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## **Relative bioavailability of the flavonoids quercetin, hesperetin and naringenin given simultaneously through diet**

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1 **Title**

2 Relative bioavailability of the flavonoids quercetin, hesperetin and naringenin given  
3 simultaneously through diet

4

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6 Relative bioavailability of flavonoids in humans

7

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29  
30 **Authors' contribution**  
31 KSK: contribution to the study design and responsible for conducting the experiment, responsible  
32 for LC-MS analysis of urine, plasma and juice, data interpretation and writing the manuscript;  
33 LB: contribution to the LC-MS analysis of plasma and juice, data interpretation and manuscript  
34 editing; PK: responsible for the LC-MS analysis of diet, contribution to the LC-MS analysis of  
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39  
40

41 **ABSTRACT**

42 The bioavailability and urinary excretion of three dietary flavonoids, quercetin, hesperetin and  
43 naringenin was investigated. Ten healthy men consumed a 'juice-mix' containing equal amounts  
44 of the three flavonoids and urine and plasma samples were collected. Resulting mean plasma  
45  $AUC_{0-48h}$  and  $C_{max}$  values for quercetin and hesperetin were similar, whereas the  $AUC_{0-48h}$  of  
46 naringenin, and thus the relative bioavailability, were higher after consumption of the same dose.  
47 The study consolidates a significant lower urinary excretion of quercetin ( $1.5\% \pm 1\%$ ) compared to  
48 hesperetin ( $14.2\% \pm 9.1\%$ ) and naringenin ( $22.6\% \pm 11.5\%$ ) and shows that this is not due to a  
49 lower bioavailability of quercetin, but rather reflects different clearance mechanisms.

50

51 **Keywords:** bioavailability, biokinetics, humans, quercetin, hesperetin, naringenin.

52

53 To compare the impact of dietary important flavonoids it is necessary to study them in the  
54 same food matrix and at similar realistic doses. The present study investigate the bioavailability  
55 and urinary excretion of the flavonoids, quercetin, hesperetin and naringenin in a 48h intervention  
56 study with a single dose (6.3 mL/kg bw) of 'juice-mix' containing the three flavonoids.

57 Complete urine and plasma samples were obtained from 10 healthy men, 21-28 years.  
58 The study was approved by the ethics committee of Copenhagen and Frederiksberg municipality  
59 (J.No.(KF)01-161/01). The 'juice-mix' was provided to fasting subjects in the morning, along  
60 with a standardized flavonoid-free diet (0-24 h), after which the volunteers kept a flavonoid-free  
61 diet (24-48 h). Blood and urine samples were collected as previously described (Nielsen *et al.*  
62 2006). Flavonoid aglycones were quantified in the 'juice-mix' (30 mg/L quercetin, 28 mg/L  
63 naringenin and 32 mg/L hesperetin) and flavonoid glycosides were identified according to  
64 Breinholt *et al.* (2003).

65 Flavonoids in plasma were completely hydrolysed as described in Nielsen *et al.* (2006).  
66 Samples were added 25  $\mu$ L aqueous ascorbic acid (20 mg/mL) and 0.5% formic acid to pH = 4  
67 and applied to Evolute ABN columns (25 mg, Mikrolab, Denmark). The eluted flavonoid  
68 aglycones were evaporated to dryness and re-dissolved in 200  $\mu$ L 0.5% formic acid, 10%  
69 methanol and added 250 ng  $^{13}$ C-daidzein as external standard.

70 Determination of flavonoids in urine is essentially described elsewhere (Nielsen *et al.*,  
71 2006), except for the inclusion of SPE (Isolute SPE 100) before injection on the LC-MS system.

72 Statistical analyses were performed using Wilcoxon matched pair tests (SPSS version  
73 14.0). The relative bioavailability,  $AUC_{0-48h}$ , was calculated with the linear trapezoidal rule.

