Food Restriction Reduces Aflatoxin B₁ (AFB₁)-DNA Adduct Formation, AFB₁-Glutathione Conjugation, and DNA Damage in AFB₁-Treated Male F344 Rats and B6C3F₁ Mice¹,²

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ABSTRACT The objective of this study was to examine effects of food restriction (FR) on the metabolic activation of aflatoxin B₁ (AFB₁) in rats and mice, which are AFB₁-sensitive and -resistant rodent species, respectively. Forty percent FR [60% of ad libitum (AL) food consumption] reduced the metabolic activation of AFB₁ in both rats and mice, causing formation of hepatic AFB₁-DNA adducts to be 43% and 31% lower, respectively. The AFB₁-DNA adduct 8,9-dihydro-8-(N⁷-guanyl)-9-hydroxyaflatoxin B₁ (AFB₁-N⁷-Gua) was predominantly formed in rat liver DNA; the formation of the ring-open analogue of AFB₁-N⁷-Gua, AFB₁-formamidopyrimidine (AFB₁-FAP), was predominantly found in mouse liver DNA. In contrast to the in vivo results, the in vitro AFB₁-DNA adduct formation mediated by the microsomes of liver, kidney or lung from FR-mice was greater than the formation of AFB₁-DNA adducts mediated by the tissue microsomes from the AL-mice. Food restriction induced hepatic glutathione S-transferase (GST) activity, as measured by the formation of AFB₁-glutathione conjugates (AFB₁-SG), in both rats and mice; AFB₁-SG was also formed in mouse kidney. Food restriction–induced GST activity assayed in an in vitro system, using [³H]AFB₁-8,9-epoxide and glutathione (GSH) as substrates, was also found when mouse kidney and lung cytosolic fractions were used. Food restriction inhibited the AFB₁-induced DNA double strand breaks in mouse kidney. The reduction of levels of AFB₁-DNA adduct formation in mouse kidney was comparable to the degree of AFB₁-induced DNA strand breakages. The results of this study indicate that the metabolic activation of AFB₁ can be modulated by FR through the alteration of the formation of AFB₁-DNA adducts and AFB₁-SG conjugation. However, species and tissue specificities exist regarding the metabolic activation of AFB₁. J. Nutr. 127: 210–217, 1997.

KEY WORDS: • rats • mice • food restriction • aflatoxin B₁-DNA adducts • aflatoxin B₁-glutathione conjugates

Nutritional modification of carcinogenesis has become an active area of research, in part because the growing awareness that dietary excess, deficiencies and imbalances can play a major role in the etiology or modulation of cancer. Food restriction (FR) extends life span and decreases the prevalence of spontaneous and chemically induced cancers in laboratory animals (Allaben et al. 1991, Pariza and Boutwell 1987, Tannenbaum 1942). The effects of FR on chemically induced tumors have been reported for various target tissues, including the aflatoxin B₁ (AFB₁)-induced liver tumors in rats (Newberne and Rogers 1986). The mechanisms of the reduction of tumor incidence by FR are not known. However, the finding that FR inhibits tumor formation induced by the indirect-acting carcinogen (requiring metabolic activation) methylnitrosourea (Pollard and Luckert 1985), led us to focus our studies on the role FR may play in the modulation of neoplastic disease by inducing alterations in activities of drug-metabolizing enzymes, both phase I and phase II, of animals.

