Bioavailability of rutin and quercetin in rats

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Abstract Quercetin is a powerful antioxidant which is widely distributed in edible plants, mainly as glycosides such as rutin. It has been reported to be absorbed in mammals, but its metabolism needs further investigation to evaluate its possible physiological effects. We compared the evolution of the absorption of quercetin and rutin in rats fed with supplemented diets. Rutin was absorbed more slowly than quercetin because it must be hydrolysed by the cecal microflora, whereas quercetin was absorbed from the small intestine. Conjugated derivatives of quercetin, and its methylated forms isorhamnetin and tamarixetin, were recovered in plasma from rats receiving the two kinds of experimental diets after the first meal, but after 10 days, no traces of tamarixetin were detected anymore. The rate of elimination of quercetin metabolites seems very low, and high plasma concentrations are easily maintained with a regular supply of quercetin or rutin in the diet.

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Key words: Quercetin; Rutin; Flavonol metabolism; Dietary antioxidant; (Rat)

1. Introduction

Flavones and flavonols are secondary metabolites of plants which are regularly consumed by humans. Depending on dietary habits or countries, the daily intake has been estimated between 3 and 70 mg, essentially quercetin (60–75%) [1,2]. The major sources, which differ somewhat between countries, are tea, wine, onions, apples or berries; but fruits and vegetables in general also provide noticeable amounts. Flavonoids are non-energetic and are not considered as indispensable as are vitamins, yet the supply of some of them, or maybe of a complex mix, may have a positive effect on health. Two recent epidemiological studies support the view that flavones and flavonols protect against cardiovascular mortality [1,2]. Also, they have been shown to inhibit the growth of various cancer cell lines in vitro, and to reduce tumor development in experimental animals [3]. A lot of studies have focused on the antioxidant effect of flavonoids, since it is now established that antioxidants are involved in the prevention of various pathologies, but their capacity to chelate metal ions or to influence enzymatic activities could also be involved in their metabolic effects [4,5]. In addition to those experiments performed to determine the different effects of flavonoids on cellular constituents, we need to examine their absorption and metabolism to evaluate their real physiological impact.

Flavonoids usually occur as glycosides in dietary plants. Thus, the question arises as to whether glycosylated forms could be absorbed to the same extent as aglycone forms from the digestive tract. It is generally considered that when flavonols are supplied as glycosides in the diet, they are first hydrolyzed by the digestive microflora before being absorbed [6]. However, this has been recently questioned by Hollman et al. [7] who reported that, in ileostomy patients, quercetin glycosides from onions were more readily absorbed than the aglycone form. The present study was designed to compare the absorption of rutin and quercetin in rats, and to examine the influence of adaptation to a diet containing quercetin on its absorption and metabolism.

2. Materials and methods

2.1. Chemicals

Quercetin, rutin (quercetin-3β-D-rutinoside) and β-glucuronidase/sulfatase were purchased from Sigma (St. Louis, MO). Diosmetin, isorhamnetin and tamarixetin were from Extrasynthese (Genay, France).

2.2. Animals and diets

Ninety male Wistar rats (IFFA-CREDO, L'Arbresle, France) weighing approximately 170 g were randomly divided into three groups. Rats were housed, two per cage, in temperature-controlled rooms (22°C), with a dark period from 8:00 to 20:00 h and access to food from 08:00 to 16:00 h. Sixty rats called 'non-adapted rats' were fed semi-purified diets: a control diet for 5 days (73% wheat starch, 15% casein, 6% mineral mixture, 1% vitamin mixture, 5% corn oil), and then a single experimental meal, containing either 0.2% quercetin or 0.4% rutin. The other 30 rats, called 'adapted rats' were fed the 0.2% quercetin diet for 10 days; the daily food intake (22.5 g/day) and weight gain (6.1 g/day) were unaffected by the flavonoid supplementation. Animals were maintained and handled according to the recommendations of the Institutional Ethic Committee (INRA), in accordance to the decree No. 87-848.

2.3. Sampling procedure

At 2, 4, 8, 12, and 24 h after the beginning of the experimental meal, 6 rats of each group were killed. They were anesthetized with sodium pentobarbital (40 mg/kg body weight). Blood was withdrawn from the portal vein (2 ml) and from the abdominal aorta (2 ml) into heparinized tubes. Plasmas were acidified with 10 mM acetic acid to prevent losses of flavonoids since the pH of plasma increases with time, due to decomposition of bicarbonate, and flavonoids are unstable at a pH higher than 7.4. After blood sampling, cecal contents were drained by finger pressure into microfuge tubes and immediately frozen.

