Urinary isoflavone phytoestrogens in German children and adolescents – A longitudinal examination in the DONALD cohort

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Scope: In light of concerns about hormonally active agents, it is important to assess human exposure to such compounds, especially in children as a susceptible subgroup. Estrogenic plant constituents are present in the human diet in varying levels, in particular the isoflavones daidzein (DAI) and genistein (GEN). We aimed to examine age-dependent and secular trends in phytoestrogen exposures and to investigate equol (EQ) excretion of German children using biomarker analysis in 24-h urine samples from a longitudinally designed study.

Methods and results: The concentrations of DAI, its metabolite EQ and GEN were determined by GC-MS analysis in 24-h urines (510 samples) collected between 1985 and 2000 in 90 (47 boys) German children (6–18 years old), who are participants in the Dortmund Nutritional and Anthropometric Longitudinally Designed study. The results from the urinary biomarker analysis indicate isoflavone exposures at quite variable levels in German children: Analyte concentrations in over 500 urine samples cover the range reported previously in adults on typical German diet and with soy intake. EQ, the DAI metabolite produced by the gastrointestinal microflora, was detected in a high fraction of all samples, with 28/90 children (31%) excreting EQ in all their urines, and 62/90 children (68%) in at least one sample. Interestingly, when multiple urines obtained from individuals at different ages (6–18 years) were analyzed, EQ formation did not appear to be a constant trait over time. When stratified by sex, DAI, EQ and GEN concentrations (ng/mL) in urines and excretion rates (µg/day) were similar in boys and girls. Total isoflavone excretion rates (µg/day) increased during childhood (6–12 years) (p < 0.02) and were constant during adolescence (13–18 years) (p = 0.6). No clear trend for changes in dietary isoflavone exposure over the total study period was seen (p = 0.7).

Conclusions: In conclusion, biomarkers in urine of German children and adolescents indicate a frequent, but widely variable dietary isoflavone intake and suggest no secular increase (1985–2000) in the exposure to isoflavone phytoestrogens among German children and adolescents.

Keywords: Biomarkers / Daidzein / Equol / Genistein / Phytoestrogens

1 Introduction

Human exposure to hormonally active compounds of natural or anthropogenic origin occurs mainly with food [1, 2]. An important group of dietary estrogens is phytoestrogens [3, 4], in particular the isoflavones daidzein (DAI) and genistein (GEN). DAI and GEN are found at highest levels in soybeans and soy products, but are also present in numerous other edible plants and in fruits [3, 5]. Although estrogenic potency is rather weak by comparison to steroids [6], isoflavones may affect the endocrine system of humans when ingested in sufficient quantities and during stages of low endogenous hormone levels such as childhood and menopause [3, 4]. Because of potential health benefits (reviewed in [7, 8]), phytoestrogens have received considerable attention, also as an alternative to hormone replacement therapy. Yet, isoflavone-rich supplements due to their
estrogenic activity may present a risk in breast cancer survivors [9, 10], and their increased use is regarded with caution [8, 11].

Moreover, based on animal studies isoflavone phytoestrogens qualify as potential endocrine disruptors [3, 12, 13], and fetal exposure is documented in rodents [14, 15] and also in humans [16]. Of special interest are isoflavone exposures early in life, since infants on soy-based formula have a much higher intake of DAI and GEN than breast-fed infants or those receiving cow milk [8, 17]. This is also reflected in rather high blood [18], and about 500-fold higher isoflavone urine levels in infants on soy formula [19, 20]. While much attention has been paid to infants and soy isoflavone exposure in early life [11, 17], little is known about isoflavone exposure in childhood and adolescence in the general population although measures of long-term phytoestrogen exposure in relation to health outcome (adverse or beneficial) later in life are of interest.

