Effects of dietary broccoli on human in vivo drug metabolizing enzymes: evaluation of caffeine, oestrone and chlorzoxazone metabolism

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Ingestion of cruciferous vegetables may prevent chemically induced carcinogenesis by their influence on specific cytochrome P450 enzymes (CYP) and phase II drug metabolizing enzymes in humans and rodents. Thus CYP enzymes are involved in transformation of procarcinogens, mutagens, steroid hormones and a large variety of other endogenous and exogenous components. In order to learn more about the influence of cruciferous vegetables on drug metabolizing enzymes in man two CYP enzymes previously suggested to be induced by vegetables were selected in an in vivo experiment in humans. Sixteen healthy non-smoking subjects, two females and 14 males, were exposed to three different types of diets and afterwards assayed for CYP1A2 catalysed caffeine metabolites and for CYP2E1 catalysed 6-hydroxylation of chlorzoxazone. Further, 2-hydroxyoestrone:16α-hydroxyoestrone ratios were determined in urine by means of a monocular antibody-based enzyme immunoassay. The three dietary periods were: (A) a customary home diet; (B) a 6 day standard diet avoiding well-known dietary inducers and inhibitors of CYP; (C) a 12 day dietary supplement to the standard diet of 500 g/day broccoli. The average 6-hydroxychlorzoxazone:chlorzoxazone ratio decreased by 21% (P < 0.05) after diet B compared with diet A in a 2 h plasma sample after ingestion of 500 mg chlorzoxazone. The ratio increased by 19% after diet C, however, this was not statistically significant. The caffeine metabolic ratio (CMR) was determined in urine 6 h after ingestion of 100 mg caffeine. The mean CMR increased by 5.5% when changing from diet A to diet B. When changing to diet C the mean CMR increased a further 19% (P < 0.0005). The average 2-hydroxyoestrone:16α-hydroxyoestrone ratio decreased by 1.3% when comparing diet A with diet B. Daily broccoli intake increased the ratio by 29.5% (P < 0.05). A low correlation of CMR with the 2-hydroxyoestrone:16α-hydroxyoestrone ratio indicates that human CYP1A2 and other CYP enzymes involved in oestrone 2-hydroxylation are induced by dietary broccoli. On the other hand, the catalytic activity of CYP2E1 is not affected to the same degree by dietary broccoli.

Abbreviations: I3C, indole-3-carbinol; CYP, cytochrome P450 (nomenclature according to Nebert et al.; ICZ, indole[3,2-c]carbazole; 1,7-DMX, 1,7-dimethylxanthine; I-MX, 1-methylxanthine; CMR, caffeine metabolic rate = (AFMU + 1-MX + 1-MU)/1,7-DMFU; AFMU, 5-acetylamino-6-formylamino-3-methyluracil; 1-MU, 1-methyluric acid; 1,7-DMU, 1,7-dimethyluracil acid; CV, coefficient of variation.

Introduction

Intake of a diet rich in fruit and vegetables, particularly the yellow–green vegetables, is associated with a lower risk of certain types of cancer compared with the common diet (1). Cruciferous vegetables of the genus Brassica contain several chemical compounds that may modulate the carcinogenic process. The compounds may act as anti-oxidants or as inhibitors and/or inducers of phase I and phase II enzymes. One such compound, indole-3-carbinol (I3C*), a degradation product of glucobrassicin, is proposed to be responsible in part for the anti-carcinogenic potential of cruciferous vegetables (2,3). Consumption of I3C by animals and humans increases the activity of cytochrome P450 (CYP; 4) enzymes and a variety of phase II drug metabolizing enzymes such as glutathione S-transferases and UDP-glucuronosyl transferase (5,6). This induction may be due to binding of I3C to the Ah receptor (7,8), however, the affinity of I3C for the Ah receptor is very low compared with I3C-derived indole[3,2-b]carbazole (ICZ) (9). ICZ is formed in small amounts from I3C in the stomach under acidic conditions. Flavonoids, other probably anti-carcinogenic substances, induce and/or inhibit specific glutathione S-transferases and CYP enzymes (10,11). The CYP1A enzymes are known to be induced by cruciferous vegetables in rat tissue (12) and in humans (13–15). Other CYP enzymes, such as CYP2A (16), CYP2B1/2 (17,18), CYP2C11 (18) and CYP2E1 (19,20), appear to be induced by cruciferous vegetables or by I3C. No enhanced level of CYP3A has been observed as a result of a cruciferous diet (16,18), however, large amounts of I3C may induce CYP3A (21).

