Comparison of CYP Induction by Coleus forskohlii Extract and Recovery in the Small Intestine and Liver of Mice

Kaori Yokotani, a Yuko Yamazaki, b Fumio Shimura, c and Keizo Umegaki* a

a Department of Food Safety and Management, Showa Women’s University; 1–7–57 Taishido, Setagaya-ku, Tokyo 154–8533, Japan; b Department of Food & Nutrition, Jumonji University; 2–1–28 Sugasawa, Niiza, Saitama 352–8510, Japan; and c Graduate Schoool of Human Life Sciences, Jumonji University; 2–1–28 Sugasawa, Niiza, Saitama 352–8510, Japan.

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We examined CYP induction and recovery at various doses of Coleus forskohlii extract (CFE) to assess potential drug interactions by a mechanism involving intestinal CYP. Mice were administered diets with various doses of CFE up to 0.5% (equivalent to 700–800 mg/kg body weight) for 2 weeks, then CFE was withdrawn for 3 d. Changes in CYP activities and mRNA expression in the small intestine and liver were then evaluated. CFE induced CYP in the small intestine at a higher dose compared to the liver; CYP3A was induced at 0.5% and 0.005% CFE in the small intestine and liver, respectively. There was no sex difference in CFE dose for CYP induction. CYP induction quickly reverted after withdrawal of CFE, especially for CYP3A, in the small intestine; whereas, a gradual recovery was observed in the liver. In conclusion, CFE induced CYP in the small intestine and liver; however, a higher dose of CFE was needed for the small intestine. Moreover, the induction was soon recovered, suggesting actual interactions of CFE with prescription drugs are unlikely to occur through CYP in the small intestine.

Key words Coleus forskohlii extract; CYP; sex difference; small intestine; induction; recovery

INTRODUCTION

Dietary supplements are commonly used in contemporary society. 1) Supplements composed of herbal ingredients have been well accepted, as consumers perceive the term “natural” to indicate safety, and herbal ingredients have historically been used in complementary and alternative medicines. 2) However, herbal ingredients have encountered problems such as misidentification, production errors, and contamination. 3) In addition, herbal ingredients are composed of multiple chemicals that remain unidentified; thus, in general, they are produced without standardization. 4) In this context, safety concerns have been raised in regards to hepatic failure 5) and interactions with drugs. 6, 7)

In the case of hepatic failure, the use of dietary supplements for weight loss is most commonly identified, 8) and a causality assessment method has been developed. 9) On the other hand, interactions between herbal supplements and drugs are often difficult to clarify because of the numerous combinations among them. Nevertheless, consumers continue to ingest herbal supplements and prescription drugs concomitantly. 10) Some consumers take herbal supplements to treat diseases without disclosure to their conventional health care provider. 11, 12) In this context, it is important to conduct basic research to clarify interactions between herbal ingredients and drugs.

For orally administered drugs, herb–drug interactions occur at the level of absorption in the intestinal tract, and during metabolism in the small intestine and liver through CYP. 13, 14) It is well known that there are different types of CYP that affect herb–drug interactions. Also, it has been reported that CYP expression is organ-specific and differs among species, such as humans and mice. 15, 16) In humans, 30% of CYP expressed in the liver is CYP3A4, which metabolizes about 50% of the drugs, and CYP3A4 and CYP2C9 are the major constituents in the small intestine. 13, 15) By clarifying the relationship between CYPs involved in drug metabolism and the types of CYP affected by herbal supplements, it would be possible to better describe herb–drug interactions. The herb–drug interaction is well characterized for some herbs such as St. John’s wort. 10) A number of clinical trials have shown that St. John’s wort attenuates drug efficacy by inducing CYP in the small intestine and liver, and P-glycoprotein in the small intestine. 16) It was also revealed that the compound involved in the interaction is hyperforin, while a St. John’s wort extract with low hyperforin content was not thought to interact with the drugs. 17) This finding indicates the importance of intake dose to clarify the interaction between herbs and drugs. In the small intestine, ingested compounds are present at high concentrations, which may markedly induce or inhibit CYPs in the region.

