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Energy Restriction Does Not Compensate for the Reduced Expression of Hepatic Drug Processing Genes in Mice with Aging

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Non-standard Abbreviations: AhR, aryl hydrocarbon receptor; Aldh, aldehyde dehydrogenase; Bcrp, breast cancer resistance protein; Bsep, bile salt export pump; CAR, constitutive androstane receptor; Ces, carboxylesterase; Cyp, cytochrome P450; Cpr, cytochrome P450 reductase; DPGs, drug processing genes; Ent1, equilibrative nucleoside transporter 1; ER, energy restriction; Gst, glutathione S-transferase; HNF-4 α , hepatocyte nuclear factor 4 α ; Mdr, multidrug resistance protein; Mrp, multidrug resistance-associated protein; Nrf2, nuclear factor erythroid 2-related factor 2; Ntcp, sodium/taurocholate-cotransporting polypeptide; Oat, organic anion transporter; Oatp, organic anion-transporting polypeptide; Oct, organic cation transporter; Ost, organic solute transporter; Pon, paraoxonase; PPAR α , peroxisomal proliferator-activated receptor α ; PXR, pregnane X receptor; Rgn, regucalcin; RXR α , retinoid X receptor α ; Sult, sulfotransferase; Udp-gdh, UDP-glucose dehydrogenase; Udp-gpp, UDP-glucose pyrophosphorylase; Ugt, UDP-glucuronosyltransferase.

Abstract

Liver is the major organ that eliminates xenobiotics from the body, a process that is accomplished by a series of drug processing genes (DPGs). These genes encode transporters on both basolateral and apical membranes of hepatocytes, as well as phase-I and phase-II enzymes. The current study compares the expression of hepatic DPGs in adult and aged mouse livers and explores the potential effects of energy restriction (ER) on these genes during aging. Out of 79 quantified hepatic DPGs, 52 were expressed lower in 24-month-old aged mice than in 12-month-old adult mice. Furthermore, the mRNA expression of multiple xenobiotic-activated transcription factors also decreased with age. Six-month ER exerted less of an effect on the hepatic DPGs than did aging. ER increased the mRNAs of 2 and decreased the mRNAs of 9 DPGs in adult mice. In aged mice, ER increased the mRNAs of 10 and decreased the mRNAs of 5 DPGs. The only mRNA that was increased by both ER and aging was *Gstm3*. ER increased the mRNAs of *Cyp2b10*, *Ugt1a9*, *Gsta1*, and *Oatp1a4* only in adult mice, and decreased the mRNAs of *Aldh6a1*, *Pon3*, *Ugt1a1*, *Sult1a1*, and *Atp8b1* only in aged mice. In summary, the reduced mRNA expression of hepatic DPGs in aged mice indicates diminished drug processing capability, whereas ER did not compensate for the global reduction of hepatic DPG expression in aged mice. The hepatic transcription factors are likely to mediate the changes in hepatic DPG expression during aging and ER.

Introduction

The geriatric population is rapidly increasing in the world. The proportion of persons aged 60 or older is projected to reach 22% of the global population in 2050, with 33% in the more developed regions, and 20% in the less developed regions (United Nations, Population ageing and development 2009, Available at: <http://www.un.org/esa/population/publications/ageing/ageing2009chart.pdf>). Healthcare for this population presents a major medical and economic problem. The elderly population is more susceptible to various diseases, and they often take several drugs at the same time. Liver, the major organ for drug elimination from the body, exhibits decreased volume, diminished hepatobiliary functions, declined phase-I drug metabolism capability, and increased or decreased expression of a variety of proteins in the elderly (Schmucker, 2005).

Liver metabolizes endogenous and exogenous compounds (nutrients and drugs), as well as maintains energy balance in the body. Numerous liver functions are accomplished by proteins encoded by a series of drug processing genes (DPGs), which include basolateral uptake transporters, phase-I and phase-II enzymes, as well as apical and basolateral efflux transporters.

Hepatocytes have transporters on both their basolateral and apical sides to remove xenobiotics from intestine-derived portal blood and to excrete them or their metabolites into bile or blood. Uptake solute carriers include organic anion-transporting polypeptides (Oatps), sodium/taurocholate-cotransporting polypeptide (Ntcp), organic cation transporters (Octs), organic anion transporters (Oats), etc. Apical efflux transporters, including the multidrug resistance proteins (Mdrs), multidrug

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resistance-associated proteins (Mrps), bile salt export pump (Bsep), and breast cancer resistance protein (Bcrp), etc., transport compounds into bile. Several Mrps, such as Mrp3 and 4, are located on the basolateral membrane of hepatocytes and transport chemicals back into blood.

Phase-I enzymes in the liver are mainly cytochrome P450s (Cyps), aldehyde dehydrogenases (Aldhs), carboxylesterases (Ces's), and paraoxonases (Pons). These enzymes catalyze oxidation, reduction, and hydrolysis reactions. Phase-II enzymes often conjugate phase-I metabolites to make them more water soluble to be excreted by efflux transporters. In typical phase-II reactions, glucuronic acid, sulfate, or glutathione are added to these compounds by UDP-glucuronosyltransferases (Ugts), sulfotransferases (Sults), and glutathione S-transferases (Gsts), respectively.

The expression of hepatic DPGs is regulated by multiple xenobiotic-activated transcription factors (Klaassen and Slitt, 2005). These transcription factors detect xenobiotics in hepatocytes and accordingly turn on/off the expression of certain DPGs. In general, these changes in gene expression will facilitate the elimination of xenobiotics. Included among these hepatic xenobiotic transcription factors are pregnane X receptor (PXR), constitutive androstane receptor (CAR), peroxisomal proliferator-activated receptor α (PPAR α), aryl hydrocarbon receptor (AhR), and nuclear factor erythroid 2-related factor 2 (Nrf2).

Energy restriction (ER) has been extensively investigated as an intervention that both extends lifespan and delays age-related diseases in laboratory animals (Anderson and Weindruch, 2007). It has been proposed that caloric restriction might produce beneficial effects by reprogramming energy metabolism (Anderson and Weindruch, 2007).

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However, the exact effects of ER on hepatic drug metabolism remain unknown. It has been reported that a 35% reduction of dietary calories protects male rats from a lethal dose of thioacetamide, due to enhanced tissue repair (Ramaiah et al., 1998), although the induction of hepatic microsomal Cyp2e1 by ER led to exaggerated hepatotoxicity at a low dose of thioacetamide (Ramaiah et al., 2001).

The current study is designed to determine the changes of hepatic DPGs during aging, as well as what effects ER has on these liver genes in adult and aged mice. To fulfill this purpose, adult (6-month) and aged (12-month) male C57BL/6 male mice were placed on either a control or energy restricted dietary program for 6 months. The expression of hepatic DPGs, including transporters, phase-I and phase-II enzymes, as well as key xenobiotic transcription factors, was determined. Data generated from mice in this study may provide information for the prediction of drug efficacy, pharmacokinetics, and toxicity in elderly as well as energy-restricted human populations.

Materials and Methods

Animals. The 6-month energy restriction feeding procedure was previously described (Colman et al., 2007). Briefly, male C57BL/6 mice at 6-month (adult) and 18-month (aged) of age were separated into control (C) and energy restricted (ER) groups, respectively. For the control adult and aged groups, the initial energy intake was 90% of the mean *ad libitum* energy intake and was gradually decreased to 75% of the *ad libitum* intake by the end of the experimental period. The ER groups were restricted in energy intake to ~55% of the *ad libitum* during the 6-month experimental period. AIN-93M diet (TestDiet, Richmond, IN; #5801-M), containing 12.7% protein, 4.1% fat, 5.0% fiber, and 73% carbohydrates, was used as the control diet. A modified AIN-93M diet enriched 67% in vitamins and minerals was given to the adult and aged ER groups to ensure that the ER mice received approximately the same amounts of micronutrients as the control groups (Reeves et al., 1993). By the end of the study, the adult mice were 12-month old, and the aged mice were 24-month old. Livers were harvested from 9 AM to 12 PM in the morning, snap frozen with liquid nitrogen, and stored at -80°C.

