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).

Diet	Standard		High sucrose		High fat	
CFE treatment	−	+	−	+	−	+
Average daily food intake (g)	4.8±0.08	4.7±0.07 [0.98]	4.8±0.08	4.7±0.10 [0.99]	3.6±0.04	3.6±0.10 [0.99]
Calculated CFE dose (mg/kg body weight)	0	380±10.5	0	364±4.6	0	375±5.8
Final body weight (g)	39.0±1.1	38.2±1.1 [0.98]	40.1±0.57	39.9±0.90 [0.99]	39.2±0.35	39.6±1.1 [1.0]
Liver weight (g)	1.51±0.07	3.21±0.22 [2.1][a]	1.55±0.04	2.71±0.25 [1.8][a]	1.42±0.04	2.22±0.15 [1.6][a,b]
(%/body weight)	3.87±0.084	8.41±0.51 [2.2][a]	3.86±0.11	6.75±0.50 [1.7][a, b]	3.61±0.093	5.61±0.30 [1.6][a,b]

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 $p<0.05$.
[b] Significant difference from standard diet with 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).

Diet	Standard		Low-protein		High-protein	
CFE treatment	−	+	−	+	−	+
Average daily food intake (g)	4.3±0.090	4.3±0.15 [1.0]	4.4±0.082	4.5±0.16 [1.0]	4.4±0.099	4.5±0.14 [1.0]
Calculated CFE dose (mg/kg body weight)	0	330±7.9	0	364±11.8	0	344±12.8
Final body weight (g)	40.6±1.3	39.1±1.3 [0.96]	37.5±0.89	36.3±1.1 [0.97]	41.1±1.2	38.9±1.2 [0.95]
Liver weight (g)	1.61±0.03	2.94±0.24 [1.8][a]	1.43±0.03[b]	2.47±0.22 [1.7][a]	1.50±0.052	2.57±0.19 [1.7][a]
(%/body weight)	3.96±0.10	7.48±0.50 [1.9][a]	3.82±0.092	6.76±0.45 [1.8][a]	3.66±0.098	6.63±0.49 [1.8][a]

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 without CFE at $p<0.05$.