For portal blood flow measurement, bromosulfophtalein in saline (5 mmol/l) was infused at 50 μl/min into a mesenteric vein: dilution of the marker in the portal vein allows determination of the blood flow.

2.4. HPLC analysis

Plasmas were spiked with 25 μmol/l diosmetin, used as internal standard, and acidified (to pH 4.9) with 0.1 volume of 0.58 mol/l acetic acid solution. Solutions were treated for 30 min at 37°C in the presence of 5×10^6 units/l β-glucuronidase and 2.5×10^5 units/l sulfatase, then treated with 8.5 volumes of acetone and centrifuged. Supernatants were evaporated to a volume equivalent to twice the initial volume of plasma. The recovery of this method has been checked (>85%) using pure flavonoids (quercetin, diosmetin, iso-

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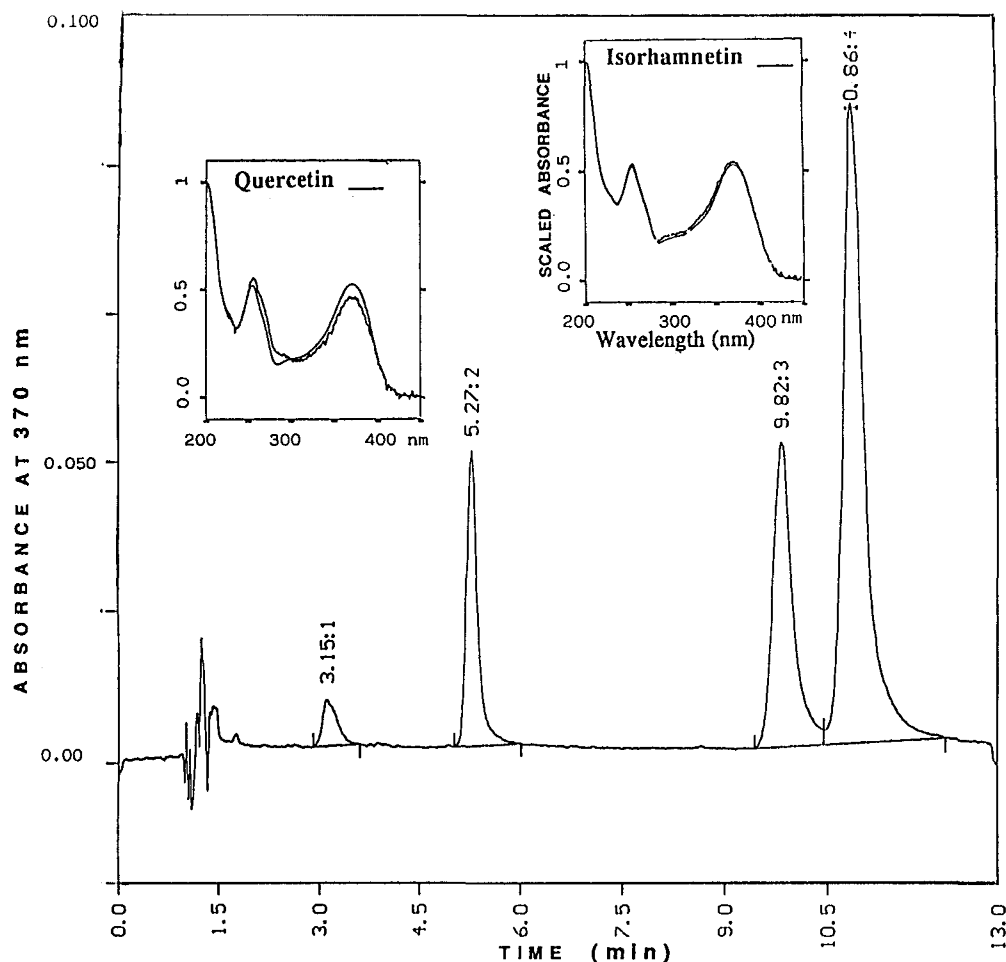

