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Effect of Nine Diets on Xenobiotic Transporters in Livers of Mice

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Abstract

1. Lifestyle diseases are often caused by inappropriate nutrition habits and attempted to be treated by polypharmacotherapy. Therefore, it is important to determine whether differences in diet affect the disposition of drugs. Xenobiotic transporters in the liver are essential in drug disposition.

2. In the current study, mice were fed one of 9 diets for 3 weeks. The mRNAs of 23 known xenobiotic transporters in livers of mice were quantified by microarray analysis, and validated by branched DNA assay. The mRNAs of 15 transporters were altered by at least one diet. Diet-restriction (10) and the atherogenic diet (10) altered the expression of the most number of transporters, followed by western diet (8), high-fat diet (4), lab chow (2), high-fructose diet (2) and EFA-deficient diet (2), whereas the low n-3 FA diet had no effect on these transporters. Seven of the 11 xenobiotic transporters in the Slc family, three of 4 in the Abcb family, two of 4 in the Abcc family and all 3 in the Abcg family were changed significantly.

3. This first comprehensive study indicates that xenobiotic transporters are altered by diet, and suggests there are likely diet-drug interactions due to changes in the expression of drug transporters.

Keywords

Disposition; drugs; mRNA expression; microarray

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Declaration of interest

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Introduction

Lifestyle diseases, which include atherosclerosis, asthma, some cancers, chronic liver diseases, chronic obstructive pulmonary disease, type 2 diabetes, heart disease, metabolic syndrome, chronic renal failure, osteoporosis, stroke, and obesity can be caused by daily habits that have been characterized by inappropriate relationship of people with the environment (Sharma and Majumdar, 2009). In 2005, lifestyle diseases accounted for 60% of all projected deaths worldwide, which was an estimated 35 million people. China has projected to have a 17% increase in deaths and estimated to spend about 558 billion international dollars from 2005 to 2015 on lifestyle diseases (<http://www.who.int/dietphysicalactivity/workplace/en/>). Lifestyle diseases are becoming an important economic burden all over the world.

Improper dietary habits are the most important factor contributing to lifestyle diseases (Sharma and Majumdar, 2009). In developed countries, processed, low-nutrient, high-calorie foods are a major part of the daily diet. It is reported that the average dietary intake of calories in the United States between 1971 and 2004 increased 22% among women and 10% in men, primarily due to the increased consumption of refined carbohydrates, starches, and sugar-sweetened beverages. Eating healthier diets could prevent at least \$71 billion per year in the USA in medical costs, lost productivity, and lost lives according to the U.S. Department of Health (Lustig, 2006; Popkin *et al.*, 2006).

Lifestyle diseases are often attempted to be treated by long-term polypharmacotherapy. In addition, drugs are used in people that are on various diets. Therefore, it is of importance to determine whether differences in diet affect the disposition of drugs.

Liver is an extremely important organ in the disposition of drugs and environmental chemicals. For many chemicals to be metabolized by the liver, they first need to be transported into the liver, where they are often biotransformed by phase-I and phase-II enzymes to more water-soluble chemicals before being transported out of the liver into the canaliculi to be excreted into the bile, or across the basolateral membrane to be transported into the blood and eliminated in the urine (Fig. 1). Uptake transporters on the basolateral membrane of hepatocytes, which include organic anion-transporting polypeptides/solute carrier organic anion transporter family (Oatps/Slco), sodium taurocholate cotransporting polypeptide/solute carrier family 10 member 1 (Ntcp/Slc10a1), organic cation transporter 1/solute carrier family 22 member 1 (Oct1/Slc22a1), organic anion transporter 2/solute carrier family 22 member 7 (Oat2/Slc22a7), concentrative nucleoside transporter 2/solute carrier family 28 member 2 (Cnt2/Slc28a2), and equilibrative nucleoside transporter 1/solute carrier family 29 member 1 (Ent1/Slc29a1), which together remove organic anions, cations and nucleosides from the portal blood and transfer them into the liver. Efflux transporters on the canalicular membrane of hepatocytes export xenobiotics into bile by multidrug resistance protein (Mdr) 1, multidrug resistance protein 2/ATP-binding cassette subfamily b member 4 (Mdr2/Abcb4), bile salt export pump (Bsep)/Abcb11, Multidrug resistance associated proteins/ATP-binding cassette subfamily C member 2 (Mrp/Abcc2), breast cancer resistance protein/ATP-binding cassette subfamily G member 2 (Bcrp/Abcg2), Cnt1, transporter heterodimer ATP-binding cassette subfamily G