Since the phytoestrogen metabolite equol (EQ) has a higher affinity for estrogen receptors than DAI, it has been proposed that biological effects from soy isoflavones may correlate with EQ-producing status of individuals, and that EQ excretion may serve as a biomarker for isoflavone effectiveness [21]. EQ is formed by the gut microflora from its precursor DAI, and the extent of this metabolism is apparently highly variable among populations and individuals, and also influenced by dietary components such as carbohydrates and fat [22, 23].

Overall, we know currently little about (i) age-related differences in isoflavone exposure in Western countries, and practically nil in contemporary cohorts of children or adolescents in Germany. This is the first study to examine this aspect. Moreover, since soy flour has been widely adopted in the preparation of numerous processed foods prominent in the Western diet in the past decades [24], it is also of interest to study (ii) possible long-term temporal changes in isoflavone exposure. We investigated these two longitudinal aspects by means of isoflavone biomarkers in 24-h urines of male and female German children and adolescents (age 6–18), participants of the DONALD Study, who have previously been examined for a longitudinal study of melatonin production rates during growth [26]. Of those, only children and adolescents aged 6–18 years were included (n = 76, 445 urine samples). These urine samples were grouped according to the following three time periods: 1985–1989, 1990–1994 and 1995–2000. Fourteen additional DONALD participants (n = 65 urine samples) were selected (6 boys, 8 girls) in order to replenish the 1995–2000 time period and to assure a similar sex and age distribution across all three periods. Therefore, the current study population comprised 90 subjects (47 boys) with 510 urine samples.

2.2 Sample selection

The present sample was in first place selected from 84 healthy participants (age 3–18 years) of the DONALD Study with at least three complete 24-h urine samples (n = 621), who have previously been examined for a longitudinal study of melatonin production rates during growth [26]. Of those, only children and adolescents aged 6–18 years were included (n = 76, 445 urine samples). These urine samples were grouped according to the following three time periods: 1985–1989, 1990–1994 and 1995–2000. Fourteen additional DONALD participants (n = 65 urine samples) were selected (6 boys, 8 girls) in order to replenish the 1995–2000 time period and to assure a similar sex and age distribution across all three periods. Therefore, the current study population comprised 90 subjects (47 boys) with 510 urine samples.

2.3 Urinary collection and isoflavone measurements

Parents and children were carefully instructed on how to collect complete 24-h urine samples. All micturitions were stored immediately in preservative-free, Extrann-cleaned 1 L plastic containers at ≤−12 °C before they were transported. At the research institute, the containers are stored at ≤−20 °C until analysis. For the purpose of this analysis, completeness of the 24-h urines was ascertained via the criteria, daily creatinine excretion rates > 0.1 mmol kg⁻¹ d⁻¹ [27]. Creatinine was measured by the Jaffé method with a creatinine analyzer (Beckman-2; Beckman Instruments, Fullerton, CA). Samples that were reported or found to contain incomplete micturitions were excluded from analysis. The urine samples were thawed immediately before analysis and were analyzed for the concentration of isoflavones (DAI, GEN and EQ) using a previously validated sample preparation and GC-MS method [28]. In brief,
urine aliquots (200 μL) were mixed with hydrolysis buffer and internal standards, then incubated overnight for hydrolysis of conjugates with pure β-glucuronidase and sulfatase enzyme. The aglycone analytes (DAI, EQ, GEN) were then extracted with solid-phase extraction cartridges. Eluates were evaporated and residues dissolved in derivatization reagent prior to GC-MS analysis; see [28] for all details. The limits of detection were 3.5 ng/mL for DAI, 5.3 ng/mL for GEN and 3.8 ng/mL for EQ (the gut bacterial metabolite of DAI). The 24-h excretion of isoflavones was calculated by multiplying the analyte concentrations with the 24-h urine volumes. The sum of urinary DAI, GEN and EQ excretion was used as marker of the overall daily isoflavone excretion.