2-Hydroxylation of oestrogens is a metabolic pathway mediated by several CYP enzymes and is induced by I3C in animals (2,17,22). Several studies of human tissue have shown that 2-hydroxylation of 17α-oestradiol is mediated primarily by CYP3A4 and CYP1A2 (23–25) and to a minor extent by CYP2C9 and other CYP enzymes (26,27). Furthermore, CYP3A4 is the dominant form in human liver (28). In vivo studies of humans show that 2-hydroxylation of oestrogens is enhanced in smokers compared with non-smokers (29). Michnovicz et al. (30) showed a 50% increase in 2-hydroxylation of oestrogen in humans after a 7 day period of I3C exposure, which may lead to a decrease in oestriol formation and explain the reduced risk of malignant tumour formation (31,32).

In the present study we examined the effects of dietary broccoli on CYP1A2 and CYP2E1 activities and on 2- and 16α-hydroxylation of oestrone in a group of volunteers. It is possible to determine human hepatic CYP1A2 activity by metabolic conversion of caffeine. Caffeine can be N-demethylated at three positions, of which formation of 1,7-dimethylxanthine (1,7-DMX) is a specific metabolic pathway of CYP1A2 (33). 1,7-DMX is further metabolized by CYP1A2 to 1-methylxanthine (1-MX). The so-called urinary caffeine metabolic ratio (CMR) [5-acetylamino-6-formylamino-3-methyluracil (AFMU) + 1-MX + 1-methyluric acid (1-MU)]
17-dimethyluric acid (17-DMU)] is a measurement of human hepatic CYP1A2 activity (34,35). Chlorzoxazone, a muscle relaxant drug, is metabolized by 6-hydroxylation, mediated by ethanol-inducible CYP2E1 (36). The ratio of 6-hydroxychlorzoxazone to chlorzoxazone in a plasma sample 2 h after oral ingestion of chlorzoxazone was recently shown to be a good marker for changes in CYP2E1 levels in studies where subjects act as their own references (37). The levels of 2- and 16β-hydroxyoestrone were assayed immunochromatically in urine.

**Materials and methods**

**Chemicals**

All chemicals used were of the highest available commercial purity. Chlorzoxazone (250 mg tablets) and caffeine (100 mg tablets) were from DAK (Nycop Med Ltd, Denmark). AFMU was synthesized as previously described (38). 6-Hydroxychlorzoxazone was extracted from an overnight urine sample from a subject who had ingested 500 mg chlorzoxazone before bedtime. 500 ml urine was mixed with 100 ml 12 M HCl and the acidified urine refluxed at 90°C for 60 min. The now black solution was cooled to room temperature and extracted six times with 500 ml ethylacetate. The organic phase was then reduced to 50 ml under reduced pressure at 40°C. The red organic phase was kept at room temperature for 12 h, after which a white precipitate was separated from the solution. The solution was reduced to 2-3 ml syrup. The syrup was briefly washed in 100 ml cold water and then dissolved in 5 ml ethylacetate, resulting in a bright red solution containing ~75% 6-hydroxychlorzoxazone as judged by HPLC. 6-Hydroxychlorzoxazone was isolated and purified (>99%) by means of a semi-preparative HPLC column (Ultrasphere ODS, 150X10 mm i.d., 5 μm; Beckman Instruments) eluted with 30% acetonitrile at 5 ml/min with UV detection at 280 nm. A reddish powder (50 mg) was isolated after evaporation of acetonitrile, freeze drying and recrystallization from hot CH3OH. The structure was confirmed by means of 1H NMR, giving four signals [CDCl3/d2-DMSO (50:50) + TMS]: δ 6.85 (s, s), 6.93 (s, s), 9.58 (s, b), 11.10 (s, b), in agreement with Peter et al. (36).