RNA Preparation. Total liver RNA was prepared with RNA-Bee reagent (Tel-Test Inc., Friendswood, TX) from frozen tissue following the manufacturer's instructions. Purified RNA was stored at -80°C. RNA concentrations were quantified with a Nanodrop nd-1000 spectrophotometer (Thermo Scientific, Wilmington, DE) and diluted to a final concentration of 50 ng/μl. The quality of RNA was determined by agarose gel electrophoresis. Five individual samples from each group were used for gene expression assays.

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Multiplex Suspension Array. The mRNA levels of hepatic genes were determined by QuantiGene P2.0 technology (Panomics/Affymetrix Inc., Fremont, CA), as previously described (Zhang et al., 2009). Briefly, 0.5 µg RNA samples were loaded into 96-well plates that contained X-MAP beads coated with capture probes, label extenders, and blockers. Following several incubation and washing steps, streptavidin-conjugated *R*-phycoerythrin fluorescence was analyzed by a Bio-Plex 200 system array reader interfaced with Bio-Plex data manager software 5.0 (Bio-Rad, Hercules, CA). The majority of the genes quantified in the current study have been previously characterized by our lab (Cheng et al., 2005; Alnouti and Klaassen, 2006; Alnouti et al., 2006; Buckley and Klaassen, 2007; Alnouti and Klaassen, 2008b; Alnouti and Klaassen, 2008a; Cheng and Klaassen, 2009). Details of the plex sets used in the current study can be found at www.panomics.com with the following catalog numbers: 21073, 21150, 21152, 21153, 21174, and 21175. Messenger RNA expression level of each gene was presented as a ratio to the loading control gene Rpl13a in the same panel, except for panels 21152 and 21153, where Gapdh was used as the loading control.

Statistical Analysis. All data were analyzed using one-way analysis of variance (ANOVA) followed by Duncan's multiple range test ($p \leq 0.05$).

Hierarchical Cluster Analysis. JMP7 (SAS Institute, Cary, NC) was used to distinguish the hepatic gene expression pattern across age-feeding groups. Hierarchical clustering was obtained from either drug processing genes, including phase-I enzymes, phase-II enzymes, and transporters, or transcription factors. The highest expression of a specific gene is represented by the darkest region of the gray scale spectrum, whereas the lowest

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expression is indicated by the lightest region. The spectrum is specific to the expression to each individual gene. The relative intensities of gray scale should not be used to compare the expression levels of different genes.

Results

Effects of aging and ER on the mRNA levels of hepatic uptake transporters.

The body composition changes after the 6-month energy restriction in these mice were reported previously (Colman et al., 2007). In brief, the 12-month-old adult control mice weighed 30.5 g with 21.5% body fat; the adult ER mice weighed 24.4 g with 13.4% body fat; the 24-month-old aged control mice weighed 32.1 g with 14.5% body fat; and the aged ER mice weighed 28 g with 12.8% body fat.

Multiple uptake transporters are expressed on the basolateral membranes of hepatocytes. Aging caused significant reduction in the mRNA expression of organic anion-transporting polypeptide (Oatp) 1a1, 1b2, 2b1, organic cation transporter (Oct) 1, sodium taurocholate co-transporting polypeptide (Ntcp), and equilibrative nucleoside transporter 1 (Ent1) (Fig. 1). Among these uptake transporters, Oatp1a1 was reduced most markedly with age. The 24-month-old mice only expressed about 15% of the hepatic Oatp1a1 mRNA as the 12-month-old mice.

The 6-month ER decreased the mRNA of Oatp1a1 by 96% in adult and 91% in aged mouse livers. In addition, ER also decreased ~30-50% of the hepatic expression of Oatp1b2 mRNA in both age groups and the apical aminophospholipid flippase Atp8b1 mRNA in aged mice. However, ER increased Oatp1a4 mRNA 92% in adult but not in aged mouse livers. In summary, the expression of most hepatic uptake transporters decreased with aging; ER decreased the mRNA levels of Oatp1a1 and 1b2 at both ages, and Atp8b1 in aged mice only, whereas it increased Oatp1a4 mRNA expression in adult mice.

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Effects of aging and ER on the mRNA expression of phase-I enzymes in mouse livers. As shown in Fig. 2, mRNA levels of major hepatic drug metabolising cytochrome P450s (CyPs), including Cyp1a1, 1a2, 3a11, and 4a14, decreased with age, except for Cyp2b10. These CyPs decreased 50-75% between 12-month and 24-month of age. The mRNA expression of cytochrome P450 reductase (Cpr), the common electron donor of the CyPs, was not changed by aging in mouse livers. Six months of ER increased the mRNA expression of Cyp2b10 and 4a14 by 1140 and 81%, respectively, in adult mouse livers, but not in aged mice. The hepatic expression of Cyp3a11 and 4a14 tended to be enhanced by ER in both age groups, but significant induction was only observed in Cyp4a14 mRNA in livers of adult mice. Cyp2e1, another important member of CyPs in liver, was not influenced by either aging or ER at the mRNA level in the current study (data not shown). In summary, the majority of hepatic CyPs studied decreased by aging, and ER enhanced the expression of Cyp2b10 and 4a14 in adult mouse livers, but to a lesser extent in aged mouse livers.

The mRNA expression of most hepatic carboxylesterases (Ces's) decreased with age except Ces1e1 (Fig. 3). For example, the hepatic mRNA of Ces1b2, 1d1, and 2a6 were reduced 65-82%, and Ces1, 1b1, 3a2, and 5b1 were decreased 28-42% in aged mice. ER increased the hepatic expression of Ces1d1 (84%) in aged mouse livers and decreased the mRNA level of Ces3a2 (40%) in adult mouse livers. The hepatic expression of other Ces's was not significantly influenced by ER in either age group.

Aldehyde dehydrogenases (Aldhs) were abundantly expressed in mouse liver. Aging decreased the mRNA expression of most of the Aldhs (Fig. 4), but not Aldh1b1 and

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Aldh2. In aged mouse livers, the mRNA of Aldh3a2 and 6a1 decreased 63 and 45%, respectively, compared to adult mice. About 30% less mRNA of Aldh1a1, 1a7, 4a1, 7a1, 8a1, and 9a1 was expressed in aged mice. In comparison to aging, ER had less of an effect on Aldh mRNA levels; however ER decreased Aldh6a1 mRNA in aged mice. In summary, hepatic Aldh mRNA levels were markedly decreased by aging, but less influenced by ER.

Aging decreased the expression of both paraoxonase (Pon) 1 and 3 (~30%) in the liver as shown in Fig. 4. ER further decreased the Pon3 mRNA 30% in the aged group, but did not influence its expression in livers of adult mice.

Effects of aging and ER on the mRNA expression of phase-II enzymes in mouse livers. The mRNA levels of major hepatic conjugation enzymes were quantified, including 7 UDP-glucuronosyltransferases (Ugts), UDP-glucose pyrophosphorylase (Udp-gpp), and UDP-glucose dehydrogenase (Udp-gdh); 2 sulfotransferases (Sults) that can be detected in male mouse liver, as well as 2 isoforms of 3'-phosphoadenosine-5'-phosphosulfate synthase (Papss); and 13 glutathione S-transferases (Gsts).

Compared to adult mice (12 mo), aged mice (24 mo) expressed 25-55% less of the following hepatic enzymes involved in glucuronidation: Ugt1a6, 1a9, 2b1, 2b35, 2b36, 3a1, 3a2, Udp-gdh, and Ugp-gpp (Fig. 5). ER decreased the mRNA levels of Ugt 1a1 (27%) and 2a3 (38%) in aged mouse livers, Ugt3a1 (42%) in adult mouse livers, but increased Ugt1a9 (53%) expression in adult mouse livers. In addition, the aged ER

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group tended to express less Ugt1a6, 2b1, 2b34, 2b36, 3a1, and 3a2 mRNA than aged control-feeding group, but these differences were not statistically significant. Ugt1a5 was the only Ugt that was not significantly influenced by either aging or ER (data not shown). In summary, aging decreased the mRNA levels of 9 out of 13 hepatic glucuronidation enzymes examined in the present study, whereas ER influenced the expression of 4 Ugts in the two age groups.