Fig. 1. HPLC chromatogram from a rat adapted to a quercetin diet, after enzymatic treatment to hydrolyze the conjugated metabolites. Peak are as follows: (1) unknown, (2) quercetin, (3) diosmetin (internal standard), (4) isorhamnetin. In inset is shown the scan of peaks 2 and 4, using a diode array detector: the spectrum of the plasma peak was compared to that of an authentic standard (smooth scan).

rhamnetin, tamarixetin) in plasma. For analysis, 20 µl of each preparation was injected onto a 150×4.6 m Hypersil BDS C18-5µ column (Life Sciences International, Cergy, France). The mobile phase consisted of 73% water/H_3PO_4 (99.5:0.5) (solvent A) and 27% acetonitrile (solvent B), with flow rate 1.5 ml/min, and the UV detector was set at 370 nm. A Beckman 168 diode array detector was also used to record the absorption spectra of flavonoids, and compared to authentic standards.

Cecal contents were extracted with 9 volumes of acetone/HCl (50 mM) then briefly sonicated. Supernatants were analysed with the same HPLC system as for plasma, but the conditions of elution were different: with 80% A/20% B until 2 min, then in gradient conditions to 40% A/60% B within 8 min, next isocratically up to 12 min. These conditions allowed a determination of rutin (tr = 3.4 min) and quercetin (tr = 8 min).

3. Results

Fig. 1 shows a representive chromatogram of plasma after enzymatic hydrolysis, from a rat adapted to a quercetin diet. The two major identified peaks were quercetin (Tr = 5.27 min) and isorhamnetin (Tr = 10.86 min). For quantification, diosmetin (Tr = 9.82 min) was used as internal standard. The inset shows the absorbance spectrum recorded on a diode array detector: it appears that the spectra of the sample and of the standard are perfectly superimposable (Similarity Index: SI quercetin = 0.985, SI isorhamnetin = 0.992). In some samples, tamarixetin (Tr = 11.45) was also detected, and it also displayed a high SI. In rats receiving a single experimental meal, the kinetics of intestinal absorption of rutin were delayed, relative to that of quercetin (Fig. 1A). 2 h after the beginning of the meal, a significant concentration of quercetin metabolites (16 ± 6 µmol/l) was recorded in plasma from rats fed the quercetin diet. By contrast, no trace of quercetin metabolites was detected at this time in the plasma from rats fed a rutin diet. With this diet, measurable concentrations were obtained only 4 h after the beginning of the meal. Later, plasma concentrations of quercetin metabolites were higher in rats fed the quercetin diet than in those fed the rutin diet; 24 h after the beginning of the meal, high levels were still present in plasma for the two types of diet (35 ± 2 and 51 ± 3 µmol/l, for the rutin and quercetin group respectively).

The absorption of quercetin was measured by arterio-venous differences (portal vein-abdominal aorta concentration) (Fig. 1B). After the beginning of the meal, the portal blood flow increased progressively, from 12 ml/min at 2 h to 14 ml/min at 12 h, without significant influences of the diet. During the early digestion period (2 h), quercetin absorption was detected only in rats fed the diet containing 0.2% quercetin. Later, in this group, the absorption flux was high (about 50 nmol/min) and relatively constant between 4 h and 12 h after the beginning of the meal. In rats fed the rutin diet, the ab-

