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Vitamin E Status and Metabolism in Adult and Aged Aryl Hydrocarbon Receptor Null Mice

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Abstract

The aryl hydrocarbon receptor (AhR) is involved in regulation of mechanisms for detoxification of xenobiotics, as well as vitamin A metabolism. Vitamin E is a fat-soluble nutrient whose metabolism is initialized via the cytochrome P450 system. Thus, AhR absence could alter hepatic regulation of α -tocopherol metabolism. To test this hypothesis, we assessed vitamin E status in adult (2–5 m) and old (21–22 m), wildtype and AhR-null mice. Plasma α -tocopherol concentrations in AhR null mice (2.3 ± 1.2 $\mu\text{mol/L}$, $n = 19$) were lower than those of wildtype mice (3.2 ± 1.2 , $n = 17$, $P = 0.0131$); those in old mice (3.2 ± 1.2 , $n = 20$) were higher than those of adults (2.2 ± 1.0 , $n = 16$, $p = 0.0075$). Hepatic α -tocopherol concentrations were not different between genotypes, but were nearly double in old (32 ± 8 nmol/g, $n = 20$) as compared with adult mice (17 ± 2 , $n = 16$, $p < 0.0001$). Hepatic Cyp3a concentrations in AhR-null mice were greater than those in wildtypes ($p = 0.0011$). Genotype ($p = 0.0047$), sex ($p < 0.0001$) and age ($p < 0.0001$) were significant modifiers of liver α -tocopherol metabolite (α -CEHC) concentrations. In general, Cyp3a concentrations correlated with hepatic α -tocopherol ($r = 0.3957$, $p < 0.05$) and α -CEHC ($r = 0.4260$, $p < 0.05$) concentrations. Since there were no significant genotype differences in the hepatic α - or γ -tocopherol concentrations, AhR null mice did not have dramatically altered vitamin E metabolism. Since they did have higher hepatic α -CEHC concentrations, these data suggest metabolism was up-regulated in the AhR null mice in order to maintain the hepatic tocopherol concentrations similar to those of wildtypes.

Keywords

alpha tocopherol; gamma tocopherol; carboxyethyl hydroxychromanol (CEHC); xenobiotic metabolism

INTRODUCTION

Vitamin E includes the antioxidant molecules, tocopherols and tocotrienols, and has the general structure of a chromanol ring with a side chain. Tocopherols and tocotrienols include α , β , γ , and δ isomers, which differ by the number of methyl groups on the chromanol ring. The degree

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of saturation in the side chain distinguishes tocopherols from tocotrienols; tocotrienols have three double bonds, whereas tocopherols have a saturated side chain (phytyl tail) [1]. α -Tocopherol is preferentially retained by the body, largely as a function of the hepatic α -tocopherol transfer protein, while other vitamin E forms are actively metabolized and excreted [1].

Vitamin E is the only fat-soluble vitamin that does not accumulate to toxic levels in the body, likely as a result of mechanisms of hepatic xenobiotic metabolism [2]. Metabolism occurs via ω -oxidation of the side chain, followed by several rounds of β -oxidation. The major metabolite of vitamin E, carboxyethyl hydroxychroman (CEHC), is then excreted in the urine [3] and/or bile [4]. The ω -oxidation of the vitamin E side chain is catalyzed by a cytochrome P450 (CYP) protein. Both CYP3A and 4F have been identified as possible candidates for this step [5–8]. The regulation of vitamin E metabolism remains unclear because CYP4F, although shown to ω -hydroxylate the tocopherol side chain [6,7], CYP4F protein did not increase as measured by western blots in response to increased liver concentrations of either α -tocopherol in rats [9, 10] or γ -tocopherol in mice [11]. In contrast, the amount of Cyp3a protein was correlated with hepatic α -tocopherol concentrations in mice fed high γ -tocopherol-diets [11], while in vitamin E-injected rats CYP3A protein, not CYP4F protein, was increased with increased hepatic α -tocopherol [9,10].

The pregnane X receptor (PXR) is an up-stream regulator of various xenobiotic pathways [12], including regulation of CYP3A [13]. Initially, non- α -tocopherol forms of vitamin E were thought to mediate vitamin E metabolism through PXR because tocotrienols, compared with tocopherols, were more effective at binding to PXR in an in vitro assay [8]. However, the Cyp3a gene increased in mice consuming a high α -tocopherol diet [14,15] a phenomenon that was not observed with tocotrienol feeding [14], suggesting that PXR was not involved in regulating vitamin E metabolism.

The Aryl Hydrocarbon Receptor (AhR) is also involved in controlling xenobiotic metabolism [16]. AhR is a cytoplasmic, ligand-activated receptor that binds numerous endogenous and exogenous compounds [17]. Certain xenobiotic molecules, for example polycyclic aromatic hydrocarbons and some dioxins, have particularly strong affinities for the AhR. Once bound, the activated AHR moves into the nucleus of a cell, where it forms a heterodimer with the AhR nuclear translocator (Arnt). Together, AhR and Arnt bind to the xenobiotic-responsive element (XRE) and induce the transcription of xenobiotic metabolizing enzymes [17]. While AhR is most often associated with control of CYP1A and 1B proteins, treatment with the AhR ligand, Sudan III, decreased basal and clofibric acid-induced CYP2B, CYP3A and CYP2C11 protein, activities and mRNA expression [18]. Thus, the lack of the AhR may alter xenobiotic metabolism, as well as increase cross-talk between various downstream xenobiotic responses [19].