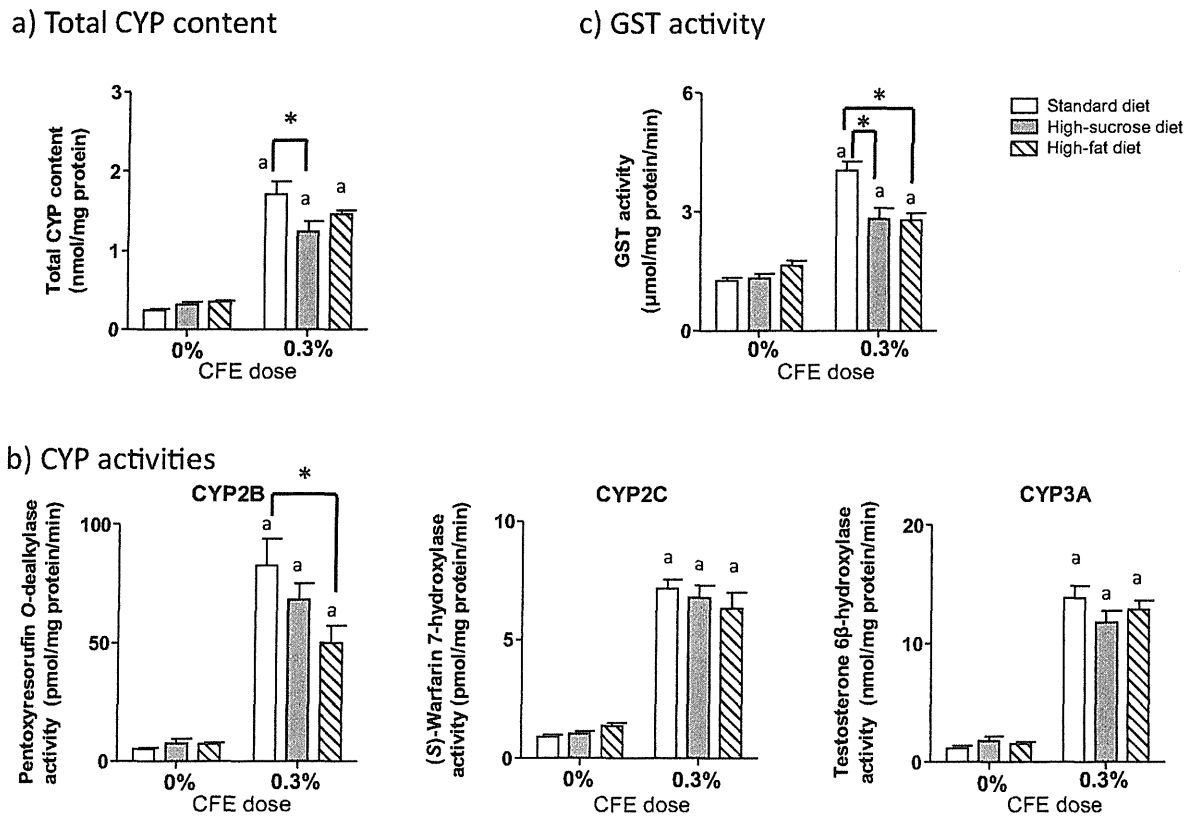

Fig. 2. Hepatic drug-metabolizing enzymes in mice fed a semi-purified standard, high-sucrose, or high-fat diet with and without 0.3% *C. forskohlii* extract (CFE). Mice were fed a semi-purified standard (10% sucrose and 7% soybean oil), high-sucrose (62.9% sucrose) or high-fat diet (29.9% soybean oil) 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). [a] Significant difference from the respective diet without CFE at $p<0.05$. * Significant difference between two groups at $p<0.05$.

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% CFE for 18 d. In mice fed a high-sucrose (62.9% sucrose), a high-fat (29.9% soybean oil), or a semi-purified standard diet (10% sucrose and 7% soybean oil), final body weight did not differ among the groups (Table 3). In addition, liver weight and hepatic drug-metabolizing enzymes did not differ among the groups without CFE treatment (Fig. 2). Calculated intakes of CFE in the CFE-treated groups were similar because daily food intake did not differ. In the CFE-treated groups, the ratio of liver to body weight was higher in the standard diet groups than in the high-sucrose and high-fat diet groups. Similar phenomena were observed for the total CYP content and the activities of CYP2B and GST (Fig. 2). These findings indicate that dietary effects on drug metabolizing enzymes became clearer with the treatment with CFE.

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;