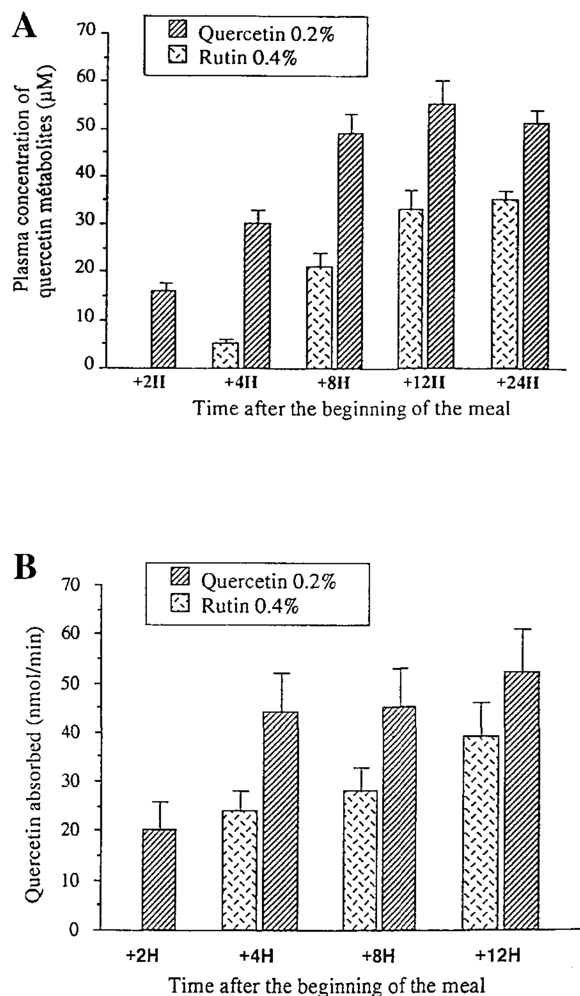

Fig. 2. Evolution of the plasma concentration of quercetin metabolites (A) and of the absorption of quercetin (B) in rats receiving a single meal containing 0.2% quercetin or 0.4% rutin. Values are means ± S.E.M. ($n = 6$).

sorption flow was markedly lower than in rats fed the quercetin diet, but it increased significantly between 4 and 12 h.

Two hours after the beginning of the experimental meal, neither quercetin nor rutin were detected in the cecal contents (data not shown); thereafter, the rutin or quercetin which was not absorbed in the small intestine accumulated in the cecum. With the rutin diet, the two forms (rutin and quercetin) were recovered in the cecum (Fig. 2) but, at 4 h, the concentration of the aglycone was still lower than that of the glycoside. This was reversed from 8 h after the beginning of the meal, and at 24 h, only quercetin could be found in the cecum. In rats fed the quercetin diet, quercetin accumulation was similar to the sum of rutin+quercetin in rats fed the rutin diet.

In Fig. 3, the evolution of quercetin metabolites during a 24 h period was followed in rats fed a 0.2% quercetin diet, either receiving it as the first experimental meal or after 10 days of adaptation. In non-adapted animals, there was a progressive accumulation of metabolites in the plasma, then a plateauing concentration (about 50 µmol/l) was observed (Fig. 3A). In contrast, the concentration of plasma metabolites was poorly affected during the digestion period in rats adapted to the 0.2% quercetin diet. In these animals, the presence of a relatively high concentration of quercetin metabolites in the plasma might depress the efficiency of flavonol intestinal absorption. Thus, whatever the sampling time (4, 8 and 12 h after the beginning of the experimental meal), the intestinal absorption of quercetin was higher in non-adapted rats than in adapted rats (Fig. 3B).

In the plasma from non-adapted rats, after deconjugation, quercetin was recovered in three forms: quercetin, 3′-O-methyl derivative (isorhamnetin), 4′-O-methyl derivative (tamarixetin). 2 h after the beginning of the 0.2% quercetin experimental meal, quercetin was the major circulating form, then its level plateaued at 10–12 µmol/l (Fig. 4A). Also, there was a progressive accumulation of isorhamnetin, and at 24 h the isorhamnetin/quercetin ratio was about 4. The concentration of tamarixetin increased during the first 12 h, then it progressively declined and after 24 h, this derivative was no more detected in plasma. Similar changes in quercetin and its methyl derivative concentrations were observed with rats fed the 0.4% rutin meal, but the magnitude of the changes was lower than before (Fig. 4B). In plasma from rats adapted to the quercetin diet, the isorhamnetin/quercetin ratio was always near 5, and tamarixetin was found in non-measurable concentrations (Fig. 5).