In contrast to most phase-I and -II enzymes, the hepatic expression of sulfation-related enzymes were not significantly influenced by aging, but by ER (Fig. 6). Due to the female-dominant expression of hepatic Sults, only a few Sults were detected in male mouse livers in this study. In aged mice, the 6-month ER markedly decreased mRNAs of Sult1a1 (33%) and 1d1 (51%), as well as 3'-phosphoadenosine-5'-phosphosulfate synthase 2 (Papss2; 32%). However, ER induced 38% of the expression of Papss2 in adult mouse livers. The hepatic expression of Papss1 was not significantly influenced by either aging or ER. In general, ER had a more predominant effect on the hepatic sulfation enzymes than did aging.

Figure 7 shows the mRNA expression of hepatic Gsts with aging and ER. Aging decreased the mRNA levels of 5 Gsts (Gsta4, m1, m4, t1, and t2) out of the 12 quantified in this study. In adult mouse livers, Gsta1, a4, m3, and Mgst3 were induced by ER, whereas, in aged mice, only Gstm3 was induced by ER. Among all the Gsts, ER increased Gsta1 and Gstm3 the most in both age groups, which were increased ~200 and ~270%, respectively. In contrast, Mgst1 was stably expressed regardless of age and energy intake. In summary, the mRNA of several hepatic Gsts was decreased during

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aging, whereas ER can increase the expression of some Gsts in adult and aged mouse livers.

Effects of aging and ER on the mRNA levels of hepatic efflux transporters. The mRNA expression of most efflux transporters was decreased by aging (Fig. 8). Approximately 50% reduction occurred at the mRNA level of the major apical efflux transporters, including multidrug resistance-associated protein 2 (Mrp2), breast cancer resistance protein (Bcrp), the half cholesterol transporters ATP-binding cassette subfamily G members 5 and 8 (Abcg5 and 8), multidrug resistance protein 2 (Mdr2), bile salt export pump (Bsep), as well as basolateral efflux transporters Mrp3 and 6. The aged mice expressed 26% less multidrug and toxin extrusion transporter (Mate) mRNA than adult mice. The mRNA of organic solute transporter β (Ost β) was expressed 74% lower in 24-month-old aged mice than in 12-month-old adult mice, but the difference was not statistically significant. The partner of Ost β , Ost α , was expressed at a very low level in liver (data not shown). For basolateral transporters, the mRNA levels of Mrp4 and cholesterol efflux transporter Abca1 were not changed during aging. Six-month of ER decreased the expression of Bsep, but increased that of Ost β in adult mouse livers. On the basolateral membrane, ER markedly increased the expression of Mrp4 only in adult mice and decreased Abca1 mRNA only in aged mice. In brief, hepatic efflux transporters were expressed less in aged mice, and ER increased the expression of Ost β and Mrp4 in adult mice, but decreased the mRNAs of Bsep and Abca1 in adult and aged mice, respectively.

Expression of drug metabolism-related transcription factors in energy-restricted

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adult and aged mouse livers. In aged mouse livers, the majority of xenobiotic and drug metabolism-related transcription factors decreased ~30% compared to adults. These transcription factors are the constitutive androstane receptor (CAR), peroxisomal proliferator-activated receptor α (PPAR α), nuclear factor erythroid 2-related factor 2 (Nrf2), and hepatocyte nuclear factor 4 α (HNF-4 α). The pregnane X receptor (PXR) tended to be expressed lower in the livers of aged control or ER mice compared to corresponding adult groups. The senescence marker regucalcin (Rgn) was also decreased ~30% in 24-month-old aged mouse livers compared to 12-month-old adult mice. In comparison to the aforementioned transcription factors, the mRNAs of retinoid X receptor α (RXR α) and aryl hydrocarbon receptor (AhR) were not changed by aging, but were decreased 30% and 57% by ER, respectively, only in aged mouse livers. ER also significantly decreased the mRNA expression of HNF4 α in both age groups. In summary, the expression of hepatic drug metabolism-related transcription factors was lower in aged than adult mice, whereas long-term ER decreased the mRNAs of HNF4 α in adult mice, as well as RXR α and HNF4 α in aged mouse livers.

Discussion

Aging is a biological process characterized as a time-dependent functional decline in various tissues, including liver. Aged cells lose the capability to withstand external and internal challenges, leading to increased morbidity and mortality (Yu, 1996). Energy restriction has been shown to exert various beneficial effects in attenuating aging and age-related diseases (Anderson and Weindruch, 2007; Anderson et al., 2009). However, studies of ER effects on liver functions are either general, such as the whole genome profiling of hepatic gene expression (Cao et al., 2001), or focus on energy metabolism, such as glucose metabolism (Hagopian et al., 2003), or several specific drug metabolizing enzymes (Agrawal and Shapiro, 2003). The current study was designed to reveal how aging influences the hepatic drug processing system and whether long-term energy restriction can counteract the changes caused by aging.

A hierarchical analysis clearly shows that the majority of hepatic DPGs decreased at the mRNA level in aged mouse livers compared to adult mice (Fig. 10). These results in mice are consistent with those in aged rats characterized by full-genome Affymetrix arrays (Lee et al., 2008). Among 79 DPGs examined in the current study, 52 were expressed significantly lower in aged than in adult mouse livers. These genes encode enzymes involved in phase-I and phase-II drug metabolism, as well as transporters that function in the uptake and efflux of xenobiotics in hepatocytes. The reduced expression of these fundamental genes in liver provides a molecular explanation for the diminished hepatic drug processing capability in the elderly described previously (Klotz, 2009).

The expression of DPGs is regulated by multiple transcription factors, which are

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considered critical in xenobiotic pharmacokinetics and toxicity. For example, the activation of nuclear receptor CAR by chemicals like phenobarbital, an anticonvulsant drug, induces various enzymes and transporters, thereby increasing the hepatic activities of drug metabolism and transport (Kodama and Negishi, 2006). CAR target genes include phase-I enzymes (e.g., Cyp2b, 2c, and 3a), phase-II enzymes (e.g., Sults, Ugts, and Gsts) (Ueda et al., 2002), as well as transporters such as Mrp2 and Mrp4 (Kast et al., 2002; Assem et al., 2004). Similar to CAR, PXR (Timsit and Negishi, 2007), AhR (Nebert et al., 2004), Nrf2 (Nguyen et al., 2003), PPAR α (Barbier et al., 2004), HNF4 α (Chiang, 2009), and RXR α , the common heterodimer partner for many nuclear receptors (Brtko, 2007), can also be activated by certain drugs and accordingly regulate the expression of a battery of hepatic DPGs. PPAR α can be activated by a large class of drugs, such as fibrates (Barbier et al., 2004). Interestingly, the knock-out mouse model revealed that PPAR α is important in maintaining longevity, delaying various nonneoplastic spontaneous aging lesions, and preventing the onset of hepatocellular carcinomas (Howroyd et al., 2004).

As shown by hierarchical analysis (Fig. 11), the mRNA expression of these transcription factors, except for AhR, decreases in aged mouse livers, which might account for the declined basal expression of hepatic DPGs in aged mice. Given the fact that numerous therapeutic drugs and xenobiotics can activate these transcription factors, the diminished expression of these transcription factors might lead to altered drug response and processing capability in aged mice.

Due to its well-known anti-aging function, the effect of ER on these hepatic DPGs was

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investigated in aged mice. Surprisingly, the 6-month ER only influenced very few DPGs (11 out of 79 DPGs) in aged mouse liver. More interestingly, the majority (9 out of 11) of the changed genes were decreased instead of being increased by ER. These genes encode the apical aminophospholipid flippase Atp8b1, phase-I enzymes Aldh6a1 and Pon3, phase-II enzymes Ugt1a1, 2a3, Sult1a1, 1d1, and Papss2, as well as basolateral efflux transporter Mrp6. Of note, mRNAs of 3 out of the 4 quantified sulfation-related enzymes were decreased by ER, suggesting that ER might reduce hepatic sulfation capability in aged mouse livers. The only two genes that were increased by ER at the mRNA level in aged mice were the phase-I enzyme Ces1d1 and the phase-II enzyme Gstm3. Based on these data, ER is not capable to compensate for the age-induced decrease in hepatic DPG expression, at least at the mRNA level.

