Available online at www.sciencedirect.com
ScienceDirect
Journal of Nutritional Biochemistry 18 (2007) 236–249
Journal of Nutritional Biochemistry

Effect of dietary fat on metabolism and DNA adduct formation after acute oral exposure of F-344 rats to fluoranthene☆

Stormy A. Walker, Amma B. Addai, Malcolm Mathis, Aramandla Ramesh*

Department of Pharmacology, Meharry Medical College, Nashville, TN 37208, USA

Received 22 February 2006; received in revised form 28 March 2006; accepted 6 April 2006

Abstract

Adverse health effects such as cancer and toxicity may be attributed to consumption of chemically contaminated food rich in fat. This leads to a larger intake and retention of lipophilic toxic chemicals in the body with an increase in risks to human health. The objective of this study was to characterize the effect of dietary fat on disposition and metabolism of fluoranthene (FLA), a polycyclic aromatic hydrocarbon compound. FLA was administered to F-344 rats in monounsaturated (peanut oil), polyunsaturated (corn oil) and saturated (coconut oil) fats at doses of 50 and 100 μg/kg via oral gavage. Blood, small intestine, liver, lung, testis, adipose tissue, urine and feces were collected at various time points' post-FLA exposure. Samples were analyzed by reverse-phase high-performance liquid chromatography for FLA parent compound and metabolites. DNA was isolated from the tissues and subjected to ^{32}P-post labeling to measure FLA–DNA adducts. The concentrations of unchanged FLA (FLA parent compound) and its metabolites showed an increase for the saturated fat treatment group compared with mono- and polyunsaturated fat groups. The FLA–DNA adduct concentrations were high in tissues of rats that received FLA through saturated fat. The toxicokinetic parameters, concentrations of FLA metabolites and FLA–DNA adduct showed a dose-dependent increase, and this increase was statistically significant ($P<.05$) for saturated fat. These findings clearly demonstrate that the high residence time of FLA parent compound in saturated fat allows extensive metabolism, contributing reactive metabolites of FLA that bind with DNA and causing marked damage in a long-term exposure scenario.

Keywords: Fluoranthene; Polycyclic aromatic hydrocarbons; Dietary fat; Metabolism; Fluoranthene–DNA adducts

1. Introduction

Epidemiological surveys have revealed that about 80% of all human cancers are caused by environmental and lifestyle-related factors. Out of these, approximately 35% of the cancer deaths in the Western world and 20% of those in developing countries may be attributed to a variation in diet [1]. Chemically contaminated diet is a matter of great public concern in view of its possible role in cancer. Diet-associated cancers include stomach, colorectal, breast, kidney, prostate and ovarian cancers [2]. Although fat comprises a very important energy component of diet, more fat in the diet translates to larger intake and retention of toxic chemicals with an accompanying increase in risks to human health. The extent to which dietary fat determines the bioavailability of ingested dose and its metabolic fate will have implications for the toxicity and mutagenicity of hazardous chemicals in the environment. In order to delineate the impact of diet on toxicity and to predict human risks of intake of carcinogenic chemicals through diet, it is important to determine the bioavailability and kinetics of toxic chemicals ingested through lipid-rich diets.

Chemical contamination of diet arises from both unprocessed and processed foods. Environment is the prime source of contamination for unprocessed food of plant (vegetables and fruits) and animal (meat and dairy products) origin. Food processing and preservation techniques such as

Abbreviations: CYP, cytochrome P-450; FLA, fluoranthene; FLA 2, 3-diol, fluoranthene 2, 3-dihydrodiol; 2, 3 DFLA, *trans*-2, 3-dihydroxy-1, 10b-epoxy-1, 2, 3, 10b-tetrahydrofluoranthene; HPLC, high-performance liquid chromatography; 3(OH) FLA, 3-hydroxyfluoranthene; 8(OH) FLA, 8-hydroxyfluoranthene.

☆ The authors declare that they have no competing financial interest or any form of conflict of interest with the institution or funding agency during the course of this study.

* Corresponding author. Division of Cancer Biology, Department of Biomedical Sciences, Meharry Medical College, Nashville, TN 37208, USA. Tel.: +1 615 327 6163; fax: +1 615 327 6632.

E-mail address: aramesh@mmc.edu (A. Ramesh).

0955-2863/$ – see front matter © 2007 Elsevier Inc. All rights reserved.
doi:10.1016/j.jnutbio.2006.04.001

charcoal broiling, deep frying, combustion gas heating and smoking, when used for meats with high fat content, generate contaminants such as polycyclic aromatic hydrocarbons (PAHs), a family of toxic and mild-to-potent carcinogenic chemicals, through pyrolysis [3].

Fluoranthene (FLA) is a member of the PAH family of compounds that are formed from both natural and man-made sources. FLA originating from man-made sources makes it quantitatively the most significant and most ubiquitous one in the environment. FLA has been classified as both a genotoxic agent and a suspected human carcinogen by the International Programme on Chemical Safety [4]. This chemical is one of the important dietary PAH contaminants, registering high concentrations in lipid-rich diets of both plant and animal origin with a dietary intake of 1–2 μg/day [3]. The oral cancer risk ($1{:}10^4$ excess lifetime cancer risk for oral exposure) for FLA was calculated as 50 μg/kg body weight [5]. FLA was also reported to cause lung and liver tumors [6], apoptosis in T cells [7], decrease in white blood counts and tubular casts in kidneys [8]. When inhaled or ingested, FLA becomes activated in biological systems and, as a consequence, the reactive metabolites formed cause toxicity [9]. These metabolites also bind to DNA in exposed tissues and form adducts. The formation of reactive metabolites and carcinogen–DNA adducts are critical steps in carcinogenesis and are considered as important biomarkers during the initiation stage for carcinogenesis [10]. Therefore, an understanding of the influence of diet on DNA adduct formation in target organs in animals is important in identifying the organ systems that are more vulnerable to damage from intake of PAHs such as FLA when diets are altered.

Due to the widespread availability of FLA in the environment and the great potential for human exposure through diet, the metabolic fate of this compound subsequent to ingestion of different types of dietary fats merits investigation. Therefore, the objective of this particular study was to characterize the effect of dietary fat on disposition and metabolic fate of FLA.

2. Materials and methods

2.1. Chemicals

FLA, peanut oil, corn oil, coconut oil, Tween 80, tricaprylin, sodium dodecyl sulfate (SDS) halothane and potato apyrase were purchased from Sigma Chemical Company (St. Louis, MO). Lithium chloride, urea, sodium phosphate (monobasic and dibasic), methanol, chloroform and ethanol were purchased from Fisher Scientific Company (Kennesaw, GA). Sucrose, EDTA and Tris–HCl were purchased from Curtin Matheson Scientific Inc. (Houston, TX). Lithium formate was purchased from Alfa Aesar Chemical Company (Ward Hill, MA). Polyethylenimine-cellulose thin-layer chromatography (TLC) plates were purchased from Bodman Chemical Company (Aston, PA). Spleen phosphodiesterase, DNase and alkaline phosphatase were purchased from Worthington Biochemical Corporation (Freehold, NJ). T4 polynucleotide kinase was purchased from New England Biolabs Inc. (Beverly, MA). Micrococcal nuclease, nuclease P1, calf thymus DNA, polydeoxyadenylic acid, polydeoxycytidylic acid and polydeoxythiaminic acid were purchased from Pharmacia Biotech Inc. (Piscataway, NJ). Deoxyguanosine monophosphate (3′; dGMP) was purchased from Midland Certified Reagent Company (Midland, TX). Labeled (^{3}[H]) FLA was purchased from Chemsyn Labs (Lenexa, KS). Labeled ATP (γ-^{32}P-ATP) was purchased from Amersham Corporation (Arlington Heights, IL). FLA metabolite standards were obtained from the National Cancer Institute Chemical Carcinogen Repository (Midwest Research Institute, Kansas City, MO). Because FLA and its metabolite standards are suspected carcinogens and mutagens, they were handled in accordance with NIH guidelines [11].

2.2. Animals and exposure

Eight-week-old male F-344 rats (Harlan Laboratory, Indianapolis, IN), weighing approximately 200 g, were used in these studies. The animals were housed in a controlled environment (21±2°C; humidity, 30–70%) in groups of three per cage, maintained on a 12:12 h light/dark cycle (lights on at 0600 h) and allowed free access to rat chow (5001 Lab Meal; Purina Ralston Co.) and water. All animals were allowed a 7-day acclimation period prior to being randomly assigned to a control (n=6 per time point) or treatment group (n=6 per time point).

Treatment consisted of two doses (50 and 100 μg/kg) of FLA (97% pure, unlabeled) mixed with [G-^{3}H] FLA (specific activity, 770 mCi/mmol; ~97% purity) and dissolved in research grade peanut, corn and coconut oils. These oils containing FLA were added to a finely pulverized rat chow mentioned above and thoroughly mixed. Five milliliters of oil was added to 1 g of rat chow. This yields a fat/protein ratio of 1:5, typically found in a rodent diet [12], and a ratio of 1.5:4, found in certain items of human diet [13]. In other words, the percentage of fat in the test meal has the same relative content comparable with what humans may be exposed to (i.e., when grilled or barbecued burgers are eaten). These homogenized test meals (1 ml total volume) were administered orally via gavage, while animals similarly dosed with FLA (without the oils) in Tween 80/isotonic saline (1:5) were used as positive control. A separate group of animals received the Tween 80/isotonic saline (1:5) without FLA, which served as negative control. The above FLA doses were chosen as they represent the concentrations of FLA in food (grilled meat, smoked fish and sausage; reviewed in Ref. [3]) and soil from contaminated sites [14]. The main ingredient of test meals (peanut, corn and coconut oils) was selected depending on their fatty acid categories. For example, peanut oil represents monounsaturated dietary fats (48%; composition of fat type/100 g of food), corn oil represents polyunsaturated dietary fats

(58%) and coconut oil represents the saturated dietary fat (85%; [13]). The fat content in the test meals used in this study was higher than that found in American adult diet (34% fat, of which 14–18% is saturated fat [15]), to account for an increased meat consumption of certain segments of population [16].