2.4 Statistical analysis

All statistical tests were performed using SAS procedures (version 9.1, SAS Institute, Cary, NC). Descriptive data are given as mean ± SD or median with inter-quartile range when appropriate. Sex differences for anthropometric and urinary characteristics were tested by using unpaired t-test.

For the longitudinal analysis of time trends and age dependence of 24-h total isoflavone excretion, the mixed linear model PROC MIXED was used (Year of urine collection and age were both included as continuous variables). Models for age dependency were run separately in prepubertal/early pubertal (6–12 years) and pubertal children (13–18 years) adjusted for sex and year of urine collection. Models for time trends were each run for the three 5-year intervals (1985–1989, 1990–1994, 1995–2000) adjusted for sex and age. This model allowed for the inclusion of age covariates in the mean structure and repeated measurements in the same subjects. In contrast to other models, e.g. PROC GLM for balanced longitudinal data, the PROC MIXED model uses all the available data and not only the complete cases [29]. The level of significance was set at p < 0.05.

3 Results

Anthropometric and urinary characteristics of a subset of German children (n = 90) of the DONALD cohort are presented in Table 1. Boys included in the present analyses (n = 47) were 9.1 (± 2.8) years old at the visit of the first urine collection. Girls included in the analyses (n = 43) were 9.6 (± 2.7) years old at the visit of the first urine collection. Each individual in this subcohort of children and adolescents had provided at least three complete 24-h urines between 1985 and 2000. When stratified by sex, anthropometric data (weight, height, body surface, BMI and BMI-SDS) were not significantly different between boys and girls. Also urinary volume, body weight-related creatine output and isoflavone (DAI, EQ, GEN) levels were not significantly different between boys and girls. When isoflavone analyte concentrations (ng/mL) were converted into excretion rates

| Table 1. Anthropometric and urinary characteristics of a sample of 90 healthy children and adolescents at the visit of the first urine collection (1985–2000) stratified by sex |
|-------------------------------------------|----------------|-------------------|
| Age (year) | 9.1 ± 2.8c) | 9.6 ± 2.7 |
| Year of birth | 1979 ± 4 | 1978 ± 4 |
| Birth weight (g) | 3439 ± 477 | 3354 ± 447 |
| **Anthropometric data** | &nbsp; | &nbsp; |
| Weight (kg) | 33.4 ± 14.1 | 34.5 ± 10.7 |
| Height (cm) | 137.4 ± 18.7 | 140.3 ± 16.3 |
| BMI (kg/m²) | 16.9 ± 2.5 | 17.0 ± 2.0 |
| BMI-SDSc) | −0.02 ± 0.86 | −0.08 ± 0.71 |
| **Urinary data** | &nbsp; | &nbsp; |
| Urine volume (mL/day) | 643 (448, 862) | 601 (424, 844) |
| Creatinine (mmol/kg weight) | 0.19 ± 0.04 | 0.17 ± 0.04 |
| DAI concentration (ng/mL) | 152 (71, 319) | 106 (55, 202) |
| DAI 24-h excretion (μg/day) | 100 (40, 221) | 61 (38, 107) |
| GEN concentration (ng/mL) | 75 (42, 198) | 51 (36, 97) |
| GEN 24-h excretion (μg/d) | 51 (31, 130) | 33 (26, 50) |
| EQ concentration (ng/mL) | 9.1 (5.8, 25.5) | 7.4 (5.8, 10.5) |
| EQ 24-h excretion (μg/d) | 5.3 (3.5, 16.7) | 4.7 (3.6, 7.6) |

a) Sex differences were tested with unpaired t test for continuous variables and chi-square test for categorical variables.
b) 18 boys and 10 girls have no measurable EQ and 3 boys and 3 girls have no measurable GEN.
c) Mean ± SD (all such values).
d) SDS, SD scores according to the German reference curve for BMI [43].
e) Median: 25th and 75th percentile in parentheses (all such values).
on the basis of each person’s 24-h urine volume, the only significant difference was a lower DAI excretion in girls than in boys (Table 1). All subjects excreted DAI, and only 3 boys and 3 girls had no measurable GEN levels in urine. The DAI metabolite EQ was apparently formed in many but not all children, with urines of 18 boys and 10 girls showing no measurable EQ levels.