Previously, AhR-null mouse livers were shown to be nearly half the size of livers from wildtype mice. AhR-null mouse livers exhibited abnormal hepatic vasculature and fibrosis [20]; the hepatic fibrosis increased with age [21]. Importantly, AhR-null mouse livers contained elevated vitamin A concentrations, specifically, retinoic acid, retinol, and retinyl palmitate [22]. These alterations in vitamin A metabolism were shown to be a cause of the liver fibrosis in that vitamin A depletion prevented fibrosis in Ahr-null mice [23]. Subsequently, vitamin A metabolism was found to be under AhR-mediated regulation of Cyp2c39 [24].

Given the role of the AhR in regulation of the metabolism of a fat-soluble vitamin, and the lack of clarity in the regulation of vitamin E metabolism, the objective of this study was to determine the ability of AhR status to alter vitamin E concentrations and metabolism, in adult and old mice, and the extent to which this ability differs according to gender. We hypothesized that (1)

tissue α -tocopherol levels will be increased in AhR-null mice compared to wild type mice due to a decrease in vitamin E metabolism in AhR-null mice, and (2) the difference in tissue α -tocopherol levels between AhR-null mice and wild type mice would increase with age as vitamin E concentrations accumulate over time.

MATERIALS AND METHODS

Animal Protocol

Male and female AhR-null mice (B6.129-AhR^{tm1Bra}/J) and age and gender-matched wild type littermates (C57B1/6J; provided by Jackson Laboratories, Bar Harbor, ME, maintained by Laboratory Animal Research Center, Oregon State University, Corvallis, OR) were maintained on a 12 hour light/dark schedule and fed Harlan Teklad rodent diet 8604 (vitamin E (*all rac*- α -tocopheryl acetate, 90 IU/kg diet; no γ -tocopherol was added)) throughout their lives; food and water were given *ad libitum*. Old mice ages were between 21 and 22 months old; adult mice ages were between 2 and 5 months. Mice were not fasted. They were sedated with CO₂ gas, followed by exsanguination. Blood samples were collected with ethylene diamine tetra acetic acid (EDTA) and placed on ice. Plasma was obtained by centrifugation of blood samples for 10 min at 1500 $\times$ g (4 °C), frozen in liquid nitrogen, and stored at -80 °C until analysis. Tissues were quickly removed, rinsed and dried, then frozen in liquid nitrogen and stored at -80 °C until analysis.

Plasma Cholesterol and Triglyceride Measurements

Plasma total cholesterol and triglyceride concentrations were determined using Infinity Reagent Kits (Thermo Electron, Melbourne, Australia) and detected using a Beckman DU[®] 640 spectrophotometer. Plasma total lipids (mmol/L) are calculated as the sum total cholesterol and triglycerides.

Tissue cholesterol measurements were determined in hexane extracts (following tissue saponification as described for vitamin E below) using the Amplex[®] Red Cholesterol Assay Kit (Invitrogen Molecular Probes, Eugene, OR) and detected using an Applied Biosystems Cytofluor multi-well plate reader, series 4000 (Foster City, CA).

Vitamin E and CEHC analyses

Tissue and plasma α - and γ -tocopherols were measured using a modification of the method described [25]. Tissue samples (~50 mg) or plasma samples (20 μ l) were saponified with alcoholic potassium hydroxide with 1% ascorbic acid. Vitamin E was extracted in hexane and measured using a C18 isocratic reverse phase column with a Shimadzu HPLC and electrochemical detection. Tocopherols were quantitated by comparison to standard curves generated from peak areas of known amounts of authentic standards.

Mouse liver CEHC concentrations were isolated from liver homogenates following addition of an internal standard (trolox), as described previously [26]. Briefly, following homogenization, CEHCs were hydrolyzed with β -glucuronidase/sulfatase for 60 min at 37°C, then CEHCs were extracted in diethyl ether, an aliquot dried under N₂ and the residue resuspended in 1:1 H₂O:methanol. Extracted α - and γ -CEHCs were analyzed by a reverse phase liquid chromatography method using a gradient of (A) methanol or (B) H₂O, each containing 0.1% acetic acid. CEHCs were detected using a Micromass (Manchester, England) ZQ 2000 single-quadrupole MS with an electrospray ionization source. Single ion recording (SIR) mass-to-charge (m/z) ratios were obtained for the ions of interest. Quantitation was performed using external standards with trolox as an internal standard. The working linear range is 0.2 to 20 pmol CEHC injected with a low limit of detection of 0.08 pmol injected.

Western blot analyses

Tissue samples (~50 mg) were homogenized on ice in 1 mL ice-cold RIPA buffer (50 mM Tris-HCl, pH 8.0, 150 mM NaCl, 0.01% SDS, 0.5% sodium deoxycholate [wt/vol], 1% Igepal [vol/vol]) with freshly added protease inhibitor cocktail (Calbiochem, San Diego, CA) using a Potter-Elvehjem-type homogenizer. Homogenates were centrifuged for 15 min. at $1,000 \times g$ (4°C) and supernatants were stored at -80 °C for western blot analysis.