All aspects of this study were conducted according to U.S. Environmental Protection Agency Health Effects Testing Guidelines (40 CFR 798) affecting waste generation, storage and handling practices (40 CFR 792). This study is also in compliance with the Occupational Safety and Health Administration regulations (29 CFR 1910-1450) related to substance-specific and general occupational health and safety standards. Unused test substance was stored in a hazardous waste container for proper disposal.

2.3. Sample collection and processing for metabolite analysis

The animals were fasted for 12 h prior to dosing. Soon after administration, the rats were housed individually in polycarbonate metabolic cages to allow for separate collection of urine and feces. Animals were sacrificed every hour from 1 to 12 h post-FLA exposure. Blood and organs/tissues of interest were collected at each time point, transferred into cryovials and stored at −70°C until analyzed. Plasma from blood samples was harvested by centrifugation at 2900×*g* for 25 min and stored frozen at −70°C until analyzed.

Up to 1 g of tissue (jejunum, liver, lung, testis and adipose) or feces or 1 ml of plasma or urine was homogenized with 2 vol of Tris–sucrose–EDTA buffer (Tris 0.10 M, sucrose 0.25 M, EDTA 0.10 M; pH 7.4) using a Polytron homogenizer (Brinkmann Instruments, Westbury, NY) for 2 min. Twenty microliters of SDS was added to the homogenate and vortexed for 1 min. The homogenate was extracted utilizing methanol (40 ml), deionized water (10 ml) and chloroform (15 ml) solution. Centrifugation was performed at 2000×*g* for 20 min to facilitate separation of aqueous and organic phases. The organic phase was passed through anhydrous sodium sulfate to remove moisture. A naphthalene surrogate was used to determine the extraction efficiency, and the recovery of naphthalene was 90%. The organic phase was dried under N_2 using a Meyer Analytical Evaporator (Organomation Associates Inc., Berlin, MA) and resuspended in 0.5 ml of methanol. Particulates from the elutes were removed by passing through Acrodisc filters (0.45 μm; 25 mm diameter, Gelman Sciences, Ann Arbor, MI). The final extracts were transferred to amber color screw top vials to prevent photodegradation and stored at 4°C until analyzed by a high-performance liquid chromatograph.

2.4. High-performance liquid chromatography (HPLC)

The metabolites were resolved by an HPLC Model 1050 (Agilent Technologies, Wilmington, DE) equipped with an HP1046 programmable fluorescence detector. The chromatograph was hooked to a 2D ChemStation (Model 1050; Agilent Technologies) for instrument control, data acquisition and analyses. Using an automatic sampler, 50 μl of sample was injected onto a C_{18} reverse-phase column (ODS Hypersil, 5 μm, 200×4.6 mm; Hewlett Packard). The column (temperature, 33°C) was eluted for 45 min at a flow rate of 1.0 ml/min with a ternary gradient of water/methanol/ethanol (40%:40%:20%) for 20 min, followed by the same gradient at a ratio of 30:46:24 for 10 min, 100% methanol for 10 min and returning to the initial gradient of 40:40:20 for 5 min. The excitation and emission wavelengths for the fluorescence detector were 244 and 410 nm, respectively. Identification of the metabolites was accomplished by comparison of retention times and peak areas of the samples with those of standards using HPLC 2D ChemStation software. To ensure the authenticity of HPLC detection of metabolites, we collected fractions of the HPLC eluate for scintillation counting in 2.0 ml of Econo-Safe cocktail (Research Products International Corporation, Illinois) using a Beckman Model LS6000 counter (Beckman Coulter Instruments Inc., Fullerton, CA). Radiochromatograms for each sample were reconstructed from the counted fractions. Metabolite peaks in the HPLC chromatogram were compared with scintillation counts. The coinciding peaks were compared with the standard chromatogram. The peaks that matched were considered as indicative of the confirmation of identity of metabolites. The detection limit (evaluated by a minimum signal-to-noise ratio of 3) of FLA metabolites by HPLC was ~300 fmol/total sample on column. All analyses were performed in triplicate with each tissue sample. The variability among triplicates was less than 10%. To make sure that the oil samples used for preparation of test diets were free from FLA contamination, we also analyzed the samples as detailed above.

2.5. Toxicokinetics

FLA toxicocokinetic parameters in plasma and lung tissues were analyzed using PK solutions 2.0 (Summit Research Services, Ashland, OH) software. The biological half-life ($t_{1/2}$) of FLA was calculated by a linear regression of the log plasma concentration versus the time curve. The area under the curve (AUC) was calculated by measuring the area under the blood FLA concentration–time curve. The mean residence time (MRT) was determined as AUC/area under the first moment of curve (AUMC). The volume of distribution (V_d) was calculated by considering the volume of FLA in the body, assuming that it is present throughout the body; FLA remains at the same concentration as in plasma. The total body clearance (Cl) was computed as the ratio of FLA dose and AUC. The elimination rate constant (K_d) is a ratio of Cl and V_d. Bioavailability (F) was determined by calculating the ratio of AUC obtained by oral administration to AUC obtained by intravenous administration and the ratio of intravenous dose of FLA to oral dose. The results were expressed as percentage.

Table 1
Effect of dietary lipid on toxicokinetic parameters of 50 and 100 μg FLA/kg body weight orally administered to F-344 male rats

Parameter	Monounsaturated fat (μg/kg)		Polyunsaturated fat (μg/kg)		Saturated fat (μg/kg)	
	50	100	50	100	50	100
AUC (mg/ml/h)	0.015±0.005	0.020±0.008	0.018±0.002	0.028±0.002	0.025±0.004*	0.038±0.004*
Biological half-life ($t_{1/2}$; h)	2.8±0.028	3.2±0.028	3.2±0.031	3.7±0.030	3.7±0.035*	4.7±0.037*
Volume of distribution (V_d; ml/kg)	0.14±0.019	0.24±0.015	0.21±0.012	0.27±0.012	0.27±0.014*	0.36±0.018*
Clearance (Cl; ml/h/kg)	0.013±0.008	0.018±0.009	0.020±0.002	0.028±0.006	0.031±0.004*	0.051±0.004*
MRT (h)	5.0±0.018	5.5±0.018	5.5±0.013	6.1±0.013	6.5±0.015*	7.0±0.015*
Elimination rate (K_d; h)	0.09±0.007	0.075±0.007	0.095±0.002	0.10±0.002	0.11±0.003	0.14±0.003*
Bioavailability (F; %)	68±1.0	72±1.3	65±1.5	70±1.6	59±1.1*	63±1.2*

Values represent mean±S.E. (n=6).
* Statistical significance (P<.05) of toxicokinetic parameter values compared with monounsaturated fat.

2.6. DNA isolation

DNA was isolated from liver, lung, intestine and testicular tissues of rats exposed to FLA through diets containing monounsaturated, polyunsaturated and saturated fat. DNA isolation was carried out by using the Stratagene DNA isolation kit. The concentration of DNA in tissues was determined via spectrophotometry. Using the above method, the DNA yield from 1 g of tissue was >250 μg.

2.7. ^{32}P-postlabeling

The methods of Gupta [17] and Gupta and Randerath [18] were used for analysis of DNA adducts, which are briefly described as follows. DNA (5 μg) from organs of interest was digested to 3′-dNPs at 37°C for 2 h with 5 μg each of micrococcal nuclease and spleen phosphodiesterase. The nucleotides were enriched by adding 4 μg/μl P1 nuclease to the digest and incubated at 37°C for 60 min. The DNA digests were diluted to 50 μl (to yield 0.14 μg/μl DNA) with water. The digests were then ^{32}P-postlabeled by incubation with 2.3 μl [γ-^{32}P] ATP (225 μCi/μl) and 1.2 μl of T4 polynucleotide kinase (3 U/μl) at 37°C for 30 min. Potato apyrase (20 mU/μl) was added to destroy residual [γ-^{32}P] ATP, and the mixture was incubated at 37°C for a further 10 min.

2.8. TLC and autoradiography

The labeled adducts were separated and quantified by TLC as described below. The postlabeled samples (1 μl) were applied to 20×20 cm polyethylenimine-cellulose TLC

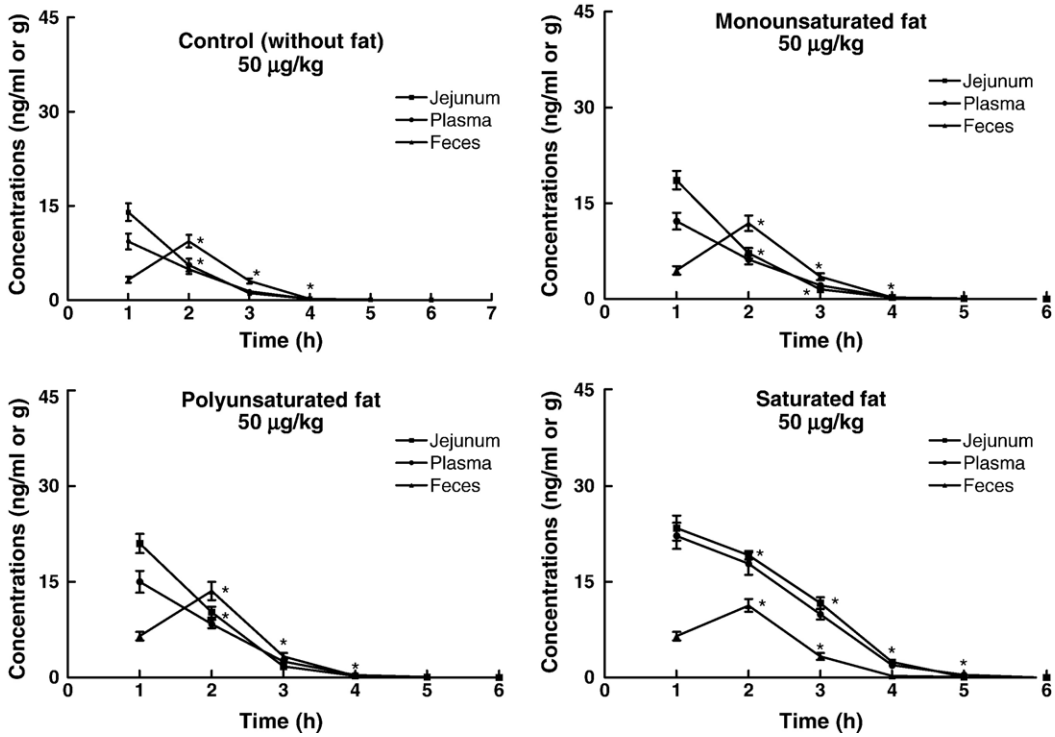

Fig. 1. Effect of monounsaturated, polyunsaturated and saturated fat on time-course distribution of unmetabolized FLA in plasma, jejunum, colon and feces of F-344 rats that received 50 μg FLA/kg body weight via oral gavage. Reverse-phase HPLC analysis of FLA parent compound/metabolites was performed with an Agilent Technologies HPLC system coupled to a fluorescence detector as described in Section 2. Control rats were dosed with FLA (without dietary fat) in Tween 80/isotonic saline (1:5). Values represent mean±S.E. (n=6). Asterisks denote statistical significance (P<.05) at the respective time point compared with 1 h postexposure.