4. Discussion

The present study demonstrates noticeable differences in the rate of absorption and of appearance of quercetin in blood plasma, when provided as rutin or as quercetin. When rats were fed semi-purified diets containing either forms of this flavonol, quercetin was more rapidly absorbed than the aglycone moiety of rutin. Furthermore, quercetin appeared in detectable amounts in the blood circulation, long before the bolus had reached the cecum. By contrast, in rats fed a rutin diet, quercetin metabolites could be found in blood plasma only after a significant breakdown of rutin to quercetin by the cecal microflora. Quercetin concentrations measured in cecal contents were lower in rats fed a rutin diet than in those fed a quercetin diet. Thus, with a quercetin diet, the possibilities of absorption of this flavonol appear greater than with a rutin diet, since quercetin is available for digestive absorption both in the small intestine and in the large bowel. During the post-absorptive period, whilst absorption in the small intestine was declining (due to transit of digesta and dilution of remaining digesta by endogenous materials), flavonol absorption took

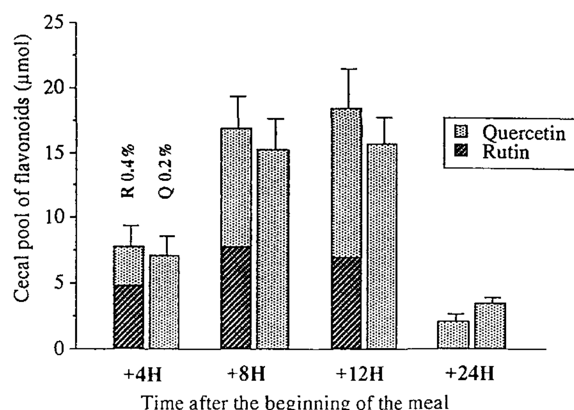

Fig. 3. Evolution of the cecal pools of rutin and quercetin in rats receiving a single meal containing 0.2% quercetin or 0.4% rutin. Values are means ± S.E.M. ($n = 6$).

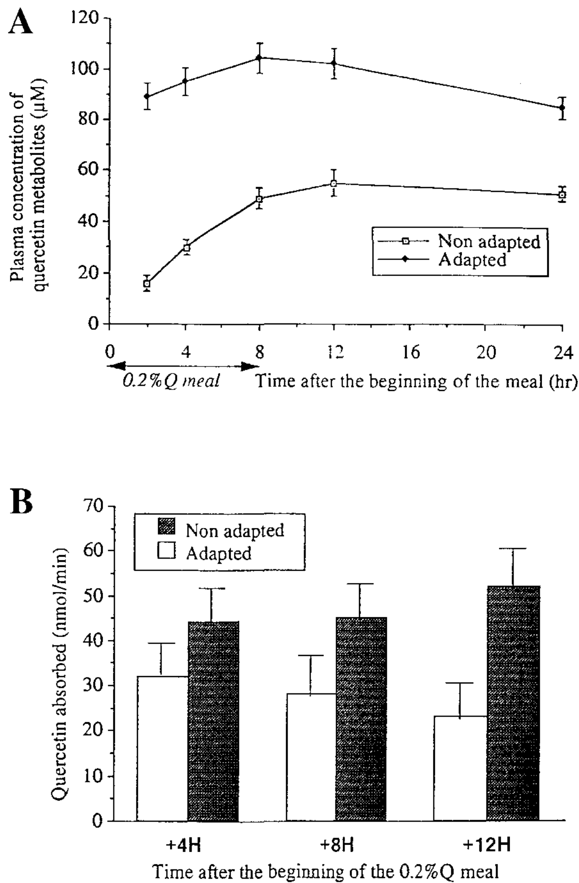

Fig. 4. Evolution of the plasma concentration of quercetin metabolites (A) and of the absorption of quercetin (B) in rats adapted or not to a 0.2% quercetin diet. Values are means ± S.E.M. ($n = 6$).

place in the large intestine, and this phenomenon was observed with the rutin diet as well as with the quercetin diet. During this period (12–24 h after the beginning of food intake), rutin was completely hydrolyzed to quercetin. After 24 h, some quercetin was still present in the cecum, which suggests that its absorption may spread over the total dark/light cycle. These data indicate that, in rats, rutin in contrast to quercetin, is not absorbed from the small intestine. However, it has recently been reported, in ileostomized subjects, that mono- and di-glycosyl derivatives of quercetin could also be absorbed by the small intestine [7]. Whether this reflects species differences, or corresponds to modifications of the digestion in ileostomized patients (for example bacterial colonization) is still not clear. Nevertheless, in the same study, rutin was less effectively absorbed than quercetin. Thus it is conceivable that the relatively high absorption of quercetin glycosides from onions is not due to glycosylation, but rather to factors present in complex foodstuffs.