Total protein concentrations were determined using Coomassie Plus reagent, according to manufacturer's instructions (Pierce, Rockford, IL). Equal amounts of protein were added to lauryl dodecyl sulfate (LDS) loading buffer (Invitrogen, Carlsbad, CA), denatured at 70° C for 10 minutes, and resolved by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) using 10% Bis-Tris gels (Invitrogen). Proteins were transferred to a polyvinylidene fluoride membrane (Invitrogen). Blots were blocked in Tris-buffered saline with 0.05% Tween-20 (TBST) and 3% non-fat milk for 2 hours at room temperature. Fresh TBST with 3% non-fat milk and primary antibody was incubated on membranes overnight at 4°C at the following concentrations: Cyp3A2 (Chemicon, Temecula, CA) 1:4000, Cyp4F2 (a generous gift from Jerome M. Lasker, Institute for Biomedical Research, Hackensack University Medical Center), and actin (Santa Cruz) 1:1500. Blots were washed three times in TBST and incubated with the appropriate near infrared fluorophore-conjugated secondary antibody (Li-Cor Biosciences, Lincoln, NE) for 45 min at room temperature, protected from light. Blots were again washed three times with TBST. Protein bands were detected and quantified using a Li-Cor® Odyssey® infrared imaging system and the analysis software provided (Li-Cor Biosciences). Cyp protein levels in each sample were normalized to their respective actin protein levels. Insect microsomes expressing the specific protein of interest, i.e., CYP3A or CYP4F, were run on each gel as a positive control.

Statistical Analysis

Data are expressed as mean $\pm$ SD. ANOVA was performed using JMP Statistical Discovery Software (SAS Institute, Cary, NC) to evaluate effects attributed to age, genotype and sex. Results for each age, genotype and sex group are reported in the tables. If a three-way, or two-way interaction was found to be statistically significant, then a Tukey's HSD (Honestly Significant Differences) Test was performed. Where no overall significant interaction was found, main effects and associated means $\pm$ SD are reported in the text. The results were considered to be statistically significant at $P < 0.05$.

RESULTS

Body and liver weights

Body weights of old AhR null (33 ± 8 g, $n=11$) and old wildtype (38 ± 10 , $n=9$) mice were greater than those of adult AhR null (25 ± 3 , $n=8$) and adult wildtype (21 ± 5 , $n=8$) mice (age $\times$ genotype interaction ($p=0.038$); Tukey HSD $p < 0.05$). Males (35 ± 10 , $n=17$) weighed more than females (26 ± 7 , $n=19$) (age effect, $p < 0.0001$, Table 1).

Liver weights were not significantly different between genotypes (Table 1). Livers from old mice (2.0 ± 1.0 g, $n=20$) were larger than those from adult mice (1.1 ± 0.2 , $n=16$, age effect, $p < 0.0002$); livers from male mice (2.0 ± 1.1 , $n=17$) were larger than those from female mice (1.2 ± 0.4 , $n=19$, sex effect, $p < 0.0019$). However, when liver weights were expressed per body weight, AhR null mice (47 ± 15 mg/g, $n=19$) had smaller livers per body weight than did wildtype mice (56 ± 9 , $n=17$, $p=0.0158$, genotype effect). This is expected based on AhR null effects on hepatic vasculature limiting liver growth [20].

Hepatic cholesterol concentrations were not statistically different between genotypes whether expressed per gram of liver or for the entire liver tissue (Table 1). Liver cholesterol concentrations ($\mu\text{mol/g}$ liver) were lowest in adult males (0.82 ± 0.07) compared with old males (1.86 ± 0.35), adult females (1.76 ± 0.43) or old females (1.61 ± 0.27 ; age $\times$ gender interaction ($p=0.0001$), Tukey HSD, $p<0.05$). When expressed per liver, old males (4.9 ± 2.3 μmol) had the highest cholesterol values compared with adult males (1.1 ± 0.2), adult females (1.6 ± 0.4) or old females (2.4 ± 0.8 , age $\times$ gender interaction ($p=0.0016$), Tukey HSD, $p<0.05$).

Taken together these data show that old female AhR mice were smaller than old wildtype mice, Relative to body size, livers from AhR null mice were smaller than wildtype livers. Neither of these factors had an effect on hepatic cholesterol concentrations. Females and old male mice had the highest cholesterol concentrations; old males had the most cholesterol per whole liver.

Plasma Vitamin E and Lipid Concentrations

Plasma α - and γ -tocopherol concentrations in AhR null and wildtype, adult and old, male and female mice were measured to assess vitamin E status (Table 2). Plasma α -tocopherol concentrations in AhR null mice (2.3 ± 1.2 $\mu\text{mol/L}$, $n=19$) were less than those of wildtype mice (3.2 ± 1.2 , $n=17$, genotype effect, $p=0.0131$). Additionally, plasma α -tocopherol concentrations in adult mice (2.2 ± 1.0 , $n=16$) were less than those in old mice (3.2 ± 1.2 , $n=20$, age effect, $p=0.0075$). Plasma γ -tocopherol concentrations in old mice (0.14 ± 0.06 , $n=20$) were almost double those of adult mice (0.08 ± 0.04 , $n=16$, age effect, $p=0.0015$).

