Influence of Dietary Macronutrients on Induction of Hepatic Drug Metabolizing Enzymes by Coleus forskohlii Extract in Mice

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Summary From studies in mice, we have reported that Coleus forskohlii extract (CFE), a popular herbal weight-loss ingredient, markedly induced hepatic drug metabolizing enzymes, especially cytochrome P450 (CYP), and interacted with co-administered drugs. This study was designed to examine how the induction of drug metabolizing enzymes by CFE was influenced by different levels of macronutrients in the diet. Mice were fed a non-purified diet or semi-purified diet with and without CFE (0.3–0.5%) for 14–18 d, and changes in the ratio of liver weight to body weight, an indicator of hepatic CYP induction, and hepatic drug metabolizing enzymes were analyzed. The ratio of liver weight to body weight, content and activities of CYPs, and activity of glutathione S-transferase were higher in a semi-purified standard diet (AIN93G formula) group than in high sucrose (62.9%) and high fat (29.9%) diet groups. Different levels of protein (7%, 20%, and 33%) in the diets did not influence CFE-induced CYP induction or increase the ratio of liver weight to body weight. The effect of CFE on the ratio of liver weight to body weight was higher with a semi-purified diet than with a non-purified diet, and was similar between dietary administration and intragastric gavage when the CFE dose and the diet were the same. There was a positive correlation between CFE-induced CYP induction and the content of starch in the diets, suggesting that dietary starch potentiates CFE-induced CYP induction in mice. The mechanism of enhanced CYP induction remains unclear.

Key Words Coleus forskohlii, cytochrome P450, macronutrients, administration route, dietary starch

Coleus forskohlii extract (CFE) is a popular herbal ingredient for commercial weight-loss dietary supplements (1). C. forskohlii is native to India (2), where it has been used for centuries in Ayurvedic medicine to treat various diseases of the cardiovascular, respiratory, gastrointestinal and central nervous systems (3). CFE contains the diterpene forskolin as in Fig. 1, which increases cAMP concentrations via the activation of adenylyl cyclase, resulting in various therapeutic effects against asthma and idiopathic congestive cardiomyopathy (4, 5). Theoretically, an increase in cAMP induced by forskolin will enhance lipolysis leading to elevated fat degradation and physiological fat utilization, and thus promote fat and weight loss. It has been shown that forskolin increases both cAMP accumulation and lipolysis in fat cells (6, 7), and CFE standardized with forskolin reduces fat accumulation in ovariectomised rats (8) and induces favorable effects on body fat in overweight women and obese men (9, 10).

Currently, drug–herb interactions are becoming a source of serious concern in relation to adverse effects, because consumers of herbal supplements often take prescribed drugs concomitantly (11–13) and health professionals might be unaware of possible interactions (14, 15). A decrease in efficacy or an increase in the adverse effects of prescribed drugs might interfere with appropriate medical care and have a fatal outcome. Drugs are metabolized by the Phase I and Phase II enzymes; the former is catalyzed by cytochrome P450 (CYP) enzymes, and the latter is catalyzed conjugation enzymes such as glutathione S-transferase (GST) and UDP-glucuronosyltransferase (16). Interactions between some herbal ingredients, such as St John’s wort (17) and ginkgo biloba (18), have been documented and shown to be mediated by CYPs, but those for other herbal ingredients remain unknown. We previously showed that feeding mice a diet containing CFE (standardized with 10% forskolin) dose- and time-dependently induced hepatic CYPs and GST enzymes (19). Significant induction of the hepatic CYP content and CYP2C activity was evident at an intake dose of 0.05%; the CFE dose was 60 mg/kg body weight in mice and corresponded to about 5 mg/kg body weight of a human equivalent dose when calculated using the body surface normalization method (20). We also reported the interaction
of warfarin and CFE in mice in vivo, where CFE attenuated the anticoagulant action of warfarin via induction of hepatic CYPs, especially CYP2C, which is involved in active (S)-warfarin metabolism (21). Furthermore, we showed that CFE induced CYPs in vivo and directly inhibited CYP2C activity in vitro as well. In both in vivo and in vitro studies, the effect of forskolin, a biologically active marker, was negligible, indicating the contribution of unknown substances in the CFE (19, 22).

