**Short communication**

**High concordance of daidzein-metabolizing phenotypes in individuals measured 1 to 3 years apart**

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Particular intestinal bacteria are capable of metabolizing the soya isoflavone daidzein to equol and/or O-desmethylangolensin (O-DMA), and the presence of these metabolites in urine after soya consumption are markers of particular intestinal bacteria profiles. Prevalences of equol producers and O-DMA producers are approximately 30–50 % and 80–90 %, respectively, and limited observations have suggested that these daidzein-metabolizing phenotypes are stable within individuals over time. Characterizing stability of these phenotypes is important to understand their potential as markers of long-term exposure to particular intestinal bacteria and their associations with disease risk. We evaluated concordance within an individual for the equol-producer and O-DMA-producer phenotypes measured at two time points (T1, T2), 1–3 years apart. Phenotypes were ascertained by analysing equol and O-DMA using GC-MS in a spot urine sample collected after 3 d soya (source of daidzein) supplementation. In ninety-two individuals without recent (within 3 months before phenotyping) or current antibiotics use, 41 % were equol producers at T1 and 45 % were equol producers at T2, and 90 % were O-DMA producers at T1 and 95 % were O-DMA producers at T2. The percentage agreement for the equol-producer phenotype was 82 and for the O-DMA-producer phenotype was 89. These results indicate that these phenotypes are stable in most individuals over time, suggesting that they provide a useful biomarker for evaluating disease risk associated with harbouring particular intestinal bacteria responsible for, or associated with, the metabolism of the soya isoflavone daidzein.

**Equol: O-Desmethylangolensin: Soya: Daidzein**

The soya isoflavone daidzein is metabolized to equol and O-desmethylangolensin (O-DMA) by particular intestinal bacteria. Although the specific bacteria responsible have not yet been definitively identified, there are several lines of evidence from in vitro and animal studies to indicate that intestinal bacteria, and not endogenous host metabolism, are responsible for this conversion (Chang & Nair, 1995; Blair et al. 2003; Bowey et al. 2003; Atkinson et al. 2004). For example, it has been observed that, in vitro, microbiota in faeces from equol producers can convert daidzein to equol, whereas microbiota from non-producers does not (Chang & Nair, 1995; Atkinson et al. 2004) and in vivo, that germ-free animals do not produce equol (Bowey et al. 2003). Equol and O-DMA can be absorbed from the gastrointestinal tract into host circulation and excreted in urine. Thus, urinary excretion of equol and O-DMA are markers of particular intestinal bacterial profiles. Approximately 30–50 % of individuals harbour the bacteria capable of producing equol (equol producers) and 80–90 % of individuals harbour the bacteria capable of producing O-DMA (O-DMA producers; reviewed in Atkinson et al. 2005). Because intestinal bacteria are involved in hormone metabolism in the gut (Adlercreutz et al. 1976; Lombardi et al. 1978; Jarvenpaa et al. 1980), the variability in bacterial daidzein metabolism may be associated with hormone-related disease risk; we review this in detail elsewhere (Atkinson et al. 2005).

Observations from a study of Japanese men suggest that these daidzein-metabolizing phenotypes are stable in individuals over time. In forty men, 85 % retained their equol-producer phenotype when measured at two time points approximately 1·5 years apart (Akaza et al. 2004); the O-DMA-producer phenotype was not evaluated. The objective of the present study was to evaluate

**Abbreviations:** Cr, creatinine; O-DMA, O-desmethylangolensin.

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within-individual concordance of equol-producer and O-DMA-producer phenotypes measured at two time points, 1–3 years apart, in a population of men, women and children living in the USA. Understanding the stability of these phenotypes over time will provide insight into their importance as markers of long-term exposure to particular intestinal bacteria.

Materials and methods

Individuals who had previously participated in a family study of daidzein metabolism (Frankenfeld et al. 2004a) were approached to participate in a follow-up study. In order to achieve our target sample of 100 individuals, 182 individuals were mailed approach letters and consent forms. Urine samples were received from 112 of the 122 individuals who consented to participate in the follow-up. The Institutional Review Board at Fred Hutchinson Cancer Research Center approved all procedures and informed, written consent was obtained from all participants.