Plasma lipids are often used to correct variations in circulating vitamin E concentrations [27]. Cholesterol concentrations (mmol/L) were higher in old mice (1.9 ± 1.0 , $n=20$) than in adult mice (1.4 ± 0.6 , $n=16$, $p=0.0019$, age effect); higher in male mice (2.3 ± 0.8 , $n=17$) than in female mice (1.1 ± 0.3 , $n=19$, sex effect, $P<0.0001$, Table 2). Triglyceride concentrations (mmol/L) were approximately double in old wildtype (2.1 ± 1.0 , $n=9$) compared with adult wildtype (1.3 ± 0.3 , $n=8$), old AhR null (1.0 ± 0.2 , $n=11$) or in adult AhR null mice (1.0 ± 0.1 , $n=8$, Tukey, HSD, $p<0.05$, Figure 1). Plasma lipids (sum of the triglyceride and total cholesterol concentrations) (mmol/L) were lower in AhR null mice (2.6 ± 0.8 , $n=19$) compared with wildtype (3.5 ± 1.2 , $n=17$, genotype effect, $p=0.0003$); lower in adult (2.6 ± 0.7 , $n=16$) compared with old (3.3 ± 1.2 , $n=19$, age effect, $p=0.0003$); and lower in female (2.5 ± 1.0 , $n=19$) compared with male mice (3.5 ± 0.9 , $n=17$, sex effect, $p<0.0001$).

When normalized to plasma lipid concentrations, plasma α -tocopherol per lipids showed a significant age $\times$ sex $\times$ genotype interaction ($p<0.05$), but differences were not sufficiently large to reach statistical differences for paired comparisons (Table 2). Plasma γ -tocopherol per lipids did not reach any statistical differences.

Taken together these data show that plasma α -tocopherol and triglycerides increase with age in wildtype mice, but only α -tocopherol increases in the AhR null mice (Figure 1). Thus, the old AhR mice compared with their adult counterparts (Tukey, HSD, $p<0.05$) have nearly double the plasma α -tocopherol when expressed per triglycerides (age $\times$ genotype, $p=0.019$).

Tissue Vitamin E Concentrations

Liver— α -Tocopherol concentrations expressed per gram of liver were nearly double in old mice (32 ± 8 nmol/g , $n=20$) compared with adults (17 ± 2 , $n=16$, $p<0.0001$, main effect), and were greater in females (28 ± 10 , $n=19$) than in males (22 ± 9 , $n=17$, $p=0.0291$, main effect); there were no genotype differences (Table 3). When expressed per liver, hepatic α -tocopherol concentrations were more than triple in old (62 ± 37 nmol , $n=20$) compared with adult (18 ± 3 , $n=16$, $p<0.0001$, main effect) mice, as well as being higher in male (49 ± 45 , $n=17$) than in

female (36 ± 22 , $n=19$, $p=0.0370$, main effect) mice; there were no significant genotype differences.

Hepatic γ -tocopherol concentrations were higher in old female (1.3 ± 0.2 nmol/g, $n=11$) than in old male (1.0 ± 0.2 , $n=9$) mice; both old male and female liver concentrations were greater than those of either adult female (0.7 ± 0.1 , $n=8$) or adult male (0.6 ± 0.1 , $n=8$) mice (Tukey HSD, $p<0.05$). When expressed per liver, livers from old mice (2.2 ± 1.2 nmol, $n=20$) contained three times as much γ -tocopherol as did those from adult mice (0.7 ± 0.1 , $n=16$, main effect of age, $p<0.0001$). Livers from female (1.4 ± 0.8 nmol, $n=19$) contained less γ -tocopherol than did those from male mice (1.8 ± 1.5 , $n=17$, main effect of sex, $p=0.0111$).

It has previously been suggested that increasing vitamin E concentrations with age is due to the accumulation of lipids [28]. Therefore, hepatic cholesterol concentrations were measured in the same extracts as used for vitamin E assessment and were then used to normalize vitamin E concentrations. However, cholesterol concentrations varied differently than did vitamin E such that this data manipulation did not yield meaningful information (data not shown).

Lung—Females (19 ± 4 nmol/g, $n=19$) had higher lung α -tocopherol concentrations than did males (15 ± 4 , $n=17$, main effect of sex, $p=0.0022$), but again there were no genotype differences (Table 3). With respect to lung γ -tocopherol concentrations, old female mice (0.8 ± 0.1 mmol/g, $n=11$) had the highest levels as compared with old male (0.6 ± 0.2 , $n=9$), adult male (0.6 ± 0.2 , $n=8$) or adult females (0.6 ± 0.1 , $n=8$; Tukey HSD, old females > old males or adult males or adult females, $p<0.05$; main effects of age ($p=0.0007$) and of sex $p=0.0415$).

Spinal Cord—Spinal cord α - and γ -tocopherol concentrations were each higher, respectively, in old (α -tocopherol: 6.4 ± 2.0 nmol/g, γ -tocopherol: 0.2 ± 0.1) compared with adult mice (α -tocopherol: 2.7 ± 0.8 , γ -tocopherol: 0.1 ± 0.1 ; main effect of age, α -tocopherol, $p<0.0001$ and γ -tocopherol, $p=0.0151$).

Liver Cytochrome P450 Concentrations

Vitamin E metabolism is initiated by an ω -hydroxylation step thought to be carried out by either CYP3A or CYP4F [5–8]. Therefore, these two P450 proteins were quantified using western blot analysis and expressed relative to actin (Figure 2).

Hepatic Cyp3a concentrations in AhR null (2.7 ± 0.4 arbitrary units relative to actin, $n=15$) were higher than those in wildtype (2.3 ± 0.4 , $n=15$, $p=0.0011$, genotype effect) mice. Cyp3a concentrations in female (2.7 ± 0.4 , $n=13$) were higher than in male mice (2.4 ± 0.5 , $n=17$, $p=0.0029$, sex effect).

