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## **Free fruit at workplace increases total fruit intake: a validation study using 24h dietary recall and urinary flavonoid excretion**

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1 **Title**

2 Free fruit at workplace intervention increases total fruit intake: a validation study using 24h  
3 dietary recall and urinary flavonoid excretion

4

5 **Running title**

6 Biomarker validation of workplace fruit intervention

7

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29

30 **Key words:** Validation, fruit, vegetables, biomarker, flavonoids, 24h dietary recall

31

### 32 **Authors' contribution**

33 KSK: contributed to the study design and carrying out the study, responsible for statistical  
34 calculations, data interpretation and writing the manuscript; LB: responsible for determination of  
35 flavonoids in urine by LC-MS, contributed to manuscript editing and carrying out the study; SA:  
36 responsible for carrying out the study, contributed to manuscript editing; TC: responsible for  
37 calculation of dietary intake, contributed to manuscript editing; SER: contributed to study design  
38 and manuscript editing; LOD: responsible for study design, contributed to manuscript editing.  
39 None of the authors had any conflicts of interest.

40

## Abstract

41 **Objective:** To validate 24h dietary recall of fruit intake by measuring the total 24h excretion of  
42 ten different flavonoids in 24h urine during an intervention with free fruit at workplaces.

43 **Design and methods:** Employees at workplaces offering a free fruit program, consisting of daily  
44 free and easy access to fresh fruit, and controls employees at workplaces with no free fruit  
45 programme were enrolled in this validation study (n=103). Dietary intake was assessed by using  
46 a 24h dietary recall questionnaire at baseline and approximately 5 months later. Ten flavonoids,  
47 quercetin, isorhamnetin, tamarixetin, kaempferol, hesperetin, naringenin, eriodictyol, daidzein,  
48 genistein and phloretin, were measured using HPLC–electrospray ionisation–MS. **Results:** The  
49 24h urinary excretion of total flavonoids and the estimated intake of fruits were significantly  
50 correlated ( $r_s = 0.31$ ,  $p < 0.01$ ). The dietary intake of citrus fruits and citrus juices was significantly  
51 correlated with total excretion of citrus specific flavonoids ( $r_s = 0.28$ ,  $p < 0.01$ ), and orange was  
52 positively correlated with naringenin ( $r_s = 0.24$ ,  $p < 0.01$ ) and hesperetin ( $r_s = 0.24$ ,  $p < 0.01$ ).

53 Phloretin in urine was correlated with apple intake ( $r_s = 0.22$ ,  $p < 0.01$ ) and also with overall  
54 estimated intake of fruit ( $r_s = 0.22$ ,  $p < 0.01$ ). **Conclusion:** The present study shows that a 24h  
55 dietary recall can be used as a valid estimate of the intake of fruits in agreement with an objective  
56 biomarker of fruit intake in free fruit at workplace interventions.

## INTRODUCTION

57           Meta-analyses of observational studies have repeatedly shown that a high fruit and  
58 vegetable intake is associated with a reduced risk of cardiovascular disease, even though  
59 causality and mechanism remain to be demonstrated (Riboli *et al.* 2003; Dauchet *et al.* 2006; He  
60 *et al.* 2007; Duijnhoven *et al.* 2009). Based on these results, authorised food based dietary  
61 guidelines recommend to eat more fruits and vegetables, e.g. the '5 to 10 a day' programme in  
62 Canada, the 'go for 2&5' programme in Australia or the '6 a day' programme in Denmark. Fruit  
63 and vegetable intake is however still inadequate among the majority of the population in many  
64 countries (Ashfield-Wall *et al.* 2004; Lassen *et al.* 2004; Guenter *et al.* 2006; Ovesen *et al.* 2007;  
65 Kimmens *et al.* 2009). The average intake of fruits and vegetables in Denmark is currently 434  
66 g/day with only 16% of the population consuming an amount, corresponding to the Danish  
67 recommendations of 600 g fruits and vegetables per day for adults and children above 10 years  
68 old (Astrup 2005; Fagt *et al.* 2008).

69           It has been shown that simple provision of information is not enough in order to  
70 achieve significant changes in the dietary behaviour of a population (Stockley *et al.* 2001;  
71 Eertmans *et al.* 2001). Free-fruit campaigns on worksites are therefore well suited as an  
72 environmental strategy with the potential for increasing the daily fruit intake in a given  
73 population. This study will investigate whether a 24h dietary recall is a valid tool to measure, if a  
74 free fruit intervention at workplace will increase fruit intake among employees.