Users of weight loss supplements may have an extreme meal with different macronutrient compositions. There are 4 popular weight loss diets: Atkins (very low in carbohydrate), Zone (low in carbohydrate), Ornish (very high in carbohydrate), and LEARN (Life style, Exercise, Attitude, Relationships and Nutrition) (23). These differences in dietary macronutrients may influence drug-metabolizing enzymes (24). Rats with protein-calorie malnutrition decreased hepatic CYP levels (CYP1A2, 2C11, 2E1 and 3A1/2) (25), and rats fed a high-sucrose diet exhibited decreased hepatic content of CYP1A1, CYP3A2 and GST activity (26). It has also been shown that a diet deficient in carbohydrate remarkably enhanced liver mixed-function oxidase activity and the metabolism of carbon tetrachloride in rats (27). Based on these findings, it is important to determine how dietary macronutrients influence CFE-induced hepatic CYP induction.

In this study in mice, we examined how induction of drug metabolizing enzymes, especially CYPs, was influenced by CFE with regard to route of administration and dietary conditions that differ in macronutrient compositions. In our previous study, CYP induction by CFE was well correlated with an increase in the ratio of liver weight to body weight (22). Therefore, we measured CYP content and activities in the liver as well as the ratio of liver weight to body weight as a reliable indicator of CYP induction. The present study in mice had two benefits: one was to clarify dietary conditions that can minimize possible drug–CFE interactions via CYP induction, and the second was to establish experimental diet conditions that can readily be used to seek unknown substances in CFE that induce CYPs in vivo.

MATERIALS AND METHODS

Materials. Powdered CFE standardized with 10% forskolin was prepared as follows. Dried roots of C. forskohlii obtained from Bangalore in southern India were crushed and supercritically extracted under CO2 gas. The forskolin-rich extract (20–30%) was mixed with dextrin to a forskolin concentration of 10%. These processes were performed by Tokiwa Phytochemical Co. Ltd. (Chiba, Japan). CFE comprised: water, 5.6%; protein, 0.3%; lipids, 22.7%; ash, 2.2%; and carbohydrates, 69.2%. For CYP enzyme assays, resorufin, pentoxyresorufin, (S)-warfarin, 7-hydroxycoumarin, testosterone, 6β-hydroxytestosterone, and corticosterone, and glutathione were purchased from Sigma-Aldrich Inc. (St Louis, MO, USA). NADPH was purchased from Oriental Yeast Co., Ltd. (Tokyo, Japan). All other reagents were purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan).

Experimental diets. Non-purified commercial rodent diet (CE-2) was supplied by CLEA Japan, Inc. (Tokyo, Japan). The non-purified diet comprised: water, 88 g/kg diet; crude protein, 252 g/kg diet; crude lipids, 44 g/kg diet; total ash, 70 g/kg diet; crude fiber, 44 g/kg diet and soluble non-nitrogenous matter, 502 g/kg diet according to the manufacturer’s information. A semi-purified standard diet was prepared based on the composition of the AIN93G formula of Reeves et al. (28). Various semi-purified diets with different compositions of macronutrient were prepared as shown in Table 1. The high-starch diet that differed only in the source of the carbohydrate and the high-fat diet were isonitrogenous per kilocalo-

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Standard</th>
<th>High sucrose</th>
<th>High fat</th>
<th>Low protein</th>
<th>High protein</th>
</tr>
</thead>
<tbody>
<tr>
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<td>0</td>
<td>205.082</td>
<td>641.436</td>
<td>417.536</td>
</tr>
<tr>
<td>Sucrose</td>
<td>100</td>
<td>629.486</td>
<td>126</td>
<td>120</td>
<td>80</td>
</tr>
<tr>
<td>Casein</td>
<td>200</td>
<td>200</td>
<td>250</td>
<td>70</td>
<td>330</td>
</tr>
<tr>
<td>Soybean oil</td>
<td>70</td>
<td>70</td>
<td>292</td>
<td>70</td>
<td>70</td>
</tr>
<tr>
<td>Cellulose</td>
<td>50</td>
<td>50</td>
<td>63</td>
<td>50</td>
<td>50</td>
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<td>Vitamin mixture (AIN93G)</td>
<td>10</td>
<td>10</td>
<td>13</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
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<td>35</td>
<td>35</td>
<td>44</td>
<td>35</td>
<td>35</td>
</tr>
<tr>
<td>l-Cystine</td>
<td>3</td>
<td>3</td>
<td>3.76</td>
<td>1.05</td>
<td>4.95</td>
</tr>
<tr>
<td>Choline hydrogen tartrate</td>
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<td>2.5</td>
<td>3.14</td>
<td>2.5</td>
<td>2.5</td>
</tr>
<tr>
<td>Tertiary butylhydroquinone</td>
<td>0.014</td>
<td>0.014</td>
<td>0.018</td>
<td>0.014</td>
<td>0.014</td>
</tr>
</tbody>
</table>
Dietary Macronutrient and CYP Induction by C. forskohlii Extract