Only sex was a significant modifier of Cyp4f concentrations. Female mice (6.1 ± 1.0 relative to actin, arbitrary units, $n=14$) had greater Cyp4f concentrations than did male mice (4.3 ± 0.4 , $n=17$, $p<0.0001$, sex effect). Previously, female rats have been reported to have higher CYP4F2 concentrations [29].

Liver α -CEHC Concentrations

Genotype ($p=0.0047$), sex ($p<0.0001$) and age ($p<0.0001$) were significant modifiers of liver α -CEHC concentrations. AhR null (153 ± 179 pmol/g liver, $n=18$) had greater α -CEHC concentrations than wildtype mice (79 ± 57 , $n=16$); females (168 ± 170 , $n=19$) had greater α -CEHC concentrations than did males (53 ± 32 , $n=14$); and old (172 ± 158 , $n=20$) had greater α -CEHC concentrations than did adults (38 ± 43 , $n=13$). Hepatic γ -CEHC concentrations were below levels of detection.

DISCUSSION

The mechanisms of vitamin E metabolism have not been fully elucidated. AhR, a modulator of xenobiotic metabolism, was considered a possible regulator of vitamin E metabolism because it controls vitamin A metabolism [22–24]. We, therefore, anticipated that AhR null mice would have increased plasma and tissue α -tocopherol concentrations with a reduced ability to metabolize vitamin E. Contrary to our expectations, we found that plasma α -tocopherol concentrations in AhR null mice were less than those of wildtype mice, as were their plasma lipid concentrations. Indeed, genotype showed no statistically significant effects on liver α - or γ -tocopherol concentrations. And, there were no obvious differences in liver cholesterol concentrations between genotypes; discounting any derangement of liver cholesterol metabolism. Importantly, we also found that plasma triglycerides were lower in the old AhR null mice. This finding is consistent with the report by Minami et al. [30], who showed that AhR null mice not only have low plasma triglycerides, they also have up-regulated lipoprotein lipase mRNA levels, suggesting that AhR regulates circulating triglyceride levels.

Perhaps the most interesting and physiologically relevant findings on vitamin E status from this study were the effects of age and sex. Vitamin E metabolism has been recognized as a major determinant of circulating tocopherol concentrations in that the non- α -tocopherol forms of vitamin E are readily metabolized [6]. Moreover, γ -tocopherol metabolism has been found in humans to proceed at a faster rate in women than in men [31,32]. In the present study, mouse plasma γ -tocopherol was higher in old compared with adult mice, but there was no sex effect. However, liver γ -tocopherol concentrations were higher in old female mice than the other groups, but γ -CEHC concentrations were undetectable, therefore, no comment can be made about γ -tocopherol metabolism.

Tissue α -tocopherol concentrations were higher in old mice than in adult mice, as well as being higher in female mice than in males. The elevated Cyp4f and 3a levels found in females may reflect important gender differences in hepatic tocopherol metabolism. CYP4F2 was previously been reported to be increased in females compared with male rats [29], while CYP3A is well-known to be expressed at higher concentrations in female mice [33] and in women [34]. AhR null mice had significantly higher hepatic Cyp3a concentrations compared to wildtypes ($p=0.0011$, genotype effect). Cyp3a concentrations were also correlated with hepatic α -tocopherol ($r=0.3957$, $p<0.05$) and α -CEHC ($r=0.4260$, $p<0.05$) concentrations. Since there were no significant genotype differences in the hepatic α - or γ -tocopherol concentrations, AhR null mice did not have dramatically altered vitamin E metabolism. However, since they did have higher hepatic α -CEHC concentrations, these data suggest that α -tocopherol metabolism was somewhat up-regulated in AhR null mice, and that elevated Cyp3a may play a role in increased α -tocopherol metabolism. Alternatively, hepatic excretion of α -CEHC may have been limited. Minami et al. [30] reported that AhR null mice have less than 50% of the gene expression of Solute carrier family 22, member 7 (Slc22a7, also known as OAT2). Although the transporter for CEHCs are not known, OATS are important organic ion transporters in the membrane [35], opening the possibility that OAT2 could be involved in CEHC trafficking.