75           Estimation of fruit and vegetable intake is complicated by a tendency to over-report  
76 intake of healthy foods (Gibson 1990). Furthermore food registration methods are often based on  
77 self-reported data, which implies the risk for subjective bias (Kipnis *et al.* 2001). Biological  
78 markers of exposure as an alternative to the more traditional dietary assessment tools have been  
79 used for many years to provide semi-quantitative indexes of the exposure to individual food

80 constituents or food groups like fruits or vegetables (Spencer *et al.* 2008). A combination of  
81 several different flavonoids quantified in 24h urine samples has recently been shown to correlate  
82 more strongly to fruit intake, than plasma carotenoids. Therefore, at the present, a combination of  
83 urinary flavonoids seems to be the best performing objective biomarker of fruit intake (Nielsen *et*  
84 *al.* 2002; Brantsæter *et al.* 2007; Mikkelsen *et al.* 2007). This biomarker has therefore been used  
85 in a workplace based intervention study providing free fruit or no free fruit to employees as  
86 validation of a 24h dietary recall.

87

## 88 **MATERIALS AND METHODS**

### 89 **Workplaces and subjects**

90           Eight comparable workplaces in the Copenhagen area signed up for the study. Five  
91 were enrolled as intervention workplaces and the remaining three workplaces, without any actual  
92 consideration of free-fruit at the workplace, were enrolled as control workplaces. At each  
93 workplace, collaboration with a contact person was established. Recruitment of the employees,  
94 interested in participating in the study, occurred through this contact. Altogether 146 employees  
95 were enrolled at baseline in the free fruit at work intervention study. Of the 146 employees, a  
96 total of 103 accepted participation in the validation study. Pregnant and lactating women, and  
97 individuals, not expecting to be at the particular workplace 5 months later were excluded. The  
98 study protocol was accepted by the Ethics Committee of the Copenhagen and Frederiksberg  
99 municipalities (J. No. KA-20060047).

100

### 101 **The intervention**

102           Workplaces entered the study at distinct points in time, starting between June and  
103 September 2006. Assessments were made both at baseline and at termination approximately 5  
104 months later. The intervention was a fruit program, consisting of a fruit basket placed where  
105 employees had free and easy access, i.e. in the reception or in a kitchenette. There was at least  
106 one piece of fruit per employee each day. The fruit intervention was not accompanied with  
107 informational, counselling or other health promotion activities in order to make a minimal  
108 difference to the controls.

109

### 110 **Dietary assessment**

111 Dietary intake was assessed by a 24h dietary recall questionnaire, which was a  
112 modified form of the dietary recall questionnaire from the Danish National Dietary Survey 2000–  
113 2002 (Lyhne *et al.* 2005) The recall was performed by trained interviewers both at baseline and  
114 after 5 months. The software program, General Intake Estimation System (GIES, version 1.0,  
115 Technical University of Denmark, National Food Institute, Division of Nutrition 2008), was used  
116 to calculate dietary intake.

117

### 118 **Urine samples**

119 Urine samples were collected by the employees through 24 hours at baseline and 5  
120 months later in order to determine the concentration of flavonoids as an objective biomarker for  
121 fruit intake. The collection of the 24h urine samples started in the morning the day before  
122 completion of the 24h recall questionnaires and lasted until the next day at the same hour. Hence,  
123 the 24h urine sample and the 24h dietary recall questionnaire covered the same period of time.  
124 All urine samples were collected as previously described by Krogholm *et al.* (2004). The  
125 employees were instructed on how to collect the urine and in the importance of providing  
126 complete samples. Completeness of the urine collection was checked by oral questionnaires and  
127 study diaries. Anthropometric measurements (height, bodyweight, and waist circumference) were  
128 also performed, and will be published elsewhere.