Overall, the percentage of urines with detectable (> LOD 3.5 ng/mL) EQ levels was about 49%. EQ concentrations in urines from boys and girls of different ages at urine collection are depicted in Fig. 1, indicative of a wide range of EQ concentrations, with peak values of nearly 200 ng/mL in some samples. The longitudinal design of the DONALD study enabled us to monitor isoflavone and EQ excretion over time in individuals who had provided urines on several occasions. Examples of such profiles are given in Fig. 2, for a female with EQ in all urines collected between age 10 and 17 (Fig. 2A), a male with EQ in nearly all urines with variable levels between age 6 and 18 (Fig. 2B), and another male that is apparently a poor EQ excreter between age 6 and 11, but, a rather high EQ excreter from age 12 to 18 (Fig. 2C). Interestingly, the EQ levels were often unrelated to concurrent DAI and GEN levels reflecting high or low isoflavone intake. In other words, the extent of EQ formation was variable and apparently not only dependent on phytoestrogen precursor (DAI) ingestion.

Despite the observed intra- and inter-individual variability in EQ excretion (Figs. 1 and 2), it is obvious that this metabolite can account for an important fraction of the total urinary isoflavones. EQ as a component of total urinary biomarkers was thus included in a further analysis for possible age-related changes and secular trends of phytoestrogen isoflavone exposure in the DONALD cohort.

The results of urinary biomarker data, grouped according to age at sample collection, for children and adolescents between 6–10, 11–14 and 15–18 years are depicted in Fig. 3. There appeared to be small decline in DAI concentrations (mg/day) on the basis of each person’s 24-h urine volume.

![Figure 1](image1.png)

**Figure 1.** EQ concentrations (ng/mL) measured in 24-h urines of boys and girls from the DONALD cohort show a considerable variation, ranging from ≥3 ng/mL to nearly 200 ng/mL. The dotted line indicates the threshold of 20 ng/mL proposed to define an individual as EQ excreter [39].

![Figure 2](image2.png)

**Figure 2.** Urinary biomarker analysis of three individuals in different years. Profiles in 24-h urine samples from (A) a female between age 10 and 17, from (B) a male between 6 and 18, and from (C) another male between age 6 and 18 show variable excretion of EQ in different years of sampling, and apparently unrelated to the amount of its precursor DAI in that year.
with increasing age (Fig. 3A), yet a small increase in DAI daily excretion rates after adjusting analyte concentrations by 24-h urine volumes (Fig. 3B). The additional longitudinal analysis of age dependence of 24-h total isoflavone excretion (by mixed linear model PROC MIXED) revealed the following: The 24-h total isoflavone excretion increased across age among the prepubertal and early pubertal children (age 6–12 years), and was constant throughout adolescence (13–18 years) (Fig. 4). Overall, this biomarker data document frequent, albeit variable, dietary isoflavone phytoestrogen exposure in German children from infancy to adulthood.

Table 2 compiles the anthropometric and urinary characteristics of the 90 children stratified by urine collection cohort to investigate possible secular trends in phytoestrogen exposure between 1985 and 2000. As expected, the weight, height and mean urine volumes increase over the three sample collection periods (1985–1989, 1990–1994, 1995–2000) along with a shift in the mean age of participants (from 10 to 15 years) at urine collection. The median concentrations and 24-h excretion rates of DAI, EQ and GEN, depicted in Fig. 5, were similar in the urine samples of the three collection cohorts. Urinary isoflavone and metabolite levels (Fig. 5A) and excretion rates (Fig. 5B) showed a wide range of values among German adolescents probably related to variable dietary intake, yet no clear trend for changes in the collection cohorts between 1985 and 2000.