In adult mice, ER increased the mRNA levels of 10 hepatic DPGs and decreased 5 others. Of note, except for Gstm3, the hepatic DPGs changed by ER in adult mice are different from those in aged mice. ER enhanced the expression of phase-I enzymes Cyp2b10 and 4a14, but suppressed Ces3a2 in adult mice. ER might enhance glutathione conjugation in adult mice as the mRNA expression of several Gsts (Gsta1, a4, m3, and Mgst3) was increased after 6-month ER, but had less of an effect on sulfation (increased Papss2) and glucuronidation (decreased Ugt3a1) in adult mouse livers. For basolateral transporters, ER increased the mRNA of uptake transporter Oatp1a4 and efflux transporter Mrp4, but decreased those of Oatp1a1 and 1b2. For apical transporters, the bile acid efflux transporters Bsep and Ost β were decreased and increased by ER, respectively. Due to the important physiological roles of these hepatic transporters (Kusuhara and Sugiyama, 2009), altered disposition of endo- and

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xenobiotics is expected in adult mice undergoing ER. In summary, ER has a significant influence on some hepatic DPGs and could influence hepatic drug metabolism in adult mice.

Hepatic transcription factors might play a role in different ER-induced expression profiles of the aforementioned hepatic DPGs in adult and aged mice. For example, Cyp2b10 mRNA is increased by ER only in adult mice, but not in aged mouse livers (Fig. 2), which corresponds with high and low expression levels of CAR in adult and aged mice, respectively (Fig. 9). CAR was previously reported to be activated by fasting and energy restriction in mice, and prevented ER-induced weight loss by decreasing circulating thyroid hormone (Maglich et al., 2004). Other transcription factors, such as PPAR α (Corton et al., 2004), which responds to both energy status and drugs in the liver, could also mediate the ER-induced changes in hepatic DPGs. Although the transcription factors themselves were not significantly influenced by ER at the mRNA level (Fig. 9), ER could influence hepatic drug metabolism through the activation of numerous hepatic transcription factors.

In addition to the changes of mRNA expression of hepatic drug metabolism genes revealed in mice by the current study, investigators have shown protein expression and activities of various hepatic enzymes and transporters in aged and/or energy-restricted rats. Although little reduction was observed in the activity of most Cyp isoforms in aged male rats, the male-dominant Cyp2c11 isoform decreased ~70% in mRNA, protein, and catalytic activity between 5- and 23-month of age (Agrawal and Shapiro, 2003). Because Cyp2c11 comprises approximately 50% of total hepatic P450s in male rats, a

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dramatic decline in drug processing capacity would be expected in aged male rats. In humans, age-associated decrease in the predominant human isoform CYP3A4/5 may account for the decline in the metabolism of many drugs in the elderly (Hunt et al., 1992).

Exposure to certain xenobiotics is known to induce numerous hepatic DPGs. Low-dose phenobarbital treatments (1 mg/kg and 10 mg/kg) induced the expression of Cyp2b1, 2b2, and 3a1 to the same degree in young and aged rats (Agrawal and Shapiro, 2003). However, exposure to toluene (1 g/kg) decreased the transcription of more phase-II enzymes and efflux transporters in 24- than in 4-month old rats (Lee et al., 2008). Long-term caloric restriction induced several Cyp isoforms in rats (Leahey et al., 1989b), and attenuated the age-related decrease in Gst activity towards 1,2-dichloro-4-nitrobenzene, but had no effect on age-related changes in Ugt and Sult activities towards hydroxysteroids in aged rats (Leahey et al., 1989a). These studies suggest that aging and ER might alter the hepatic metabolism of some chemicals but not others.

Taken together, aging reduces the mRNAs of the majority of hepatic DPGs and xenobiotic-related transcription factors, which might lead to decreased hepatic drug processing capability in aged mice. In comparison, ER only increases or decreases the expression of a small portion of hepatic enzymes and transporters in an age-dependent manner. Thus, ER is not sufficient to counteract the effects of aging on hepatic drug metabolism gene expression. However, ER could influence the disposition of certain drugs in mice through its influences on the expression of certain hepatic DPGs. Results generated by this study suggest that age and dietary habit can both influence hepatic

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drug processing system in mice. Hypothesizing that similar influences occur in human, then age and dietary habit should be taken into consideration for clinical drug prescriptions.

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Footnotes

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Figure Legends

Fig. 1. Effects of aging and energy restriction on the mRNA levels of uptake transporters in mouse livers. Adult groups were 12-month of age and aged groups were 24-month of age. C, control; ER, energy restricted. Data are expressed as mean \pm SE of 5 mice per group. *, statistically different from same-age control group; †, statistically different from adult mice on the same diet. $p \leq 0.05$, one-way ANOVA followed by Duncan's test. The same illustrations are applied to the following figures.

Fig. 2. Effects of aging and energy restriction on the mRNA expression of cytochrome P450s (Cyps) in mouse livers.

Fig. 3. Effects of aging and energy restriction on the mRNA levels of carboxylesterases (Ces's) in mouse livers.

Fig. 4. Effects of aging and energy restriction on the mRNA expression of aldehyde dehydrogenases (Aldhs) and paraoxonases (Pons) in mouse livers.

Fig. 5. Effects of aging and energy restriction on the mRNA expression of enzymes involved in hepatic glucuronidation in mouse livers.

Fig. 6. Effects of aging and energy restriction on the mRNA expression of sulfotransferases (Sults) and PAPS synthases (Papss) in mouse livers.

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Fig. 7. Effects of aging and energy restriction on the mRNA expression of glutathione S-transferases (Gsts) in mouse livers.

Fig. 8. Effects of aging and energy restriction on the mRNA expression of efflux transporters in mouse livers.

Fig. 9. Effects of aging and energy restriction on the mRNA expression of drug metabolism-related transcription factors in mouse livers.

Fig. 10. Hierarchical cluster analysis of effects of aging and energy restriction on mRNA levels of drug processing genes in mouse livers. Each column represents an age-feeding group, and each row shows a particular gene with its name indicated on the right. The darkness of the region is positively correlated with the expression level. Each spectrum is specific to the scale of mRNA expression for each gene on an individual basis. Adult, 12-month old; Aged, 24-month old; C, control; and ER, energy restricted. The same illustrations are also applicable to Fig. 11.

Fig. 11. Hierarchical cluster analysis of effects of aging and energy restriction on the mRNA expression of hepatic transcription factors related to drug metabolism.