The metabolic activation of xenobiotics and drugs is catalyzed by cytochrome P450 (CYP)-dependent drug-metabolizing enzymes. Any factor altering the activity of these enzymes may affect the metabolic activation of certain carcinogens. Such alterations may influence both the pharmacokinetics and the efficacy of various drugs as well as the metabolic activation of toxic xenobiotics. Drug-metabolizing enzymes are involved not only in the initiation of neoplastic disease but also in the acute cellular activation and detoxification of many chemicals and drugs. Furthermore, the alteration of activities of drug-metabolizing enzymes by FR may affect the metabolism, utilization, and efficacy of drugs needed. The effect of FR on the metabolic activation of xenobiotics in laboratory animals can be estimated by measuring either the specific enzyme activities (activation or detoxification) or
changes in carcinogen-DNA adduct formation. Previous studies demonstrated that FR alters xenobiotic metabolizing enzyme activities (Chou et al. 1993a, Koizumi et al. 1987, Leakey et al. 1989, Sachan and Das 1982), decreases 7,12-dimethylbenz[a]anthracene (DMBA) binding to dermal DNA in mice (Pashko and Schwartz 1983) and reduces the binding of AFB1 to hepatic nuclear DNA in rats (Chou et al. 1993a). Results indicate that the effect of FR on the initiation stage of carcinogenesis could differ in different species of animals and that FR may play an important role in the modulation of neoplastic diseases. Here, we report results of a study on the effects of FR on AFB1 activation in mice, a species less sensitive to AFB1-induced carcinogenesis than rats because of the high glutathione S-transferase (GST) activity in mouse liver. A species comparison between mice and rats, in terms of the formation of the major AFB1-DNA adducts and AFB1-glutathione conjugates (AFB1-SG), was also conducted.

Aflatoxin B1 is a potent food carcinogen and has been implicated epidemiologically as a causative agent in human liver cancer (Busby and Wogan 1984, Wogan 1992). Aflatoxin B1 requires metabolic activation to exert its genotoxicity. Metabolic activation of AFB1 (Fig. 1) by microsomal xenobiotic metabolizing enzymes, both in vivo and in vitro, results in the formation of AFB1-8,9-epoxide, which binds to cellular metabolizing enzymes, both in vivo and in vitro, resulting in (Croy et al. 1978, Lin et al. 1977, Shaulsky et al. 1990). Genes and carcinogenesis. Aflatoxin B1-induced mutagenic activities (both phase I and phase II) is tested.

FIGURE 1
Metabolic activation of aflatoxin B1 (AFB1) and the formation of total AFB1-DNA adducts. Abbreviations used: AFB1-epoxide, 8,9-dihydro-8,9-oxide-AFB1; AFB1-N7-Gua, 8,9-dihydro-8-(N7-guanyl)-9-hydroxyaflatoxin B1; AFB1-FAP, AFB1-formamidopyrimidine; CYP, cytochrome P450; GSH, glutathione; GST, glutathione S-transferase.

EXPERIMENTAL METHODS

Chemicals. Tritiated aflatoxin B1 ([1H]AFB1, specific radioactivity 555 GBq/mmol, purity >98.7%) was purchased from Moravek Biochemicals (Brea, CA). Unlabeled AFB1, tritiated glutathione ([1H]GSH, specific radioactivity 1.63 GBq/μmol), unlabeled GSH, calf thymus DNA, NADPH, ribonuclease A, protease K, dATP, dGTP, dCTP, dTTP, Klenow fragment were purchased from Sigma Chemical (St. Louis, MO). The [1H]AFB1 and [1H]GSH were diluted with unlabeled AFB1 and GSH, respectively, to obtain the desired specific radioactivity. α-[35S]PdCTP (specific radioactivity 111 GBq/μmol) was purchased from Amersham (Arlington Height, IL). Dimethylsulfoxide was synthesized according to the method of Adam et al. (1989). Other reagents required for DNA purification and HPLC analyses were HPLC grade.