members 5 and 8 (Abcg5/g8), and multidrug and toxin extrusion transporters/solute carrier family 47 member 1 (Mate1/Slc47a1). Organic solute transporter (Ost) α , Mrp/Abcc3, 4 and 6 are efflux transporters on the basolateral side of hepatocyte that transfer the drug metabolites back to blood. Additional information on transporters is provided in two reviews (Klaassen and Aleksunes, 2010; Klaassen and Lu, 2008).

Energy required for endo- and xenobiotic processing originates from the metabolism of carbohydrates and lipids, therefore, nutrition may have a global impact on genes involved in metabolism (Osada, 2013). Altering the nutrients in diets of laboratory animals is a good method to investigate the effects of various diets, which are important in lifestyle diseases, especially when done at the same time to exclude complex genetic and husbandry variables. Previous studies have reported that mice fed a high-fat diet from 6 weeks of age had increased Mrp3/Abcc3 and 4 and decreased Mrp1/Abcc1 (More and Slitt, 2011); Western diet (high-fat/high-cholesterol diet) induced higher mRNA expression of hepatic Abcg5 and 8 in mice (Zhang *et al.*, 2012); Atherogenic diet decreased Abcg8 transcripts (Cote *et al.*, 2013); Diet-restriction for 6 months decreased Oatp1a1 and 1b2, while it increased Oatp1a4 in liver of mice (Zhang *et al.*, 2010). However, no studies have comprehensively addressed how the expression of xenobiotic transporters is altered by numerous diets in the same experiment.

Given the rising occurrence of lifestyle disease and the long term polypharmacotherapy of lifestyle diseases, the present study was designed to determine whether xenobiotic drug transporter expression levels are altered in livers of mice fed 9 various diets. Research on the influence of various dietary habits on the expression of xenobiotic transporters in liver will provide a rationale to guide the use of drugs during different dietary conditions.

Methods

Ethics statement

The housing facility is an American Animal Associations Laboratory Animal Care-accredited facility at the University of Kansas Medical Center, and all procedures were approved in accordance with the Institutional Animal Care and Use Committee guidelines.

Animals

Male C57BL/6 mice (22 $\pm$ 2 g, 8-weeks old, n=5) were obtained from Charles River Laboratories, Inc. (Wilmington, MA). Animals were housed in a temperature-, light-, and humidity-controlled environment. Lab chow (#8604; Teklad rodent diet; 14% calories from fat), EFA deficient diet (#84224), high-fat diet (#97070; 59.9% calories from fat), western diet (42% calories from fat, and 0.2% cholesterol, #88137), atherogenic rodent diet (#02028, 42.6% calories from fat; cholesterol 1.3%, 0.5% cholic acid), high-fructose diet (#89247 60% fructose diet), AIN-93M purified diet (#94048; 10.2% calories from fat), and low n-3 FA diet (#00235 + 7% sunflower oil) (Levant *et al.*, 2006) were all purchased from Harlan Laboratories (Madison, WI), and diet restriction performed by providing 75% of the #8604 diet consumed by *ad libitum* feeding (Varady *et al.*, 2007) were given to mice for 3 weeks, and all mice drank water *ad lib*. The *ad libitum* daily feed intake was approximately 4 g/

mouse. Therefore, for diet restriction, each mouse was given approximately 2.7–3.0 g of food per day. The general characteristics of each diet were described previously (Renaud *et al.*, 2014). All mice were euthanized in the morning (8:00–10:00 A.M.) when liver samples were collected. Mice were not fasted before liver sample isolation. Livers were collected and frozen in liquid nitrogen, and stored at -80°C before use. These mouse livers were previously used to examine the influence of diets on expression of genes involved in lipid metabolism, oxidative stress, and inflammation (Renaud *et al.*, 2014).