Figure 1

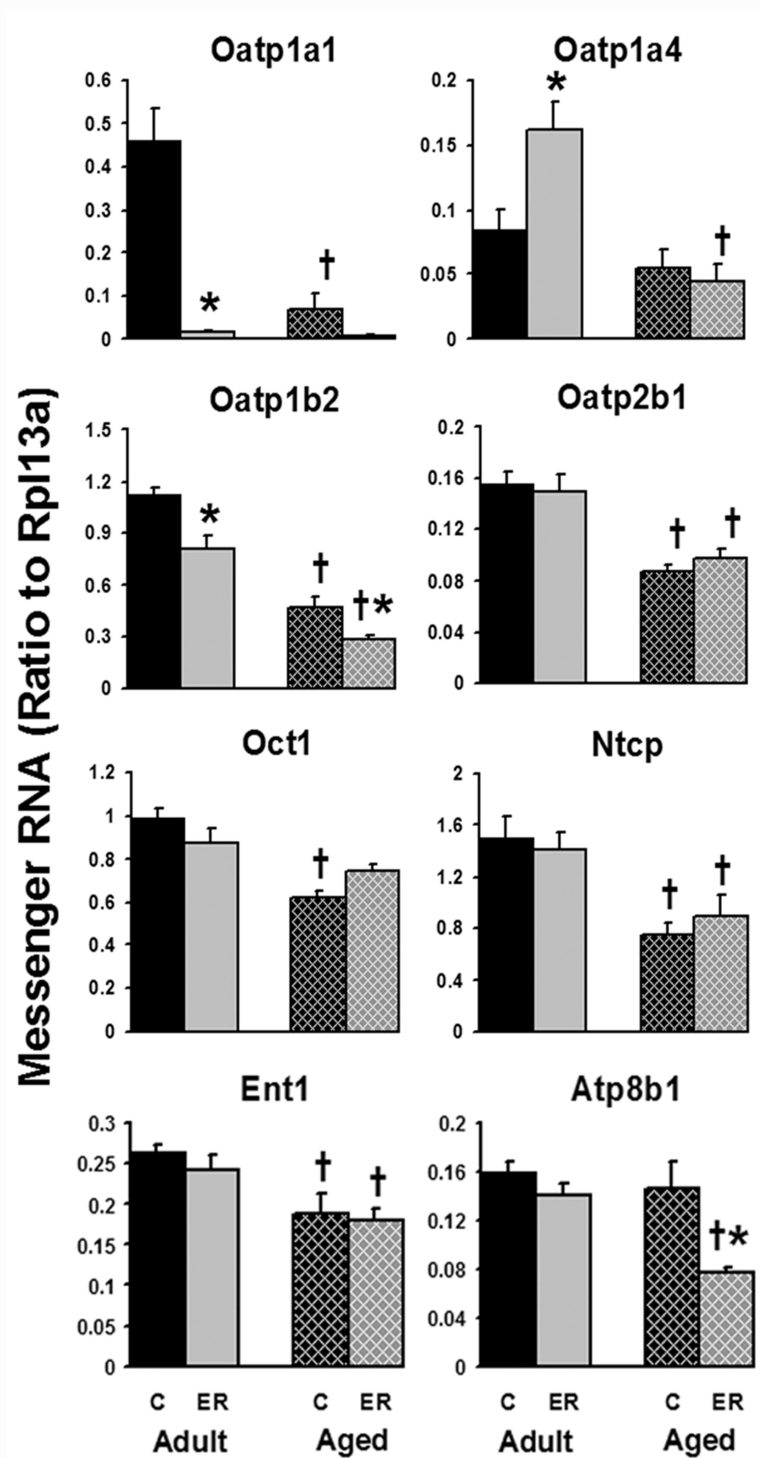


Figure 2

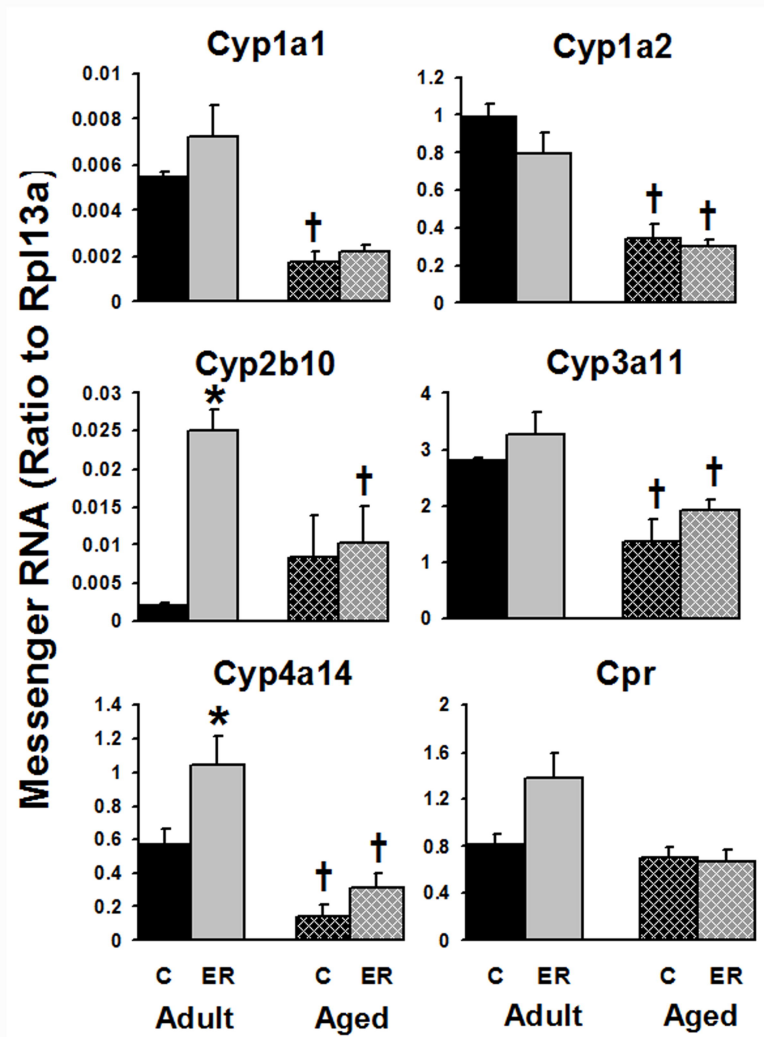


Figure 3

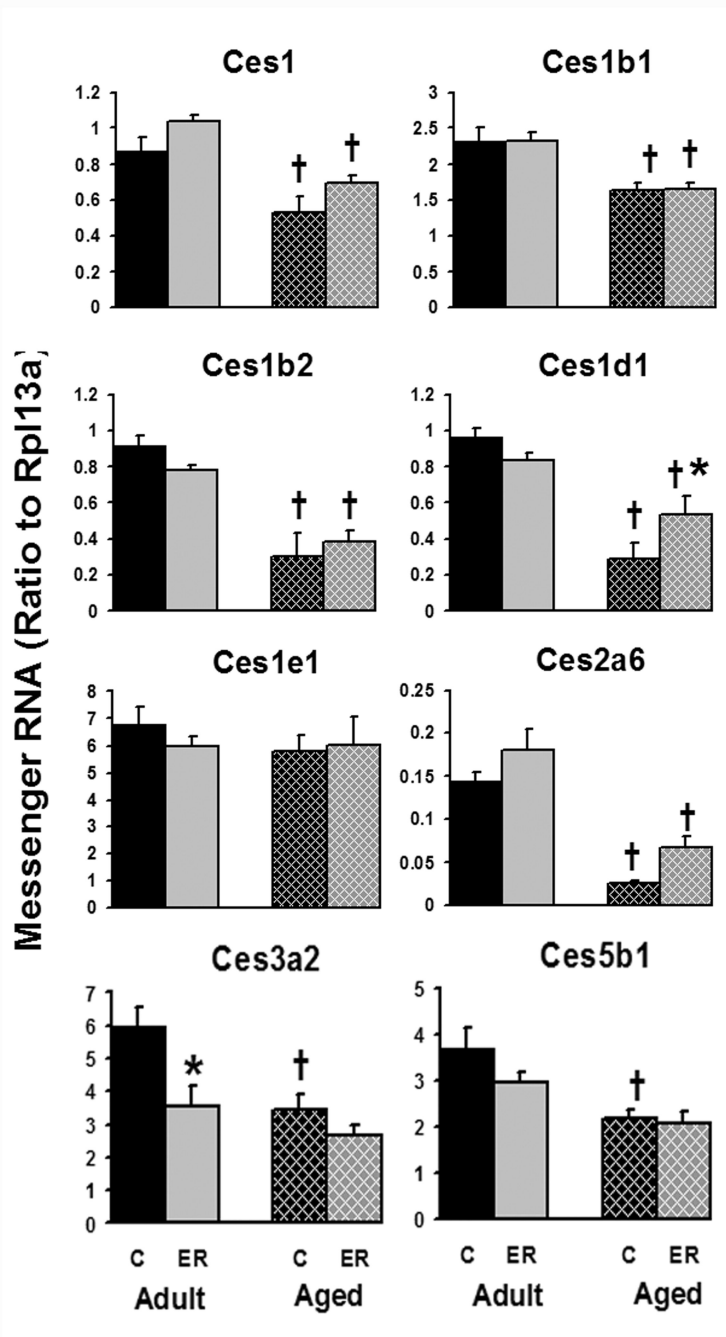


Figure 4