Animals and diets. Weanling male B6C3F1 mice and Fischer 344 rats from the breeding colony of the National Center for Toxicological Research were housed individually. Free access (AL) was given to a standard NIH-31 open formula diet (Purina Mills, Richmond, IN) with the following composition (g/kg): fish meal (60% protein), 90; soybean meal (48.59% protein), 50; alfalfa meal (17% protein), 20; corn gluten meal (60% protein), 20; ground whole hard wheat, 355; ground #2 yellow shelled corn, 210; ground whole oat, 100; wheat middlings, 100; brewer dried yeast, 10; soybean oil, 15; sodium chloride, 0.5; decalcium phosphate, 15; ground limestone, 5; vitamin premixes, 5. The FR animals were fed 60% of the AL diet using a powdered diet form. FR at the age of 10 wk; mice started FR at the age of 16 wk. At 16 wk of age, groups of AL and FR rats (four per group) received a single dose of [3H]AFB1 [0.1 mg AFB1/kg body wt, specific radioactivity, 7.7 GBq/mmol, dissolved in dimethylsulfoxide (DMSO), 10.5 mol/L]. The control groups of rats were treated with 10.5 mol/L DMSO only. At 20 wk of age, the FR animals (four animals per group) were treated with a single dose (1, 2, 5, 10 μg/kg body wt, respectively) of [3H]AFB1 (specific radioactivity, 5.5 GBq/mmol, dissolved in 10.5 mol/L DMSO) injected intraperitoneally. Control groups were treated with vehicle only. At various time points, animals were killed by
hypoxygenation with CO2, followed by exsanguination. Livers, kidneys, lungs and other tissues were removed immediately, rinsed with cold saline, and stored at -70°C for further analysis.

Preparation of subcellular fractions. Tissues were homogenized with cold sucrose-Tris buffer, pH 7.4, containing 0.25 mol/L sucrose, 25 mmol/L KC1, 10 mmol/L MgCl2, and 50 mmol/L Tris-HCl. Nuclei prepared by washing with 0.5% Triton X-100 in buffer were used for DNA isolation and AFB1-DNA adduct analysis. DNA was purified with RNase A and protease K treatment, followed by phenol and chloroform-isoamyl alcohol (24:1) extractions, and assayed according to the method of Beland et al. (1984). Microsomal and cytosolic fraction were prepared by differential centrifugation (Chou et al. 1988) and stored at -70°C prior to use. Protein concentrations were determined by the method of Lowry et al. (1951) with bovine serum albumin as standard.

Preparation of aflatoxin B1-8,9-epoxide. Aflatoxin B1-epoxide was prepared from the peroxidation of AFB1, by dimethylfumurate, which was prepared as previously described (Baertschi et al. 1989, Chen et al. 1995). Briefly, approximately 1.5 equivalent weight of freshly prepared dimethylfumurate (concentration range from 0.05 to 0.12 µmol/L) in anhydrous acetone was added to AFB1 solution (dissolved in anhydrous acetone). The reaction mixture was stirred at room temperature for 20 min, and the resulting AFB1-8,9-epoxide was obtained. Solvent and excess dimethylfumurate were removed by vacuum evaporation. The residue was redissolved in anhydrous acetone used for the preparation of AFB1-DNA adducts and AFB1-SG conjugation products. [3H]Aflatoxin B1-8,9-epoxide was similarly prepared as described using [3H]AFB1 as the starting material.

Analysis of aflatoxin B1-DNA adduct. Standard AFB1-N7-Gua adduct, AFB1-8,9-dihydrodiol adduct and AFB1-7,8-dihydroxy-9,10-epoxy-7,8,9,10-tetrahydro-AFB1 adduct were used as controls for the quantification of specific AFB1-N7-Gua and AFB1-8,9-dihydroxy-9,10-epoxy-7,8,9,10-tetrahydro-AFB1 adducts. AFB1-epoxide adducts were separated by a reversed-phase HPLC equipped with a Bondapak C-18 column (3.9 x 300 mm) eluted with 3.5 mol/L acetonitrile in water. The structural confirmation was accomplished by mass and NMR spectrometry (Baertschi et al. 1989, Chen et al. 1995, Shaulsky et al. 1990). The total [3H]AFB1-DNA binding of in vivo samples was determined by measuring the radioactivity in the modified DNA. The [3H]AFB1-N7-Gua adducts obtained from in vivo samples were identified by co-chromatography with the synthesized standard on a reversed-phase HPLC column (Waters, µBondapak C18, 38 x 300 mm) eluted with a linear gradient of 1.75 to 5.25 mol/L of acetonitrile for 40 min and quantified by measuring the radioactivity of bound [3H]AFB1. In vitro microsomal-mediated binding of [3H]AFB1 to calf thymus DNA was determined by using a 1.0 mL reaction mixture containing 1.0 mg of DNA, 20 nmol of [3H]AFB1 (specific radioactivity 7.4 GBq/mmol), 0.1 mol/L sodium phosphate, pH 7.4, 0.65 mol/L NaDH, 3 mol/L MgCl2, and 1 mg of liver (or other tissue) microsomal protein from AL or FR rats. After 30 min of incubation, DNA was extracted and purified. The total binding and specific AFB1-DNA adducts were measured as described.