Total RNA isolation

Total liver RNA was isolated using RNeasy Lysis reagent (Qiagen, Crawfordsville, IN) per the manufacturer's protocol, and concentrations were quantified with a NanoDrop Spectrophotometer (NanoDrop Technologies, Wilmington, DE) at 260 nm. Formaldehyde-agarose gel electrophoresis was used to evaluate the integrity of these total RNA samples, which were confirmed by visualization of the 18s and 28s rRNA bands.

Microarray and data analysis

Gene expression in mice livers of the nine diets was determined using the Affymetrix Mouse 430.20 arrays at the KUMC Microarray Core Facility. The cRNAs of three individual mice fed each diet were hybridized to an individual array. Raw data CEL files were imported into the 'R' program using the "Affy" package, normalized by the Robust Multichip Averaging (GCRMA) package, with the output data being $\log_2$ transformed. The probes with intensities higher than $\log_2 100$ in at least one group were selected for further analysis. Gene annotations were obtained using GeneSpring (Agilent Technologies, Santa Clara, CA), and gene symbols were obtained from the mouse 4302 package (Wu *et al.*, 2012). Accession number of the microarray data is GSE51885 (Gene Expression Omnibus database).

Branched DNA Assay for Oatp1a1 and 1a4

The expression of Oatp1a1 and 1a4 ($n=5$) were validated by the branched DNA assay (QuantiGene high volume branched DNA signal amplification kit; Panomics/Affymetrix). The assay was performed as described previously (Cheng *et al.*, 2005).

Statistical analysis

Differential expression of microarray data was determined using the limma package in R (compared to data from mice fed AIN purified diet, $P<0.05$). All values are expressed as mean $\pm$ S.E.M. Hierarchical clustering of mRNAs of xenobiotic transporters in the livers of mice that exhibited changes in at least 1 diet was compared to the AIN-93M purified diet by the 'R' program using the "Pheatmap" package. Average values of three replicates per age are given by colored squares. High mRNA abundance is represented in red, whereas low mRNA abundance is in blue.

Results

Uptake transporter mRNAs in livers of mice fed various diets

All four Slco (a subfamily of Slc) xenobiotic transporters in the livers of mice were changed significantly by the various diets (Fig. 2). Oatp1a1/Slco1a1 mRNA was essentially decreased to non-detectable levels in livers of mice on the restricted diet, and was decreased 62% in livers of mice fed the atherogenic diet. In contrast to the abolition of Oatp1a1 mRNA in the mice on the restricted diet, there was a dramatic 20-fold increase of Oatp1a4/Slco1a4 mRNA in mice on diet restriction. A marked 80% decrease of Oatp1a4/Slco1a4 mRNA was observed in livers of mice fed the high-fructose diet. Oatp1b2/Slco1b2 was increased (87%) in mice on the western diet. Oatp2b1/Slco2b1 was slightly decreased (23%) in liver of mice fed the high-fructose diet.

Most of the xenobiotic uptake transporters of the Slc10a, 15a, 22a, 28a and 29a families, including Oct1/Slc22a1, Cnt2/Slc28a2 and Ent1/Slc29a1, did not change significantly in liver of mice fed the nine diets (data not shown). Compared with the purified diet, the mRNA of Ntcp/Slc10a1 decreased 60% in mice fed the atherogenic diet, and the mRNA of Oat2/Slc22a7 in livers of mice was decreased markedly by lab chow (73%), diet-restriction (91%) and the atherogenic diet (64%).

Efflux transporter mRNAs in livers of mice fed various diets

Canalicular efflux transporters—The expression of efflux transporters in hepatocytes is shown in Fig. 3. Feeding mice the various diets significantly altered the mRNA expression of 3 of the 4 transporters for the ATP-binding cassette sub-family B, namely Mdr1a/Abcb1a, Mdr2/Abcb4 and Bsep/Abcb11. The mRNA of Mdr1a/Abcb1a was increased markedly (18-fold) in mice on a restricted diet, and also increased (3-fold) in livers of mice fed the atherogenic diet. Mdr2/Abcb4 mRNA was increased by the high-fat diet (64%), atherogenic diet (100%) and western diet (72%). Bsep/Abcb11 mRNA was decreased (40%) by diet restriction and increased (81%) by the atherogenic diet. The mRNAs of Mdr1b/Abcb1b in livers of mice was not altered.