Small amounts of 6-hydroxychlorzoxazone, kindly donated by Dr Steffen Loft (Department of Pharmacology, University of Copenhagen, Denmark), were used as an HPLC reference during the isolation of 6-hydroxychlorzoxazone.

**Protocol**

Caffeine and chlorzoxazone metabolism were determined 1 day apart, in order to avoid drug interaction. Chlorzoxazone (500 mg) was administered 30 min before breakfast on days 1, 6 and 19 after an overnight fast and 12 h caffeine abstention. Two and four hours after chlorzoxazone ingestion blood samples were taken and plasma was frozen and stored at -80°C until analysis. 6-Hydroxychlorzoxazone was isolated and purified (>99%) by means of a semi-preparative HPLC column (Ultrasphere ODS, 150X10 mm i.d., 5 μm; Beckman Instruments) eluted with 30% acetonitrile at 5 ml/min with UV detection at 280 nm. A reddish powder (50 mg) was isolated after evaporation of acetonitrile, freeze drying and recrystallization from hot CH3OH. The structure was confirmed by means of 1H NMR, giving four signals [CDCl3/d2-DMSO (50:50) + TMS]: δ 6.85 (s, s), 6.93 (s, s), 9.58 (s, b), 11.10 (s, b), in agreement with Peter et al. (36).

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**Volunteers**

Sixteen subjects, 14 male and two female, mean age 26 years (range 21-35), mean weight 76 kg (60-100), mean height 183 cm (164-196), volunteered to take part in the study. They were all non-smokers, exercised for 2 h/week (0-6) and were all healthy according to a questionnaire. None of the women were using oral contraceptives or were pregnant during the period of the experiment. Each volunteer acted as his or her own reference, as in a previous study (38). The study had the permission of the local ethical committee and the participants were informed of every detail of the scope of the present study. Eighteen subjects volunteered at the beginning of the study, however, two male subjects dropped out in the middle of the experimental period for personal reasons.

**Diet**

Several lifestyle factors, such as alcohol (37), tobacco smoke, exercise, drugs such as oral contraceptives (15) and dietary compounds, may affect CYP-mediated metabolism. Therefore, in order to study the isolated effects of the broccoli diet the volunteers were chosen by selecting individuals with excessive environmental exposure. The selected group of individuals were offered a standard diet for 6 days, avoiding known affecters of the enzymes. This diet was also made up so that dietary effects on glutathione S-transferases were minimized, for another part of the study.

The study period consisted of a 20 day intervention period in which the
Fig. 1. The individual mean ± SD values of three CYP assays determined in 16 subjects based on triplicate analyses during different dietary periods. The hydroxycholorzoxazone:chlorzoxazone ratio is a marker for CYP2E1. CMR is a CYP1A2 marker and the 2-hydroxyoestrone:16α-hydroxyoestrone ratio is a non-specific CYP marker. The CYP markers are determined under three different dietary conditions: a customary home diet, a 6 day standard diet and 12 day supplement with 500 g/day broccoli. Bars indicate the mean ratio with SD determined on the basis of triplicate analyses. Asterisks indicate significance when the mean value for one dietary period is compared with that for the following dietary period. Subjects 4 and 13 are females. (A) The 6-hydroxy-cholorzoxazone:chlorzoxazone ratio determined in a 2 h plasma sample after ingestion of 500 mg chlorzoxazone. (B) The CMR (AFMU + 1-MX + 1-MU/1,7-DMU) ratio determined in urine spots 6 h after ingestion of 100 mg caffeine. (C) The 2-hydroxyoestrone:16α-hydroxyoestrone ratio determined by means of a monoclonal antibody-based enzyme immunoassay in urine.
of 6-hydroxychlorzoxazone, phenacetin and chlorzoxazone were 5.1, 8.1 and 12.8 min respectively. The detection limit was 500 ng/ml. The CV was <5% for chlorzoxazone, 6-hydroxychlorzoxazone and the 6-hydroxychlorzoxazone/chlorzoxazone ratio.