Analysis of aflatoxin B1-glutathione conjugate. The authentic standard of AFB1-SG (or [3H]AFB1-SG) was prepared by the reaction of AFB1-8,9-epoxide (or [3H]AFB1-8,9-epoxide) with GSH catalyzed using GST of rat liver cytosol as enzyme source (Chen et al. 1995). An aliquot was prepared by a reaction of AFB1-8,9-epoxide with calf 10 mol/L sodium phosphate buffer, pH 7.4. Aflatoxin B1-epoxide in acetonitrile was gradually added with a microsyringe into 1 mL of AFB1-8,9-epoxide reaction mixture with vigorous stirring. The reaction mixture (pH 7.4) contained 50 µmol of sodium phosphate, 5 µmol of GSH and 1 g/L liver cytosolic protein. After 5 min of incubation at room temperature, 50 µL of 2 mol/L acetic acid was added, and the mixture was centrifuged to remove the precipitated cytosolic proteins. The supernatant was filtered through a 0.45-µm filter, and the filtrates were stored at -70°C until analyzed by HPLC. The [3H]AFB1-SG samples were analyzed by HPLC eluted with a linear gradient of 0-7 mol/L of acetonitrile-H2O over a period of 40 min (1 mL/min). The HPLC was monitored by a photodiode-array detector (Waters model 996) at 362 nm wavelength. The fractions containing [3H]AFB1-SG were collected, and the radioactivity was measured by a liquid scintillation counter. The analysis of [3H]AFB1-SG of in vivo samples was performed by applying 30 µL of respective cytosolic samples on HPLC, and the radioactivity of the fractions containing [3H]AFB1-SG was measured as described. The cysteic GST activity was expressed as micromoles of [3H]AFB1-SG per milligram of protein per minute.

Assay of DNA strand breaks. To determine the effect of FR on AFB1-induced DNA strand breaks, random oligonucleotide-primed synthesis DNA fragmentation assay was employed (Basnakian and James 1994). Mouse kidney (200 mg) was powdered with liquid nitrogen and digested with proteinase K (0.1 g/L) at 50°C for 12 h. The genomic DNA was isolated according to the method of Ausbel et al. (1989). DNA was dialyzed against 100 volumes of 10 mmol/L Tris-HCl-1 mmol/L EDTA buffer (TE buffer), pH 7.4, overnight. The DNA concentration was diluted to 25 g/L with TE buffer. A 50-µL DNA solution was incubated at 10°C for 5 min to denature the DNA and then cooled on ice. Ten microliters of each DNA sample was placed in a 96-well polystyrene plate on ice, and a 15-µL reaction mixture (containing 2.5 µL of 0.5 mmol/L dGTP, dATP and dTTP mixture, 0.5 unit of Klenow fragment, 2.5 µL of Klenow buffer, 0.45 µL of 33 mmol/L dCTP, and 18.5 kbq of [3H]dCTP) was added to each sample well. The plate was incubated at 16°C for 30 min. The reaction was stopped by cooling the sample on ice, and 25 µL of 12.5 mmol/L EDTA, pH 8.6, was added. An aliquot (5 µL) from the incubation mixture was applied on DESII filter paper. The filter paper was rinsed five times in 100 mL of 0.5 mol/L sodium phosphate buffer and twice with water. After drying, the radioactivity on the paper was counted in a scintillation counter.