129

130

### 131 **Determination of urinary flavonoids**

132           The flavonoids were determined by LC-MS after enzymatic hydrolysis of  
133 conjugates, as described by Nielsen *et al.* (2000) with slight modifications. In short, 2 mL  
134 aliquots of each urine sample were initially added 500 ng of <sup>13</sup>C *O*-desmethylangolensin (<sup>13</sup>C  
135 ODMA) internal standard (20 ng/μL DMSO) and enzymatically hydrolyzed. The hydrolyzed  
136 samples were evaporated to dryness under vacuum. The urine samples were then dissolved with  
137 50 μL 100% methanol and 2.0 mL 0.5% formic acid and added on an Isolute SPE 100 column  
138 preconditioned with 2.0 mL 100% methanol and 2.0 mL 0.5% formic acid. The column was  
139 washed and the flavonoids eluted and evaporated as described in Nielsen *et al.* (2000). After  
140 evaporation, the samples were redissolved in 25 μL 100% methanol and 200 μL 0.5% formic  
141 acid and added 500 ng of <sup>13</sup>C-daidzein as an additional standard (20 ng/μL DMSO). The entire  
142 amount of supernatant was injected into the LC-MS system.

143

### 144 **Reagents and standards**

145           Enzymes used for the hydrolysis of the flavonoid glycosides in urine samples were  
146 arylsulphatase (*Aerobacter aerogenes*, 16.8 standard units/mL) from Sigma Chemicals Co. (St.  
147 Louis, MO, USA) and β-glucuronidase (*Escherichia coli*, >200 standard units/mL) obtained from  
148 Boehringer Mannheim (Mannheim, Germany). Methanol and acetonitrile were of HPLC grade  
149 and obtained from Rathburne Ltd. (Walkerburn, United Kingdom). The flavonoid standards  
150 quercetin, kaempferol, isorhamnetin, tamarixetin and naringenin were obtained from Aldrich  
151 (Steinheim, Germany). Hesperetin, eriodictyol and phloretin was purchased from Sigma  
152 Chemical Co. (St. Louis, MO, USA). Genistein and daidzein were obtained from LC  
153 Laboratories, (Woburn, MA). The isotopically labelled internal standards, 3 x <sup>13</sup>C *O*-

154 desmethylangolensin ( $^{13}\text{C}$  ODMA), and 3 x  $^{13}\text{C}$  daidzein were obtained from School of  
155 Chemistry, University of St. Andrews, UK. Dimethylsulfoxid (DMSO) (Urasol) was purchased  
156 from Merck & Co., Inc. The Isolute SPE 100 was purchased from Mikrolab, Aarhus, Denmark.  
157 All standards were HPLC grade. The concentration of flavonoids in urine samples were  
158 determined as single determinations based on calibration curves generated by spiked blank urine  
159 samples as described by Nielsen *et al.* (2000).

160

## 161 **Statistical analysis**

162           The concentration of flavonoids in urine and intake of fruit and flavonoid-rich foods  
163 did not have a normal distribution even after logarithmic transformation, so nonparametric tests  
164 were used in the statistical analysis of the data. Wilcoxon two related samples test were  
165 performed in the intervention (n=34) and control (n=45) group separately to evaluate changes  
166 from baseline to after 5 months in the urinary excretion of flavonoids and in intakes of fruit, and  
167 individual flavonoid-rich foods. The Mann-Whitney *U* tests were performed to compare the two  
168 groups (control and intervention) on one variable at the time. Spearman correlation ( $r_s$ ) was used  
169 to examine associations between the intake of fruits, flavonoid-rich foods and the excretion of the  
170 flavonoid biomarkers in urine (n=158). The analyses were made using the SPSS package  
171 program version 14.0 (SPSS, Inc., Chicago, IL). P values < 0.05 (two-tailed) were regarded as  
172 statistically significant.

173

## 174 **RESULTS**

175

### 176 **Participants and food intake**

177                   Altogether 103 employees were enrolled at baseline in the validation study. Of  
178 these, 99 employees did complete the 24h dietary recall and 24h urine collection at baseline. At  
179 the second measurement, after 5 months, the 24h dietary recall and the collection of 24h urine  
180 sample was completed by 79 out of the 99 employees. Main causes of drop-out or exclusion were  
181 unexpected termination of employment (16 individuals) and incomplete 24h urine collection.  
182 Data from the 79 employees were analysed using Wilcoxon two related samples test and the  
183 Mann-Whitney *U* tests.