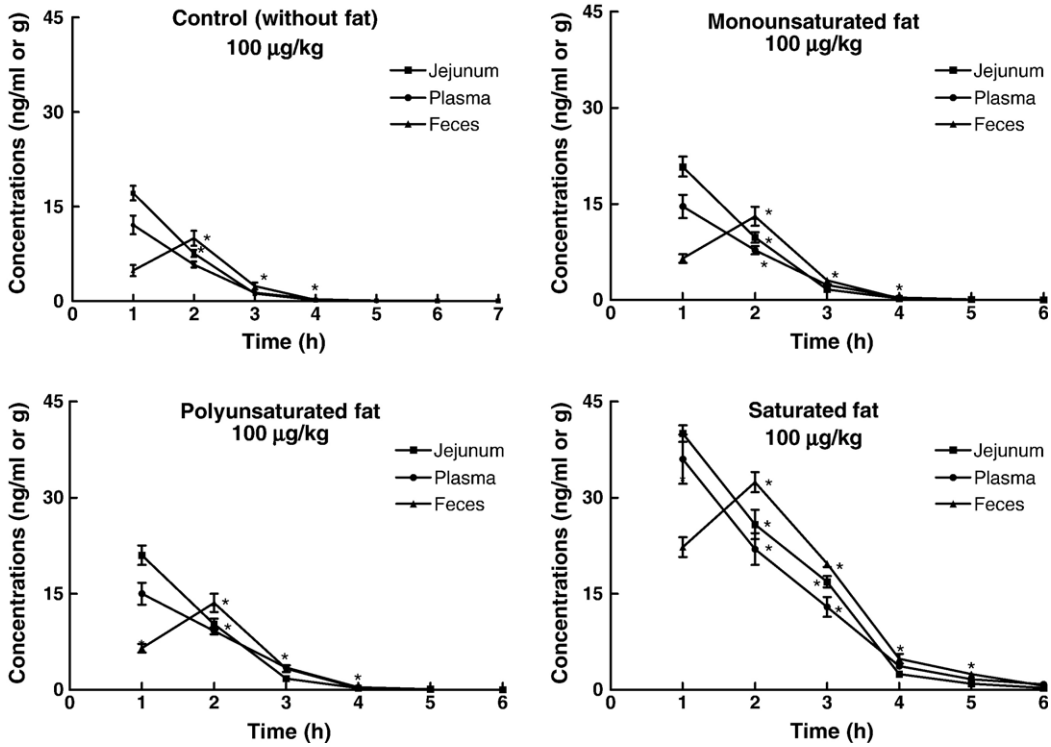

Fig. 2. Effect of monounsaturated, polyunsaturated and saturated fat on time-course distribution of unmetabolized FLA in plasma, jejunum, colon and feces of F-344 rats that received 100 µg FLA/kg body weight via oral gavage. Reverse-phase HPLC analysis of FLA parent compound/metabolites was performed with an Agilent Technologies HPLC system coupled to a fluorescence detector as described in Section 2. Control rats were dosed with FLA (without dietary fat) in Tween 80/isotonic saline (1:5). Values represent mean±S.E. ($n=6$). Asterisks denote statistical significance ($P<.05$) at the respective time point compared with 1 h postexposure.

plates. A paper wick was stapled to the top of TLC plates. The plates were developed overnight in 1 M NaH_2PO_4, pH 6. The plates were washed twice with deionized water, dried and developed in 3.5 M lithium formate and 7 M urea, pH 3.5, from bottom to the top of the plate. The plates were again washed in water, air-dried and developed at a right angle to the previous direction of development in 0.8 M lithium chloride, 0.5 M Tris–HCl and 7 M urea, pH 8.0. The plates were not washed but air-dried and developed in the first direction with 1.7 M NaH_2PO_4, pH 6.0. The plates were again washed in water, dried and wrapped in Saran wrap. The adducts were detected by autoradiography at −80°C using a Kodak XAR-5 film (Sigma Chemical Company) and a Fisher intensifying screen as an image enhancer.

After the end of exposure, the adduct spots corresponding to the autoradiogram were excised and counted in a scintillation counter (Beckman Coulter Instruments). Areas adjacent to the adduct spots were counted in the same way, and the background radioactivity was subtracted from the sample counts. The total nucleotides were analyzed by a diluted DNA digest (2 ng) in parallel with adducts. Subsequently, the normal nucleotides were separated by PEI-cellulose TLC in 0.3 M lithium chloride, pH 8.0.

2.9. Identification and quantitation of FLA–DNA adducts

Adduct levels were calculated by relative adduct labeling and represented as femtomoles per microgram of DNA. The FLA metabolites, especially fluoranthene 2,3-dihydrodiol (FLA 2,3-diol) and *trans*-2,3-dihydroxy-1,10b-epoxy-1,2,3,10b-tetrahydrofluoranthene (2,3 DFLA), were incubated with 40 µM DNA and subjected to cochromatography with unknown adduct sample. A multidimensional TLC system mentioned above was used for this purpose. Those unknown adducts that exhibited equivalent mobility (comigration) with that of known standard were mapped and identified.

To further identify whether the adducts are deoxyadenosine or deoxyguanosine (dG) ones, we mixed solutions of respective nucleotides (40 µM each of 3′-deoxyadenosine monophosphate and 3′-dGMP) with equal volumes of each one of the metabolite standards for 12 h at 37°C. The modified nucleotides were subjected to ^{32}P-postlabeling analysis and run in parallel with unknown adduct samples.

2.10. Statistical analyses of data

A two-way analysis of variance (ANOVA) was used for the determination of statistical differences in total metabolite concentrations in plasma, tissues or excreta for each time point and for each treatment group. The two-way ANOVA was also used for the determination of statistical differences in FLA–DNA adduct concentrations in some target tissues. Since there is intra- and interorgan variation with regard to composition of metabolites and DNA adducts, individual metabolite/adduct types were tested

for statistical differences among different time points. All pairwise multiple comparisons were performed using the Student–Newman–Keuls method. The criterion for statistical significance was $P<.05$.

3. Results

3.1. Effect of dietary lipid on toxicokinetic parameters

The result of toxicokinetic studies for FLA is shown in Table 1. Our studies demonstrated that the quantitative and qualitative distribution of FLA in plasma and target tissues depend on the kinetic parameters of this compound as discussed below.

The parameters such as volume of distribution (V_d), clearance (Cl) and elimination rate constant (K_d) were lower, whereas the MRT and biological half-life ($t_{1/2}$) were higher for FLA administered through test meal containing saturated fat. On the other hand, FLA administered through test meal containing monounsaturated and polyunsaturated fats showed a reverse trend. These observations were similar for both the doses (50 and 100 μg/kg) used in this study. Within each dose group, the differences in toxicokinetic parameters such as AUC, $t_{1/2}$, V_d, Cl and MRT were statistically significant ($P<.05$) between monounsaturated and saturated fat. Between the two dose groups, although the values for toxicokinetic parameters showed an increase at 100 μg/kg compared with 50 μg/kg, the differences were statistically significant ($P<.05$) in the case of $t_{1/2}$, V_d and Cl for FLA administered through saturated fat.

3.2. Effect of dietary lipid on time-course distribution of parent FLA compound

Control rats that received FLA in Tween 80/isotonic saline (1:5) had a not so marked disposition of FLA and its metabolites compared with their counterparts that received FLA through dietary fat.

The FLA parent compound was detected only in plasma, intestine and feces for the three types of dietary lipid used at both doses. A time-course distribution of FLA parent compound in plasma and tissues following oral exposure is shown in Figs. 1 and 2 for both 50 and 100 μg/kg doses, respectively.

Peak levels of FLA parent compound in plasma and intestine were recorded 1 h after exposure, followed by a gradual decrease, and after 4 h, no more parent compound was seen. On the contrary, the concentrations of FLA parent compound from feces samples showed a peak at 2 h after exposure and showed a gradual decline thereafter.