Statistical analysis. Data were expressed as means ± SEM. Differences between the AL and FR groups were evaluated using one-way ANOVA. Differences were considered statistically significant when P < 0.05.

RESULTS

Effect of food restriction on the body and liver weights and on in vivo metabolic activation of aflatoxin B1. After 6 wk of food restriction, rats and mice had 33% and 18% lower body weights and 16% and 21% lower relative liver weights, respectively (P < 0.05, Table 1). Food restriction reduced the in vivo AFB1-DNA binding in liver and kidney of both animal species (Table 1). The total hepatic AFB1-DNA binding in rats (the AFB1-sensitive species) was seven- to ninefold greater than that in mice (the AFB1-resistant species). The total AFB1-DNA binding in rat liver, the target tissue for AFB1-induced cancer, was 300-fold greater than that in rat kidneys; however, in mouse liver the total AFB1-DNA binding was comparable to that in mouse kidney (Table 1). The in vivo [3H]AFB1-DNA binding was not detected under our experimental conditions in lung nuclear DNA isolated from either AL or FR mice.

After hydrolysis of [3H]AFB1-DNA, the radioactivity of specific AFB1-DNA adducts, AFB1-N7-Gua and AFB1-FAP, was on average 67% (62 to 75%) of the total AFB1-DNA binding. Three h after dosing, the AFB1-N7-Gua was the predominant DNA adduct formed in rat liver, and the AFB1-FAP adduct (a ring open form derived from AFB1-N7-Gua) was the predominant DNA adduct found in both rat and mouse livers (Table 1). Food restriction reduced both AFB1-N7-Gua and AFB1-FAP adduct formation in rat liver; however, it reduced the formation of AFB1-FAP only in mouse liver (Table 1).

The formations of the two specific AFB1-N7-Gua and AFB1-FAP adducts in liver and kidney from AL and FR mice were proportional to dose (Fig. 2). At a dose greater than 5 mg AFB1/kg body wt, the major form of AFB1-DNA adducts de-
Effects of 6 wk of food restriction (FR) on the body weights, relative liver weights, hepatic and renal aflatoxin B$_1$ (AFB$_1$)-DNA adduct formation, and AFB$_1$-glutathione (AFB$_1$-SG) conjugation of male B6C3F$_1$ mice and F344 rats.$^1$,$^2$

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<td><strong>Body weight, g</strong></td>
<td>33.4 ± 2.5</td>
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<td>224.2 ± 5.5*</td>
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<td><strong>Relative liver weight, g/100g body wt</strong></td>
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<td>28.8 ± 1.7*</td>
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<td>11.7 ± 2.0</td>
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<td>12.3 ± 0.8*</td>
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$^1$ Values are means ± SEM, n = 26 (body weight and relative liver weight), n = 4 (AFB$_1$-N$^7$-Gua, AFB$_1$-FAP and AFB$_1$-SG). * Significantly different from the value for the corresponding AL group (P < 0.05).

$^2$ At the age of 10 wk, rats (body weight 228.2 ± 9.1g, n = 26) received food restriction for 6 wk. Mice (body weight 27.1 ± 1.6g, n = 26) received food restriction for 6 wk starting at the age of 14 wk. AL mice, AL rats = mice or rats fed the NIH-31 diet with ad libitum access; FR mice, FR rats = mice or rats fed 60% of the food intake of the AL animals. Abbreviations used: AFB$_1$-N$^7$-Gua, 8,9-dihydro-8-(N$^7$-guanyl)-9-hydroxyaflatoxin B$_1$; AFB$_1$-FAP, AFB$_1$-formamidopyrimidine; AFB$_1$-SG, AFB$_1$-glutathione conjugates; NA, not available.