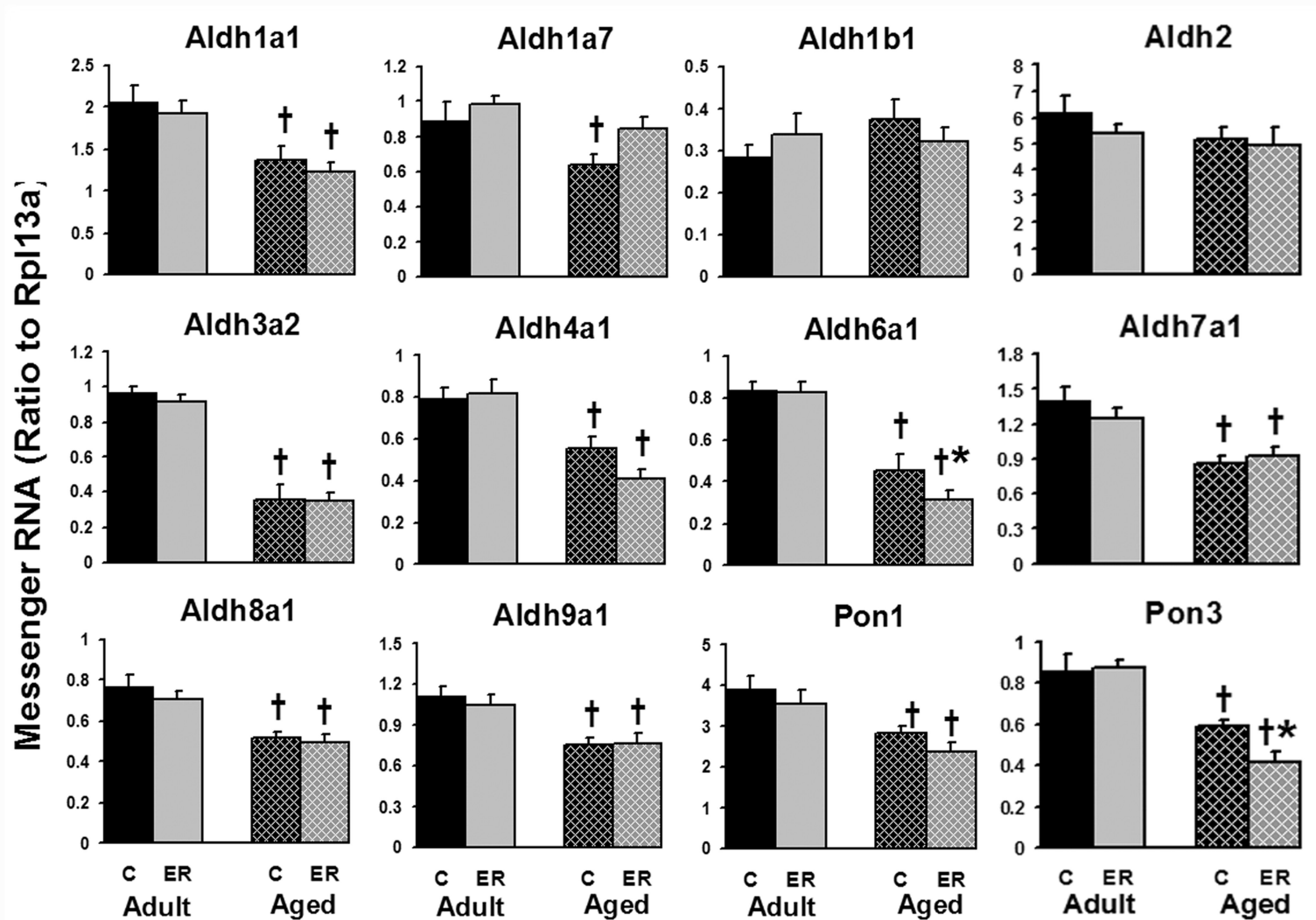


Figure 5

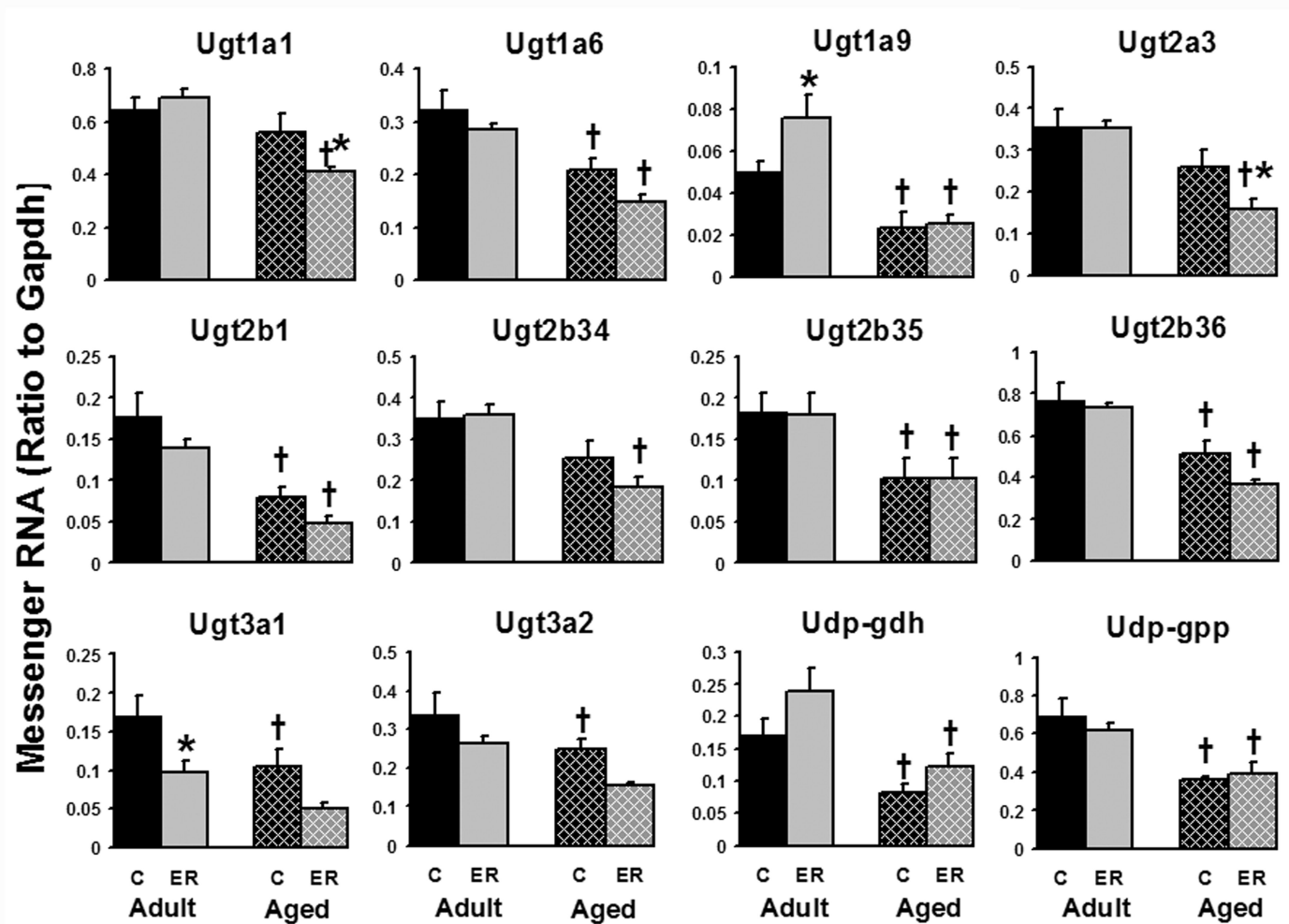


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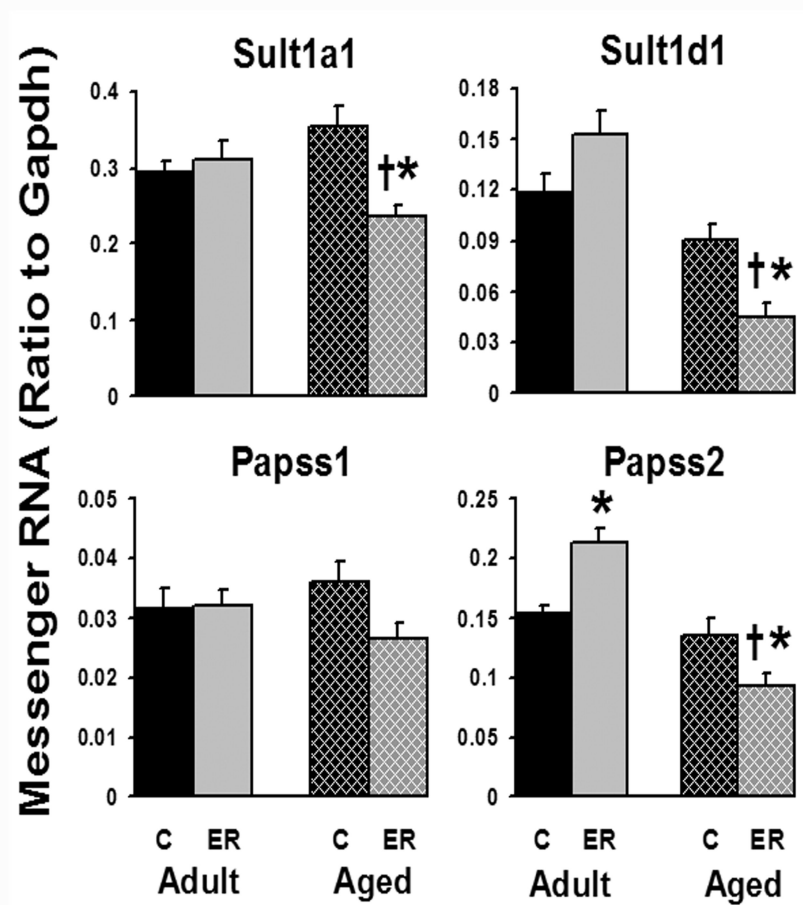