Within each dose group, the FLA parent compound concentrations were high in intestine, followed by feces and plasma. These differences, however, were not statistically significant. Between the two dose groups, the FLA parent compound concentrations showed an increase at 100 μg/kg

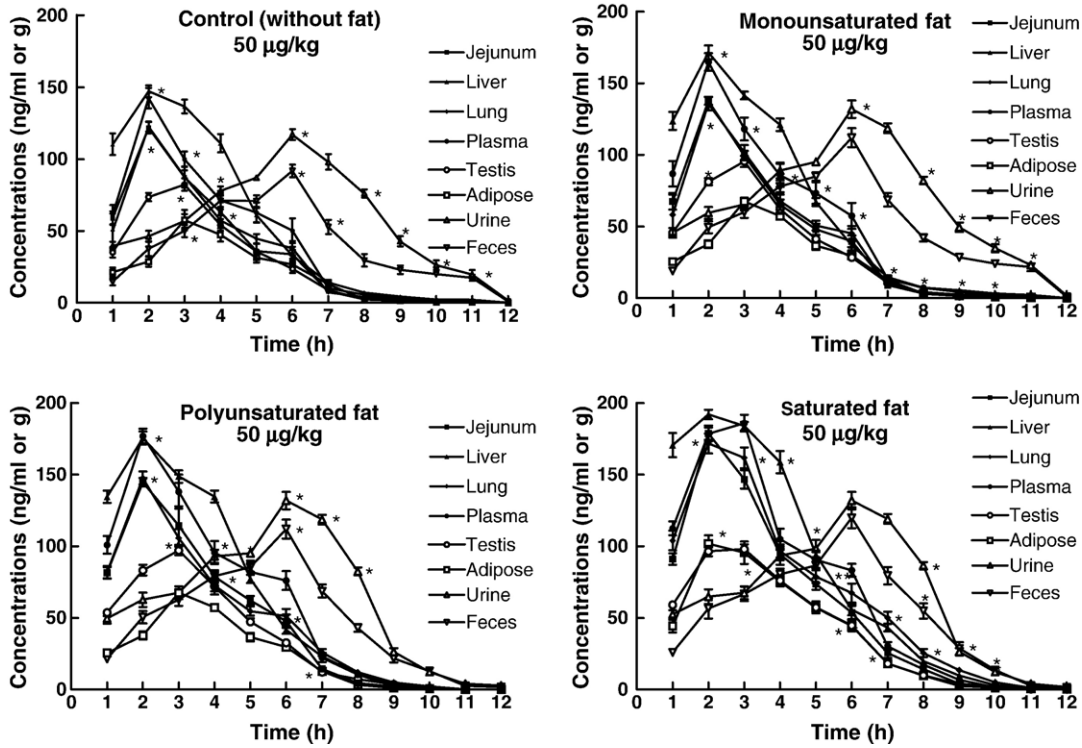

Fig. 3. Effect of monounsaturated, polyunsaturated and saturated fat on time-course distribution of FLA metabolite concentrations in plasma, jejunum, colon, liver, lung, testis, adipose, urine and feces of F-344 rats that received 50 μg FLA/kg body weight via oral gavage. Reverse-phase HPLC analysis of FLA parent compound/metabolites was performed with an Agilent Technologies HPLC system coupled to a fluorescence detector as described in Section 2. Control rats were dosed with FLA (without dietary fat) in Tween 80/isotonic saline (1:5). Values represent mean±S.E. ($n=6$). Asterisks denote statistical significance ($P<.05$) at the respective time point compared with 1 h postexposure.

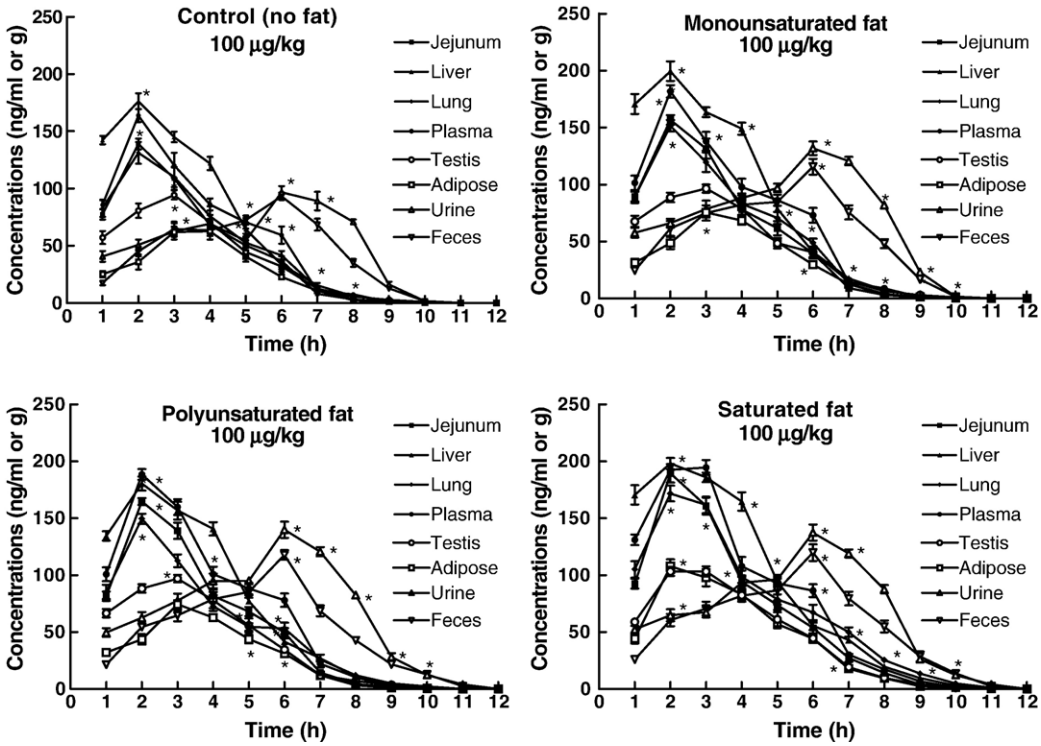

Fig. 4. Effect of monounsaturated, polyunsaturated and saturated fat on time-course distribution of FLA metabolite concentrations in plasma, jejunum, colon, liver, lung, testis, adipose, urine and feces of F-344 rats that received 100 μg FLA/kg body weight via oral gavage. Reverse-phase HPLC analysis of FLA parent compound/metabolites was performed with an Agilent Technologies HPLC system coupled to a fluorescence detector as described in Section 2. Control rats were dosed with FLA (without dietary fat) in Tween 80/isotonic saline (1:5). Values represent mean ± S.E. ($n = 6$). Asterisks denote statistical significance ($P < .05$) at the respective time point compared with 1 h postexposure.

compared with 50 μg/kg for all the three dietary lipids used. However, the increase was statistically significant ($P < .05$) only for FLA administered through saturated fat. At the 50-μg/kg dose, there is no significant difference in concentrations of parent compound among monounsaturated, polyunsaturated and saturated fats, whereas a reverse trend was seen in the case of the 100-μg/kg dose.

3.3. Effect of dietary lipid on time-course distribution of FLA metabolites

The results for time-course distribution of FLA metabolites in plasma, tissues, urine and feces following oral administration are presented in Figs. 3 and 4 for 50 and 100 μg/kg, respectively.

The FLA metabolite concentrations in plasma, intestine, liver and lung peaked at 2 h post-FLA administration and decreased gradually thereafter. This trend was consistent for the three types of dietary lipid at both doses used. On the other hand, FLA metabolite concentrations in adipose tissue and testis peaked at 3 h, but there is no significant difference in concentrations between 2 and 3 h postexposure.

The metabolite concentrations in urine and feces showed a gradual increase, reaching a peak at 6 h postexposure and decreased thereafter. The metabolite concentrations in plasma and tissues were very low compared with urine and feces 8 h post-FLA exposure.

Within each dose group, the FLA metabolite concentrations were high in liver, followed by plasma, intestine, urine, feces, lung, adipose and testis samples. These differences, however, were statistically significant between urine and the rest of the samples and between feces and the rest of the samples, only in the case of FLA administered through saturated fat. Between the two dose groups, the FLA metabolite concentrations showed an increase at 100 μg/kg compared with 50 μg/kg for all the three dietary lipids used. The increase was statistically significant ($P < .05$) among various organs and body fluids only at time points of their peak metabolite concentrations for FLA administered through saturated fat.

3.4. Effect of dietary lipid on distribution of FLA metabolite types

The following were the predominant metabolites identified: (a) *trans*-2,3-dihydrohydroxy FLA (FLA-*trans*-2,3-dihydrodiol), (b) 2,3 DFLA, (c) 3-hydroxyfluoranthene [3(OH) FLA] and (d) 8-hydroxyfluoranthene [8(OH) FLA]. The first two are reactive metabolites, and the last two are detoxification products. The percentage composition of individual metabolite types among the total metabolites varied with the lipid type in which FLA was administered.

Metabolite percentages from monounsaturated fat were shown as representative examples for mono- and polyun-