In this administration route study, the daily dose of CFE dissolved in 0.5% (w/v) carboxymethylcellulose with 0.5% CFE or given it daily by intragastric gavage in 50 mmol/L Tris-HCl buffer (pH 7.4) containing 0.9% (w/v) NaCl, homogenized and centrifuged at 105,000 \( \times g \) at 4°C for 60 min to prepare microsomal and cytosol fractions. The total CYP content and the activities of various CYP enzymes were determined using the microsomal fraction, and glutathione S-transferase (GST) activity was determined using cytosol fraction, as described previously (29). The subtypes of CYP enzymes examined and the corresponding CYPs were pentoxyresorufin O-dealkylase, CYP2B; (S)-warfarin 7-hydroxylase, CYP2C; and testosterone 6β-hydroxylase, CYP3A. Protein concentration was determined using a BCA protein assay kit (Pierce, Rockford, IL, USA).

Statistical analyses. Data are presented as means and standard error (SE) for individual groups and were analyzed statistically using one-way ANOVA (in the non-purified diet versus semi-purified diet and CFE administration route studies) and two-way ANOVA (in the macronutrient studies) with Tukey’s multiple comparison test. Differences at \( p<0.05 \) were considered significant. All statistical analyses were performed using Prism 5.0 (GraphPad Software Inc., La Jolla, CA, USA).

RESULTS

Dietary treatment of CFE with non-purified and purified diets and by different administration routes

Mice were fed a 0.5% CFE in a non-purified commercial rodent diet (CE-2) or semi-purified standard diet for 2 wk. In the semi-purified diet groups, food intake was lower, but body weight was higher compared with the non-purified diet groups (Table 2). This discrepancy might be due to high bioavailability of ingredients in the semi-purified diet compared with crude natural ingredients used in the non-purified diet. In the CFE-treated groups, liver weight in the semi-purified diet group was higher, but the increase in the ratio of liver weight to body weight did not differ between the two CFE groups, which could be caused by the low dose of CFE in the semi-purified diet group. When mice were fed the same semi-purified standard diet, increases in liver weight and

<table>
<thead>
<tr>
<th>Diet treatment</th>
<th>Non-purified</th>
<th>Semi-purified (standard)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CFE treatment</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Average daily food intake (g)</td>
<td>5.8±0.21</td>
<td>5.4±0.18 [0.93]</td>
</tr>
<tr>
<td>Calculated CFE dose (mg/kg body weight)</td>
<td>0</td>
<td>845±26.0</td>
</tr>
<tr>
<td>Final body weight (g)</td>
<td>31.4±0.42</td>
<td>30.3±0.30 [0.96]</td>
</tr>
<tr>
<td>Liver weight (g)</td>
<td>1.44±0.05</td>
<td>2.08±0.05 [1.4]</td>
</tr>
<tr>
<td>(%/body weight)</td>
<td>4.58±0.11</td>
<td>6.87±0.10 [1.5]</td>
</tr>
</tbody>
</table>

Mice were fed a non-purified diet or semi-purified diet with and without 0.5% C. forskohlii extract (CFE) for 2 wk. Values are expressed as mean and SE (n=5). Number in brackets indicates the increase in the ratio for its respective diet group without CFE.

a Significant difference from non-purified diet without CFE at \( p<0.05 \).

b Significant difference from non-purified diet with CFE at \( p<0.05 \).

c Significant difference from semi-purified diet without CFE at \( p<0.05 \).

Animal experiments. Male 4-wk-old ICR mice (CLEA Japan, Inc.) were housed at a constant temperature (23±1°C) with a 12-h light-dark cycle in polypropylene cages. After acclimation for 1 wk, the mice were divided into treatment groups (5–6 mice per group) and were administered CFE as follows.

