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NOTE

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The difference of chows affects mouse physiological conditions

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ABSTRACT. Acetaminophen-induced liver injury in mice is a model system of human acetaminophen overdose and oxidative stress *in vivo*. The system is technically established, and we usually obtain severe liver damage in the treated mice; however, it is possible that the degree of liver damage is affected by the type of chow fed to mice. Thus, in this experiment, we investigated the effect of different chows on mice by comparing acetaminophen-induced liver damage, liver antioxidant level, and serum amino-acid concentrations. The results showed that differences in chows, even standard ones, affected mouse physiological conditions, with the response to oxidative stress greatly affected.

KEYWORDS: acetaminophen, chow, glutathione, oxidative stress

We have been studying acetaminophen (APAP)-induced liver injury in mice. This is a model system of human APAP overdose and oxidative stress *in vivo* [5]. In the liver, APAP is metabolized to N-acetyl-p-benzoquinone imine (NAPQI), a highly active chemical, and this binds to essential molecules and inactivates them. For example, the antioxidant glutathione (GSH) is inactivated by binding of NAPQI, which results in increasing oxidative stress. Liver toxicity is evaluated by the quantity of serum alanine aminotransferase (ALT) produced in hepatocytes and released from the damaged cells. In APAP-treated mice, ALT titers sometimes exceed 10,000 IU/l, which sharply contrasts with less than 30 IU/l for untreated mice.

The method for causing APAP-induced liver injury in mice is technically simple and well established, and we usually obtain high ALT titers in treated mice; however, we were aware of the possibility that the severity of liver damage was affected by the type of chow fed to mice. Furthermore, many reports have shown that foods affect physiological and even mental conditions [2, 10, 13]; thus, in this experiment, we investigated the effect of different chows on mice comparing APAP-induced liver damage, liver antioxidant level, and serum amino-acid concentrations.

Three-week-old C57BL/6 male mice were purchased from Sankyo-Lab Service (Tsukuba, Japan). The mice were fed with CE-2 (CLEA Japan, Inc., Tokyo, Japan) or FR-1 chow (Funabashi Farm Co., Ltd., Chiba, Japan) for 7–9 weeks. These are standard chows sold by each company and the compositions are shown in Supplementary Table 1. For the experiment of APAP-induced liver injury, mice were fasted 10–12 hr before APAP injection but were allowed access to water. The APAP purchased from Sigma-Aldrich (St. Louis, MO, USA) was suspended in 50% Milli-Q water and 50% propylene glycol, and was intraperitoneally injected into mice (600 mg/kg). Mice were sacrificed at 16 hr after APAP injection to collect liver tissues and sera. Serum ALT levels were measured by an automated analyzer (Hitachi 7140; Hitachi Instruments Service Co., Tokyo, Japan). The ALT values in the sera of mice fed with CE-2 (CE-2 mice) and those fed with FR-1 (FR-1 mice) [mean ± standard error of the mean (SEM)] were 14,150 ± 2,931 and 891 ± 336 IU/l, respectively (Fig. 1). The value for CE-2 mice was significantly higher than that for FR-1 mice ($P<0.01$), indicating that CE-2 mice had more hepatic damage than FR-1 mice. Given that the APAP administration in mice is a model system of inducing oxidative stress *in vivo* [5], this result suggested that food affected the response to oxidative stress, with higher sensitivity of CE-2 than FR-1 mice.