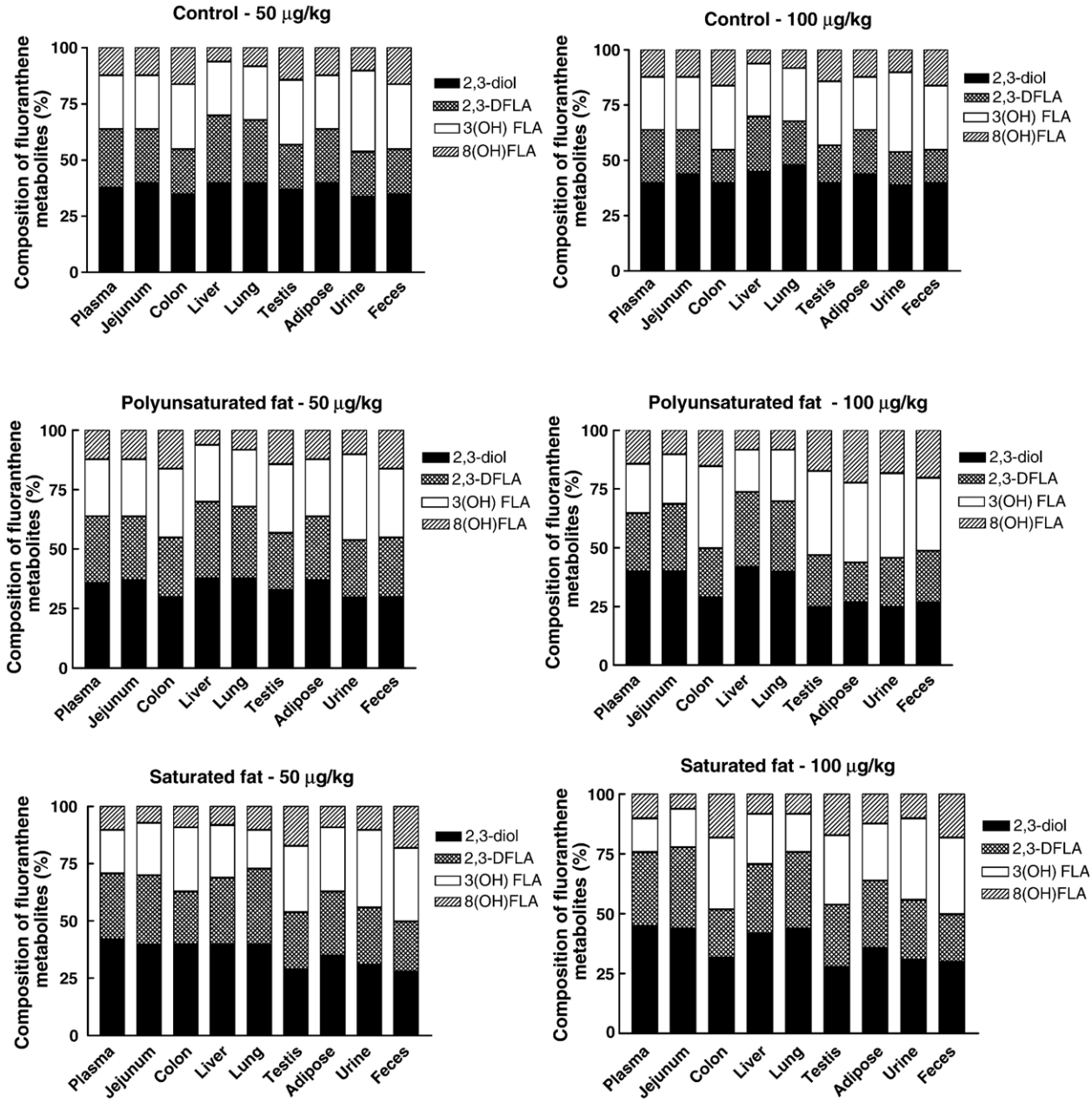

Fig. 5. Effect of monounsaturated, polyunsaturated and saturated fat on percent distribution of FLA metabolite concentrations in plasma, jejunum, colon, liver, lung, testis, adipose, urine and feces of F-344 rats that received 50 and 100 μg FLA/kg body weight via oral gavage. Values are expressed as the percentage of individual metabolite types among the total metabolites [sum of individual concentrations of FLA 2, 3-diol, 2, 3 DFLA, 3(OH) FLA and 8(OH) FLA] formed ($n = 6$ for each sample).

saturated fats inasmuch as the composition of individual metabolites among the total metabolites were not different.

The percentage composition of metabolite types in plasma, tissues, urine and feces following FLA administration in the three dietary lipids is presented in Fig. 5 for both doses. The FLA metabolites showed an organ-specific distribution. Among the reactive metabolites, the FLA 2, 3-diol is the most predominant one followed by 2,3 DFLA.

The data on qualitative distribution of various FLA metabolites showed an interesting trend. Within each dose group, the FLA 2,3-diol was the predominant metabolite in plasma and tissue samples. On the other hand, the 3(OH) FLA was the predominant metabolite in urine and feces samples. These two metabolites' percentages were comparable in testis samples. The 8(OH) FLA constituted lesser percentages relative to the other metabolite types in all the samples analyzed. The levels of FLA 2,3-diol and 2,3 DFLA were high in plasma and tissues of rats that received FLA through saturated fat, followed by those that received FLA through poly- and monounsaturated fats. On the other hand, the levels of 3(OH) FLA (the major detoxification product of FLA) were low in plasma of rats that ingested

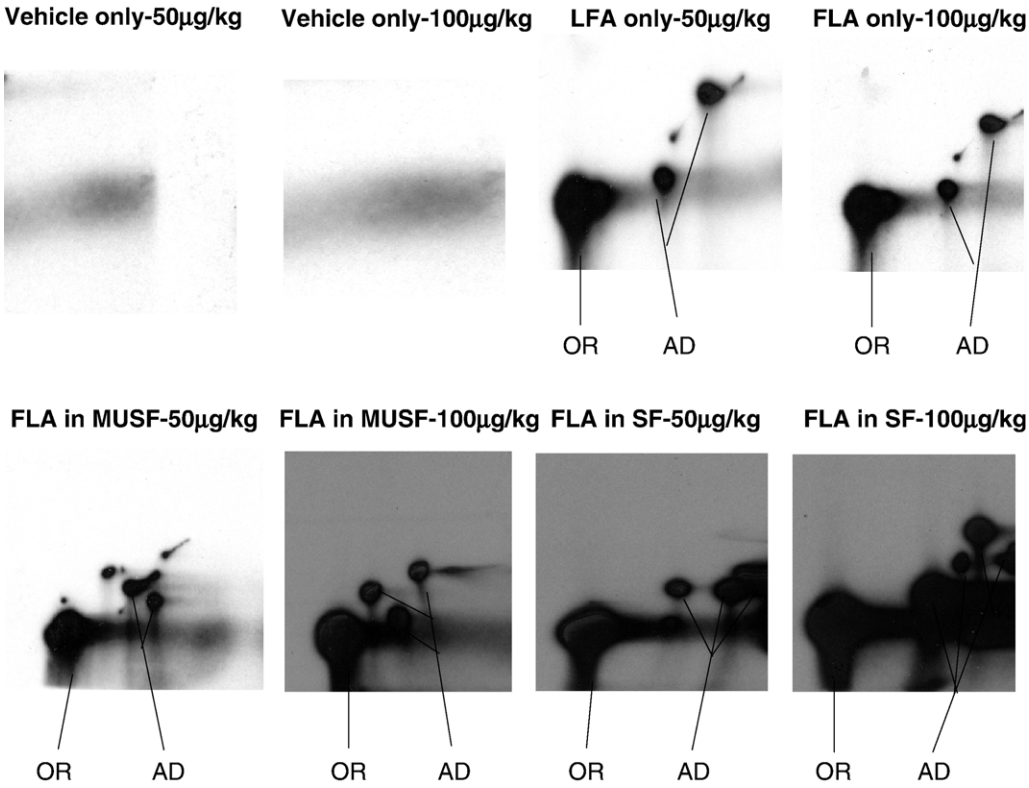

Fig. 6. FLA–DNA adduct patterns on autoradiograms of TLC maps. Adducts from jejunum tissues were shown as a representative example. Tissue samples were taken 2 h after administration of FLA in adult male rats. DNA was extracted from these samples; FLA-derived adducts were ^{32}P-post labeled, and TLC was performed as described in Section 2. Films were exposed for 24 h at −80°C. "OR" refers to origin of adducts (AD). Adducts are marked in the panels. No adducts were detected in tissues from rats that were administered vehicle only. MUSF and SF represent monounsaturated and saturated fats, respectively.

saturated fat compared with those that ingested unsaturated fat. While the difference between reactive metabolites and the rest is statistically significant ($P<.05$) in the case of saturated fat, the differences were not marked in the case of unsaturated fats. Between the two dose groups, the FLA reactive metabolite types showed high percentages at 100 µg/kg compared with 50 µg/kg for all the three dietary lipids used. The increase was statistically significant ($P<.05$) between reactive metabolites and the rest in the case of saturated fat.

3.5. Effect of dietary lipid on time-course distribution of FLA–DNA adducts

The metabolized FLA was able to bind with DNA as reflected by the FLA–DNA adducts that were detected in target tissues. Few FLA–DNA adducts were detected in tissues of control animals (positive controls; their levels were low) compared with treatment groups (rats that received FLA through dietary fat). No FLA–DNA adducts were detected in tissues of negative controls (rats that received neither FLA nor dietary fat). Representative TLC maps of FLA–DNA adducts in target tissues are shown in Fig. 6. Adduct profiles from monounsaturated fat were shown as representative examples for mono- and polyunsaturated fats inasmuch as their adduct profiles were not different.

The FLA–DNA adduct concentrations in rat tissues that were administered with FLA in the three fat types were shown in Fig. 7. Within each dose group, the FLA–DNA adduct concentrations were high in lung, followed by intestine, testis and liver samples. These differences, however, were statistically significant between liver and the rest of the samples. Also, the adduct concentrations among the three dietary lipid groups were statistically significant ($P<.05$) in each dose group. Between the two dose groups, the FLA–DNA adduct concentrations showed an increase at 100 µg/kg compared with 50 µg/kg for all the three dietary lipids used. However, the increase was statistically significant ($P<.05$) in the case of intestine, lung and testis tissues of rats that were administered FLA through saturated fat. The results for distribution of FLA–DNA adduct concentrations over time in target tissues following oral administration are presented in Figs. 8 and 9. The FLA–DNA adduct concentrations in intestine, liver, lung and testis peaked at 2 and 3 h post-FLA administration and decreased gradually thereafter. This trend was consistent for the three types of dietary lipid at both doses used. The differences in adduct concentrations among the various post-FLA exposure time points were statistically significant ($P<.05$ at 2 and 3 h compared with the 1-h time point). The adduct concentrations in the abovementioned tissues were

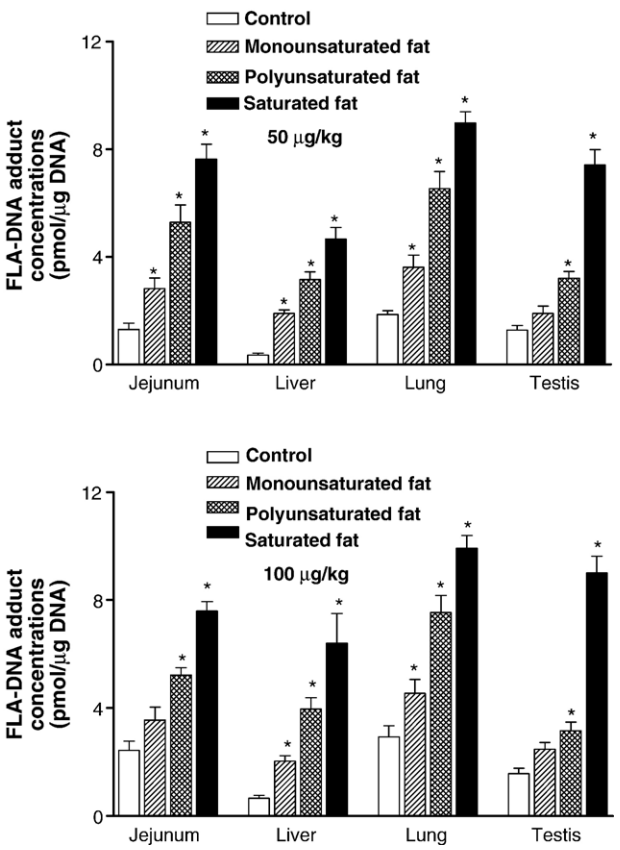

Fig. 7. FLA–DNA adduct total concentrations in jejunum, liver, lung and testis of F-344 rats exposed to FLA through diet. Tissue samples were taken 2 h after administration of FLA in adult male rats. DNA was extracted from these samples; FLA-derived adducts were ^{32}P-post labeled, and TLC was performed as described in Section 2. Each bar shows the mean adduct concentration and standard error of analysis from six individual rats. The asterisk denotes the level of statistical significance ($P<.05$) between FLA administered through monounsaturated and saturated fat for the respective dose group.

not detectable beyond 6 h post-FLA exposure in any of the dietary lipids or FLA doses used.