The canalicular efflux transporter of the ATP-binding cassette sub-family C is Mrp2/Abcc2, whose mRNA was increased by diet restriction (100%), high-fat diet (73%), atherogenic diet (94%), western diet (100%) and EFA-deficient diet (43%).

The mRNA of all three xenobiotic efflux transporters in the ATP-binding cassette sub-family G including Bcrp/Abcg2, Abcg5, and Abcg8 were changed significantly by the various diets. Bcrp/Abcg2 mRNA was decreased by diet restriction (40%) and increased by western diet (34%). Abcg5 and 8 were increased similarly in livers of mice on the restricted diet (200%), atherogenic diet (1000%), and western diet (300%), respectively.

Mate1/Slc47a1 is a canalicular efflux transporter of Slc family and the mRNA of this transporter was higher in mice on the high-fat diet (73%) and western diet (54%) than the purified diet. Cnt1 is also a canalicular efflux transporter of Slc family, and the mRNA of this transporter is not significantly changed.

Basolateral efflux transporters—Osta, Mrp3/Abcc3, Mrp4/Abcc4 and Mrp6/Abcc6 are basolateral efflux transporters, and the mRNA of Mrp3/Abcc3 was higher in the mice on lab chow (300%), diet-restriction (400%), high-fat diet (200%), atherogenic diet (200%), and western diet (200%).

Validation of microarray data by branched DNA assay

Because the changes of Oatp1a1 and 4 are the most obvious in the present study, the mRNA of these two genes in liver of mice fed the nine diets (n=5) quantified by microarray were validated with the branched DNA assay. In general, the fold change of these two genes had the same trend by these two methods (data not shown).

Hierarchical cluster analysis of mRNA profiles for transporters in liver of mice fed the various diets

Of the 23 xenobiotic transporters (Table 1) in liver of mice that were investigated in this study, 15 of them were significantly altered by at least 1 diet (Fig. 4). Transporters that were quantitatively altered the most in the current study were Oatp1a1/Slc1a1 (decreased to undetectable level) and Oatp1a4/Slc1a4 (20-fold increase) in livers of mice fed the restricted diet, followed by Mdr1a/Abcb1a (18-fold increase) and Oat2/Slc22a7 (91% decrease) in livers of mice on diet restriction; an increase of Mrp3/Abcc3 (2–4 fold) was observed in mice fed five diets; decreases of Oat2/Slc22a7 in mice fed lab chow (73%) and the atherogenic diet (64%); and a marked decrease (79%) of Oatp1a4/Slc1a4 in mice fed the high-fructose diet. The other 8 genes including Mdr1b/Abcb1b, Mrp4/Abcc4, Mrp6/Abcc6, Oct1/Slc22a1, Cnt1/Slc28a1, Cnt2/Slc28a2, Ent1/Slc29a1 and Osta were not significantly altered by any of the diets.

There were also differences in the numbers of various diets to alter the expression of xenobiotic transporters in the liver. Diet-restriction (10 transporters) and the atherogenic diet (10 transporters) altered the expression of the most xenobiotic transporters in the livers of mice, followed by the western diet (8 transporters), high-fat diet (4 transporters), lab chow (2 transporters), high-fructose diet (2 transporters) and EFA-deficient diet (2 transporters). The low n-3 FA diet had no influence on these transporters.

Discussion

The present study comprehensively characterized the mRNA profiles for 23 known xenobiotic transporters in livers of mice fed nine various diets. Compared with the AIN-93M purified diet, changes in the mRNA expression of drug transporters in livers of mice fed lab chow, diet-restriction, high-fructose, high-fat, atherogenic, western, low n-3, and EFA-deficient diet were examined simultaneously, which yielded information about diverse nutrition conditions related to lifestyle diseases.