Next, we determined the liver GSH concentrations, because they were expected to be related to the tolerance to oxidative stress in the liver caused by APAP overdose [5]. Liver pieces collected from male mice fed with CE-2 or FR-1 chow for 8 weeks were homogenized by BioMasher (Nippi, Tokyo, Japan) and lysed in 1 ml of 5% 5-sulfosalicyclic acid dihydrate (SSA) on ice. Protein concentrations in tissue homogenates were determined by using a BCA assay kit (Thermo Fisher Scientific, Inc., Waltham, MA, USA). Then, the samples were centrifuged at 8,000 *g* for 10 min at 4°C, and the supernatants diluted in Milli-Q water to 0.5%

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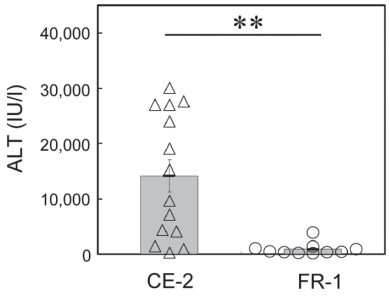

Fig. 1. Alanine aminotransferase (ALT) titers of mice fed different chows. Purchased C57BL/6 male mice at age 3 weeks were fed with CE-2 (n=14) or FR-1 chow (n=10) for 7–9 weeks, and treated with acetaminophen (APAP) overdose (600 mg/kg). At 16 hr after treatment, sera were collected and ALT titers determined. Data are shown as mean ± SEM. **P<0.01, Student's t-test.

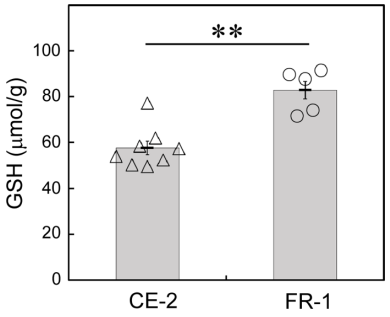

Fig. 2. Liver glutathione (GSH) concentrations in male mice fed with CE-2 (n=8) and FR-1 chow (n=5) for 8 weeks. Data are shown as mean ± SEM. **P<0.01, Student's t-test.