**Determination of 2-hydroxyoestrone and 16α-hydroxyoestrone in urine**

2- and 16α-hydroxylation of oestrone in urine was measured by means of a commercial monoclonal antibody-based enzyme immunoassay kit (Estramet™, Immuno Care Corp., Bethlehem, PA; 41). To prevent degradation of unstable oestrone metabolites the urine samples were treated as in the caffeine metabolism assay. The concentration of urinary 2- and 16α-hydroxyoestrone were measured in triplicate. Optical density development on the elisa plates were read kinetically 5, 10, 15 and 20 min after addition to the plates and end points were read after 60 min. The CV of positive control samples was <15%.

**Statistics**

The effects of diet on individual variations in data were evaluated by a t-test for independent samples. The effect on the population was calculated by Friedman's two-way analysis of variance. Probability values of \( P < 0.05 \) were considered to indicate statistical significance. The average differences in the ratios expressed in percentages were calculated as \( \Delta \% \). The CV was calculated as SD/mean×100.

**Results**

Sixteen healthy non-smoking subjects changed their diet from a customary home diet (diet A) to a standard diet (diet B) for 6 days. Then for an additional 12 days the subjects ingested 500 g/day broccoli as a supplement to the standard diet (diet C). At the end of each dietary period the subjects were assayed for caffeine and chlorzoxazone metabolism and urine samples were taken to assay for oestrogen metabolism. Seven subjects were also assayed for caffeine and oestrogen metabolism after 7 days on the broccoli diet (diet C). Data are presented as mean values ± SD.

**Chlorzoxazone assay**

The ratio of 6-hydroxychlorzoxazone to chlorzoxazone was determined as a marker for CYP2E1 catalytic activity 2 and 4 h after ingestion of 500 mg chlorzoxazone (Table I). In the 2 h plasma sample the average ratio decreased by 21% \( (P < 0.05) \) on a shift from diet A to diet B for 6 days. However, introduction of broccoli into the diet (diet C) for 12 days caused an increase in the average ratio by 19% (not significant). Large individual responses to the standard diet and the broccoli diet were observed (Figure 1A). In the 4 h plasma sample values of the 6-hydroxychlorzoxazone/chlorzoxazone ratio were ~75% higher than in the 2 h plasma ratio, however, the tendency was the same \( (r^2 = 0.65) \). The average 6-hydroxychlorzoxazone/chlorzoxazone ratio decreased by 15% on a shift from diet A to diet B for 5 days. When shifting to diet C for 12 days the average ratio increased by 4% compared with diet B, however, none of the alterations in the 4 h plasma samples were significant.

**Caffeine assay**

The CMR was determined in urine 6 h after ingestion of 100 mg caffeine (Figure 1B). The mean CMR increased by 5.5% \( (5.98 ± 3.11 \text{ to } 6.31 ± 3.28) \) on a change from diet A to diet B for 6 days. However, when broccoli was included in the diet (diet C) for 12 days the mean CMR increased by 19%, to 7.50 ± 3.47 \( (P < 0.0005) \). However, the changes in CMR during the dietary shifts corresponded to large individual alterations. When changing from diet A to diet B for 6 days the 5.5% increase covered a range of 24% decrease to 41% increase, however, the 19% increase after diet C covered the range 0–82% increase in CMR (Table II).

In the seven subjects who in addition were assayed after 7 days of dietary period C the CMR increased by 11.4% \( (4.67 ± 1.62 \text{ to } 5.20 ± 2.48) \) when they changed from diet A to diet B for 6 days. The CMR increased by 21% to 6.28 ± 1.73 \( (P < 0.05) \) after 7 days on diet C, however, the CMR increased by 20.5% to 6.25 ± 2.51 \( (P < 0.01) \) compared with diet B after 12 days on diet C. Comparing the CMR after 7 and 12 days of diet C no difference was observed \( (0.5\%, P < 0.26) \).

**2/16α-hydroxyoestrone assay**

2- and 16α-hydroxyoestrone were determined by means of a monoclonal antibody-based enzyme immunoassay in urine samples in triplicate analyses (Figure 1C). However, large standard deviations were observed during measurement of the ratios. The concentrations of 2- and 16α-hydroxyoestrone in urine samples are given in Table III. The mean 2-hydroxyoestriol:16α-hydroxyoestrone ratio decreased by 1.3% when comparing the effects of diet A with those of diet B. After 12 days of dietary period C the ratio increased by 29.5% \( (P < 0.05) \) compared with diet B.