The additional longitudinal analysis of secular trends in total isoflavone excretion (by mixed linear model PROC MIXED) showed the following: There was a significant increase in 24-h total isoflavone phytoestrogen excretion from 1985 to 1989, no significant change between 1990 and 1994, and a significant decrease from 1995 to 2000 (Fig. 6).

4 Discussion

Our study provides novel and longitudinal data on urinary isoflavone biomarkers in German children and adolescents, participants of the DONALD study. The results demonstrate isoflavone exposures at quite variable levels in German children: Analyte concentrations in all urine samples from boys and girls cover the range reported previously in a small group of adults on typical German diet [28], and upper end concentrations resemble those of adults with soy intake [30, 31]. The predominant isoflavone excreted in children’s urine was DAI, with median DAI concentrations being twice as high as those for GEN, and much higher than those for EQ (Figs. 3 and 5). This pattern, seen in all three age groups in our cohort is in accordance with results from other studies, such as spot urine analysis in adult women on Western diet, viz the Norfolk cohort of the European Prospective Investigation of Cancer and Nutrition, EPIC [32]. Their median urinary concentrations (DAI 88.1, GEN
Table 2. Anthropometric and urinary characteristics of the sample of 90 healthy children and adolescents (510 urine) stratified by urine collection cohort

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<thead>
<tr>
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<tbody>
<tr>
<td>Age at measurement (ys)(^a)</td>
<td>10.2 (8.0, 12.9)</td>
<td>13.0 (11.1, 15.0)</td>
<td>15.0 (13.0, 17.0)</td>
</tr>
<tr>
<td>n of urine measurements</td>
<td>164(^b)</td>
<td>197(^c)</td>
<td>149(^d)</td>
</tr>
<tr>
<td>n of individuals (boys)</td>
<td>69 (35)</td>
<td>70 (35)</td>
<td>53 (28)</td>
</tr>
<tr>
<td>Anthropometric at urine measurements(^e)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>37.8 (28.1, 49.0)</td>
<td>50.3 (38.0, 62.6)</td>
<td>56.3 (45.4, 64.1)</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>146 (132, 161)</td>
<td>163 (147, 170)</td>
<td>169 (158, 175)</td>
</tr>
<tr>
<td>BMI (kg/m(^2))</td>
<td>17.5 (16.0, 20.3)</td>
<td>19.1 (17.0, 21.6)</td>
<td>19.4 (17.9, 21.5)</td>
</tr>
<tr>
<td>BMI-SDS(^e)</td>
<td>0.19 (−0.40, 0.63)</td>
<td>0.08 (−0.35, 0.67)</td>
<td>−0.05 (−0.77, 0.51)</td>
</tr>
<tr>
<td>Urinary data(^f)</td>
<td></td>
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<tr>
<td>Urine volume (mL/day)</td>
<td>660 (471, 885)</td>
<td>841 (637, 1140)</td>
<td>1040 (818, 1350)</td>
</tr>
<tr>
<td>Creatinine (mmol/kg weight)</td>
<td>0.17 (0.16, 0.20)</td>
<td>0.18 (0.16, 0.20)</td>
<td>0.18 (0.16, 0.21)</td>
</tr>
<tr>
<td>DAI concentration (ng/mL)</td>
<td>123 (59, 253)</td>
<td>137 (65, 270)</td>
<td>105 (43, 218)</td>
</tr>
<tr>
<td>DAI 24-h excretion (µg/day)</td>
<td>84 (42, 165)</td>
<td>117 (61, 218)</td>
<td>101 (47, 237)</td>
</tr>
<tr>
<td>GEN concentration (ng/mL)</td>
<td>63 (35, 150)</td>
<td>71 (40, 152)</td>
<td>54 (26, 116)</td>
</tr>
<tr>
<td>GEN 24-h excretion (µg/day)</td>
<td>45 (24, 96)</td>
<td>66 (30, 134)</td>
<td>53 (27, 129)</td>
</tr>
<tr>
<td>EQ concentration (ng/mL)</td>
<td>7.7 (5.3, 16.0)</td>
<td>9.0 (6.2, 21.9)</td>
<td>8.1 (6.1, 17.0)</td>
</tr>
<tr>
<td>EQ 24-h excretion (µg/day)(^f)</td>
<td>4.9 (3.5, 10.9)</td>
<td>8.9 (5.1, 15.2)</td>
<td>9.0 (6.4, 16.4)</td>
</tr>
</tbody>
</table>