All the adducts isolated from target tissues were identified as the dG ones [10b-(deoxyguanosin-N^2-yl)-1,2,3-trihydroxy-1,2,3,10b-tetrahydrofluoranthene].

4. Discussion

The small intestine, by virtue of an enormous surface area, is one of the largest body part in terms of area, which comes into contact with the environment [19,20]. Exposure of this organ to foodborne xenobiotics or their metabolites leads to disruption in physiological processes and cause toxicity. Subsequent to oral intake, while majority of these compounds are absorbed directly into portal circulation, lipophilic toxicants and dietary lipids are transported to the systemic circulation by chylomicrons (lipoproteins secreted by the small intestine) via the lymph [21] and, thus, play an important role in lymphatic pathway [22]. A linear correlation between the uptake of benzo(a)pyrene (BaP) by isolated chylomicrons (ex vivo) and corresponding intestinal lymphatic transport (in vivo) has been shown by Gershkovich and Hoffman [23].

In the present study, the absorption of FLA from the gastrointestinal tract of rats appears to be enhanced when it is administered via vehicles possessing lipophilic and hydrophobic properties (dietary fats) compared with controls, as demonstrated by Vetter et al. [24] for BaP. Association of PAHs with chylomicrons may have an influence on the pharmacokinetics of these chemicals and their toxicity, as shown for several hydrophobic chemicals [25]. Hence, studies were undertaken to see the extent of absorption and elimination of lipid-incorporated FLA.

The lipophilic nature of FLA [26] probably facilitates its absorption through the gastrointestinal tract. The low residence time and high clearance values for FLA in mono- and polyunsaturated fat indicate that this chemical is rapidly bioavailable to organs. However, the high residence time and low clearance values for FLA in saturated fat relative to unsaturated fat reflect delays in the elimination of FLA ingested through saturated dietary lipid. The absence of a significant difference between unsaturated fats in residence time as well as clearance of FLA suggests that FLA ingested through these fats has similar elimination characteristics. The bioavailability data obtained for FLA in the unsaturated fat group (65–72%) in the present study are comparable with those reported for mice (bioavailability, 62.4%; [27]) that were intragastrically administered with FLA, the difference in doses notwithstanding. FLA in saturated fat is more likely to stay in the body for a longer period of time at high doses. The difference in FLA elimination characteristics between unsaturated and saturated fats was more marked at 100 μg/kg compared with 50 μg/kg. It is likely that FLA absorbed through saturated fat, that is, a fat that is mostly made of medium-chain triglycerides, is transported to the liver via portal venous system. In contrast to this, FLA absorbed through monounsaturated and polyunsaturated fats that are made of long-chain triglycerides may have been incorporated into chylomicrons for transport through the lymphatic system or peripheral circulation. That lipid type governs the toxicokinetics of ingested FLA is also supported by published reports on the association between the biotransformation enzymes and PAHs incorporated in dietary lipids. A positive correlation between the content of polyunsaturated fatty acids in the diet and hepatic and intestinal epoxide hydrolase (the key enzyme involved in FLA metabolism; [28]) activity subsequent to PAH administration through diet has been reported [29,30]. In this context, it is worth mentioning that epoxide hydrolase has been detected in several rat organs and tissues (reviewed in Ref. [31]). Since the activities of both constitutive and inducible toxic chemical-metabolizing enzymes in rats depend on the diet [32], this raises the likelihood that the disposition of FLA in body is dependent on the nature of the coadministered lipid.

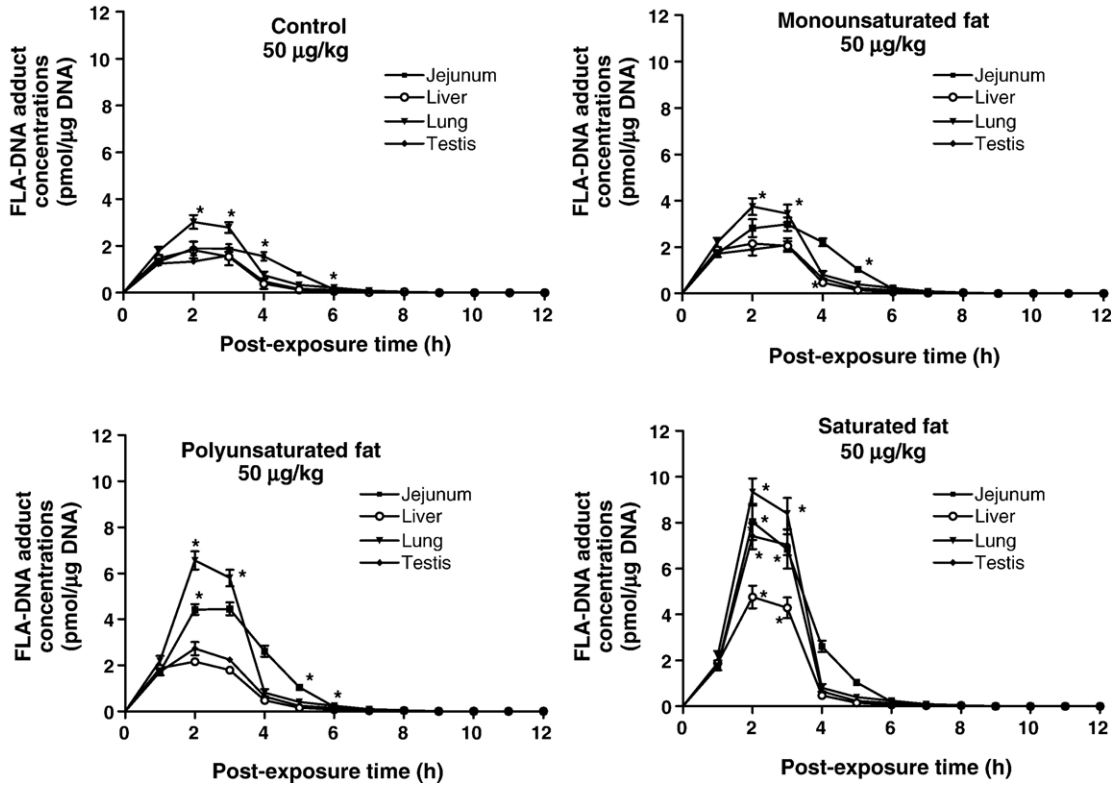

Fig. 8. FLA–DNA adduct persistence in jejunum, liver, lung and testis of F-344 rats that received 50 µg FLA/kg body weight via oral gavage. Each data point shows the mean adduct concentration and standard error of analysis from six individual rats. Rats were exposed to FLA through oral gavage. Tissue samples were taken every hour from Hour 0 to Hour 12 after cessation of exposure.

While there are no differences in the levels of unchanged FLA among the three dietary lipids at the 50-µg/kg dose, the reverse trend noticed at 100 µg/kg suggests that the presence of FLA parent compound depends on the bioavailability of the administered dose. The high dose of FLA administered may have caused a poor extraction of FLA by the gastrointestinal fluids and a capacity-limited absorption and biotransformation [33], eventually contributing to the high levels of unmetabolized FLA. Other factors that could have a bearing on the bioavailability include incorporation of FLA molecules into mixed micelles that are a function of the bile salt–lipid aggregates [34] in the small intestine as well as the extent of lymphatic absorption [35]. In other words, the uptake of FLA by chylomicrons in the enterocytes is governed not only by the FLA dose but also by the nature of coadministered lipid [19].

The high concentrations of FLA metabolites in liver are consistent with the report of Wall et al. [36], which states that liver is the principal organ of metabolism for PAHs. When the cytochrome P-450 (CYP) concentrations are taken into account, the greater weight of the liver relative to that of individual organs attributes a greater capacity for this organ in the processing of toxic chemicals [37]. Next to liver, intestine registered high concentrations of FLA metabolites. Findings of the present study are in agreement with those of Choi et al. [38] and Cavret et al. [39], who reported that intestine plays an important role in the initial processing of orally ingested PAHs.

As biotransformation of PAHs plays a vital role in determining the tissue susceptibility to these chemicals, we looked whether FLA incorporated in different dietary lipids shows any variation in types and quantities of metabolites produced. Initial metabolism of FLA through CYP oxidation yields epoxide intermediates that undergo hydrolysis, catalyzed by epoxide hydrolase, generating *trans*-dihydrodiols. These are finally oxidized by CYP enzymes to form diol-epoxides that are highly reactive [28]. Epoxide hydrolase mediates the formation of FLA 2,3-diol, an important step in the metabolism of FLA [28]. This diol undergoes further metabolism to form syn- and anti-FLA 2,3-diol-1,10b-epoxides. Published reports have further shown that FLA 2,3-diol-1,10b-epoxide contributes to significant DNA binding [40]. Other oxidation pathways of this diol may yield FLA 2,3-dione and FLA 7,8-diol [28]. These pathways appear to be minor, as we have not been able to detect either one of these metabolites in our study.