The Slc family of transporters are important in the uptake of xenobiotics into the liver. The Oatps are integral membrane proteins in the Slc family (Hagenbuch and Meier, 2003; Hagenbuch and Meier, 2004), which transport organic anions, cations and neutral compounds. Some drugs commonly used to study Oatps *in vitro* include BQ-123, [D-Pen2, D-Pen5] enkephalin, statins, and fexofenadine. In the present study, the mRNA expression of

Oatp1a1 and 1a4 had opposite patterns of expression in livers of mice fed the restricted diet and atherogenic diet (Fig. 2). Oatp1a1 and 1a4 are rodent Oatps, and might be more important in secondary bile acid metabolism and intestinal bacteria homeostasis of mice than transporting xenobiotics into the liver (Zhang *et al.*, 2013). Liver specific Oatp1b2 has a broad substrate specificity, and plays an important role in rifampicin, pravastatin, phalloidin and microcystin-LR disposition as well as transport of unconjugated bile acids (Csanaky *et al.*, 2011), and it increased in mice on the western diet. The data from the current study suggests high intake of red meat, sugary desserts, high-fat foods, and refined grains (Evers and Chu, 2008; Halton *et al.*, 2006) may alter responses of drugs and chemicals transported by the Oatps.

Other members of the Slc transporter family also play important roles in drug disposition. Ntcp/Slc10a1 is a Na⁺-dependent conjugated bile acid transporter expressed on the basolateral membrane of hepatocytes, which transports bile salts, but also transports estrone-3-sulfate, sulfobromophthalein, fluvastatin, and rosuvastatin *in vitro* (Hagenbuch and Meier, 1994; Hagenbuch *et al.*, 1991; Mareninova *et al.*, 2005). The current study showed that an atherogenic diet for 3 weeks decreased the expression of Ntcp/Slc10a1 (Fig. 2), which might be related to the cholic acid intake in mice fed this diet. Therefore, the inclusion of cholic acid and a much higher cholesterol concentration in the atherogenic diet may decrease the uptake of conjugated bile acids, and decrease the transport of these drugs into the liver. Oat2/Slc22a7 is a polyspecific anion transporter on the basolateral membrane of hepatocytes that transports anticonvulsant drugs, antibiotics, chemotherapy and endobiotics (Klaassen and Aleksunes, 2010). In addition, it has also been reported that Oat2/Slc22a7 is important in the efflux of glutamate and uptake of orotic acid (Fork *et al.*, 2011). Although Oat2/Slc22a7 appears to be expressed at low levels in livers of mice, the mRNA decreased markedly in livers of mice fed lab chow, diet-restriction, and an atherogenic diet (Fig.2), which implies that these diets might be noteworthy when drugs transported by Oat2/Slc22a7 are used.

ATP-binding cassette (ABC) transporters function to export xenobiotics out of cells. Mdr1/Abcb1 is the first member of the sub-family B of the ATP-binding cassette superfamily, also known as P-glycoprotein, which is over-expressed in tumor cells and confers multidrug resistance. Mdr1 is expressed highly in intestine, kidney, placenta, blood-brain barrier (apical), but low in liver. However, the present data indicate there is a marked increase in Mdr1a mRNA (about 18-fold) in livers of mice fed the restricted diet (Fig. 3). The effect of this phenomenon requires further investigation. Mdr2/Abcb4 is a phospholipid floppase, and mediates the efflux of phosphatidylcholine and possibly some hydrophobic drugs, including paclitaxel and vinblastine (Cui *et al.*, 2009). The mRNA of Mdr2 was increased moderately in livers of mice fed a high-fat (64%), western (72%), and atherogenic diets (100%) (Fig. 3), which might be a feedback effect of high lipid diets to enhance the transport of phospholipids to protect the canalculus from bile acids. Although Bsep/Abcb11 primarily transports bile acids, it can also transport pharmaceuticals such as pravastatin (Hirano *et al.*, 2005). Data in the present study indicate that diet restriction decreases whereas the atherogenic diet increases Bsep mRNA levels (Fig. 3).

Mrp2 and Mrp3 in the ATP-binding cassette sub-family C appears to be more easily altered by dietary habits, because 5 of the 8 diets up-regulated the mRNA of these two transporters in the current study. Mrp2/Abcc2 is on the canalicular membrane of hepatocytes and transports phase II conjugates of endo- and xenobiotics, organic anions, leukotrienes, numerous chemotherapeutics (Jedlitschky *et al.*, 2006; Klaassen and Aleksunes, 2010). In fact, most drugs in bile are transported by Mrp2. The hepatic efflux of these drugs might be higher (Fig. 3) under diet restriction, a high-fat diet, an atherogenic diet, a western diet, and an EFA-deficient diet.