a) Median: 25th and 75th percentile in parentheses (all such values).
b) In cohort 1, EQ was not measurable in 48 urines and GEN in 12 urines.
c) In cohort 2, EQ was not measurable in 48 urines, GEN in 7 urines, and DAI in 3 urines.
d) In cohort 3, EQ was not measurable in 25 urines, GEN in 13 urines, and DAI in 5 urines.
e) SDS, SD scores according to the German reference curve for BMI [43].
f) 28 children have measurable EQ excretion in all urine samples; the other 62 children have measurable EQ excretion in at least one sample.

38.9 and EQ 2.6 ng/mL) were lower than the median urinary isoflavone concentrations in the DONALD cohort (Table 1). This probably reflects the low dietary isoflavone intake in the EPIC cohort of less than 0.5 mg/day, based on dietary records [32], while the recently calculated mean dietary isoflavone intake of German prepubertal children is 3–12 mg for vegetarians and soy consumers in European countries. By comparison, the daily isoflavone intake of adult Asians consuming traditional soy-rich food, and that of infants on soy-based formula is clearly higher, in the range of about 7–47 mg/day [33]. The intake values reported from epidemiological studies among European omnivorous adults [34] are of similar magnitude. A recent overview [8] quotes total daily isoflavone intake in adults on typical Western diet in the range of 0.5 to \(\approx\) 3 mg, and between 3–12 mg for vegetarians and soy consumers in European countries. By comparison, the daily isoflavone intake of adult Asians consuming traditional soy-rich food, and that of infants on soy-based formula is clearly higher, in the range of about 7–47 mg/day [8]. In an intervention study it was observed that isoflavones from soy food are more bioavailable in children than in adults [35]. Since this may affect comparisons of dietary intake and urinary biomarkers between adults and children, the further discussion will focus on studies in children.

A pilot study on urinary biomarkers [36] and a related cross-sectional study [37] in prepubertal girls in New York City revealed that – similar to the situation in German children – phytoestrogen exposure was common in the multiethnic US cohort. Dietary intake estimates reported for isoflavones were 1.7–2.2 mg [37], and geometric mean urinary levels for DAI, GEN and EQ in New York City girls were 112, 60.4 and 10.9 µg/L, respectively [36]. These urinary biomarker concentrations were similar to those reported for children in the NHANES 1999–2000 and 2000–2001 exposure survey [37], (U.S. Center for Disease Control and Prevention, Third National Report on Human Exposures to Environmental Chemicals, 2005, http://www.cdc.gov/exposurerreport). The latter studies have all used a cross-sectional design to investigate isoflavone exposure in the general population.

To our knowledge, there are no longitudinal studies so far on isoflavone excretion in children and adolescents on Western style diet. Also, presently published data on the temporal variability of urinary isoflavone concentrations are limited, except for a study in 9-year-old girls [38] that reported a reasonable degree of temporal reliability over a short (6 months) period. Yet, most studies published so far include only a single urine sample that may or may not reflect an individual’s long-term exposure level. Our results for urinary isoflavone biomarkers in the DONALD cohort shed some light on long-term phytoestrogen exposure of German children and adolescents as well as excretion of EQ in this cohort.