In a comparison of non-purified diet and semi-purified standard diet, CFE was added at a concentration of 0.5% (w/w) to each diet, and given to mice ad libitum for 2 wk. In a comparison of the route of administration, mice were either fed a semi-purified standard diet with 0.5% CFE or given it daily by intragastric gavage of CFE dissolved in 0.5% (w/v) carboxymethylcellulose for 2 wk. In this administration route study, the daily dose of CFE was adjusted to 750 mg/kg body weight. In studies of the effect of macronutrients (i.e., starch, fat, and protein), the CFE dose was reduced to 0.3% (w/w) in the semi-purified diets and the treatment term was set at 18 d, because the dietary effects were thought to need a longer period at this CFE dose. In the study, food intake in each group was adjusted to keep a similar intake dose of CFE. At the end of each treatment, mice were anesthetized with pentobarbital and killed. Their livers were removed immediately, weighted, snap frozen with dry ice and stored at −80°C until analysis.

All procedures were in accordance with National Institute of Health and Nutrition Guidelines for the Care and Use of Laboratory Animals and were approved by the Ethical Committee in the same institute.

Analytical methods

Analysis of drug-metabolizing enzymes: The liver was rinsed with 0.9% (w/v) NaCl, homogenized in 50 mmol/L Tris-HCl buffer (pH 7.4) containing 0.25 mol/L sucrose, and separated by centrifugation at 10,000 \( \times g \) at 4°C for 30 min. The supernatant was...
Table 3. Body weight and liver weight of mice fed a semi-purified standard, high-sucrose, or high-fat diet with and without *C. forskohlii* extract (CFE).

<table>
<thead>
<tr>
<th>Diet</th>
<th>CFE treatment</th>
<th>Standard</th>
<th>High sucrose</th>
<th>High fat</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Average daily food intake (g)</td>
<td>4.8±0.08</td>
<td>4.7±0.07 [0.98]</td>
<td>4.8±0.08</td>
<td>4.7±0.10 [0.99]</td>
</tr>
<tr>
<td>Calculated CFE dose (mg/kg body weight)</td>
<td>0</td>
<td>380±10.5</td>
<td>0</td>
<td>364±4.6</td>
</tr>
<tr>
<td>Final body weight (g)</td>
<td>39.0±1.1</td>
<td>38.2±1.1 [0.98]</td>
<td>40.1±0.57</td>
<td>39.9±0.90 [0.99]</td>
</tr>
<tr>
<td>Liver weight (g)</td>
<td>1.51±0.07</td>
<td>3.21±0.22 [2.1]a</td>
<td>1.55±0.04</td>
<td>2.71±0.25 [1.8]a</td>
</tr>
<tr>
<td>(%/body weight)</td>
<td>3.87±0.08</td>
<td>8.41±0.51 [2.2]a</td>
<td>3.86±0.11</td>
<td>6.75±0.50 [1.7]a, b</td>
</tr>
</tbody>
</table>

Mice were fed a semi-purified standard (10% sucrose and 7% soybean oil), high-sucrose (62.9% sucrose) or high-fat diet (29.9% soybean oil) with and without 0.3% *C. forskohlii* extract (CFE) for 18 d. The detailed composition of the experimental diets is shown in Table 1.

Values are expressed as mean and SE (*n*=6). Number in brackets indicates the increase in the ratio for its respective diet group without CFE.

a Significant difference from its respective diet without CFE at *p* < 0.05.

Table 4. Body weight and liver weight of mice fed a semi-purified standard, low-protein, or high-protein diet with and without *C. forskohlii* extract (CFE).

<table>
<thead>
<tr>
<th>Diet</th>
<th>CFE treatment</th>
<th>Standard</th>
<th>Low-protein</th>
<th>High-protein</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Average daily food intake (g)</td>
<td>4.3±0.090</td>
<td>4.3±0.15 [1.0]</td>
<td>4.4±0.082</td>
<td>4.5±0.16 [1.0]</td>
</tr>
<tr>
<td>Calculated CFE dose (mg/kg body weight)</td>
<td>0</td>
<td>330±7.9</td>
<td>0</td>
<td>364±11.8</td>
</tr>
<tr>
<td>Final body weight (g)</td>
<td>40.6±1.3</td>
<td>39.1±1.3 [0.96]</td>
<td>37.5±0.89</td>
<td>36.2±1.1 [0.97]</td>
</tr>
<tr>
<td>Liver weight (g)</td>
<td>1.61±0.03</td>
<td>2.94±0.24 [1.8]a</td>
<td>1.43±0.03 b</td>
<td>2.47±0.22 [1.7]a</td>
</tr>
<tr>
<td>(%/body weight)</td>
<td>3.96±0.10</td>
<td>7.48±0.50 [1.9]a</td>
<td>3.82±0.092</td>
<td>6.76±0.45 [1.8]a</td>
</tr>
</tbody>
</table>

Mice were fed a semi-purified standard (20% casein), low-protein (7% casein) or high-protein (33% casein) diet with and without 0.3% *C. forskohlii* extract (CFE) for 18 d. The detailed composition of the experimental diets is shown in Table 1.