Table 2

Effects of 6 wk of food restriction (FR) on the body weights, relative liver weights, hepatic and renal aflatoxin B$_1$ (AFB$_1$)-DNA adduct formation, and AFB$_1$-glutathione (AFB$_1$-SG) conjugation of male B6C3F$_1$ mice and F344 rats. The result supports the finding that the more AFB$_1$-SG formed, the lower level of the AFB$_1$-SG seemed to be dependent on the rate of AFB$_1$ epoxidation and the activity of the GST.

The GST activity measured in the in vitro system using FR mouse cytosol as the enzyme source was 1.5-fold greater than that catalyzed by the cytosol from AL mouse liver (Table 2). Similarly, the in vitro cytosolic GST activity of FR mouse kidney and lung was also greater than those obtained when cytosolic fractions of kidney and lung from AL mice were used (Table 2). In both FR and AL mice, the liver cytosolic GST activity of mice was 30 times greater than that of rats. Mouse pulmonary AFB$_1$-metabolizing enzyme activity and GST activity measured in vitro were lower than for liver and kidney. The in vivo formation of AFB$_1$-DNA adducts and AFB$_1$-SG conjugation were not found in mouse lung nuclear DNA or in lung cytosolic fractions. However, FR increased the in vivo microsomal and cystolic AFB$_1$-DNA adduct and AFB$_1$-SG conjugation, respectively, indicating that the metabolism of AFB$_1$ in mice occurred primarily in liver and kidney.

Effect of food restriction on aflatoxin B$_1$-induced mouse DNA strand breaks. Three hours after mice received a 2 mg/kg dose of AFB$_1$, there were 60% fewer AFB$_1$-induced DNA strand breaks in the kidney DNA from FR mice than in kidney DNA of AL mice, in terms of [$^{32}$P]dCTP incorporation (113 ± 12 pmol/mg DNA vs. 59 ± 5 pmol/mg DNA, P ≤ 0.05).

The result was comparable to the data for the reduction of in vivo adduct formation and the greater detoxification of AFB$_1$ by FR mice.

**DISCUSSION**

Food restriction inhibits chemically induced tumorigenesis in various tissues of laboratory animals. Newberne and Rogers.
(1986) studied AFB1-induced hepatocarcinoma in male F344 rats fed 25% restricted diets and found that the liver tumor incidence was reduced significantly. Recently, we found that FR decreased spontaneous and AFB1-induced mutations in rat lymphocytes at the hypoxanthine guanine phosphoribosyl transferase (hprt) locus (Casciano et al. 1996). This finding agrees with our results that FR may decrease AFB1-induced carcinogenic initiation by reducing rat liver AFB1 metabolizing enzyme activity and subsequently decreasing the AFB1-DNA binding (Chou et al. 1993a). The in vivo formation of AFB1-DNA adducts involves metabolic activation (AFB1 epoxidation) and detoxification (AFB1-SG formation). Thus, the objective of this study was to generalize our hypothesis that FR reduces metabolic activation of AFB1 in both AFB1-sensitive (male F344 rats) and AFB1-resistant animals (male B6C3F1 mice) and to compare the mechanisms of the effect of FR on the metabolic activation of AFB1 in these two species. Our results demonstrate a reduction of metabolic activation of AFB1 in both FR rats and mice; however, species and tissue specificities exist regarding AFB1 epoxidation, AFB1-DNA adduct formation and AFB1-SG conjugation. Mice are less sensitive to AFB1-induced hepatocarcinogenesis, probably because of their high levels of cytosolic GST activity. The GST activities in livers of both rats and mice can be induced by FR. The FR-induced GST activity and FR-reduced CYP2C11 in rat liver (Leakey et al. 1989) resulted in the decrease of AFB1-DNA adduct formation in rats. The FR-induced mouse liver GST activity and subsequently increased formation of AFB1-SG. FR also reduced in vivo AFB1-DNA adduct formation in mouse liver, in spite of the increase in mouse liver microsomal AFB1-epoxidation induced by FR. Removal of the AFB1-DNA adducts in rat and mouse liver and kidney showed biphasic slopes (Gao and Chou 1992). The resulting AFB1-DNA adducts detected were dependent upon the levels of the reactive metabolite (AFB1-8,9-epoxide) formed and the levels of cellular GST that catalyzed the formation of the detoxification product, AFB1-SG. In addition, the total AFB1-DNA adducts may also be affected by DNA repair enzymes and the depurination of AFB1-bound purine moiety. Our results demonstrate that the reduction of the hepatic AFB1-metabolizing enzyme activities by FR resulted in the decrease of AFB1-DNA adduct formation and AFB1-induced DNA strand breaks in both
FOOD RESTRICTION REDUCES AFB₁-DNA ADDUCTS