74

75

## 76 RESULTS AND DISCUSSION

77 This study demonstrates that when subjects consume equal amounts of quercetin and  
78 hesperetin, the  $AUC_{0-48h}$ , and thus the bioavailabilities of these flavonoids, is similar. However,  
79 the  $AUC_{0-48h}$  of naringenin was significantly higher than for quercetin and hesperetin (**Figure 1**).  
80 Also plasma  $C_{max}$  reached for quercetin and hesperetin was comparable, whereas the level  
81 achieved for naringenin was significantly higher (**Table 1**). In the study design we assumed that  
82 the low and natural amounts of flavonoids in the 'juice-mix' excluded any interference with  
83 respect to pharmacokinetics of the flavonoids investigated. Previous studies dealing with  
84 flavanone and flavonol bioavailability report AUC-values in the same range (Hollman *et al.*,  
85 1997; Erlund *et al.*, 2001; Manach *et al.*, 2003; Manach *et al.*, 2005) as observed in the current  
86 study. However, the lack of blood sampling between 8-24 h in the present study may cause an  
87 overestimation of the AUCs, especially for the flavanones.

88 In the present study, we observed a 9-15 fold higher urinary excretion of naringenin and  
89 hesperetin compared to quercetin (**Table 1**) consolidating the apparent paradox that absorption  
90 and bioavailability of the three flavonoids seems quite similar, but that urinary excretion is highly  
91 different. The low urinary recovery (1.5%) of the ingested quercetin leaves a large amount of the  
92 ingested dose unaccounted for compared to hesperetin and naringenin. The most likely fate of the  
93 absorbed quercetin is excretion via bile (Matsukawa *et al.*, 2009) and further degradation to low  
94 molecular weight phenolic acids which were not analysed in the current and most previous  
95 flavonol bioavailability studies (Mullen *et al.*, 2006; Mullen *et al.*, 2008). Only small amounts of  
96 the 3'-methylated form of quercetin, isorhamnetin, were present in the 'juice-mix' (1.8 mg/500  
97 mL 'juice-mix') and of this only  $4.1 \pm 3.3\%$  of isorhamnetin was excreted in urine after 48 h,  
98 excluding extensive methylation of quercetin. Only trace amounts of tamarixetin (4'-OMe-  
99 quercetin) were found in juice, plasma and urine.

100           Hydrolysis of the flavonoid glycoside-moiety is a necessary step for absorption of  
101 flavonoids from the gut. In the present study apple juice was the source of quercetin and  
102 qualitative LC-MS investigation of the ‘juice-mix’ showed that approximately 60% of the  
103 quercetin was present as monoglycosides, 25% as aglycone and less than 15% as rutosides. It  
104 has been shown that only quercetin-3-glucoside and quercetin-4'-glucoside is absorbed in  
105 substantial quantities in the small intestine, while little quercetin-3-galactoside, and quercetin-3-  
106 rhamnoside and quercetin-3-arabinoside is absorbed in the small intestines, but are hydrolysed  
107 and absorbed in the distal part of the colon together with diglycosides such as naringin and  
108 hesperidin (Arts *et al.*, 2004). More than 99% of the hesperetin and naringenin in the ‘juice-mix’  
109 were diglycosides, presumably naringin and hesperidin. The nature of the glycoside moieties  
110 bound to the three flavonoids was thus, mainly in a form that favours absorption from the colon.  
111 The similar  $T_{max}$  for quercetin, hesperetin and naringenin in current study supports this  
112 assumption.  $T_{max}$  for quercetin in the literature is between 1-9 h, depending on sugar moiety  
113 composition (Manach *et al.*, 2005), and the intermediate  $T_{max}$  ( $3.6 \pm 1.6$  h) in the present study  
114 correlates with the mixed content of quercetin glycosides. The average  $T_{max}$  in the literature for  
115 naringenin is between 2.0–4.6 h (Manach *et al.*, 2003) and for hesperetin between 5.4–5.8 h  
116 (Manach *et al.*, 2003) and thus also in the same range as observed here.

117           In conclusion, the current study confirms a lower urinary excretion of quercetin compared  
118 to naringenin and hesperetin. The study furthermore shows that the significant lower urinary  
119 excretion of quercetin is not due to a low bioavailability of quercetin, but rather reflects different  
120 clearance mechanisms for this flavonoid.