Participants followed the same protocol of soya consumption and urine collection as described in the parent study (Frankenfeld et al. 2004a). Briefly, each participant supplemented his/her usual diet with a soya food item once per d on three consecutive days. On the morning of the fourth day, each participant collected a first-void urine sample (50–80 ml). Urinary isoflavones are stable at room temperature for 14 d (Frankenfeld et al. 1999) and analysed for isoflavonoids by GC-MS as described elsewhere (Frankenfeld et al. 2004b). Given the sensitivity of assay, urine concentrations less than 182 nmol/l (44 ng/ml) in urine) of equol and 170 nmol/l (44 ng/ml) in urine) of O–DMA were considered below the level of quantification. Equol and O-DMA producers were defined as individuals with any concentration greater than the level of quantification of equol and O-DMA, respectively.

Prior to data analysis, ten individuals were excluded because daidzein and genistein concentrations were below the level of quantification suggesting non-compliance with soya consumption (n 4), or questionnaire information was incomplete (n 4), or we were unable to match the urine sample with the questionnaire because of suspected kit swapping between family members (n 2).

For the main analysis of phenotype concordance between the parent study (T1) and the follow-up study (T2), individuals without current or recent (within the 3 months prior to phenotyping at T2) antibiotics use were analysed. A separate analysis was conducted including all individuals. Percentage agreement was defined as the number of concordant individuals divided by the total number of individuals multiplied by 100. The £statistic, a measure of agreement that corrects for agreement observed by chance defined as £ = (observed − expected proportion in agreement)/ (1 − expected proportion in agreement) (Thompson & Walter, 1988), and its standard error were calculated using Stata 8.2 (Stata Corporation, College Station, TX, USA). Frequency tables were generated to explore potential factors associated with phenotype discordance.

Results

Percentage agreement between T1 and T2 for the equol-producer phenotype was 81 (£ 0·64, SE 0·10) and for the O-DMA-producer phenotype was 89 (£ 0·27, SE 0·10; Table 1). Including individuals with current and recent antibiotics use did not markedly alter the percentage agreement: 81 for the equol-producer phenotype (£ 0·61, SE 0·10) and 86 for the O-DMA-producer phenotype (£ 0·22, SE 0·09).

There was no apparent relationship between urinary equol and O-DMA concentrations with phenotype concordance. Equol concentrations in producers had wide variation, but were overall similar in equol-producing concordant individuals (T1: mean 4·3 µg/mg creatinine (Cr) (SD 3·6), T2: mean 3·7 µg/mg Cr (SD 2·2)) and discordant individuals who were producers at T1 but not T2 (T1: mean 2·3 µg/mg Cr (SD 4·0)) and discordant individuals who were producers at T2 but not T1 (T2: mean 5·8 µg/mg Cr (SD 5·9)). O-DMA concentrations were also similar in O-DMA-producing concordant individuals (T1: mean 2·1 µg/

| Table 1 | Equol-producer and O-desmethylangolensin (O-DMA)-producer phenotypes in ninety-two individuals measured at two time points (T1 and T2), 1–3 years apart, who were not taking antibiotics or had not used antibiotics in the 3 months prior to phenotyping at either measurement |
|---|---|---|
| Phenotype at T2 | Equol producer | Equol non-producer | Totals |
| Equol-producer phenotype | | | |
| Phenotype at T1 | Equol producer | 31 (34) | 10 (11) | 41 (45) |
| | Equol non-producer | 7 (8) | 44 (47) | 51 (55) |
| | O-DMA producer | 38 (41) | 54 (59) | 92 (100) |
| O-DMA-producer phenotype | | | |
| Phenotype at T1 | O-DMA producer | 80 (87) | 7 (8) | 87 (95) |
| | O-DMA non-producer | 3 (3) | 2 (2) | 5 (6) |
| | O-DMA producer | 83 (90) | 9 (10) | 92 (100) |
Daidzein-metabolizing phenotype concordance