The relatively high concentrations of FLA 2,3-diol and 2,3 DFLA in plasma and organs of rats that received FLA through saturated fat compared with polyunsaturated and monounsaturated fats suggest that FLA administered through saturated fat is likely to generate more reactive metabolites. This assumption is also strengthened by the

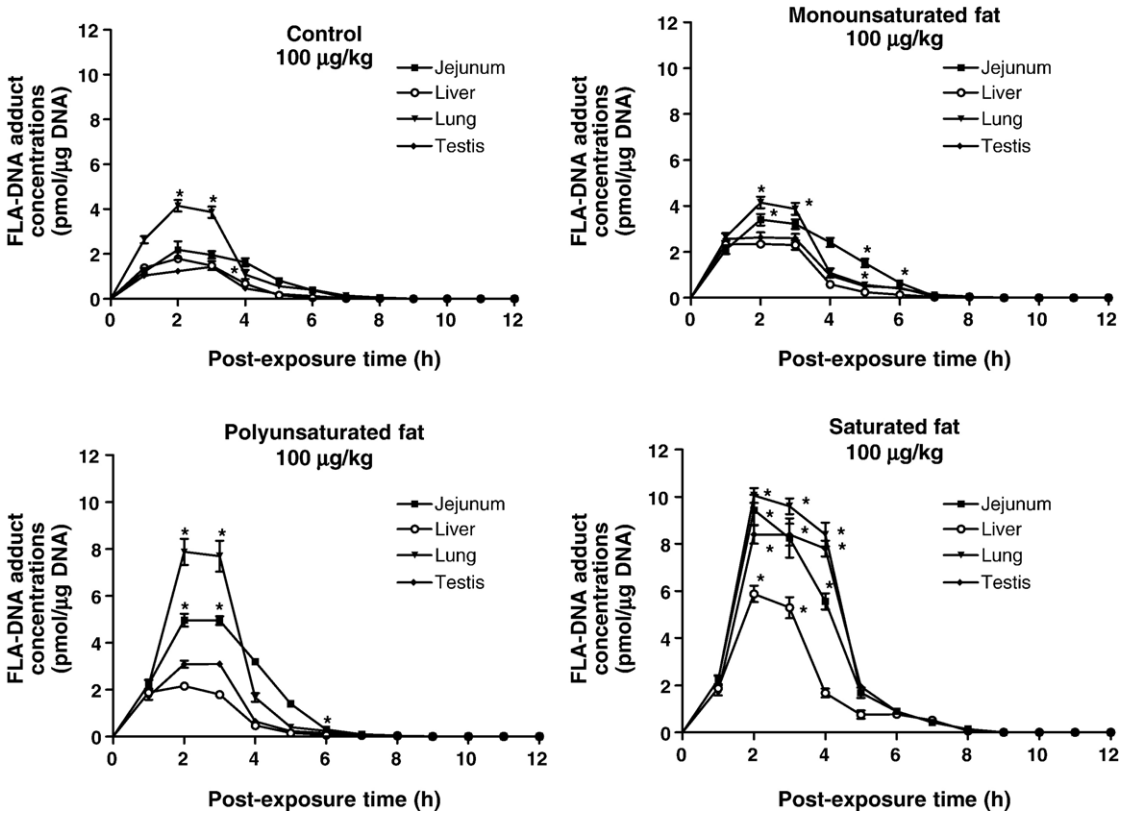

Fig. 9. FLA–DNA adduct persistence in jejunum, liver, lung and testis of F-344 rats that received 100 µg FLA/kg body weight via oral gavage. Each data point shows the mean adduct concentration and standard error of analysis from six individual rats. Rats were exposed to FLA through oral gavage. Tissue samples were taken every hour from Hour 0 to Hour 12 after cessation of exposure.

low concentrations of 3(OH) FLA, which is one of the major detoxification products of FLA in plasma and tissues of rats that ingested saturated fat relative to unsaturated fat.

Overall, the FLA metabolite types identified in our study are in agreement with those from published literature. Biotransformation studies of FLA using microsomal preparations from rodent liver tissues revealed that 1-, 3- and 8-hydroxyfluoranthenes, 2,3-dihydro-2,3-dihydroxy FLA and FLA 2,3-dione were the predominant metabolites [28,41–43]. FLA 2,3-diol, one of the predominant metabolites found in our study, has been reported to constitute 29–43% and 70–95% of the total metabolite concentrations in the rat [28] and human [44] hepatic microsomes, respectively. Hydroxyfluoranthenes are not mutagenic in their normal form but may be oxidized to their potentially mutagenic diol counterparts.

The relationship between FLA disposition and dietary fat type was further examined by looking at the DNA damage caused by FLA. One of the mechanisms through which PAHs cause toxicity/cancer is through binding with cellular macromolecules such as proteins and nucleic acids [4]. Findings of the present study on disposition of FLA metabolites and FLA–DNA adduct concentrations revealed that the differences in intake of PAH from the dietary lipid matrix and transport of this chemical to target tissues contribute to differences in DNA adduct formation.

The low FLA–DNA adduct levels in tissues of rats in the unsaturated fat treatment group indicate a decreased biotransformation of FLA in target tissues that received FLA through unsaturated fat relative to saturated fat. Published information reveals that PAHs and their derivatives have a higher affinity for high-density lipoproteins (HDLs; [45]). Hence, it is likely that the HDL-sequestered FLA and its metabolites undergo cellular internalization [45], which protects especially the reactive metabolites against hydrolysis in rats that received FLA through saturated fat. Evidence that shows that dietary lipid modulates the PAH-induced DNA damage is available from the published literature. The extent of DNA binding was found to be greater in tissues of rats that received diets containing lard and cod liver oil compared with their counterparts that were fed fat-free diet [46]. Similarly, rats whose diet had more polyunsaturated fat content registered DNA adduct formation to a greater extent [47] than their cohorts that received diets containing less amount of polyunsaturated fat. Our results are also in agreement with those of Schaffer and Milner [48] who reported an increase in dimethylbenz(a)anthracene (DMBA)–DNA adduct levels in mammary tissues of rats that were fed diets containing varying amounts of dietary fat. Diets containing 20% corn oil registered high DMBA–DNA adduct levels compared with those that received 5% and 10% corn oil. The same

studies demonstrated high adduct levels in diets supplemented with oleic and linoleic acids compared with those with corn oil alone. Using a mouse model, Curfs et al. [49] have demonstrated that high-fat cholesterol diet induced DNA damage due to BaP exposure. The increased concentrations of DNA adducts in intestine, lung and testis from the saturated fat group in the present study suggest that these organ systems are more vulnerable to damage from intake of FLA. In this context, it is worth mentioning that dietary lipids have been reported to modulate the bioavailable doses of PAHs, leading to altered DNA adduct levels [50] and DNA repair mechanisms [51]. Furthermore, the observed association of diet-elevated DNA damage in mouse [49] reiterates that the genotoxic effects of ingested PAHs such as FLA are governed by the lipid intake.

The adducts isolated from tissues in this acute study have been identified as the dG ones, derived from the anti-2,3-dihydroxy-1,10b-epoxy-1,2,3-trihydrofluoranthene (anti-FADE) metabolite. This adduct has been previously identified from in vitro and in vivo systems of rodents treated with FLA [6,9,28]. Collectively, our data and those of others [6,9,28] attest to the stability of the anti-FADE metabolite from which these adducts are derived from. The FADE generated via metabolism by metabolically competent tissues and transported to metabolically quiescent tissues may have contributed to adduct formation in these tissues.

Although FLA administered through saturated fat at 100 μg/kg yielded a greater concentration of reactive metabolites and DNA adducts, none of the tissues examined showed any signs of pathology. The exposure dose and duration may not have been high enough for FLA to exert an adverse effect on integrity of organs. However, the lack of pathological effects may change in chronic exposure conditions, as tumors have been diagnosed in extrahepatic tissues of mice that received PAHs through diet on a long-term basis [52]. Hence, detailed studies that will focus on dysregulation of gene expression including signal transduction for PAHs ingested through dietary fat in a long-term exposure regime may yield more definitive information on toxicity mechanisms from a functional standpoint.

Future studies will focus on the metabolic fate of FLA in rats that will receive FLA in dietary fat under subacute and subchronic exposure regimens. These studies are expected to shed some light on whether an increased duration of exposure to dietary-fat-incorporated FLA will contribute to a corresponding increase in retention of reactive metabolites in the system and enhance DNA damage in target tissues.

Acknowledgments

This publication was made possible through Grant No. ES012168 from the National Institute of Environmental Health Sciences, Grant Nos. GM08037 and 2R25GM59994 from the National Institute of General Medical Sciences and Grant No. RR03022 from the National Center for Research Resources. Partial financial support from the NASA-HCOP summer program to MM and AGA is also acknowledged. The contents of this article are solely the responsibility of the authors and do not necessarily represent the official views of either NIH or NASA.