**FIGURE 3** Time courses of the removal of 8,9-dihydro-8-(N⁷-guanyl)-9-hydroxyaflatoxin B₁ (AFB₁-N⁷-Gua) and its ring-open form, AFB₁-formamidopyrimidine (AFB₁-FAP), from livers and kidneys of mice that consumed food ad libitum (AL) or were 40% food restricted (FR). The mice were treated with a single dose of tritiated aflatoxin B₁ ([³H]AFB₁, 7.7 GBq/mmol) and killed 3 h after dosing. Values are means ± SEM, n = 4. *FR significantly different from AL (P < 0.05, A vs. B and C vs. D).

**TABLE 2**

Effects of food restriction (FR) on in vitro aflatoxin B₁ (AFB₁)-DNA adduct formation and on AFB₁-glutathione (AFB₁-SG) conjugation mediated by microsomes obtained from liver, kidney and lung of male B6C3F₁ mice and F344 rats¹,²

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<td>Liver</td>
<td>32.1 ± 2.32</td>
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<td>GST activity, fmol AFB₁-SG formed/(mg protein-min)</td>
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<td>Liver</td>
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<td>1.31 ± 0.09*</td>
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¹ Values are means ± SEM, n = 4. * Significantly different from the value for the corresponding AL group (P < 0.05). The AFB₁-DNA adduct was expressed as pmol AFB₁.

² AL mice, AL rats = mice or rats fed the NIH-31 diet with ad libitum access; FR mice, FR rats = mice or rats fed 60% of the amount of food consumed by AL animals for 6 wk. Abbreviations used: AFB₁-N⁷-Gua, 8,9-dihydro-8-(N⁷-guanyl)-9-hydroxyaflatoxin B₁; AFB₁-FAP, AFB₁-formamidopyrimidine; AFB₁-SG, AFB₁-glutathione conjugates; GST, glutathione S-transferase; NA, not available.
The initial DNA adduct formation may be more important to processes (Martin and Garner 1977). It has been proposed that patterns of metabolism and DNA modification with biological effects. Basic be more stable than AFB1-N7-Gua adduct (Bailey 1994, Eaton and Gallagher 1994). Thus, our finding that AFB1-N7-Gua was formed predominantly in rat liver and that the ring-open form (AFB1-N7-FAP) was predominantly generated in mouse liver may support the hypothesis. The data suggest that the rate of formation of the ring-open form from AFB1-N7-Gua under our experimental conditions may be different in these two species. The ring-open DNA adducts may occur non-enzymatically either in situ after the DNA was modified by AFB1 or during the sample preparation. However, the possibility that the formation of ring-open adducts was catalyzed by enzymes could not be excluded. Metabolites of AFB1, such as aflatoxin M1, P1 and Q1 can also be metabolically activated and bind to DNA to form minor AFB1-DNA adducts (Bailey 1994, Lutz et al. 1980, Raney et al. 1992). However, under our HPLC conditions, only two AFB1-DNA adduct peaks were identified.

Our results indicate that the reduction of the metabolic activation of AFB1 and increased activities of detoxification enzymes by FR may contribute to the reduction of AFB1-induced tumor incidence in food-restricted animals.

LITERATURE CITED
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