Figure 7

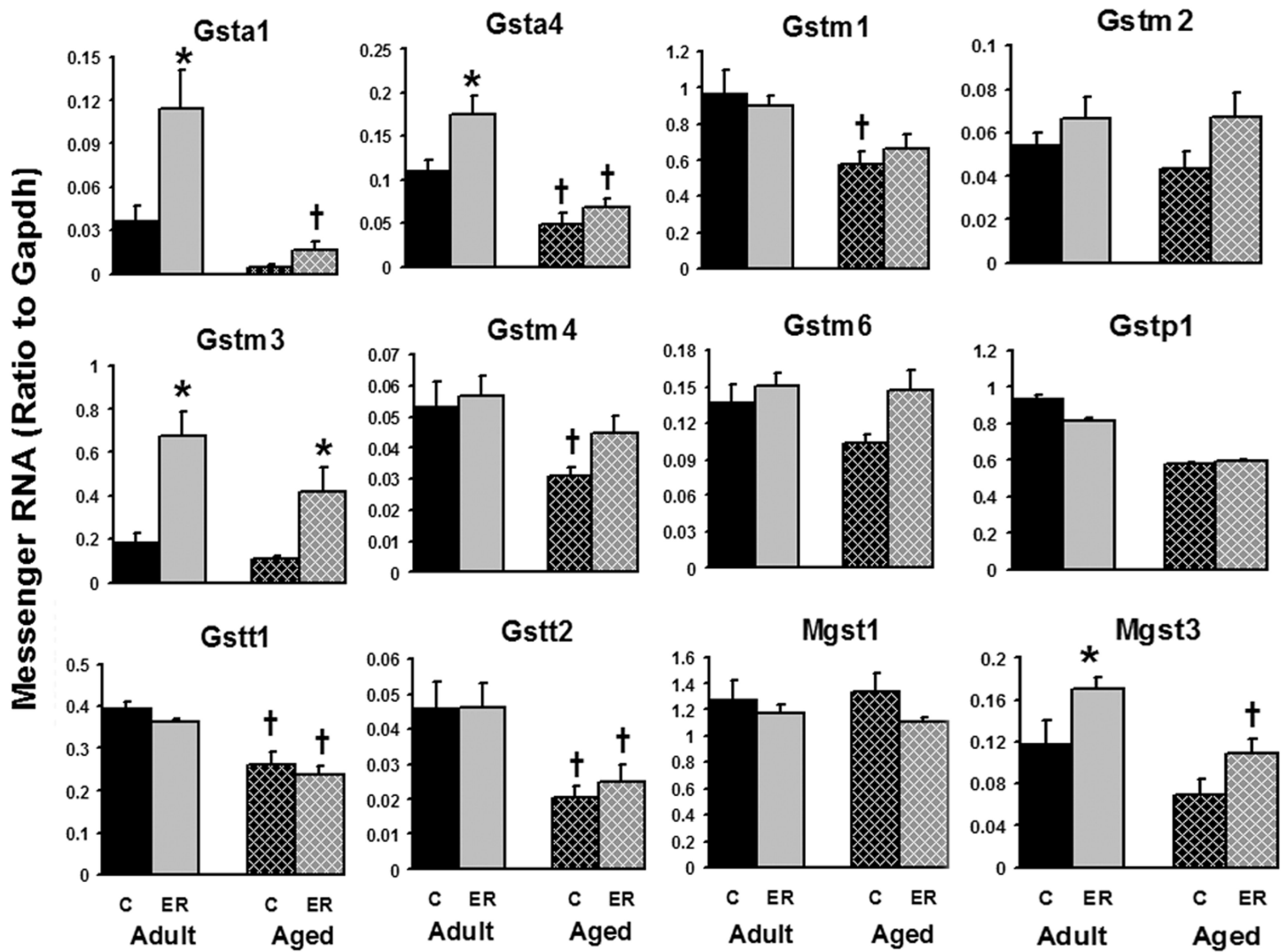


Figure 8

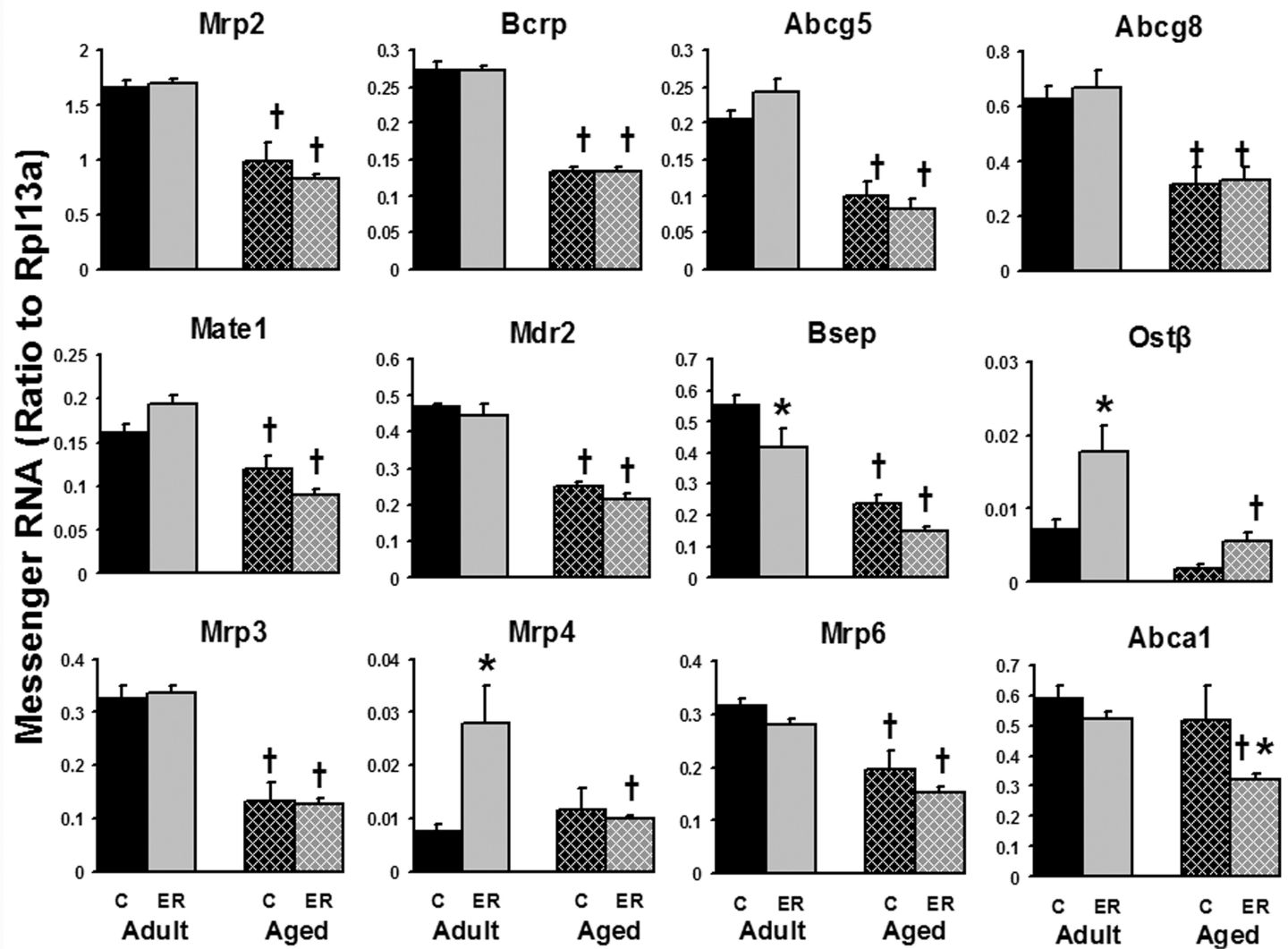


Figure 9