Whatever the absorption site (small intestine or cecum), it remains to be determined whether quercetin could be transferred across digestive mucosa by a purely passive process (because this compound is not typically lipophilic), or by a carrier mediated process. There was apparently a less efficient absorption of quercetin in rats maintained previously on a flavonol diet. Thus, as with other micronutrients, the existence of a substantial steady state level of quercetin could lead to a decrease in the rate of digestive absorption.

Previous investigations have established that: (i) about 80% of circulating plasma quercetin units is present as a methoxy derivative (isorhamnetin) and (ii) flavonol metabolites are circulating in plasma as conjugated derivatives [8]. The capacities of flavonol biotransformations, such as catechol-O-methyl transferase or various transferases catalyzing sulfo- or glucuro-conjugations, are particularly active in the liver, which is considered to be the chief organ for the metabolism of dietary flavonols [9]. However, the existence of a conjugation activity in the intestinal wall could not be ruled out. In a previous paper, we have shown that the liver exhibits the capacity to O-methylate quercetin on its 4′ hydroxyl (yielding tamarixetin), since this metabolite is found in relatively high concentrations in the bile [8]. In the present study, the presence of tamarixetin has been observed only in plasma from rats adapted to a flavonol-free diet before receiving quercetin in an experimental meal, whereas this compound was not found in plasma from rats adapted to the quercetin diet. A more effective release of isorhamnetin into the hepatic venous blood might account for its prevalence in systemic blood plas-

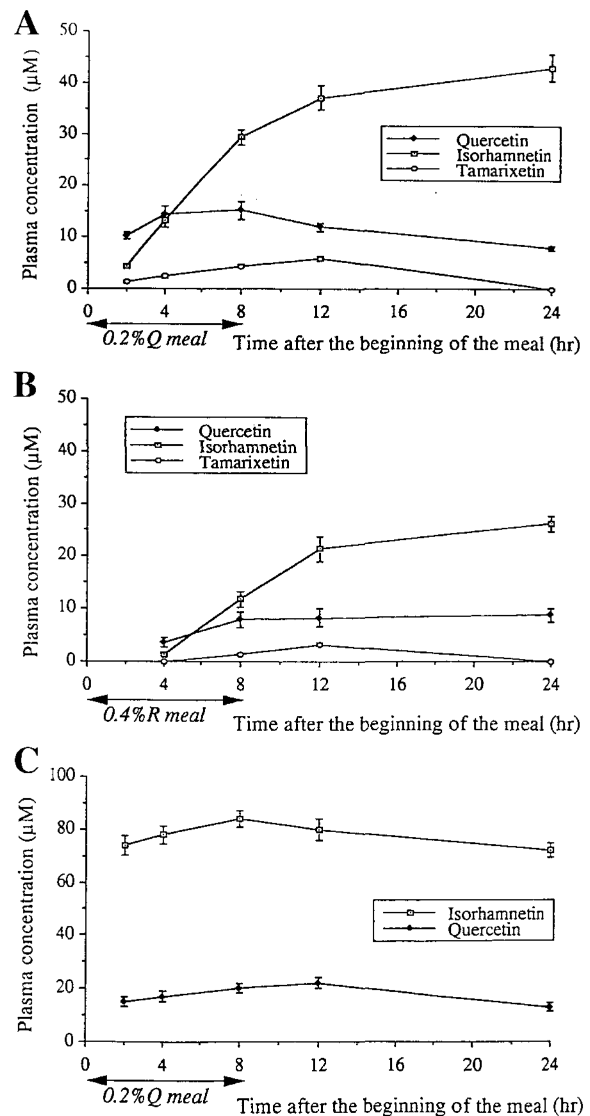

Fig. 5. Evolution of the plasma concentration of quercetin metabolites in rats receiving a single meal containing 0.2% quercetin (A), or 0.4% rutin (B), or adapted for 10 days to a 0.2% quercetin diet (C). Values are means ± S.E.M. ($n = 6$).

ma, but there is no satisfactory hypothesis to explain why tamarixetin is present only in the plasma of non-adapted rats. Conjugation and/or methylation, which probably play a role in lowering the reactivity of quercetin which arises from the presence of two -OH on the B-ring, or the presence of -OH in 3 and 5 position at the vicinity of the 4-oxo group. In this view, it is noteworthy that the concentration of quercetin conjugates seems maximal at 20 μmol/l in rats adapted to a flavonol-containing diet, and at a lower value concentration in unadapted rats. Between 12 h and 24 h after the beginning of the meal, slight changes in plasma quercetin metabolites were observed in adapted rats; thus, elimination of these compounds may be compensated for by some digestive absorption still occurring during this period.