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References

1. Traber MG, Atkinson J. Vitamin E, antioxidant and nothing more. *Free Radic Biol Med* 2007;43:4–15. [PubMed: 17561088]
2. Traber MG. Vitamin E regulatory mechanisms. *Annu Rev Nutr* 2007;27:347–362. [PubMed: 17439363]
3. Brigelius-Flohé R, Traber MG. Vitamin E: function and metabolism. *FASEB J* 1999;13:1145–1155. [PubMed: 10385606]
4. Hattori A, Fukushima T, Imai K. Occurrence and determination of a natriuretic hormone, 2,7,8-trimethyl-2-(beta-carboxyethyl)-6-hydroxy chroman, in rat plasma, urine, and bile. *Anal Biochem* 2000;281:209–215. [PubMed: 10870837]
5. Birringer M, Drozan D, Brigelius-Flohe R. Tocopherols are metabolized in HepG2 cells by side chain omega-oxidation and consecutive beta-oxidation. *Free Radic Biol Med* 2001;31:226–232. [PubMed: 11440834]
6. Sontag TJ, Parker RS. Influence of major structural features of tocopherols and tocotrienols on their omega-oxidation by tocopherol-omega-hydroxylase. *J Lipid Res* 2007;48:1090–1098. [PubMed: 17284776]
7. Sontag TJ, Parker RS. Cytochrome P450 omega-hydroxylase pathway of tocopherol catabolism: Novel mechanism of regulation of vitamin E status. *J Biol Chem* 2002;277:25290–25296. [PubMed: 11997390]
8. Landes N, Pfluger P, Kluth D, Birringer M, Ruhl R, Bol GF, Glatt H, Brigelius-Flohe R. Vitamin E activates gene expression via the pregnane $\times$ receptor. *Biochem Pharmacol* 2003;65:269–273. [PubMed: 12504802]
9. Mustacich DJ, Leonard SW, Devereaux MW, Sokol RJ, Traber MG. α -Tocopherol regulation of hepatic cytochrome P450s and ABC transporters. *Free Radic Biol Med* 2006;41:1069–1078. [PubMed: 16962932]
10. Mustacich DJ, Vo AT, Elias VD, Payne K, Sullivan L, Leonard SW, Traber MG. Regulatory mechanisms to control tissue alpha-tocopherol. *Free Radic Biol Med* 2007;43:610–618. [PubMed: 17640571]
11. Traber MG, Siddens LK, Leonard SW, Schock B, Gohil K, Krueger SK, Cross CE, Williams DE. α -Tocopherol modulates Cyp3a expression, increases γ -CEHC production and limits tissue γ -tocopherol accumulation in mice fed high γ -tocopherol diets. *Free Radic Biol Med* 2005;38:773–785. [PubMed: 15721988]
12. Zhang J, Huang W, Qatanani M, Evans RM, Moore DD. The constitutive androstane receptor and pregnane $\times$ receptor function coordinately to prevent bile acid-induced hepatotoxicity. *J Biol Chem* 2004;279:49517–49522. [PubMed: 15358766]
13. Xie W, Barwick JL, Downes M, Blumberg B, Simon CM, Nelson MC, Neuschwander-Tetri BA, Brunt EM, Guzelian PS, Evans RM. Humanized xenobiotic response in mice expressing nuclear receptor SXR. *Nature* 2000;406:435–439. [PubMed: 10935643]
14. Kluth D, Landes N, Pfluger P, Muller-Schmehl K, Weiss K, Bumke-Vogt C, Ristow M, Brigelius-Flohe R. Modulation of Cyp3a11 mRNA expression by alpha-tocopherol but not gamma-tocotrienol in mice. *Free Radic Biol Med* 2005;38:507–514. [PubMed: 15649653]
15. Mustacich DJ, Gohil K, Bruno RS, Yan M, Leonard SW, Ho E, Cross CE, Traber MG. Alpha-tocopherol modulates genes involved in hepatic xenobiotic pathways in mice. *J Nutr Biochem*. 2008 In press.
16. Xu C, Li CY, Kong AN. Induction of phase I, II and III drug metabolism/transport by xenobiotics. *Arch Pharm Res* 2005;28:249–268. [PubMed: 15832810]
17. Beischlag TV, Luis Morales J, Hollingshead BD, Perdew GH. The aryl hydrocarbon receptor complex and the control of gene expression. *Crit Rev Eukaryot Gene Expr* 2008;18:207–250. [PubMed: 18540824]
18. Shaban Z, Soliman M, El-Shazly S, El-Bohi K, Abdelazeez A, Kehelo K, Kim HS, Muzandu K, Ishizuka M, Katusaka A, Fujita S. AhR and PPARalpha: antagonistic effects on CYP2B and CYP3A, and additive inhibitory effects on CYP2C11. *Xenobiotica* 2005;35:51–68. [PubMed: 15788368]