121

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127

128 **TABLE 1**

129 Plasma and urinary biokinetic parameters for quercetin, naringenin and hesperetin after  
 130 administration of 'juice-mix' (mean  $\pm$  SD, n=10).

	Mean excreted amount ( $\mu$ g)	Accumulated relative urinary excretion (% of the dose)	T <sub>max</sub> plasma (h)	C <sub>max</sub> plasma ( $\mu$ mol/L)	C <sub>max</sub> /AUC <sub>0-48h</sub>	AUC <sub>0-48h</sub> ( $\mu$ mol* h/L)	AUC <sub>0-48h</sub> ( $\mu$ mol* h/L) <sup>1</sup>
<b>Quercetin (30 mg/L 'juice-mix')</b>			3.6 $\pm$ 1.6	0.15 $\pm$ 0.13	0.09 $\pm$ 0.02	1.77 $\pm$ 1.63	1.77 $\pm$ 1.63
0-3 h	31 $\pm$ 13	0.3 $\pm$ 0.2					
3-6 h	73 $\pm$ 29	0.9 $\pm$ 0.7					
6-12 h	74 $\pm$ 11	1.2 $\pm$ 0.9					
12-24 h	21 $\pm$ 17	1.3 $\pm$ 0.9					
24-48 h	28 $\pm$ 18	1.5 $\pm$ 1.0					
0-48 h	227 $\pm$ 142 <sup>b,c</sup>	1.5 $\pm$ 1.0 <sup>b,c</sup>					
<b>Naringenin (28 mg/L 'juice-mix')</b>			3.6 $\pm$ 1.6	0.25 $\pm$ 0.13 <sup>a</sup>	0.11 $\pm$ 0.05	2.64 $\pm$ 1.95	2.82 $\pm$ 2.09 <sup>a</sup>
0-3 h	456 $\pm$ 231	3.3 $\pm$ 1.6					
3-6 h	1390 $\pm$ 1060	13.2 $\pm$ 8.2					
6-12 h	952 $\pm$ 482	20.0 $\pm$ 10.0					
12-24 h	278 $\pm$ 282	22.0 $\pm$ 10.9					
24-48 h	83 $\pm$ 114	22.6 $\pm$ 11.5					
0-48 h	3160 $\pm$ 1612	22.6 $\pm$ 11.5					
<b>Hesperetin (32 mg/L 'juice-mix')</b>			4.9 $\pm$ 1.4	0.18 $\pm$ 0.13 <sup>b</sup>	0.09 $\pm$ 0.03	2.13 $\pm$ 1.59	1.99 $\pm$ 1.49 <sup>b</sup>
0-3 h	103 $\pm$ 78	0.6 $\pm$ 0.5					
3-6 h	802 $\pm$ 595	5.7 $\pm$ 3.6					
6-12 h	880 $\pm$ 462	11.2 $\pm$ 6.3					
12-24 h	436 $\pm$ 542	13.9 $\pm$ 8.7					
24-48 h	58 $\pm$ 103	14.2 $\pm$ 9.1					
0-48 h	2278 $\pm$ 1457 <sup>b</sup>	14.2 $\pm$ 9.1 <sup>b</sup>					

131 <sup>1</sup>Adjusted for ingested dose. Ingested dose is based on chemical analysis. The contents of flavonoids were calculated  
 132 on the basis of internal and external standards.

133 <sup>a</sup>Significantly different from quercetin (Wilcoxon, p<0.01).

134 <sup>b</sup>Significantly different from naringenin (Wilcoxon, p<0.01).

135 <sup>c</sup>Significantly different from hesperetin (Wilcoxon, p<0.01).

136

137

138 **FIGURE LEGENDS**

139

140 **FIGURE 1** Plasma time versus concentration curves for quercetin (diamond), hesperetin  
141 (triangle) and naringenin (square) after ingestion of 'juice-mix'. Values are mean + SEM, n=10.

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**FIGURE 1**