The dietary level of quercetin used in the present experiment (2 g/kg dry diet) is much higher than in human food, in which the level may be about 10–200 mg of flavonols+flavones/kg dry weight, depending on the type of plant foods consumed. This explains why quercetin is found in much lower concentrations in human plasma than in rat plasma; Hollman et al. [10] have found a plasma concentration of about 0.65 μmol/l quercetin after consumption of a meal containing 150 g of fried onions (equivalent to 64 mg of pure quercetin). This value has been obtained after acid hydrolysis, which does not provide information about the concentration of the non-conjugated form and O-methylated quercetin would not be detected with that procedure.

According to Vinson et al. [11], free quercetin is one of the most potent antioxidant to inhibit lipid peroxidation ($IC_{50} \approx 0.22$ μmol/l). Thus, concentrations of 0.65 μmol/l of quercetin found by Hollman et al. [10] should correspond to an antioxidant capacity. However, quercetin is probably present mainly as conjugated forms, extensively bound to albumin [12], and the actual antioxidant potency of these forms is still uncertain, and possibly lesser than that of the free forms.

In conclusion, studies of the biological activity of dietary flavonols appear particularly complex since it is necessary to take into consideration a group of metabolites (phenolic acids arising from microbial breakdown, conjugated and methylated derivatives) instead of merely considering the free quercetin form, which is probably present in very low concentrations (a few nmol/l) in human plasma. Furthermore, there may be inter-individual variability in the metabolism of flavonols, as shown previously for isoflavones [13].

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References

[1] M.G.L. Hertog, D. Kromhout, C. Aravanis, H. Blackburn, R. Buzina, F. Fidanza, S. Giampaoli, A. Jansen, A. Menotti, S. Nedeljkovic, M. Pekkarinen, B.S. Simic, H. Toshima, E.J.M. Feskens, P.C.H. Hollman, M.B. Katan, Arch. Intern. Med. 155 (1995) 381–386.
[2] E.B. Rimm, M.B. Katan, A. Ascherio, M.J. Stamper, W.C. Willett, Ann. Intern. Med. 125 (1996) 384–389.
[3] C. Manach, F. Régérat, O. Texier, G. Agullo, C. Demigne, C. Rémésy, Nutr. Res. 16 (1996) 517–544.
[4] N.C. Cook, S. Samman, J. Nutr. Biochem. 7 (1996) 66–76.
[5] Middleton Jr., E. and Kandaswami, C. In: Harborne, J.B., Ed. The Flavonoids: Advances in Research Since 1986. London: Chapman and Hall, 1994:619–652.
[6] J. Kühnau, World Rev. Nutr. Diet. 24 (1976) 117–191.
[7] P.C.H. Hollman, J.H.M. de Vries, S.D. van Leeuwen, M.J.B. Mengelers, M.B. Katan, Am. J. Clin. Nutr. 62 (1995) 1276–1282.
[8] C. Manach, O. Texier, F. Régérat, G. Agullo, C. Demigné, C. Rémésy, J. Nutr. Biochem. 7 (1996) 375–380.
[9] Griffiths, L.A. In: Harborne, J.B. and Mabry, T.J., Eds. The Flavonoids: Advances in Research. London: Chapman and Hall, 1982:681–718.
[10] P.C.H. Hollman, M.V.D. Gaag, M.J.B. Mengelers, J.M.P. Van-Trijp, J.H.M. DeVries, M.B. Katan, Free Rad. Biol. Med. 21 (1996) 703–707.
[11] J.A. Vinson, Y.A. Dabbagh, M.M. Serry, J. Jang, J. Agric. Food Chem. 43 (1995) 2800–2802.
[12] C. Manach, C. Morand, O. Texier, M.-L. Favier, G. Agullo, C. Demigné, F. Régérat, C. Rémésy, J. Nutr. 125 (1995) 1911–1922.
[13] G.E. Kelly, G.E. Joannou, A.Y. Reeder, C. Nelson, M.A. Waring, PSEBM 208 (1995) 40–43.