Mrp3/Abcc3 is on the basolateral membrane of hepatocytes and thus transports chemicals back into the blood. Mrp3 mRNA had relatively more dramatic increases than Mrp2/Abcc2 when fed the various diets (Fig. 3). In humans, Mrp3 is expressed at a low level and is almost absent from the basolateral membranes of hepatocytes under normal conditions, but up-regulated under cholestatic conditions to protect hepatocytes from intrahepatic toxins, whereas, in mice, although Mrp3 is expressed in the liver and is not largely induced due to the little room to up-regulated (Donner and Keppler, 2001; Kool *et al.*, 1999), the current study showed that Mrp3 is easily induced by changes of diets. It is reported that Mrp3 in mice and humans can modulate the hepatic transport of glucuronide and glutathione conjugates (Klaassen and Aleksunes, 2010). Therefore, lab chow, diet restriction, high-fat diet, atherogenic diet, and western diet may increase the export of those drugs out of hepatocytes back into the blood to be eliminated in the urine.

The transporters of the ATP-binding cassette sub-family G also had marked changes in mRNA expression by various diets (Fig. 3). The Bcrp/Abcg2 protein is an ABC half-transporter that forms a homodimer to function (Henriksen *et al.*, 2005). Substrates for Bcrp are organic anions and numerous chemotherapeutic drugs - (Klaassen and Aleksunes, 2010). The present study suggests that diet restriction may decrease the efflux of these drugs out of hepatocytes, while a western diet which is high in fat and cholesterol may increase the efflux of the drugs noted above.

Abcg5 and 8 work in concert as a heterodimer to prevent the absorption of plant sterols from the intestine (Graf *et al.*, 2003), Abcg5/8 genes are direct or indirect targets of transcription factors including the oxysterol receptors liver X receptor α and β , hepatocyte nuclear factor 4 α , and liver receptor homolog1 (Sumi *et al.*, 2007). The present study shows that Abcg5 and 8 (Fig.3) were markedly up-regulated by the atherogenic diet (200%), diet-restriction (1000%) and western diet (300%), which might indicate the activation of these nuclear receptors in livers of mice fed these three diets.

The Mate family of transporters are Slc transporters that function as efflux proteins. The members of this family are all polyspecific electro-neutral organic cation/H⁺ transporters, first identified as a bacterial drug transporter family, which are present in almost all prokaryotes and eukaryotes, and are thus one of the most conserved transporter families in nature (Damme *et al.*, 2011). Mate 1/Slc47a1 (Fig. 3) transports a variety of small hydrophilic organic cations (versus Mdr1 which transports mainly large organic cations) such as acyclovir, cephalexin, cephadrine, cimetidine, creatinine, estrone-sulfate, ganciclovir, guanidine, 1-methyl-4-phenylpyridinium, metformin, oxaliplatin, paraquat,

procainamide, tenofovir, tetraethylammonium, thiamine, topotecan in exchange for protons (Klaassen and Aleksunes, 2010). The present study suggests that a high-triglyceride and -cholesterol diet as well as high-fat and western diet, may increase the efflux of drugs that are transported by Mate 1/Slc47a1, and thus alter drug efficacy.

Xenobiotic-activated transcription factors might be influenced by changes of diets. The most obvious alteration in livers of mice fed the restricted diet was the dramatic decrease of Oatp1a1 along with increase of Oatp1a4, Mdr1a, Abcg5/8, Mrp2 and Mrp3 on mRNA level, therefore, constitutive androstane receptor (NR1I3) and pregnane X receptor (PXR, NR1I2) might be activated (Klaassen and Aleksunes, 2010), and the data are partially consistent with previous study since the age and diet formula are not completely the same in these studies (Zhang *et al.*, 2010). In addition, marked decrease of Ntcp and increase of Bsep mRNAs were observed in livers of mice fed the atherogenic diet, which might be related to farnesoid X receptor (NR1H4) activation, and is consistent with the effect of cholic acid feeding to the mice (Klaassen and Aleksunes, 2010). Although the mRNA of Oatp1a4 was not significantly increased in livers of mice fed the atherogenic diet, the mRNAs of Abcg5/8, Mrp3, and Mdr1a were markedly increased, and therefore, PXR may be activated in livers of mice fed the atherogenic diet. However, the expression of other target genes for these xenobiotic-activated transcriptional factors need to be considered when the mechanisms of up- and down-regulation of these transporters are explored in livers of mice fed the various diets.