184                   At baseline there were no significant differences between the control and  
185 intervention groups in the estimated dietary intakes of fruits, citrus fruits and citrus juices,  
186 vegetables, fruits and vegetables, apples and oranges based on 24h dietary recall questionnaires  
187 (see **Table 1**). After 5 months of intervention the total intake of fruits was higher in the group  
188 with free access to fruit at work every day, compared to the control group receiving no additional  
189 fruit (median 310 (15, 1312) g/day *v.* median 265 (0, 732) g/day,  $P < 0.01$ ) (see **Figure 1**). Also  
190 the overall change in fruit intake from baseline to after 5 months was significant higher in the  
191 intervention group (median 72 (310, 792) g/day,  $P < 0.01$ ) compared to the control group (median  
192 2 (-308, 490) g/day). As seen in **Table 1**, the median intake of citrus fruits and juices increased  
193 from 0 g/day at baseline to 50 g/day after 5 months, and this change in intake in the intervention  
194 group was significant higher than in the control group ( $P < 0.05$ ). Also the intake of fruit in the  
195 control group tended to increase over the 5 months study period (from median 193 (2, 541) g/day  
196 to median 265 (0, 732) g/day), though the change was not statistically significant (see Figure 1).

197

### 198 **Biomarkers of fruit intake**

199                   The median (P5, P95) 24h urinary excretion of total flavonoids (i.e. the sum of  
200 quercetin, isorhamnetin, tamarixetin, kaempferol, hesperetin, naringenin, eriodictyol, daidzein,

201 genistein and phloretin) was similar in the control and intervention groups at baseline (median  
202 352 (66, 1506)  $\mu\text{g}$  v. median 175 (65, 2569)  $\mu\text{g}$ ) (see **Table 1**). After 5 months of free access to  
203 fruit at workplace every day the 24h urinary excretion of total flavonoids, in the intervention  
204 group, had increased significantly from median 175 (65, 2569)  $\mu\text{g}$  to median 635 (78, 8262)  $\mu\text{g}$ .  
205 Also the amount of total citrus flavonoids (i.e. the sum of hesperetin, naringenin and eriodictyol)  
206 increased significantly after 5 months in the intervention group ( $P<0.05$ ). In the control group,  
207 the urinary excretion of total flavonoids and total citrus flavonoids was also increased  
208 significantly after 5 months ( $P<0.05$ ) (see **Table 1**).

209           The quantitatively largest amounts of flavonoids excreted in urine were the citrus  
210 flavonoids hesperetin, naringenin and eriodictyol (see **Table 1**). Kaempferol, phloretin, quercetin,  
211 genistein, daidzein, isorhamnetin and tamarixetin were excreted in lower amounts and did not  
212 change significantly in any of the two groups over time (data not shown).

213

## 214 **Correlations**

215           In the present study we use the spearman correlations coefficients for validation of  
216 the self-reported fruit intake. Spearman correlations were calculated for the employees with  
217 complete 24h dietary recall and 24h urine collection at both baseline and after 5 months,  
218 corresponding to a total of 158 employees. Spearman correlations between dietary intake  
219 estimated by 24h dietary recall and 24h urinary excretion of different combinations of flavonoids  
220 showed that total flavonoids in urine was highly and significantly correlated ( $r_s = 0.31$ ,  $P<0.01$ ,  
221  $n=158$ ) with the total intake of fruits (see **Table 2**). Adding the intake of vegetables to the fruit  
222 intake did make the strength of the spearman correlation even stronger to total flavonoids in urine  
223 ( $r_s=0.37$ ,  $P<0.01$ ). Urinary excretion of the combination of hesperetin, naringenin, phloretin and  
224 eriodictyol (referred to as the 'fruit marker'), all present in fruits or formed after fruit