Measurable concentrations of EQ (\(\geq 3.5\) ng/mL) were found in a large proportion of 24-h urines from our cohort, with no significant difference in median levels when stratified by sex (Table 1). EQ analysis in urines collected at

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different ages showed a wide range of concentrations in boys and girls (Fig. 1), and numerous samples with EQ levels \( \geq 20 \text{ng/mL} \), a threshold proposed to classify an individual as EQ excretor [39]. Studies with infants and young children indicate that the ability to produce EQ is not acquired until later in life. EQ excretion was rarely detected (only 5% \( \geq \) LOD = 3.3 ng/mL) in urine of soy formula fed US infants up to an age of about 1 year [20]. When isoflavone excretion was examined in UK infants and children from the ages of 4 months to 7 years [19], the frequency of EQ excretors across all age groups following soy challenge (about 20%) was clearly lower than reported for adults (about 35%) in the UK [22] or in the German (Berlin) cohort of the Isoheart intervention study (about 50%) [40].

In a study on isoflavone kinetics, a small UK cohort of premenopausal women received stable isotope-labeled DAI or GEN on three occasions, separated by a minimum of 2 wk. As 3 of 8 women (37.5%) who received \(^{13}\text{C}\)-DAI were found to excrete EQ on the first visit and the next two visits, this suggested that “once an equol producer, always an equol producer” [41]. Yet, whether this holds true for longer periods in life, and the question whether a person who is unable to produce EQ will ever be able to do so, remained unclear. In this regard long-term profiles of children and adolescents from the DONALD cohort who provided urines at different ages are of interest: The examples (Figs. 2A–C) illustrate that the excreted amount of EQ was variable, and not only dependent on isoflavone precursor (DAI) ingestion in that year. The longitudinal data indicate that efficacy of DAI conversion to EQ may vary within individuals over time. This could be due to changes in other dietary components that affect isoflavone metabolism in the gut and/or changes in their gut microflora [22, 23]. Moreover, dietary intake of EQ with milk may complicate an interpretation of biomarker results. Recently, organic skimmed Finnish cow milk was found to contain higher EQ concentrations than conventionally produced milk, with 411 ± 7 g/L and 62 ± 16 g/L milk, respectively [42]. Evidence that EQ ingested with milk is bioavailable and also excreted in urine comes from studies with infants whose gut microflora is not yet fully developed. EQ was present in plasma of all 4-month-old infants fed cows’ milk formula, at higher levels than in soy-formula fed infants [18]. In line with this, urinary EQ was more frequently detected in US infants fed cow milk formula (22%) than in those fed soy formula (5%) [20]. In older children and adolescents, it will be more difficult to distinguish whether urinary EQ is due to dietary intake of EQ with milk and/or endogenous production from isoflavone precursor DAI in fruits and vegetables.

As both types of EQ exposure are important, the urinary EQ concentrations and excretion rates were included along with values for the other analytes to account for total isoflavone phytoestrogen biomarkers in our cohort (Figs. 4 and 6). Data for the separate analytes (DAI, EQ, GEN), when stratified by age groups (6–10, 11–14, 15–18 years; Fig. 3)

To our knowledge, this is the first longitudinal study on isoflavone exposure in children and adolescents, in Germany or elsewhere. While the relatively high socioeconomic status of the DONALD Study participants compared with the German general population [25] can be regarded as a limitation of our study, it is nonetheless well suited to address the three aims stated in Section 1.

In conclusion, phytoestrogen excretion increased across age among the prepubertal and early pubertal children (age 6–12 years), and was constant throughout adolescence (13–18 years). There was an increase in the total isoflavone excretion from 1985 to 1989, no significant change between 1990 and 1994, and a significant decrease from 1995 to 2000, i.e. the analysis suggests no secular increase (1985–2000) in the exposure to isoflavone phytoestrogens among German children and adolescents. With regard to EQ, it is noteworthy that detection of this biomarker in urine was frequent in our cohort. Interestingly, in several individuals EQ excretion did not appear to be a constant trait over longer periods of time.

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5 References