Coleus forskohlii extract (CFE), a popular herbal ingredient, is used in weight-loss products. CFE has been used for centuries in Ayurvedic medicine to treat various diseases of the cardiovascular, respiratory and central nervous systems. 18) CFE contains a diterpene compound, forskolin, which has been shown to activate adenylate cyclase 19) to enhance lipolysis and fat loss in cell culture 20) and human studies. 21, 22) Based on the action of forskolin, CFE is generally standardized at 10% forskolin for use in dietary supplements. In vitro studies have reported conflicting results on CYP induction by CFE. 23, 24) In an in vivo mouse study, we reported that CFE induced hepatic hypertrophy and CYP induction at a dose commonly taken as a dietary supplement. 25) In addition, we have shown that CFE interacts with warfarin and tolbutamide via induction of hepatic CYP, resulting in decreased drug ef-
ficacy in mouse in vivo studies. Moreover, while forskolin was not involved in the CYP induction, many other compounds are likely involved. However, the contribution of CYP administration to CYP induction in the small intestine remains unclear.

In this study, we examined CYP induction and recovery by CFE administration in the small intestine and liver of mice from the viewpoint of intake dose. We also examined the issue of sex differences in CFE effects because CFE supplements are often consumed by women for weight-loss purposes.

**MATERIALS AND METHODS**

**Compounds** Powdered CFE standardized with 10% forskolin was obtained from Tokiwa Phytochemical Co., Ltd. (Chiba, Japan). The components were 5.6% water, 0.3% protein, 22.7% lipids, 2.2% ash, and 69.2% carbohydrates. The HPLC chromatographic profile of CFE has been reported elsewhere, and the analyzed contents of forskolin and 1,9-deoxyforskolin in the CFE sample were 10.37 and 1.71%, respectively. All other reagents were purchased from FUJIFILM Wako Pure Chemical Corporation (Osaka, Japan).

**Animal Experiment** Four-week-old male and female ICR mice (CLEA Japan, Inc., Tokyo, Japan) were housed at a constant temperature (23 ± 1°C) with a 12-h light–dark cycle in polypropylene cages. The mice were acclimatized for 1 week in individual cages, then fed the experimental diet for 2 weeks. The experimental diets were prepared by adding various doses of CFE toAIN93G semi-purified diet (Oriental Co., Ltd., Tokyo, Japan), the composition of which was as in Reeves et al. In experiment 1, male mice were fed 0, 0.005, 0.05, and 0.5% CFE diet for 2 weeks. In experiment 2, male and female mice were fed 0, 0.05 and 0.5% CFE diet for 2 weeks. In experiment 3, male mice were fed 0.5% CFE diet for 2 weeks, then fed diet without CFE for 3 d to examine the recovery of CYP induction. In these studies, body weight and food consumption were monitored and recorded every 2 d throughout the entire study. Mice were anesthetized with pentobarbital and humanely sacrificed, and then the liver, small intestine, retropertioneal fat tissue or perirenal fat were immediately removed and weighed. A sample of the small intestine was prepared according to the method reported by Kawase et al.

Portions of liver and small intestine were stored in RNAlater (Applied Biosystems, Inc., Foster City, CA, U.S.A.) for mRNA analysis. All procedures were in accordance with the National Institute of Health and Nutrition guidelines for the Care and Use of Laboratory Animals, and were approved by the ethics committee of the National Institute of Health and Nutrition (No. 1114, March 30th, 2011). The primers were designed using the free software of Universal ProbeLibrary Assay Design Center of Roche Molecular Systems, Inc., U.S.A. and synthesized by SIGMA Genosys, Hokkaido, Japan. The primer sequences are as follows (forward and reverse): GAPDH (5’-TGCACCACACACGTCTTACG-3’ and 5’-GGCATGTGTCGAGGTAAG-3’), Cyp1a1 (5’-TCTTTTGGAGAGAAGTTGAA-3’ and 5’-TACGATGAGGTCAAGTGA-3’), Cyp2a2 (5’-CCTGGACTCTCCCACAC-3’ and 5’-CGCCATCTGCTACCAC-3’), Cyp2b1 (5’-AGCGAGGACCCTCTCCTCC-3’ and 5’-CGGCCATGCCGTTGTTCTG-3’), Cyp2b2 (5’-TTGAGTGTCTTCTACGAGACTCCTT-3’ and 5’-AGCCAGAGAGCTCAAACA-3’), Cyp3a11 (5’-CTGATAATCTACCCACAGATGAA-3’ and 5’-CTCTCTCAGGTTCTAGATGTTGAA-3’), Cyp7a1 (5’-GGGGAACCCTGGGTTCTAT-3’ and 5’-GGGCTTACACAGTAACT-3’), Cyp2c29 (5’-CCACGCCTTCCTCAGTTTTA-3’ and 5’-GGCCATGAACTTCTGAG-3’)