Conclusions

Compared with the AIN-93M purified diet, 60% of the xenobiotic transporters were altered by one or more of the diets. More specifically, the mRNA expression of the largest number of xenobiotic transporters was changed by diet restriction (10 transporters) and the atherogenic diet (10 transporters), while the low n-3 FA diet had no influence on any of these xenobiotic transporters. However, all the data in the present study are at the mRNA level and caution is needed when extrapolating these results to corresponding protein activity and function levels. The present study is the first comprehensive study to indicate that xenobiotic transporters are altered by diet, and suggest there are likely diet-drug interactions as a result in the change in hepatic expression of drug transporters.

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Abbreviations

Abc	ATP-binding cassette
Bcrp	breast cancer resistance protein
Bsep	bile salt export pump
Cnt	concentrative nucleoside transporter

Ent	equilibrative nucleoside transporter
Gapdh	glyceraldehyde-3-phosphate dehydrogenase
Mate	multidrug and toxin extrusion transporter
Mdr	multidrug resistance protein
Mrp	multidrug resistance-associated protein
Oat	organic anion transporter
Oatp	organic anion-transporting polypeptide
Oct	organic cation transporter
Ost	organic solute transporter
PXR	pregnane X receptor
Slc	solute carrier
Slco	solute carrier organic anion transporter

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Figure 1. Cellular localization of uptake and efflux xenobiotic transporters in hepatocytes and cholangiocytes. The localization and orientation of uptake and efflux transporters in livers of mice are shown.

Figure 2.

Xenobiotic uptake transporters of Slco and Slc family changed significantly in mice liver fed the 9 diets. “Lab chow” represents #8604 Teklad rodent diet; “EFA deficient” represents #84224 EFA-deficient diet; “high-fat” represents #97070 high-fat diet; “western” represents #88137 western diet; “atherogenic” represents #02028 atherogenic rodent diet; “high-fructose” represents #89247 60% fructose diet; “AIN-93M purified” represents #94048 AIN-93M purified diet; “low n-3 FA” represents low n-3 FA diet (#00235 + 7% sunflower oil); The same abbreviations used in all the figures of the current study. * represent $P < 0.05$. Data are presented as mean $\pm$ S.E.M.

Figure 3.

The mRNAs of canalicular and sinusoidal efflux transporters in liver of mice fed the 9 different diets. * represent $P < 0.05$. Data are presented as mean $\pm$ S.E.M.

Figure 4.

Hierarchical cluster analysis of mRNA profiles for transporters in liver of mice fed the various diets. Average values of three replicates per age are given by colored squares. High mRNA abundance is represented in red, whereas low mRNA abundance is in blue.

Table 1**Xenobiotic transporters in livers of mice**

Gene symbol	transporters	direction	location
Abcb1a	Mdr1a	efflux	canalicular
Abcb1b	Mdr1b	efflux	canalicular
Abcb4	Mdr2	efflux	canalicular
Abcb11	Bsep	efflux	canalicular
Abcc2	Mrp2	efflux	canalicular
Abcc3	Mrp3	efflux	basolateral
Abcc4	Mrp4	efflux	basolateral
Abcc6	Mrp6	efflux	basolateral
Abcg2	Bcrp	efflux	canalicular
Abcg5	Abcg5	efflux	canalicular
Abcg8	Abcg8	efflux	canalicular
Slc10a1	Ntcp	uptake	basolateral
Slc22a1	Oct1.	uptake	basolateral
Slc22a7	Oat2	uptake	basolateral
Slc28a1	Cnt1	efflux	canalicular
Slc28a2	Cnt2	uptake	basolateral
Slc29a1	Ent1	uptake	basolateral
Slc47a1	Mate1	efflux	canalicular
Slco1a1	Oatp1a1	uptake	basolateral
Slco1a4	Oatp1a4	uptake	basolateral
Slco1b2	Oatp1b2	uptake	basolateral
Slco2b1	Oatp2b1	uptake	basolateral
Osta	Osta	efflux	basolateral