For the seven subjects assayed on the seventh day on diet C the mean 2-hydroxyoestriol:16α-hydroxyoestrone ratio decreased by 6% on a shift from diet A to diet B for 6 days. The ratio increased by 1.5% after 7 days on diet C, however, the increase in the mean 2-hydroxyoestriol:16α-hydroxyoestrone ratio was 30% after the 12 day period on diet C compared with diet B. However, none of the changes in the ratios were significant.

**Discussion**

It is well known that lifestyle factors, e.g. dietary habits, affect the development of certain cancers. Ingestion of increasing

| Table I. Dietary effects on CYP2E1-mediated 6-hydroxylation of chlorzoxazone |
|--------------------------|--------------------------|--------------------------|
|                         | Diet A                   | Diet B                   |
|                         | Mean ± SD                | Mean ± SD                |
| Chlorzoxazone (2 h)     | 13.8 ± 7.9               | 17.3 ± 7.7               |
| Hydroxychlorzoxazone (2 h) | 6.5 ± 2.6              | 6.4 ± 1.9               |
| Chlorzoxazone (4 h)     | 4.5 ± 4.5                | 4.7 ± 2.4                |
| Hydroxychlorzoxazone (4 h) | 2.5 ± 1.4             | 2.5 ± 1.3               |
| Ratio                   | 0.47 ± 0.20              | 0.37 ± 0.15              |

Plasma concentrations (mean ± SD) and range of chlorzoxazone and 6-hydroxychlorzoxazone determined 2 and 4 h after ingestion of 500 mg chlorzoxazone in 16 subjects under the three dietary conditions.

The 6-hydroxychlorzoxazone:chlorzoxazone ratios are calculated as the mean of the 16 mean ratios of 2 h plasma samples.
CMR data from 13 of the 16 subjects who participated in the present study compared with CMR data obtained from the same 13 subjects in a previous study (38). The previous study involved 33 volunteers totally and diet C in this case was a 5 day supplement with 150 g charcoal grilled hamburger for lunch. In the previous study we observed a moderate reduction in CMR in the group when shifted from diet A to diet B. In the present study we observed a moderate increase in CMR when changing from diet A to diet B.

Table II. CMR values for 13 subjects participating in two studies with comparable design within 6 months

<table>
<thead>
<tr>
<th>Subject</th>
<th>Present study</th>
<th>Previous study</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Diet A</td>
<td>Diet B</td>
</tr>
<tr>
<td>1</td>
<td>3.69</td>
<td>4.43</td>
</tr>
<tr>
<td>2</td>
<td>7.55</td>
<td>7.59</td>
</tr>
<tr>
<td>3</td>
<td>8.08</td>
<td>10.56</td>
</tr>
<tr>
<td>4</td>
<td>7.77</td>
<td>8.80</td>
</tr>
<tr>
<td>5</td>
<td>10.82</td>
<td>9.43</td>
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<tr>
<td>8</td>
<td>2.43</td>
<td>2.62</td>
</tr>
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<tr>
<td>16</td>
<td>7.24</td>
<td>6.44</td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>6.58 ± 3.1</td>
<td>6.99 ± 3.3</td>
</tr>
</tbody>
</table>

Urinary concentrations (ng/ml, mean ± SD)

Table III. Dietary effects on P450-mediated oestrone 2- and 16α-hydroxylation

<table>
<thead>
<tr>
<th>Urinary concentrations (ng/ml, mean ± SD)</th>
<th>Diet A</th>
<th>Diet B</th>
<th>Diet C</th>
</tr>
</thead>
<tbody>
<tr>
<td>2-Hydroxyoestrone</td>
<td>7.78 ± 3.86</td>
<td>7.71 ± 5.29</td>
<td>8.26 ± 5.00</td>
</tr>
<tr>
<td>16α-Hydroxyoestrone</td>
<td>5.32 ± 4.23</td>
<td>5.29 ± 3.16</td>
<td>4.73 ± 3.03</td>
</tr>
<tr>
<td>Ratio*</td>
<td>1.58 ± 0.70</td>
<td>1.56 ± 0.71</td>
<td>2.02 ± 1.07</td>
</tr>
</tbody>
</table>

Urinary concentrations (mean ± SD) of 2-hydroxyoestrone and 16α-hydroxyoestrone found under three dietary conditions.