**Analysis of mRNA Levels**

Real time RT-PCR experiments were performed according to the method previously described. Briefly, total RNA was extracted using a QuickGene RNA tissue kit SII (FujiFilm Co., Ltd., Tokyo, Japan), and the samples were subjected to real time RT-PCR using the One-Step SYBR RT-PCR kit (Perfect Real Time; TaKaRa Bio Inc., Shiga, Japan) according to the manufacturer’s protocol, and Mx3000P (STRATAGENE Co., La Jolla, CA, U.S.A.). The results were expressed as the copy number ratio of the target mRNA to that of glyceraldehyde-3-phosphate dehydrogenase (GAPDH) mRNA. The primers were designed using the free software of Universal ProbeLibrary Assay Design Center of Roche Molecular Systems, Inc., U.S.A. and synthesized by SIGMA Genosys, Hokkaido, Japan. The primer sequences are as follows (forward and reverse): GAPDH (5’-TGCACCACACACGTCTTACG-3’ and 5’-GGCATGTGTCGAGGTAAG-3’), Cyp1a1 (5’-TCTTTTGGAGAGAAGTTGAA-3’ and 5’-TACGATGAGGTCAAGTGA-3’), Cyp2a2 (5’-CCTGGACTCTCCCACAC-3’ and 5’-CGCCATCTGCTACCAC-3’), Cyp2b1 (5’-AGCGAGGACCCTCTCCTCC-3’ and 5’-CGGCCATGCCGTTGTTCTG-3’), Cyp2b2 (5’-TTGAGTGTCTTCTACGAGACTCCTT-3’ and 5’-AGCCAGAGAGCTCAAACA-3’), Cyp3a11 (5’-CTGATAATCTACCCACAGATGAA-3’ and 5’-CTCTCTCAGGTTCTAGATGTTGAA-3’), Cyp7a1 (5’-GGGGAACCCTGGGTTCTAT-3’ and 5’-GGGCTTACACAGTAACT-3’), Cyp2c29 (5’-CCACGCCTTCCTCAGTTTTA-3’ and 5’-GGCCATGAACTTCTGAG-3’)

**Statistical Analysis**

Data are presented as means ± standard error (S.E.) for individual groups, and were statistically analyzed using one-way (experiments 1 and 3) and two-way (experiment 2) ANOVA with Tukey’s multiple comparison test or Student’s t-test when two groups were compared. Differences at p < 0.05 were considered to be significant. All statistical analyses were performed using Prism 5.0 (GraphPad Software Inc., La Jolla, CA, U.S.A.).

**RESULTS**

**Comparison of CYP Induction by CFE in the Small Intestine and Liver**

Administration of various CFE doses in the diet did not affect food intake and body weight (Table 1). The calculated intake doses of CFE were 7.2, 69.7, and 697 mg/kg body weight for the 0.005, 0.05, and 0.5% CFE diets, respectively. Relative liver weight to body weight
Males; however, the increase in the ratio due to CFE treatment resulting in a similar intake dose of CFE (Table 3). Relative intake in female mice were lower than those in male mice, examined in regards to CYP induction in the small intestine was likely to occur in the liver than in the small intestine.