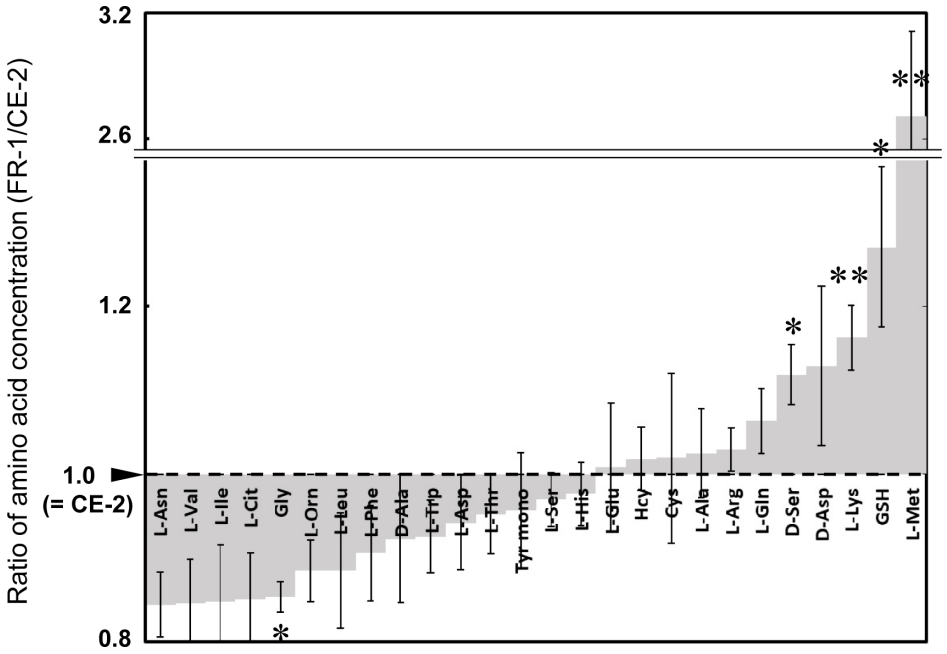

Fig. 3. The ratio of each amino-acid concentration. Serum amino-acid concentrations in male mice fed with CE-2 (n=10) or FR-1 chow (n=10) for 8 weeks were determined (Supplementary Fig. 1), and the ratio of each amino-acid concentration (FR-1/CE-2) was calculated. Amino acids: Alanine (Ala), Arginine (Arg), Asparagine (Asn), Aspartate (Asp), Cysteine (Cys), Glutamine (Gln), Glutamate (Glu), Glycine (Gly), Histidine (His), Isoleucine (Ile), Leucine (Leu), Lysine (Lys), Methionine (Met), Phenylalanine (Phe), Serine (Ser), Threonine (Thr), Tryptophan (Trp), Tyrosine (Tyr), Valine (Val), Ornithine (Orn), Citrulline (Cit), Homocysteine (Hcy), and Glutathione (GSH). Data are shown as mean ± SEM. *P<0.05, **P<0.01, Student's t-test.

SSA. The GSH concentrations of the supernatants were measured by Total Glutathione Quantification Kit (Dojindo Molecular Technologies, Inc., Kumamoto, Japan) according to the user manual. The CE-2 mice had significantly lower GSH concentrations than FR-1 mice (P<0.01) (mean ± SEM), 57.7 ± 3.0 vs 82.8 ± 3.7 μmol/g (Fig. 2), consistent with CE-2 mice having greater hepatic damage (Fig. 1). Thus, we confirmed that the liver GSH concentration was strongly related to tolerance to oxidative stress induced by APAP overdose.

To determine the systemic effect of the different chows, we measured the amino-acid concentrations in sera from male mice fed with CE-2 or FR-1 chow for 8 weeks by liquid chromatography with tandem mass spectrometry (LC-MS/MS) instrumentation as previously described [14]. The concentrations of thiol compounds were determined by our previous LC-MS/MS method with minor modifications [6, 11]. Supplementary Figure 1 shows the values in sera from CE-2 and FR-1 mice. The ratio of each amino-acid concentration (i.e. FR-1/CE-2) was calculated and the values shown as mean ± SEM; the ratios for L-methionine (Met), GSH, and L-lysine (Lys) of 2.71 ± 0.41, 1.27 ± 0.10, and 1.16 ± 0.04, respectively, were significantly high (P<0.05) (Fig. 3). Because Met is a source of antioxidant GSH [9] and Lys is a scavenger of unsaturated aldehyde acrolein, a toxic byproduct of oxidative stress

[1, 7, 16], it is reasonable that FR-1 mice could be more systemically tolerant to oxidative stress than CE-2 mice. Interestingly, the Met concentration was higher in FR-1 than CE-2 chow (7.1 vs. 4.4 g/kg) (Supplementary Table 1) and it was previously reported that Met supplementation elevated serum GSH contents, resulting in increments of tissue GSH redox potential values [3]. Concerning the other components having antioxidant effects, such as vitamin A, vitamin E, and serine [12, 15, 17], only vitamin E concentration was higher in FR-1 than in CE-2 chow (Supplementary Table 1). Thus, we concluded that higher Met and vitamin E supplementation increased antioxidant level in FR-1 mice and caused more tolerance to the oxidative stress from APAP overdose than in CE-2 mice.

Many studies have suggested that dietary Met supplementation may result in enhanced performance in pigs and chickens, especially with regard to meat yield, body weight gain, and feed conversion rate [4, 8]. Furthermore, associations between foods and behavior or mental health have been reported [2, 10, 13]. Particularly, intakes of antioxidant foods are reported to be inversely associated with depression [13]. We are also interested in the relationship between foods and body weight gain or behavior, and this is currently being investigated using CE-2 and FR-1 mice, with preliminary results indicating that FR-1 mice have greater body weight and are also more active than CE-2 mice.

In this report, we showed that differences in chows, even standard ones, could affect mouse physiological conditions. Thus, we should carefully choose chows for each animal experiment, especially for those related to oxidative stress.

POTENTIAL CONFLICTS OF INTEREST. The authors have nothing to disclose.

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