225 consumption, were also positively correlated to intake of fruits ( $r_s=0.29$ ,  $P<0.01$ ), but the  
226 correlation were stronger when including the isoflavonoids, genistein and daidzein, and the  
227 flavonols quercetin, kaempferol, isorhamnetin and tamarixetin which all are present in both fruits  
228 and vegetables ( $r_s=0.31$ ,  $P<0.01$ ) (see **Table 2**). Correlations between excretion of specific  
229 flavonoids and intake of particular foods were studied based on their known occurrence in these  
230 foods. The dietary intake of citrus fruits and citrus juices was significantly correlated with total  
231 citrus flavonoids ( $r_s=0.24$ ,  $P<0.01$ ), and oranges were positively correlated with naringenin  
232 ( $r_s=0.24$ ,  $P<0.01$ ) and hesperetin ( $r_s=0.24$ ,  $P<0.01$ ). Furthermore the total citrus flavonoids were  
233 significantly correlated to the intake of fruits in general ( $r_s=0.28$ ,  $P<0.01$ ). Phloretin in urine was  
234 correlated with apple intake ( $r_s=0.22$ ,  $P<0.01$ ) and also with overall estimated intake of fruit  
235 ( $r_s=0.22$ ,  $P<0.01$ ). Finally, some highly significant correlations between individual flavonoids  
236 were observed: Urinary excretion of hesperetin was correlated with excretion of naringenin  
237 ( $r_s=0.60$ ,  $P<0.0001$ ) and eriodictyol ( $r_s=0.58$ ,  $P<0.0001$ ), naringenin with eriodictyol ( $r_s=0.52$ ,  
238  $P<0.0001$ ) and phloretin ( $r_s=0.23$ ,  $P<0.001$ ), quercetin with phloretin ( $r_s=0.96$ ,  $P<0.0001$ ),  
239 isorhamnetin ( $r_s=1$ ,  $p<0.0001$ ) and tamarixetin ( $r_s=1$ ,  $P<0.0001$ ), and daidzein was correlated  
240 with genistein ( $r_s=0.52$ ,  $P<0.0001$ ).

241

## 242 **DISCUSSION**

243 In the present study we used the combination of several different flavonoids  
244 excreted in 24h urine as a reference biomarker to the self-reported intake of fruits and flavonoid-  
245 rich foods. The link between intake of fruits and vegetables and the urinary excretion of  
246 flavonoids has been well established in controlled intervention studies with specific foods  
247 (Nielsen *et al.* 2002; Krogholm *et al.* 2004; Brevik *et al.* 2005) and in free-living populations  
248 (Nielsen *et al.* 2002; Brantsæter *et al.* 2007; Mikkelsen *et al.* 2007). Therefore the significant

249 correlation between the urinary total flavonoids and fruit intake in the present study shows that  
250 the estimation of intake of fruits by the 24h dietary recall is a valid estimate of the true effect of  
251 the free fruit intervention programme. The correlation values and excretion levels in the present  
252 validation study are in the same range as previously reported (Nielsen *et al.* 2002; Krogholm *et*  
253 *al.* 2004; Brevik *et al.* 2005; Mikkelsen *et al.* 2007; Brandsaetter *et al.* 2007).

254 In the present study, the quantitatively largest amounts of flavonoids excreted in  
255 urine were the citrus flavonoids (hesperetin, naringenin and eriodictyol). The main reasons for  
256 this is that the urinary excretion relative to the intake of the citrus flavonoids, hesperetin and  
257 naringenin, is roughly 10 times higher than for the flavonols (Manach *et al.* 2005; Krogholm *et*  
258 *al.* 2010). Due to the high relative urinary excretion of citrus flavonoids, citrus fruit and citrus  
259 juice intake is more easily reflected by our flavonoid biomarker than the fruits containing only  
260 flavonols. This was for instance seen in the present study as highly significant correlations  
261 between intake of citrus fruits and citrus juices and total citrus flavonoids. Also the previously  
262 reported high correlation between phloretin in urine and intake of apples (Mennen *et al.* 2006;  
263 Brantsaeter *et al.* 2007) was confirmed in the present study ( $r_s = 0.22$ ,  $p < 0.01$ ). Urinary phloretin  
264 was also correlated with overall estimated intake of fruits ( $r_s = 0.22$ ,  $P < 0.01$ ), indicating that  
265 individuals consuming apples are 'high fruit consumers' in general. We could only observe weak  
266 correlations between the 24h urinary excretion of quercetin ( $r_s = 0.17$ ,  $P < 0.05$ ) and intake of apple,  
267 including apple juice. Phloretin therefore appears to be a better biomarker for apple intake than  
268 quercetin, even though apples are also a dietary important source of quercetin. Ito *et al.* (2005)  
269 has suggested, that phloretin is a metabolite of naringenin formed by reductive opening of the  
270 heterocyclic C-ring, and Mennen *et al.* (2006) has found high correlations between phloretin and  
271 naringenin ( $r_s = 0.51$ ,  $P < 0.0001$ ). In the present study the correlation between phloretin and

272 naringenin was also significant ( $r_s=0.23$ ,  $P<0.01$ ), although the association was weaker than in  
273 the study by Mennen *et al.* (2006).