The sex difference of CYP induction in the small intestine was compared using four subtype-specific substrates (Table 2). Among the four CYP activities, CYP1A1 and CYP2C were lower in the small intestine than in the liver. CYP2C activity in the small intestine was very low; specifically, the actual increase in the small intestine than in the liver. CYP2C activity in the liver was detected from the dose of 0.5% CFE. In a preliminary examination, increases in mRNA expression of Cyp2b10, Cyp2c29 and Cyp3a11 were observed from the dose of 0.5% CFE (Fig. 1). Basal mRNA expression of the Cyp1a1, Cyp1a2, Cyp1b1 and Cyp2el was higher, but that of the Cyp2b10 was lower in the liver when compared to the intestine. CFE treatment increased the mRNA expression of Cyp2b10, Cyp2c29, and Cyp3a11 in both the intestine and liver. Consistent with the CYP3A activity, CFE produced a clear elevation of Cyp3a11 levels in the intestine. The increase in the ratio of Cyp3a11 mRNA by CFE treatment was 3.9 in the small intestine and 11.8 in the liver, respectively, indicating that inductions of Cyp3a11 and CYP3A activity are more likely to occur in the liver than in the small intestine.

Sex Differences of CYP Induction by CFE in the Small Intestine and Liver (Experiment 2) Two doses of CFE were administered to male and female mice for 2 weeks, and the sex difference of CYP induction in the small intestine was examined in regards to CFE dose. Both body weight and feed intake in female mice were lower than those in male mice, resulting in a similar intake dose of CFE (Table 3). Relative liver weight to body weight was lower in females than in males; however, the increase in the ratio due to CFE treatment increased at the dose of 0.5% CFE diet. Perirenal fat did not differ among the 4 groups even at 0.5% CFE, indicating that CFE did not affect the fat tissue (data not shown).

**Table 1. Body Weight, Relative Liver Weight and Calculated Dose of CFE**

|                          | Control | 0.005% CFE | 0.05% CFE | 0.5% CFE |
|--------------------------|---------|------------|-----------|----------|
| Body weight (g)          | 32.1 ± 0.488 | 31.7 ± 0.549 | 32.2 ± 0.655 | 32.5 ± 0.511 |
| Relative liver weight (%)| 4.33 ± 0.076 | 4.17 ± 0.081 | 4.38 ± 0.068 | 7.51 ± 0.56* |
| Feed intake (g)          | 4.4 ± 0.029 | 4.5 ± 0.0048 | 4.4 ± 0.037 | 4.4 ± 0.024 |
| Calculated CFE dose (mg/kg BW) | 0 | 7.2 ± 0.10* | 69.7 ± 0.87* | 697 ± 8.3* |

Mean ± S.E. for 5 mice. * Significantly different from control (<0.05).

**Table 2. CYP Activities in the Liver and Intestine of Mice Administered Various Doses of CFE**

| CYP Activity | Control | 0.005% CFE | 0.05% CFE | 0.5% CFE |
|--------------|---------|------------|-----------|----------|
| CYP1A Liver  | 5299 ± 1013 [1.0]* | 7007 ± 1191 [1.3]* | 7545 ± 804 [1.4]* | 11487 ± 1109 [2.2]*# |
| CYP1A Intestine | 664 ± 1504 [1.0] | 717 ± 114 [1.1] | 769 ± 83 [1.2] | 1613 ± 216 [2.4]*# |
| CYP1A2 Liver | 17655 ± 1968 [1.0] | 22639 ± 1425 [1.3] | 35382 ± 4408 [2.0] | 104309 ± 7609 [5.9]*# |
| CYP1A2 Intestine | 13112 ± 2102 [1.0] | 12700 ± 1934 [0.9] | 14814 ± 524 [1.1] | 21500 ± 2841 [1.6]*# |
| CYP2C Liver | 5553 ± 802 [1.0]* | 4643 ± 1002 [0.8]* | 7301 ± 5762 [1.3]* | 8797 ± 1418 [1.6]*# |
| CYP2C Intestine | 54.0 ± 18.1 [1.0] | 74.8 ± 21.4 [1.4] | 93.0 ± 13.7 [1.7] | 177 ± 38.9 [3.3]* |
| CYP3A Liver | 3876 ± 574 [1.0] | 5666 ± 988 [1.4]* | 12768 ± 1867 [3.3]*# | 28741 ± 3291 [7.4]*# |
| CYP3A Intestine | 2921 ± 620 [1.0] | 2587 ± 424 [0.8] | 3225 ± 478 [1.1] | 6422 ± 1054 [2.2]*# |

Mean ± S.E. for 5 mice. * Significantly different from control (<0.05). # Significantly different from the intestine (<0.05). Numbers in parentheses indicate the ratio to control.