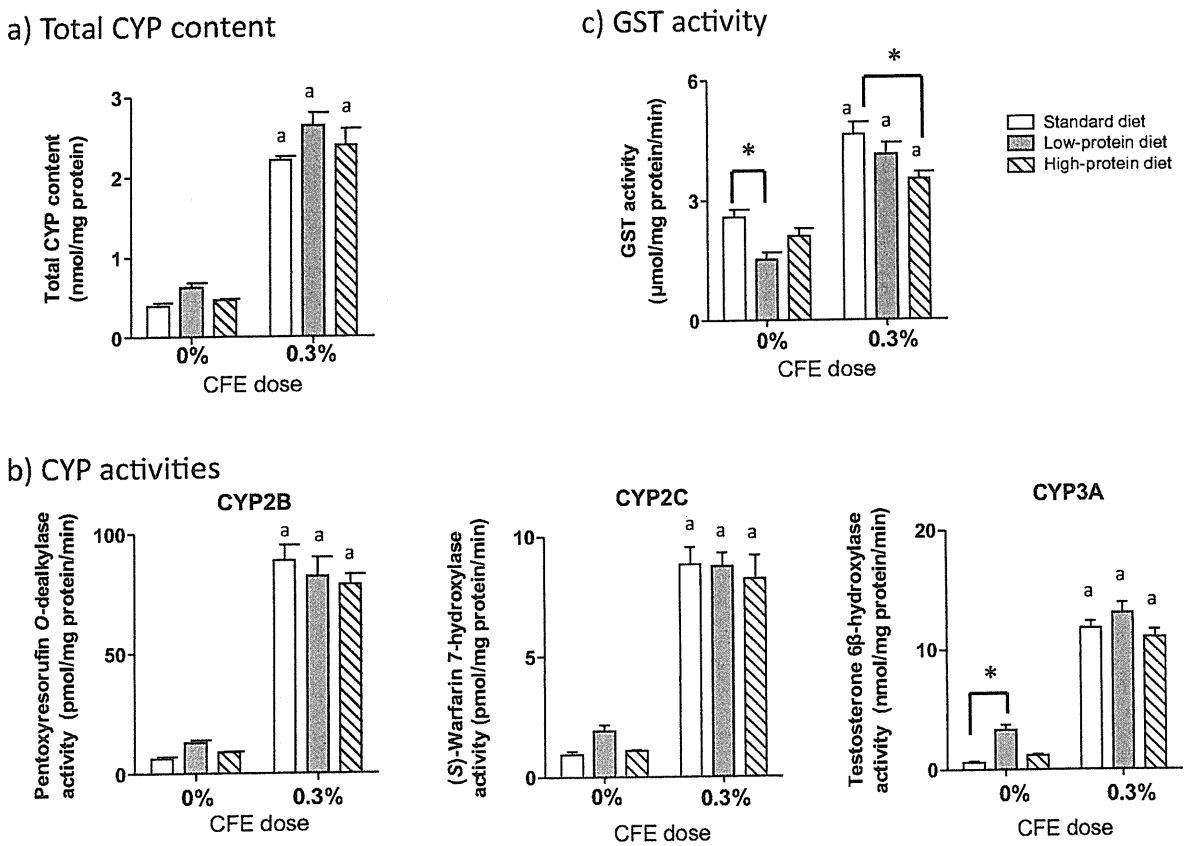

Fig. 3. Hepatic drug-metabolizing enzymes in mice fed a semi-purified standard, low-protein or high-protein diet with and without 0.3% *C. forskohlii* extract (CFE). Mice were fed a semi-purified standard (20% casein), low-protein (7% casein) or high-protein (33% casein) diet with and without 0.3% *C. 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). [a] Significant difference from the respective diet without CFE at $p<0.05$. * Significant difference between the two groups at $p<0.05$.

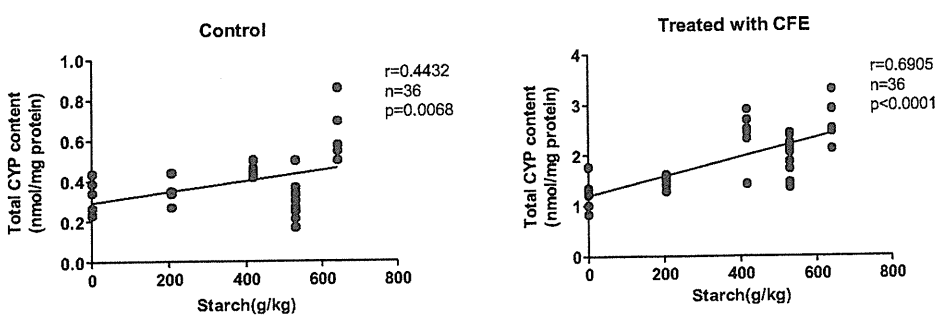

Fig. 4. Correlation between starch in the diet and hepatic CYP content in mice treated with and without *C. forskohlii* extract (CFE). Data were obtained from Figs. 2 and 3.

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-dideoxyforskoiln, 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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