Flavonoids as fruit and vegetable intake biomarkers: Development, validation and application of flavonoid biomarkers in nutritional research

Most validation studies show that the food frequency questionnaire (FFQ) is rather low in precision and accuracy, and there is an ongoing debate regarding the applicability of such self-reported data with regard to diet-disease relationships. However, no other method has so far been able to replace these dietary assessment questionnaires, and a thorough validation of the FFQ relative to one or two independent reference methods, such as objective biomarkers, is thus particularly important. If two independent reference methods are measured it is possible to use the method of triads for the validation, while calculation of the bivariate correlation coefficients is the common approach when using only one reference method. Back in 2002, a strictly controlled dietary intervention study indicated that the sum of 7 different flavonoid aglycones excreted in 24h urine samples potentially could be used as a biomarker of fruit and vegetable intake (Nielsen et al, 2002). The overall aim of the present Ph.D. thesis was to further develop and validate this potentially new fruit and vegetable biomarker and furthermore use it for the validation of self-reported dietary intake of fruits and vegetables in intervention and cohort studies. The Ph.D. thesis contains four scientific papers. Paper I provides evidence that the sum of 7 flavonoids in 24h urine respond in a linear and sensitive manner to moderate increases in the intake of fruits and vegetables, and thus consolidates that the flavonoids are a valid biomarker of fruit and vegetable intakes. In Paper II, the urinary recovery of the 7 flavonoids in morning spot urine (i.e. all urine voids from midnight including the first morning void) was also found to respond to moderate increases in the intake of fruits and vegetables. However, the association was somewhat weaker than in 24h urine samples, indicating that the 24h urinary recovery of the 7 flavonoids is a stronger biomarker of the intake of fruit and vegetables than the urinary recovery of the 7 flavonoids in morning spot urine. In Paper II, the biokinetic profiles of some of the most important dietary flavonoids are described (quercetin, naringenin and hesperetin), since there was a need for improved understanding of bioavailability and metabolism of the flavonoids included in the flavonoid biomarker assay originally developed by Nielsen et al. (2000). In Paper I-II we observed a high degree of inter-individual variation in the absorption and urinary recovery of the flavonoids, and this makes it very difficult to separate individuals according to intake by use of the flavonoid biomarker in urine. The intra-individual variation was on the contrary low, and Paper II therefore supports the assumption, that 24h urinary recovery of flavonoids has great potential as a compliance biomarker and as a biomarker of both controlled and uncontrolled changes in the intake level of fruits and vegetables achieved by an intervention. In Paper III we use the flavonoid biomarker in 24h urine samples in a workplace-based intervention study providing free fruit or no free fruit to employees as validation of a 24h dietary recall. We found that the estimation of fruit intake by the 24h dietary recall was a valid estimate of the true effect of the free-fruit intervention programme, due to highly significant correlations between 24h urinary recovery of flavonoids and the selfreported intake of fruit. One of the aims of Paper IV was to use the method of triads (Kaaks 1997, Ocké & Kaaks 1997) to validate the intake of fruits, vegetables and beverages rich in flavonoids in a population-based cohort in Denmark (referred to as the ‘Inter99’ cohort). The method of triads requires three different and independent measures of the variable of interest, and therefore, beside the FFQ, also the plasma concentration of carotenoids was measured in addition to the flavonoid biomarker. The second aim of Paper IV was to investigate whether the urinary recovery of flavonoids in morning spot urine could substitute the 24h urinary recovery of flavonoids as an alternative and more feasible biomarker of fruit and vegetable intake. Using the method of triads resulted in validity coefficients (VCs) for FFQ that were higher than the bivariate correlation coefficients between total fruit, juice, tea and vegetable intake estimated by FFQ and each of the biomarkers. This finding, of a statistically significant correlation between the Inter99 FFQ and two independent biomarkers indicates that the Inter99 FFQ provides a useful estimate of the overall dietary intake of fruits, juices, tea and vegetables in the Inter99 cohort. VCs for the FFQ ranged from 0.43-0.68 using 24h urine and from 0.35-0.64 using morning spot urine, indicating that the intake of fruits, juice, tea and vegetables was reflected by flavonoids both in morning spot and 24h urine. Collection of 24h urine is difficult and time consuming, and therefore morning spot urine may be a more convenient tool than 24h urine for validating the fruit and vegetable consumption in large population studies.

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