Sex Differences of CYP Induction by CFE in the Small Intestine and Liver (Experiment 2) Two doses of CFE were administered to male and female mice for 2 weeks, and the sex difference of CYP induction in the small intestine was examined in regards to CFE dose. Both body weight and feed intake in female mice were lower than those in male mice, resulting in a similar intake dose of CFE (Table 3). Relative liver weight to body weight was lower in females than in males; however, the increase in the ratio due to CFE treatment was observed at the dose of 0.5% CFE with no sex difference. The activities of CYP1A1, CYP1A2 and CYP3A in the liver...
were higher in females than in males (Table 4). CYP induction in the small intestine, except for CYP1A2, was detected at the dose of 0.5% CFE in both males and females. In the liver, CYP3A activity increased at the dose of 0.05% in both males and females. The result confirmed that a higher dose of CFE was needed for CYP3A induction in the small intestine compared to the liver. For both sexes, the weight of retroperitoneal fat tissue did not significantly differ due to CFE administration up to the dose of 0.5% (data not shown).

**Fig. 2. Expression of Cyp mRNA in the Small Intestine of Mice Administered CFE**

Male mice were fed 0.5% CFE diet for 2 weeks, and mRNA expression of Cyp1a1, Cyp1a2, Cyp1b1, Cyp2b10, Cyp2c29, Cyp2e1 and Cyp3a11 was compared between the small intestine and liver. Each column indicates the mean ± S.E. for 5 mice. * Significantly different from control (p < 0.05). # Significantly different from the intestine (p < 0.05).

| Table 3. Body Weight, Relative Liver Weight and Calculated Dose of CFE |
|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
|                  | Female          | Male            | Two-way ANOVA p value |
|                  | Body weight (g) | Relative liver weight (%) | Feed intake (g) | Calculated CFE dose (mg/kg BW) |
| Control         | 25.6 ± 0.51     | 4.55 ± 0.13     | 3.9 ± 0.04      | 0                |
| 0.05% CFE       | 25.5 ± 0.23     | 4.55 ± 0.11     | 3.9 ± 0.02      | 83* ± 0.9        |
| 0.5% CFE        | 26.4 ± 0.14     | 6.74* ± 0.08    | 3.9 ± 0.01      | 794* ± 6.1       |
| Control         | 30.4 ± 0.12     | 5.19 ± 0.19     | 4.6 ± 0.01      | 0                |
| 0.05% CFE       | 30.1 ± 0.21     | 4.99 ± 0.07     | 4.7 ± 0.005     | 82* ± 0.6        |
| 0.5% CFE        | 31.0 ± 0.29     | 7.40* ± 0.25    | 4.6 ± 0.02     | 799* ± 6.6       |
| Gender          | <0.0001         | <0.0001         | <0.0001         | 0.620            |
| CFE             | 0.01            | 0.750           | 0.458           | <0.0001          |
| Gender×CFE      | 0.926           | 0.860           | 0.499           |                   |
tissue and Liver (Experiment 3) Male mice were fed the 0.5% CFE diet for 2 weeks, and then CFE was withdrawn for 3 d to examine the recovery of CYP induction. The calculated intake dose of CFE was 792 mg/kg body weight. Total CYP concentration, Cyp3a11 mRNA expression and CYP3A activity were markedly increased by CFE treatment for 2 weeks (Fig. 3). The increase in CYP indicators in the small intestine quickly reverted toward control levels following withdrawal (Fig. 3). The increase in CYP indicators in the small intestine were highly unlikely. However, the possibility of CFE–drug interactions by the mechanism of hepatic CYP induction exists, as shown in the case of warfarin and tolbutamide.