19. Pascussi JM, Gerbal-Chaloin S, Duret C, Daujat-Chavanieu M, Vilarem MJ, Maurel P. The tangle of nuclear receptors that controls xenobiotic metabolism and transport: crosstalk and consequences. *Annu Rev Pharmacol Toxicol* 2008;48:1–32. [PubMed: 17608617]
20. Schmidt JV, Su GH, Reddy JK, Simon MC, Bradfield CA. Characterization of a murine Ahr null allele: involvement of the Ah receptor in hepatic growth and development. *Proc Natl Acad Sci U S A* 1996;93:6731–6736. [PubMed: 8692887]
21. Gonzalez FJ, Fernandez-Salguero P. The aryl hydrocarbon receptor: studies using the AHR-null mice. *Drug Metab Dispos* 1998;26:1194–1198. [PubMed: 9860927]
22. Andreola F, Fernandez-Salguero PM, Chiantore MV, Petkovich MP, Gonzalez FJ, De Luca LM. Aryl hydrocarbon receptor knockout mice (AHR^{-/-}) exhibit liver retinoid accumulation and reduced retinoic acid metabolism. *Cancer Res* 1997;57:2835–2838. [PubMed: 9230184]
23. Andreola F, Calvisi DF, Elizondo G, Jakowlew SB, Mariano J, Gonzalez FJ, De Luca LM. Reversal of liver fibrosis in aryl hydrocarbon receptor null mice by dietary vitamin A depletion. *Hepatology* 2004;39:157–166. [PubMed: 14752834]
24. Andreola F, Hayhurst GP, Luo G, Ferguson SS, Gonzalez FJ, Goldstein JA, De Luca LM. Mouse liver CYP2C39 is a novel retinoic acid 4-hydroxylase. Its down-regulation offers a molecular basis for liver retinoid accumulation and fibrosis in aryl hydrocarbon receptor-null mice. *J Biol Chem* 2004;279:3434–3438. [PubMed: 14623888]
25. Podda M, Weber C, Traber MG, Packer L. Simultaneous determination of tissue tocopherols, tocotrienols, ubiquinol and ubiquinones. *J Lipid Res* 1996;37:893–901. [PubMed: 8732789]
26. Leonard SW, Gumprecht E, Devereaux MW, Sokol RJ, Traber MG. Quantitation of rat liver vitamin E metabolites by LC-MS during high-dose vitamin E administration. *J Lipid Res* 2005;46:1068–1075. [PubMed: 15722564]
27. Thurnham DI, Davies JA, Crump BJ, Situnayake RD, Davis M. The use of different lipids to express serum tocopherol: lipid ratios for the measurement of vitamin E status. *Ann Clin Biochem* 1986;23:514–520. [PubMed: 3767286]
28. Traber MG, Frei B, Beckman JS. Vitamin E revisited: do new data validate benefits for chronic disease prevention? *Curr Opin Lipidol* 2008;19:30–38. [PubMed: 18196984]
29. Kalsotra A, Anakk S, Boehme CL, Strobel HW. Sexual dimorphism and tissue specificity in the expression of CYP4F forms in Sprague Dawley rats. *Drug Metab Dispos* 2002;30:1022–1028. [PubMed: 12167568]
30. Minami K, Nakajima M, Fujiki Y, Katoh M, Gonzalez FJ, Yokoi T. Regulation of insulin-like growth factor binding protein-1 and lipoprotein lipase by the aryl hydrocarbon receptor. *J Toxicol Sci* 2008;33:405–413. [PubMed: 18827440]
31. Leonard SW, Paterson E, Atkinson JK, Ramakrishnan R, Cross CE, Traber MG. Studies in humans using deuterium-labeled α - and γ -tocopherol demonstrate faster plasma γ -tocopherol disappearance and greater γ -metabolite production. *Free Radic Biol Med* 2005;38:857–866. [PubMed: 15749381]
32. Frank J, Lee S, Leonard SW, Atkinson JK, Kamal-Eldin A, Traber MG. Sex differences in the inhibition of γ -tocopherol metabolism by a single dose of dietary sesame oil in healthy subjects. *Am J Clin Nutr* 2008;87:1723–1729. [PubMed: 18541561]
33. Sakuma T, Takai M, Endo Y, Kuroiwa M, Ohara A, Jarukamjorn K, Honma R, Nemoto N. A novel female-specific member of the CYP3A gene subfamily in the mouse liver. *Arch Biochem Biophys* 2000;377:153–162. [PubMed: 10775455]
34. Scandlyn MJ, Stuart EC, Rosengren RJ. Sex-specific differences in CYP450 isoforms in humans. *Expert Opin Drug Metab Toxicol* 2008;4:413–424. [PubMed: 18524030]
35. Zhou F, You G. Molecular insights into the structure-function relationship of organic anion transporters OATs. *Pharm Res* 2007;24:28–36. [PubMed: 17103332]

Figure 1. Plasma Vitamin E and Triglyceride Concentrations

Plasma α -tocopherol ($\mu\text{mol/L}$, A), triglyceride (mmol/L, B) and α -tocopherol per triglycerides (mmol/mol, C) are shown for adult (solid bars) and old (striped bars) from AhR null and wildtype mice. Plasma α -tocopherol concentrations were higher in AhR null compared with wildtype mice ($p < 0.01$, genotype effect) and higher in young than in old mice ($p < 0.01$, age effect). Plasma triglycerides were higher in old wildtypes (a) than any other group (b; age $\times$ genotype, $P = 0.0389$, Tukey HSD, $p < 0.05$). Plasma α -tocopherol per triglycerides were nearly double in old AhR mice compared with their adult counterparts (age $\times$ genotype, $p = 0.019$; Tukey, HSD, $p < 0.05$). In each graph, bars not bearing the same letter are significantly different.

Figure 2. Hepatic Cyp3a and Cyp4f Concentrations

Hepatic Cyp3a concentrations in AhR null (arbitrary units relative to actin, n=15) and wildtype (n=15) mice, in female (striped bars, n=13) and male mice (solid bars, n=17) are shown in A; representative western blots from individual animals are shown in B. Hepatic Cyp4f concentrations in female (arbitrary units relative to actin, n=14) and male mice (n=17) are shown in C; representative western blots from individual animals are shown in D.