Messenger RNA (Ratio to Rpl13a)

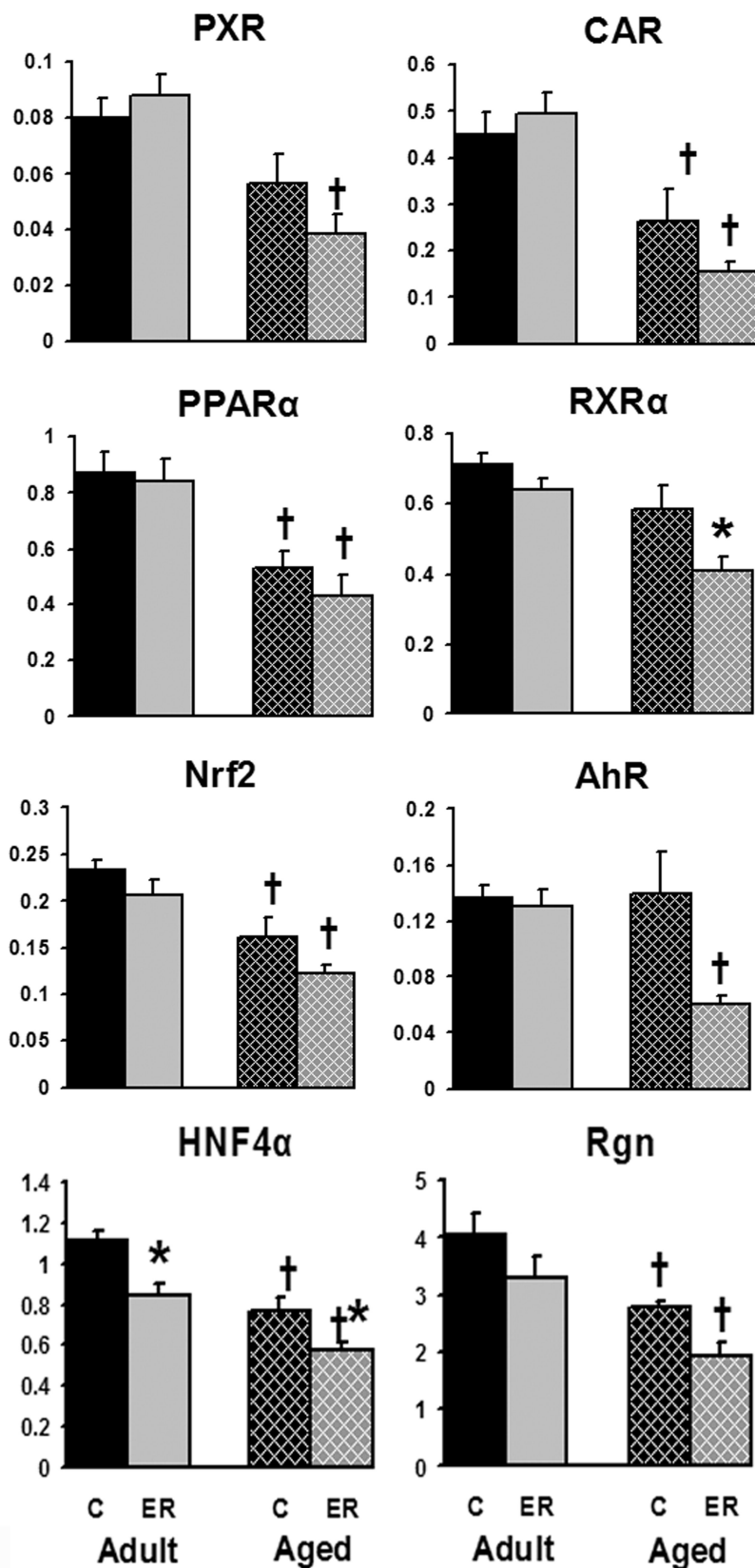


Figure 10

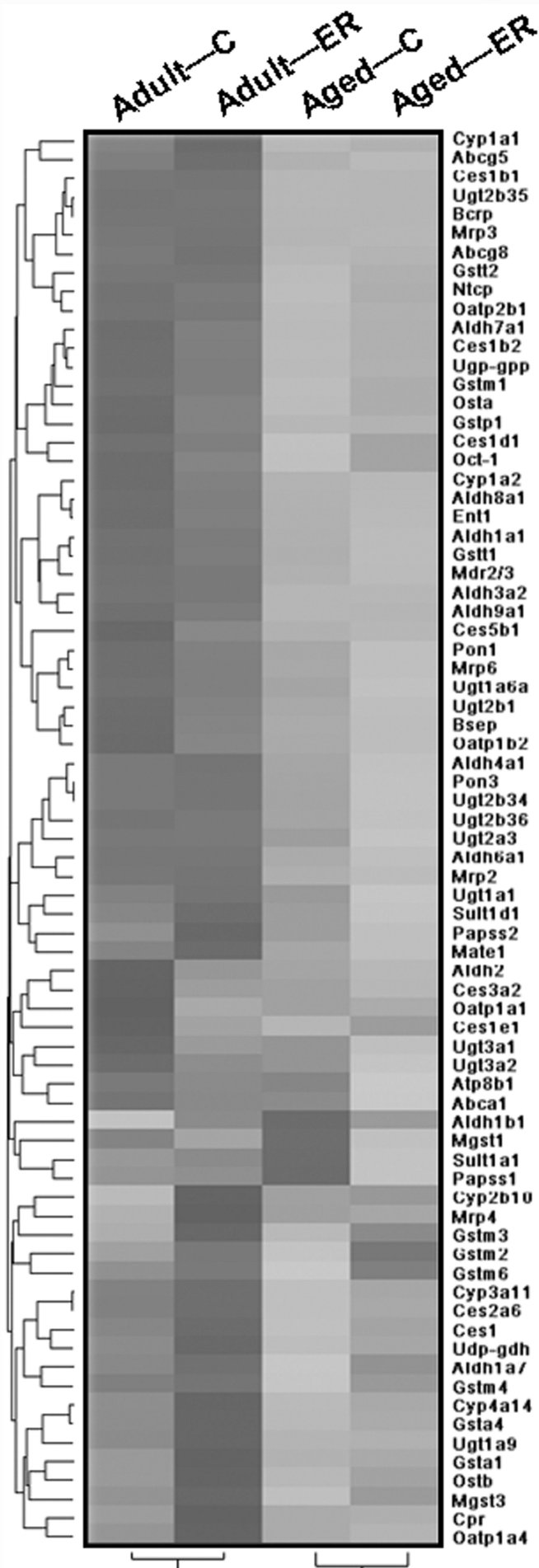


Figure 11