In the small intestine, high concentrations of CYP components are present after oral administration. On the other hand, those concentrations in the liver are likely lower due to the processes of absorption, distribution and metabolism. Nevertheless, in the present study a more than 10-fold dose of CFE was necessary to induce CYP3A in the small intestine compared to the liver. The CYP induction by CFE was soon recovered by withdrawal of CFE. This phenomenon is related to intestinal cell turnover, which is in the order of a few days. Rapid recovery of intestinal CYP has been reported for grapefruit juice, which contains furanocoumarins and inhibits CYP3A4-mediated drug metabolism in the intestine with a half-life estimated to be 8 h. Therefore, it is reasonable to consider cellular turnover as a possible mechanism for differences in the CFE dose required for CYP induction between the small intestine and the liver. CYP gene expression is transcriptionally regulated by the pregnane X receptor (PXR) and the constitutive androstane receptor. It has been reported that mouse Cyp3a11 is a PXR-regulated gene, and CFE induction of CYP has been shown to involve PXR. Differences in the CFE dose required for CYP induction between the intestine and liver may be due to differences in transcriptional regulation of the CYP gene. In an in vivo mouse study, we showed that hepatic CYP induction was promoted by CFE administration, but not by pure forskolin. Moreover, CYP was induced by multiple compounds in CFE. Further study is needed to clarify the different mechanisms of CYP induction between the liver and intestine in regards to transcriptional factors and specific compounds in CFE.

It has been shown that CYP expression is organ-specific and differs between species. In humans, about 30% of CYP is expressed as CYP3A4 in the liver and metabolizes about 50% of the drugs; further, CYP3A4 and CYP2C9 represent the major constituents in the small intestine. In the present study, mRNA expression of Cyp was higher or tended to be higher in the liver than in the small intestine, except for Cyp2b10, which was reported to be highly expressed in the small intestine and lung. Increases in the mRNA expression of Cyp2c29 and Cyp3a11 and the activities of CYP2C and CYP3A were detected in the small intestine and liver regardless of CFE treatment. However, analysis of CYP2C

| Table 4. CYP Activities in the Liver and Intestine of Mice Administered Various Doses of CFE |
|-----------------------------------------------|---------|---------|---------------|---------|---------|---------|-------|
| CYP Activity (RLU/mg protein)                 | Female  | Male    | Two-way ANOVA p value |
|                                             | Control | 0.05% CFE | 0.5% CFE | Control | 0.05% CFE | 0.5% CFE | Gender | CFE | Gender×CFE |
| CYP1A                                         |         |         |         |         |         |         |       |     |             |
| Liver                                         | 7469[1.0] | 9374[1.3] | 12654[1.7]* | 5588[1.0] | 7045[1.3] | 9185[1.6]* |       |     |             |
| ±545                                          | ±408    | ±158    | ±735    | ±967    | ±495    |             |       |     |             |
| Intestine                                      | 243[1.0] | 328[1.3] | 1043[4.3]* | 357[1.0] | 692[1.9] | 874[2.4]*  |       |     |             |
| ±92                                           | ±108    | ±176    | ±86     | ±27     | ±119    |             |       |     |             |
| CYP1A2                                        | 24828[1.0] | 47081[1.9] | 142731[5.7]* | 20683[1.0] | 29694[1.4] | 89203[4.3]* | <0.0001 | <0.0001 | 0.416  |
| ±2389                                         | ±6073   | ±8780   | ±1922   | ±3994   | ±6107   |             |       |     |             |
| Intestine                                      | 12221[1.0] | 10386[0.8] | 15948[1.3] | 12745[1.0] | 18387[1.4] | 18997[1.5] | 0.006  | 0.014 | 0.072  |
| ±1034                                         | ±2449   | ±1864   | ±1124   | ±465    | ±1651   |             |       |     |             |
| CYP2C                                         | 8258[1.0] | 6707[0.8] | 13780[1.7] | 4993[1.0] | 5154[1.0] | 10328[2.1] | 0.137  | 0.019 | 0.893  |
| ±1186                                         | ±1625   | ±4384   | ±279    | ±912    | ±2153   |             |       |     |             |
| Intestine                                      | 32.6[1.0] | 43.1[1.3] | 85.8[2.6]* | 18.1[1.0] | 33.7[1.8] | 89.6[4.9]* | 0.309  | <0.0001 | 0.492  |
| ±5.7                                          | ±5.6    | ±11.9   | ±2.7    | ±8.5    | ±8.7    |             |       |     |             |
| CYP3A                                         | 6461[1.0] | 17563[2.7]* | 4140[6.8]* | 4210[1.0] | 13980[3.3]* | 28370[6.7]* | <0.0001 | <0.0001 | 0.001  |
| ±130                                         | ±1541   | ±1385   | ±777    | ±1161   | ±3103   |             |       |     |             |
| Intestine                                      | 1540[1.0] | 1695[1.1] | 4267[2.8]* | 1754[1.0] | 3263[1.9] | 3747[2.1]* | 0.245  | <0.0001 | 0.068  |
| ±348                                          | ±506    | ±614    | ±415    | ±157    | ±409    |             |       |     |             |