274           Although the citrus flavonoids are dominating among the flavonoids measured in  
275 our biomarker, the present study consolidates the importance of including several different  
276 flavonoids and/or metabolites when using flavonoids as an overall biomarker for intake of fruits,  
277 since the combination of all the flavonoids measured in the present study, result in the strongest  
278 correlation to estimated intake of fruits ( $r_s =0.31$ ,  $P<0.01$ ), with a 95% confidence interval from  
279 0.16 to 0.45, which is acceptable when validation is the overall purpose. Adding more flavonoids  
280 and/or flavonoid metabolites present in fruits might further improve the accuracy of the flavonoid  
281 biomarker. Further research investigating this is needed. The magnitude of a given correlation  
282 between a biomarker and self-reported food intakes cannot be expected to be very high even if  
283 both methods is quite accurate, and there is at present no agreement in literature as to what  
284 constitutes a satisfactory level of correlation. The magnitude of the presented correlations  
285 between total flavonoid in urine and the estimated dietary intake of fruits, fruits and vegetables  
286 and citrus fruits and citrus juices, given the relatively small sample size and the rather narrow  
287 range in dietary intake in the present study, is rather high.

288           Being in a distinct protocol can result in changes in lifestyle and exercise, also  
289 referred to as the placebo effect. In the present study, this was reflected by an increased fruit  
290 intake in both the control and intervention group because volunteers in both groups were  
291 informed about the purpose of the study. Over the 5 months study period, the intake of fruits in  
292 the control group changed from median 193 (2, 541) g/day to median 265 (9, 732) g/day.  
293 According to the 24h dietary recall, this increase was not significant, while the urinary total  
294 flavonoid responded significantly to this change in intake. This could indicate that the 24h dietary  
295 recall is less sensitive as a marker of fruit intake than our flavonoid biomarker. With a larger

296 sample size the power would increase and the 24h dietary recall would then be a cheap and  
297 apparently accurate marker of fruit intake. In conclusion, the present study shows highly  
298 significant correlations between intake of fruit, estimated by 24h dietary recall, and the reference  
299 biomarker for fruit intake, the 24h urinary excretion of total flavonoids.

300

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389 **TABLE 1:** Median (P5, P95) dietary intake estimated by 24h dietary recall (g/day) and 24h  
 390 urinary excretion of flavonoids ( $\mu\text{g}$ ) in control and intervention group at baseline and after 5  
 391 months of a free-fruit at workplace campaign.

	Control group (n=45)			Intervention group (n=34)		
	Baseline	After 5 months	Change	Baseline	After 5 months	Change
Dietary intake estimated by 24h dietary recall (g/day)						
Fruit	193 (2,541)	267 (0,732)	2 (-308,490)	182 (0,584)	310 (15,1312)**,##	72 (310,792)§§
Citrus fruit and juice	0 (0,119)	0 (0,200)*	0 (-104, 200)	0 (0,245)	50 (0, 610)**	20 (-139,610)§
Vegetable	191 (44,594)	194 (9,521)	-19 (-334,318)	153 (29,417)	113 (4,539)#	-22 (-341,340)
Fruit and vegetables	411 (120,990)	474 (15,1049)	22 (-441,465)	322 (55,850)	470 (132,1600)**	114 (-369,1279)
Apple	61 (0,231)	61 (0,317)	0 (-125,293)	0 (0,281)	56 (0,600)*	0 (-125,511)
24h urinary excretion of flavonoids ( $\mu\text{g}/24\text{h}$ urine)						
Total flavonoids	352 (66,1506)	445 (61,4570)*	105 (-1371,3585)	175 (65,2569)	635 (78,8262)**	258 (-752,7285)
Citrus flavonoids	169 (8,1166)	317 (8,4244)**	143 (-1012,4009)	91 (8,1852)	493 (9,7899)*	156 (-816,7267)
Naringenin	72 (3,576)	210 (3,1802)*	66 (-517,1701)	70 (3,738)	226 (3,2720)*	93 (-347,2603)
Hesperetin	5 (2,521)	7 (1,2105)*	3 (-465,1724)	5 (2,1198)	177 (2,4516)**,#	73 (-1021,4172)
Eriodictyol	3 (1,135)	17 (1,154)	0 (-133,150)	3 (1,197)	24 (1,1074)	2 (-89,1071)