Values are expressed as mean and SE (*n*=6).

Number in brackets indicates the increase in the ratio for its respective diet without CFE.

a Significant difference from its respective diet without CFE at *p* < 0.05.

b Significant difference from standard diet with CFE at *p* < 0.05.
Dietary Macronutrient and CYP Induction by C. forskohlii Extract

the ratio of liver weight to body weight by the CFE treatment were similar between feeding with 0.5% CFE diet and intragastric gavage at a single dose of CFE 750 mg/kg body weight/d, a dose equivalent to that given by the 0.5% CFE diet. The ratio of liver to body weight was 4.07 ± 0.12% in the control group, 6.89 ± 0.25% in the CFE treatment by intragastric gavage group, and 7.36 ± 0.40% in CFE treatment by diet group.

Effect of macronutrients in the diet on induction of hepatic drug-metabolizing enzymes by CFE

Mice were fed semi-purified diets with different macronutrient compositions with and without 0.3% C. forskohlii extract (CFE). Mice were fed a semi-purified standard (10% sucrose and 7% soybean oil), high-sucrose (62.9% sucrose) or high-fat diet (29.9% soybean oil) with and without 0.3% C. forskohlii extract (CFE) for 18 d. The detailed composition of the experimental diets is shown in Table 1. Values are expressed as mean and SE (n=6). *Significant difference from the respective diet without CFE at p<0.05. **Significant difference between two groups at p<0.05.

In mice fed a low-protein (7% casein), a high-protein (33% casein), or a standard diet (20% casein), the ratio of liver weight to body weight was higher in the CFE-treated groups, but the values did not differ among the three CFE-treated groups (Table 4). The influence of dietary protein on the activities of CYP3A and GST was detected in low-protein and high-protein diets, but overall changes were inconsistent (Fig. 3).

Increases in a macronutrient in a diet are synonymous with a decrease in other macronutrients. We adjusted the total amount of macronutrients with starch, which was 0% to 53% in the experimental diets as in Table 1. As shown in Figs. 2 and 3, the CYP induction seemed to be high in the semi-purified standard diet, which is high in starch content. To confirm the contribution of dietary starch to CYP induction in association with and without CFE treatment, the relationship between CYP content and dietary starch levels were examined using the data in Figs. 2 and 3. There was a significant positive correlation between total CYP content in liver and starch levels in the diet (Fig. 4). The phenomenon was clearer in the CFE-treated groups; the correlation coefficient was 0.44 in the control groups and 0.69 in the CFE-treated groups. Similar positive correlation was observed between GST activity and dietary starch levels;
the correlation coefficient was 0.24 (p=0.154) in the control groups and 0.69 (p<0.0001) in the CFE-treated groups.

DISCUSSION

In the present study, we examined how dietary macronutrients influence CFE-induced hepatic drug-metabolizing enzymes, especially CYPs, in mice, and whether there is a difference in CYP induction by CFE between dietary treatment and intragastric gavage. The total content and activities of hepatic CYPs may fluctuate depending on liver sample storage, microsome preparation, and the measurement condition of CYPs. In contrast, the measurement of liver weight was simple and the increased ratio of liver weight to body weight corresponded well to the induction of CYP by CFE (22); the correlation coefficient was 0.85 (n=35, p<0.001). When the relationship between hepatic CYP content and liver weight to body weight was reanalyzed using the data from the CFE dose-response study (17), a significant positive correlation was also detected (r=0.78, n=26, p<0.001). Thus, we used the increase in the ratio
of liver weight to body weight as a simple and reliable indicator of CYP induction following CFE treatment. As a result, the induction of CYP, which was estimated by the increased ratio of liver weight to body weight, was similar between CFE administration by diet and by intragastric gavage, while it was higher in the semi-purified standard diet compared with the high-fat, high-protein, and low-protein diets. Analysis of CYP content and activities showed a similar trend. It was determined that the level of hepatic CYP and GST in CFE-treated groups was positively correlated with the level of starch in the semi-purified diet. In addition, it is worth noting that the high-starch diet in the present study was the standard diet generally used as the AIN93G formula.