The present study was performed in order to investigate the effects of dietary broccoli on important human CYP-mediated metabolism of a number of endogenous as well as exogenous chemicals in vivo.

Human CYP2E1 enzymes may be involved in the metabolism of mainly low molecular weight chemicals, including suspected procarcinogens. Plasma chlorzoxazone metabolites were assayed to determine the 6-hydroxychlorzoxazone:chlorzoxazone ratio as a marker for hepatic CYP2E1 activity. The ratio was determined both 2 and 4 h after ingestion of 500 mg 6-hydroxychlorzoxazone.

The average value of the 4 h ratio was ~75% higher than the average 2 h ratio, but both may be used as CYP2E1 markers (2 h versus 4 h, r² = 0.64). It should be emphasized that precise timing may be of some importance in studies comparing inter- as well as intra-individual variations in the 6-hydroxychlorzoxazone:chlorzoxazone ratio.

Based on the 2 h ratios we observed no significant effect of 500 g broccoli in the diet on CYP2E1-catalysed chlorzoxazone 6-hydroxylation. This study is the first attempt to evaluate the effects of cruciferous vegetables on CYP2E1 in vivo in humans and few studies have been concerned with the effects of cruciferous vegetables or compounds present therein on CYP2E1 in animals or in vitro. Vang et al. (19) observed an increase in CYP2E1 protein in rat liver and colon after feeding a broccoli diet for 7 days. Ishizaki et al. (43) showed that phenethyl isothiocyanate, a degradation product of glucobrassicin, a glucosinolate present in cruciferous vegetables, was an effective inhibitor of CYP2E1-mediated N,N-dimethylnitrosamine demethylation in vitro. Chung et al. (20) showed, in agreement with this, reduced N,N-dimethylnitrosamine demethylation in rats fed chronically with phenethyl isothiocyanate and a glucosinolate (sinigrin). However, indole-3-carbinol and I3C showed strong inducing effects on N,N-dimethylnitrosamine and 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone demethylation, an activity of CYP2E1. On the other hand, CYP1A2 may also be involved in metabolic activation of nitrosamines (44). Although we observed an average 19% increase in the ratio after dietary period C compared with diet B, this non-significant change may be caused by the occurrence of both inducing and inhibiting compounds of CYP2E1 in broccoli.

However, the only observable effect of a dietary shift was a 21% (P < 0.05) reduction of the 6-hydroxychlorzoxazone:chlorzoxazone ratio when the individuals changed from a customary home diet to the standard diet, which included absolute alcohol abstention. Human CYP2E1 is known to be affected in vivo by a number of small organic compounds, such as ethanol (37).

Human hepatic CYP1A2 activity may be assayed as the CMR value. Broccoli causes increased CYP1A2 activity, as the mean CMR value increased by 19% (P < 0.0005) after 12 days on the broccoli diet. The average increase in the CMR, however, covered a 0–82% inter-individual range of induction. This is in agreement with previous studies (13) in which comparable consumption of brussels sprouts and cabbage increased the apparent metabolic clearance rate of antipyrine and phenacetin. However, only a few studies have previously evaluated the effects of cruciferous vegetables on human in vivo CYP1A2-mediated caffeine metabolism. McDanell et al. (14) showed a 20% decrease in the plasma half-life of caffeine after a short-term Brassica diet.
comparable with the present study, Vistisen et al. (15) showed a 12% increase in the CMR in a group of nine subjects who had ingested 500 g/day broccoli for 10 days compared with a control period with ingestion of non-cruciferous vegetables.

In a previous report (38) involving 33 subjects we observed a moderate reduction in the CMR when shifting diet from a customary home diet to a standard diet comparable with diet B, whereas a small increase of 5.5% was observed in the present study. This average increase in the CMR covered individual variations of a 24% decrease to a 41% increase. Conney et al. (45) observed a decrease in the phenacetin plasma half-life in nine subjects eating a control diet. However, the authors suggest that the effect may be due to an unintended side-wind from a charcoal grill.