392 \* Significant different from baseline in same group ( $P < 0.05$ , Wilcoxon).  
 393 \*\* Significant different from baseline in same group ( $P < 0.01$ , Wilcoxon).  
 394 # Significant different from 'after 5 months' in control group ( $P < 0.05$ , Mann-Whitney U test).  
 395 ## Significant different from 'after 5 months' in control group ( $P < 0.01$ , Mann-Whitney U test).  
 396 § Significant different from change in control group ( $P < 0.05$ , Mann-Whitney U test).  
 397 §§ Significant different from change in control group ( $P < 0.01$ , Mann-Whitney U test).  
 398 Citrus flavonoids' refers to the sum of hesperetin, naringenin and eriodictyol. Total flavonoids refer to the sum of  
 399 quercetin, isorhamnetin, tamarixetin, kaempferol, hesperetin, naringenin, eriodictyol, daidzein, genistein and  
 400 phloretin.  
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404 **TABLE 2:** Spearman correlations (lower 95% confidence interval – upper 95% confidence  
 405 interval) between intake of fruit and flavonoid rich foods determined by 24h dietary recall and  
 406 24h urinary excretion of flavonoids (n=158).

	Fruits	Citrus fruits and citrus juices	Vegetables	Fruits and Vegetables	Orange	Apple
Total flavonoids	0.31** (0.16-0.45)	0.23** (0.08-0.37)	0.21** (0.06-0.36)	0.37** (0.23-0.50)	0.23** (0.08-0.37)	0.24** (0.09-0.38)
Citrus flavonoids	0.28** (0.13-0.43)	0.24** (0.09-0.38)	0.23** (0.08-0.37)	0.36** (0.22-0.49)	0.26** (0.11-0.40)	0.20* (0.05-0.35)
Hesperetin	0.29** (0.14-0.43)	0.27** (0.12-0.41)	0.13 (0.03-0.28)	0.31** (0.16-0.45)	0.24** (0.09-0.38)	0.19* (0.04-0.34)
Naringenin	0.23** (0.08-0.37)	0.19* (0.04-0.34)	0.25** (0.10-0.39)	0.33** (0.18-0.46)	0.24** (0.09-0.38)	0.19* (0.04-0.34)
Eriodictyol	0.25** (0.10-0.39)	0.08 (-0.08-0.23)	0.19* (0.04-0.34)	0.30** (0.15-0.44)	0.11 (-0.05-0.26)	0.16* (0.00-0.31)
Quercetin	0.18* (0.03-0.33)	0.07 (-0.09-0.24)	0.15 (0.00-0.30)	0.20* (0.05-0.35)	-0.06 (-0.21-0.10)	0.17* (0.01-0.32)
Phloretin	0.22** (0.07-0.36)	0.09 (-0.07-0.24)	0.12 (-0.04-0.27)	0.23** (0.08-0.37)	-0.04 (-0.20-0.12)	0.22** (0.07-0.36)
Daidzein	0.11 (-0.05-0.26)	0.12 (-0.04-0.27)	0.07 (-0.09-0.22)	0.10 (-0.06-0.25)	0.08 (-0.08-0.23)	0.03 (-0.13-0.19)
Genistein	0.12 (-0.04-0.27)	0.03 (-0.13-0.19)	0.12 (-0.04-0.27)	0.14 (-0.02-0.29)	-0.08 (-0.23-0.08)	0.12 (-0.04-0.27)
Kaempferol	0.18* (0.03-0.33)	0.08 (-0.08-0.23)	0.15 (0.00-0.30)	0.20* (0.05-0.35)	-0.06 (-0.21-0.10)	0.16* (0.00-0.31)
'Fruit marker'	0.29** (0.14-0.43)	0.24** (0.09-0.38)	0.22* (0.07-0.36)	0.37** (0.23-0.50)	0.25** (0.10-0.39)	0.22** (0.07-0.36)

407 \*\* Correlation is significant at the 0.01 level 2-tailed.

408 \* Correlation is significant at the 0.05 level 2-tailed.

409 Citrus flavonoids' refers to the sum of hesperetin, naringenin and eriodictyol. Total flavonoids refer to the sum of  
 410 quercetin, isorhamnetin, tamarixetin, kaempferol, hesperetin, naringenin, eriodictyol, daidzein, genistein and  
 411 phloretin, 'Fruit marker' refers to the sum of citrus flavonoids and phloretin.

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414 **FIGURE LEGENDS**

415

416 **FIGURE 1** Error bars reflecting the change over time (from baseline to after 5 months) in the  
417 median (95% CI) estimated intake of fruits (g/day) between control and intervention group.

**FIGURE 1**