The influence of dietary macronutrients on CYP activity has been shown in previous studies in extreme dietary conditions (25–27). Lee et al. (25) showed that hepatic CYP (CYP1A2, 2C11, 2E1 and 3A1/2) activities were decreased in the rats with protein-calorie malnutrition (feeding of 5% casein diet for 4 wk). Peters et al. (26) reported that the activities of hepatic CYP1A1 and CYP3A2 were decreased in rats fed a high-sucrose diet (60% of total calories) compared with a control diet (0% sucrose). Nakajima et al. (27) reported that the activity of hepatic mixed function oxygenase was increased in rats fed a low-sucrose diet for 3 wk, resulting in the toxicity of carbon tetrachloride. In contrast to those studies, we selected rather mild changes in the dietary macronutrient compositions in the present study, and found that content of starch in the diet correlated with the increase in drug-metabolizing enzymes, especially in the CFE-treated groups. The increases in a macronutrient in a diet were synonymous with the decrease in other macronutrients, and change in each ingredient may independently affect the drug-metabolizing enzymes. Accordingly, it will be hard to understand the dietary effect on the drug-metabolizing enzymes. Nevertheless, to the best of our knowledge, there are no reports showing the relation between CYP induction and dietary starch. Thus, this will be a first report that shows an enhanced induction of CYPs by dietary starch, and not by a diet with an extreme level of macronutrients, but by the semi-purified standard diet.

A non-purified diet is composed of natural crude ingredients that may contain substances inducing drug-metabolizing enzymes. In the present study, we observed the induction of CYPs in the semi-purified diet that was composed of isolated ingredients such as sucrose, starch and casein. Therefore, it is unlikely that unknown substance inducing CYPs was present in the ingredients such as starch. At present there is no explanation why a high starch level potentiates the induction of hepatic CYPs by CFE. We speculated that the nature of the induction of drug-metabolizing enzymes by CFE is related to such a mechanism. As shown in our previous studies (19, 21), CFE induced various drug-metabolizing enzymes such as CYP2B, CYP2C, CYP3A and GST, suggesting that the activation of transcription of drug-metabolizing enzymes is involved. Ding and Staudinger clearly showed that constituents of CFE, namely forskolin and 1,9-dideoxyforskolin, induced CYP3A gene expression through the pregnane X receptor (PXR) in cultured hepatocytes (30). Activation of nuclear receptors PXR and constitutive androstane receptor (CAR) has been shown to regulate drug-metabolizing enzymes as well as glucose and lipid metabolism (31). CFE used in the present study also induced hepatic steatosis in mice fed the semi-purified standard diet, although the effective dose was 10 times higher than the dose that induced CYPs (32). These facts suggest that changes in dietary starch level affect the induction of drug-metabolizing enzymes. CFE is composed of various substances; however, forskolin was not involved in CYP activation or hepatic steatosis (22, 32), indicating the contribution of unidentified substances. In a study of solvent fractionation of CFE, we found that the unidentified substances involved in CYP induction were mainly distributed in the diethyl ether-fraction (22). Further detailed studies are needed to clarify the mechanism of action of CYP induction and steatosis associated with CFE treatment and to identify the active substances other than forskolin in CFE. The results of the present dietary study will be helpful in guiding the in vivo studies necessary to identify these active substances.

Currently, several weight-loss diets are widely used, including Atkins, Zone, Ornish and LEARN (23). The lowest carbohydrate diet was shown to be more effective for weight loss at 12 mo in premenopausal overweight and obese women (23). If we applied the present data, intake of the lowest carbohydrate diet and CFE-containing weight loss supplement would be less vulnerable toward the induction of hepatic CYPs. On the other hand, intake of a high-starch diet and a supplement with CFE may induce CYPs, thereby potentially causing adverse events though drug–herb interactions. This may be substantiated by adverse event reports from careful examination of CFE supplement users in practice.

In conclusion, we showed that CYP induction by CFE was potentiated in mice fed a high-starch diet, corresponding to a semi-purified standard diet with the AIN93G formula, compared with low- or high-protein, and high-fat diets. The route of CFE administration, with the diet or by intragastric gavage, did not influence the induction of CYPs as long as the CFE dose and feeding diet were the same. These findings will be helpful in searches for unknown substances involved in hepatic CYP induction and steatosis and in finding a way to minimize CFE–drug interactions caused by the intake of dietary supplements with CFE.

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