Thirteen of the volunteers involved in the previous study (38) participated in the present study as well (Table III). It appears that fluctuations in the CMR values on a shift from diet A to diet B may be considered as accidental. However, it seems notable that the CMR values for some subjects differ by more than two times when comparing the individual data from the two studies. We find it difficult to explain these differences, however, the two studies differ in caffeine restriction prior to assay and the period of time between caffeine ingestion and urine sampling. In a study by Kalow and Tang (35) a more than two-fold variation in the CMR values within 8 days was reported in some subjects varying there coffee consumption. Our previous study (38) was performed in the spring and the present study in the late summer of the same year. Whether this may explain the elevated CMR values due to ingestion of fresh vegetables and to barbecuing throughout the summer is not known.

Measurement of the 2-hydroxyoestrone:16α-hydroxyoestrone ratio may have relevance in predicting development of oestrogen-dependent cancers (31,32), however, the ratio may also serve as a marker for the effects of dietary compounds, drugs and environmental exposure on the range of CYP enzymes involved in oxidative metabolism of oestrogens.

In the present study we observed an average 29.5% ($P < 0.05$) increase in the 2-hydroxyoestrone:16α-hydroxyoestrone ratio after a 12 day broccoli diet compared with a standard diet. 17α-Oestradiol 2-hydroxylation in rodents (4,17,22) and in humans is enhanced by 50% when fed 350–500 mg I3C every day for 7 days (30). CYP3A4 is the predominant CYP protein in human liver (28), however, 17α-oestradiol 2-hydroxylation is catalysed by both CYP3A4 and CYP1A2 with identical turnovers (23–25).

2-Hydroxylation of oestrogens is also enhanced by smoking (29,46,47). In agreement with this we observed an 11.5% ($P < 0.05$) increase in the 2-hydroxyoestrone:16α-hydroxyoestrone ratio in a group of six female and 14 male non-smokers after receiving charcoal grilled hamburgers for 5 days (unpublished data). Both I3C and tobacco smoking are known to enhance CYP1A2 activity, which may suggest that CYP1A2 is responsible for the induced oestrogen 2-hydroxylation. However, the lack of a correlation between the CMR and the 2-hydroxyoestrone:16α-hydroxyoestrone ratio (data not shown) indicates involvement of more enzymes than CYP1A2 in oestrone 2-hydroxylation. Human CYP2C9, CYP2C11 and CYP2E1 may also be involved in 2-hydroxylation of natural and synthetic oestrogens (26,27,48–50).

Estimation of 2-hydroxyestrone and 16α-hydroxyestrone concentrations in urine may reflect CYP activity in vivo, provided the two steroids are treated equally in the kidney. We have not estimated serum steroid concentrations and have not estimated renal clearance. However, we have calculated that the sum of the two steroids in the three dietary periods were equal (13.10, 13.00 and 12.99 ng/ml respectively), whereas the ratio between the two changed (Table II). The data may indicate that the two oestrone metabolites are treated in the kidneys by similar clearance mechanisms. Thus spot urine samples may reflect 2- and 16α-hydroxylation of oestrone in the body when subjects act as there own controls.

Nutritional factors are important in drug metabolism in man. Conney et al. (51) showed large effects on drug metabolism in human volunteers on manipulation of proteins and carbohydrates in the diet. One could speculate whether the observed increase in the average CMR value after the standard diet observed here was due to the fact that the standard diet was probably low in protein compared with the customary home diet. However, the volunteers were instructed to avoid any weight decrease and to eat extra carbohydrate during the broccoli dietary period to compensate for the reduced diet.

Analysis of chlorzoxazone metabolism during the standard dietary period showed a significant decrease of 21% in 6-hydroxylation of chlorzoxazone and an increasing tendency for chlorzoxazone 6-hydroxylation after the broccoli dietary period. Thus, if we should attribute the elevation in the CMR to energy restriction during the 20 day standard dietary period one should also expect an elevation of the 6-hydroxychlorzoxazone:chlorzoxazone ratio.

In conclusion, ingestion of broccoli may enhance CYP1A2-mediated caffeine metabolism and increase the 2-hydroxyoestrone:16α-hydroxyoestrone ratio in humans.

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