References

[1] Hill MJ. Diet and cancer: a review of scientific evidence. Eur J Cancer Prev 1995;4(Suppl 2):3–42.
[2] Woutersen RA, Appel MJ, van Garderen-Hoetmer A, Wijnands MVW. Dietary fat and carcinogenesis. Mutat Res 1999;443:111–27.
[3] Ramesh A, Walker SA, Hood DB, Guillén MD, Schneider K, Weyand EH. Bioavailability and risk assessment of orally ingested polycyclic aromatic hydrocarbons. Int J Toxicol 2004;23:301–33.
[4] IPCS. Environmental health criteria 202: selected non-heterocyclic polycyclic aromatic hydrocarbons. International Programme on Chemical Safety. Lyon (France): World Health Organization; 1998.
[5] Baars AJ, Theelen RMC, Janssen PJCM, Hesse JM, van Apeldoorn ME, Meijerinck ME, et al. Re-evaluation of human toxicological maximum permissible levels. Netherlands: National Institute of Public Health and the Environment; 2001 [RIVM report 711701025].
[6] Wang JS, Busby Jr WF. Induction of lung and liver tumors by fluoranthene in a preweanling CD-1 mouse bioassay. Carcinogenesis 1993;14:1871–4.
[7] Yamaguchi K, Near R, Shneider A, Cui H, Ju S-T, Sherr DH. Fluoranthene-induced apoptosis in murine T cell hybridomas is independent of the aromatic hydrocarbon receptor. Toxicol Appl Pharmacol 1996;139:144–52.
[8] Knuckles M, Inyang F, Ramesh A. Acute and subchronic oral toxicity of fluoranthene in F-344 rats. Ecotoxicol Environ Saf 2004;59:102–8.
[9] Gorelick NJ, Hutchins DA, Tannenbaum SR, Wogan GN. Formation of DNA and hemoglobin adducts of fluoranthene after single and multiple exposures. Carcinogenesis 1989;10:1579–87.
[10] Xue W, Warshawsky D. Metabolic activation of polycyclic and heterocyclic aromatic hydrocarbons and DNA damage: a review. Toxicol Appl Pharmacol 2005;206:73–93.
[11] NIH. Guidelines for the laboratory use of chemical carcinogens. NIH Publication No. 81-2385. Washington (DC): National Institutes of Health US, Government Printing Office; 1981.
[12] Haught D, Koepke M, Goldberg-Heavilin L, Griffin A, Cunha T. The essentials of custom diets for laboratory animals. Richmond, Indiana: Purina Mills LLC; 2003. p. 8.
[13] Holland B, Welch AA, Unwin ID, Buss DH, Paul AA, Southgate DAT. McCance and Widdowson's the composition of foods. Cambridge (England): The Royal Society of Chemistry; 1992. p. 462.
[14] Hu J, Zhang G, Liu C-Q. Pilot study of polycyclic aromatic hydrocarbons in surface soils of Guiyang city, People's Republic of China. Bull Environ Contam Toxicol 2006;76:80–9.
[15] NHANES. Daily dietary fat and total food–energy intakes: Third National Health and Nutrition Examination Survey, Phase I, 1988–1991. Morbidity and Mortality Weekly Report. 1994;43:116–7, 123–5.
[16] USDA. Agriculture fact book 2001–2002. Washington (DC): United States Department of Agriculture; 2003. p. 310.
[17] Gupta RC. Enhanced sensitivity of ^{32}P-postlabeling analysis of aromatic carcinogen: DNA adducts. Cancer Res 1985;45:5656–62.
[18] Gupta RC, Randerath K. Analysis of DNA adducts by ^{32}P labeling and thin layer chromatography. In: Friedberg EC, Hanawalt PC, editors. DNA Repair, vol 3. New York: Marcel Dekker, Inc; 1988. p. 399–418.
[19] Roth WL, Freeman RA, Wilson GE. A physiologically based model for gastrointestinal absorption and excretion of chemicals carried by lipids. Risk Anal 1993;13:531–43.

[20] Kararli TT. Comparison of the gastrointestinal anatomy, physiology, and biochemistry of humans and commonly used laboratory animals. Biopharm Drug Dispos 1995;16:351–80.
[21] Porter CJ, Charman WN. Intestinal lymphatic drug transport: an update. Adv Drug Deliv Rev 2001;50:61–80.
[22] Shen H, Howles P, Tso P. From interaction of lipidic vehicles with intestinal epithelial cell membranes to the formation and secretion of chylomicrons. Adv Drug Deliv Rev 2001;50(Suppl 1):S103–25.
[23] Gershkovich P, Hoffman A. Uptake of lipophilic drugs by plasma derived isolated chylomicrons: linear correlation with intestinal lymphatic bioavailability. Eur J Pharm Sci 2005;26:394–404.
[24] Vetter RD, Carey MC, Patton JS. Coassimilation of dietary fat and benzo(a)pyrene in the small intestine: an absorption model using the killifish. J Lipid Res 1985;26:428–34.
[25] Wasan KM, Cassidy SM. Role of plasma lipoproteins in modifying the biological activity of hydrophobic drugs. J Pharm Sci 1998;87:411–24.
[26] Librando V, Sarpietro MG, Castelli F. Role of lipophilic medium in the absorption of polycyclic aromatic compounds by biomembranes. Environ Toxicol Pharmacol 2003;14:25–32.
[27] Lipniak M, Brandys J. Toxicokinetics of fluoranthene, pyrene and benz(a)anthracene in the rat. Polycycl Aromat Compd 1993;3: 111–9.
[28] Babson JR, Russo-Rodriguez S, Wattley RV, Bergstein PL, Rastetter WH, Liber HL, et al. Microsomal activation of fluoranthene to mutagenic metabolites. Toxicol Appl Pharmacol 1986;85:355–66.
[29] Gower JD, Willis ED. The dependence of the rate of benzo(a)pyrene metabolism in the rat small intestinal mucosa on the composition of the dietary fat. Nutr Cancer 1986;8:151–61.
[30] Gower JD, Sayer JW, Willis ED. The effect of dietary lipids and antioxidants on the activity of epoxide hydratase in the rat liver and intestine. Biochem Pharmacol 1986;12:1965–9.
[31] Newman JW, Morisseau C, Hammock BD. Epoxide hydrolases: their roles and interactions with lipid metabolism. Prog Lipid Res 2005;44:1–51.
[32] Stott WT, Kan HL, McFadden LG, Sparrow BR, Gollapudi BB. Effect of strain and diet upon constitutive and chemically induced activities of several xenobiotic-metabolizing enzymes in rats. Regul Toxicol Pharmacol 2004;39:325–33.
[33] Laher JM, Rigler MW, Vetter RD, Barrowman JA, Patton JS. Similar bioavailability and lymphatic transport of benzo(a)pyrene when administered to rats in different amounts of dietary fat. J Lipid Res 1984;25:1337–42.
[34] Rahman A, Barrowman JA, Rahimtula A. The influence of bile on the bioavailability of polynuclear aromatic hydrocarbons from the rat intestine. Can J Physiol Pharmacol 1986;64:1214–8.
[35] Kelly BC, Gobas FAPC, McLachlan S. Intestinal absorption and biomagnification of organic contaminants in fish, wildlife, and humans. Environ Toxicol Chem 2004;23:2324–36.
[36] Wall KL, Gao W, te Koppele JM, Kwei GY, Kauffman FC, Thurman RG. The liver plays a central role in the mechanism of chemical carcinogenesis due to polycyclic aromatic hydrocarbons. Carcinogenesis 1991;12:783–6.
[37] Doherty MM, Charman WN. The mucosa of the small intestine—how clinically relevant as an organ of drug metabolism? Clin Pharmacokinet 2002;41:235–53.
[38] Choi SH, Nishikawa M, Sakoda A, Sakai Y. Feasibility of a simple double-layered coculture system incorporating metabolic processes of the intestine and liver tissue: application to the analysis of benzo(a)pyrene toxicity. Toxicol In Vitro 2004;18:393–402.
[39] Cavret S, Rychen G, Feidt C. In vitro intestinal transfer and metabolism of polycyclic aromatic hydrocarbons. Polycycl Aromat Compd 2004;24:513–25.
[40] Babson JR, Russo-Rodriguez S, Rastetter WH, Wogan GN. Invitro DNA-binding of microsomally-activated fluoranthene: evidence that the major product is a fluoranthene N_2–deoxyguanosine adduct. Carcinogenesis 1986;7:859–65.
[41] Rice JE, Bedenko V, Lavoie EJ, Hoffman D. Studies on the metabolism of fluoranthene and 3-methylfluoranthene. In: Cooke M, Denouś AJ, editors. Polynuclear aromatic hydrocarbons: formation, metabolism and measurement. Richland: Battelle Press; 1983. p. 1009–20.
[42] Polcaro C, Nicoletti I, Ossicini L, Caponecchi G, Cozzi R, Fiore M, et al. Chromatographic and cytogenetic analysis of in vivo metabolites of fluoranthene. J Chromatogr 1988;448:127–33.
[43] Walker SA, Whitten LB, Seals GB, Lee WE, Archibong AE, Ramesh A. Inter-species comparison of liver and small intestinal microsomal metabolism of fluoranthene. Food Chem Toxicol 2006;44:380–7.
[44] Day BW, Sahali Y, Hutchins DA, Wildschütte M, Pastorelli R, Nguyen TT, et al. Fluoranthene metabolism: human and rat liver microsomes display different stereoselective formation of the trans-2, 3-dihydrodiol. Chem Res Toxicol 1992;5:779–86.
[45] Busbee DL, Norman JO, Ziprin RL. Comparative uptake, vascular transport, and cellular internalization of aflatoxin B1 and benzo(a)-pyrene. Arch Toxicol 1990;64:285–90.
[46] Willis ED. Effects of dietary lipids on the metabolism of polycyclic hydrocarbons and the binding of their metabolites to DNA. Biochem Soc Trans 1983;11:258–61.
[47] Gower JD. The oxidation of benzo(a)pyrene 7, 8-dihydrodiol mediated by lipid peroxidation in the rat intestine and the effect of dietary lipids. Chem Biol Interact 1987;63:63–74.
[48] Schaffer EM, Milner JA. Impact of dietary fatty acids on 7, 12-dimethylbenz[a]anthracene-induced mammary DNA adducts. Cancer Lett 1996;106:177–83.
[49] Curfs DMJ, Beckers L, Godschalk RWL, Gijbels MJJ, van Schooten FJ. Modulation of plasma lipid levels affects benzo(a)pyrene-induced DNA damage in tissues of two hyperlipidemic mouse models. Environ Mol Mutagen 2003;42:243–9.
[50] Stavenow L, Pessah-Rasmussen H. Effects of polycyclic aromatic hydrocarbons on proliferation, collagen secretion and viability of arterial smooth muscle cells in culture. Artery 1988;2:94–108.
[51] Martinet W, Knaapen MW, De Meyer GR, Herman AG, Kockx MM. Oxidative DNA damage and repair in experimental atherosclerosis are reversed by dietary lipid lowering. Circ Res 2001;7:733–9.
[52] Culp SJ, Gaylor DW, Sheldon WG, Goldstein LS, Beland FA. A comparison of the tumors induced by coal tar and benzo(a)pyrene in a 2-year bioassay. Carcinogenesis 1998;19:117–24.