Mean ± S.E. for 5 mice. *Significantly different from control (p < 0.05). Numbers in parentheses indicate the ratio to control.
activity in the intestine may not be reliable due to its low activity in this tissue. Zhang et al.\textsuperscript{38} have reported the reduced expression of CYP1A, 2B, 2C and 3A in the small intestine in mice fed a semi-purified diet (AIN93G diet) used in the present study. The high induction ratio of Cyp2c29 by 0.5% CFE treatment was observed in both the small intestine and liver. We did not confirm CYP protein levels, and the actual CYP type induced by CFE is unclear. This may be related to the discrepancy that administration of 0.5% CFE to mice induced CYP1A2 activities, but not Cyp1a2 mRNA expression in the liver and intestine. However, the CYP3A activity analyzed using CYP3A substrate was high in both the liver and intestine, and was highly induced by CFE administration. Also, Cyp3a11 mRNA expression in the intestine as well as in the liver was highly induced by CFE. The enhanced expression of Cyp3a11 mRNA is consistent with our previous evaluation of the liver.\textsuperscript{25} Thus, it is concluded that Cyp3a11 is the CYP type induced by CFE administration in the small intestine; however, further detailed study is warranted to confirm this.

CFE is a popular herbal ingredient used in weight-loss products. As the users of CFE-containing dietary supplements tend to be predominantly female,\textsuperscript{33} it is pertinent to know if there are sex differences in CYP induction by CFE administration in both the liver and intestine. Renaud et al.\textsuperscript{14} showed sex differences in gene expression in 29 of the CYPs in mice, with 24 being higher in females than males. In the present study, some CYP activities were higher in females than males with and without CFE administration; however, CYP3A induction in the intestine was detected at the dose of 0.5% CFE with no sex difference.

CFE contains forskolin, an active compound shown to activate adenylyl cyclase\textsuperscript{19} to enhance lipolysis and fat loss in cell culture.\textsuperscript{20} The enhanced lipolysis by forskolin is thought to be the underlying mechanism of the weight-loss effect of CFE. However, there are inconsistencies in the weight-loss effect observed in human studies.\textsuperscript{21,22} Although we have shown that a very high dose of CFE (5%) decreased visceral fat weight, we could not detect decreases in fat tissue weight by CFE at doses up to 0.5% in the present study. According to these findings, CFE at a realistic intake dose may have no effect on fat loss, at least in mice. On the other hand, the intake of CFE dietary supplements induced diarrhea at a dose of 1000 mg/d in humans.\textsuperscript{32,33} The mechanism of diarrhea induction by CFE is via increased c-AMP by forskolin in the
intestine, leading to water secretion. Interestingly, we did not observe diarrhea in any mice even at a dose of 5% CFE; however, fatty liver was detected with this treatment. These findings suggest the existence of marked species differences in the effect of CFE.

In conclusion, we showed that CFE induced CYP in the small intestine as well as in the liver in this study. However, for CYP induction in the small intestine, a higher CFE dose was needed, and CYP induction was recovered soon after withdrawal of CFE. Thus, the actual interaction of CFE with prescription drugs is unlikely to occur through a CYP-mediated mechanism in the small intestine. The CFE-induced CYP induction in the liver, especially for CYP3A, occurred at a dose likely obtainable from dietary supplements. Thus, health professionals should carefully monitor their patients for adverse events related to CFE-containing supplements and prescription drugs, in particular drugs metabolized by CYP3A.

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Conflict of Interest The authors declare no conflict of interest.

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