450 is extensive, the *in vivo* metabolism by these Phase I enzymes is less important. *In vivo,* the flavonoids having only one free hydroxyl group in the B-ring are mainly excreted unchanged, whereas flavonoids with none or two hydroxyl groups (3'4'-OH) are metabolised by some extent to the derivatives with one free hydroxyl group, by hydroxylation or methylation reactions. This suggests a systemic preference for 4'-hydroxylated flavonoids, which is supported by the findings of a higher excretion of the citrus flavanones and of phloretin in humans on their habitual diet.

The evidence for absorption of intact flavonoid glycosides is weak. An emerging amount of data showing e.g. β-glucosidase activities in the small intestine, the absence of intact glycosides in plasma or urine, strongly indicate that only the free aglycons of the flavonoids are being absorbed.

The present knowledge also points to a higher and more rapid absorption of flavonoids originating from glucosides than from other glycosides or than the free aglycons itself, probably due to the mechanisms outlined in Fig. 6. Thus, dietary sources containing high amounts of glucose bound flavonoids are thus more likely to have potential health protective effects than foods with other flavonoid glycosides.

An issue that still remains to be resolved is the fraction of the flavonoids that actually are being absorbed. There are some indications from the ileostomy studies of an absorption of 50–80% of an oral quercetin dose, however these studies have shown very low quercetin levels in urine and plasma [21, 30]. Although the extent of bilary excretion of flavonoids is by in large unknown in humans, it seems unlikely, that such a high absorption is not reflected in the plasma level of quercetin. A possible explanation could be tissue storage or yet unknown metabolites escaping detection. Otherwise, the micro flora normally present in the large intestine may have settled in the lower part of the small intestine in the ileostomy patients, resulting in hydrolysis of the quercetin glycosides and in ring cleavage of the aglycon.

Using new and sensitive methodologies it is now possible to determine low levels of flavonoids present in urine or plasma from normal subjects on their habitual diet [40]. This may be a very strong tool to provide more insight in the health protective effects of flavonoids, since e.g. flavonoid excretion levels could be correlated to disease incidence in cohort or case control studies. Furthermore, with the uncertainties related to food registration in epidemiological studies a valid biomarker for fruit and vegetable intake is greatly warranted. The flavonoids are

present in almost all fruits and vegetables, and they may therefore be useful as a marker for fruit and vegetable consumption as indicated by recent findings. Until now, research has mainly focused on the flavonol quercetin, due to its antioxidant potency and early reports showing a relation between quercetin intake and decreased risk of heart disease [43]. However, new studies suggest that other flavonoids than the flavonols may be more important to human health, since they seem to be more bioavailable, and thus have a higher potential to protect against various mechanisms involved in disease development.

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