Body and Liver Weights; Liver Cholesterol Concentrations

Table 1

Sex	Age	Genotype	Number (n=)	Body Weight (g)	Liver Weight (g)	Liver Per Body Weight (mg/g)	Cholesterol (μmol/g)	Cholesterol Per Liver (μmol)
Male	adult	AhR null	4	27.3±0.9	1.19±0.08	43.4±2.8	0.81±0.08	0.97±0.15
		Wildtype	4	25.2±1.4	1.40±0.13	55.9±5.8	0.82±0.07	1.15±0.15
	old	AhR null	5	39.7±8.7	2.49±1.31	59.9±22.2	1.89±0.44	4.79±2.54
		Wildtype	4	43.2±9.1	2.78±1.27	59.5±14.6	1.82±0.25	5.05±2.32
Female	adult	AhR null	4	22.6±2.0	0.88±0.12	39.0±3.2	1.78±0.57	1.56±0.53
		Wildtype	4	16.4±2.7	0.92±0.01	57.6±11.4	1.73±0.32	1.60±0.29
	old	AhR null	6	28.1±2.8	1.21±0.31	43.4±11.0	1.59±0.34	1.92±0.57
		Wildtype	5	32.6±6.9	1.73±0.27	54.0±7.1	1.63±0.18	2.86±0.69
Statistical Comparisons								
Age				P<0.0001	P=0.0002		P=0.0004	P<0.0001
Sex				P<0.0001	P=0.0025		P=0.0038	P=0.0284
Genotype						P=0.035		
Age × sex							P=0.0001	P=0.0016
Age × genotype				P=0.0175				
Sex × genotype								
Age × sex × genotype								

For body weights, old AhR null and wildtype mice were greater than those of adult AhR null and wildtype mice (Tukey HSD, p<0.05); male mouse body weights were greater than those of females (Tukey HSD, p<0.05). Adult males had lower liver cholesterol concentrations (μmol/g liver) than did old males or either adult or old females (Tukey HSD, p<0.05). For cholesterol (μmol) per liver, old males had more liver cholesterol than did adult males, or either adult or old females (Tukey HSD, p<0.05).

Table 2

Plasma Vitamin E and Lipid Concentrations

Sex	Age	Genotype	Number (n=)	α -Tocopherol (μ mol/L)	γ -Tocopherol (μ mol/L)	Lipids (mmol/L)	α -Tocopherol/Lipids (mmol/mol)	γ -Tocopherol/Lipids (mmol/mol)
Male	adult	AhR null	4	2.2 $\pm$ 0.9	0.07 $\pm$ 0.06	1.53 $\pm$ 0.34	1.4 $\pm$ 0.4	0.04 $\pm$ 0.03
		Wildtype	4	2.4 $\pm$ 0.8	0.10 $\pm$ 0.04	2.10 $\pm$ 0.66	1.2 $\pm$ 0.3	0.05 $\pm$ 0.02
	old	AhR null	5	3.1 $\pm$ 0.8	0.12 $\pm$ 0.04	1.49 $\pm$ 0.21	2.0 $\pm$ 0.3	0.08 $\pm$ 0.03
		Wildtype	4	3.9 $\pm$ 1.9	0.15 $\pm$ 0.11	1.93 $\pm$ 0.34	2.1 $\pm$ 1.3	0.08 $\pm$ 0.08
Female	adult	AhR null	4	1.1 $\pm$ 0.3	0.08 $\pm$ 0.00	1.89 $\pm$ 0.12	0.6 $\pm$ 0.1	0.04 $\pm$ 0.00
		Wildtype	4	3.2 $\pm$ 1.0	0.07 $\pm$ 0.06	2.14 $\pm$ 0.36	1.6 $\pm$ 0.6	0.03 $\pm$ 0.02
	old	AhR null	6	2.7 $\pm$ 1.4	0.13 $\pm$ 0.04	2.17 $\pm$ 0.43	1.3 $\pm$ 0.7	0.06 $\pm$ 0.02
		Wildtype	5	3.4 $\pm$ 0.9	0.16 $\pm$ 0.04	3.70 $\pm$ 1.18	1.0 $\pm$ 0.3	0.05 $\pm$ 0.01
Statistical Comparisons								
Age				P=0.0075	P=0.0015	P=0.0406		P=0.0114
Sex						P=0.0009	P=0.0021	
Genotype				P=0.0131		P=0.0011		
Age $\times$ sex						P=0.0121		
Age $\times$ genotype								
Sex $\times$ genotype								
Age $\times$ sex $\times$ genotype							P=0.0461	

Plasma lipids are the sum triglycerides and total cholesterol; old females > old males or adult males or adult females (Tukey HSD $p < 0.05$). Plasma α -tocopherol/lipids, groups were not significantly different.

Table 3

Liver, Lung and Spinal Cord α - and γ -Tocopherols (nmol/g)

Sex	Age	Genotype	Number (n=)	Liver	Lung	Spinal cord
α-Tocopherol (nmol/g)						
Male	adult	AhR null	4	15.7±1.3	16.3±2.1	2.4±0.5
		Wildtype	4	14.4±1.8	11.6±4.1	2.6±0.7
	old	AhR null	5	27.0±7.6	15.7±4.5	5.9±1.5
Female	adult	Wildtype	4	30.9±9.1	16.0±2.6	5.1±1.5
		AhR null	4	18.5±2.3	18.8±0.5	3.1±1.4
	old	Wildtype	4	18.2±1.9	18.0±2.4	2.6±0.2
		AhR null	6	36.4±5.1	17.7±2.5	6.8±1.9
		Wildtype	5	33.8±10.0	21.1±5.8	7.6±2.7
Statistical Comparisons						
Age				P<0.0001		P<0.0001
Sex				P=0.0291	P=0.0022	
Genotype						
Age × sex						
Age × genotype						
Sex × genotype						
Age × sex × genotype						
γ-Tocopherol (nmol/g)						
Male	adult	AhR null	4	0.66±0.08	0.62±0.09	0.11±0.03
		Wildtype	4	0.62±0.04	0.56±0.21	0.15±0.11
	old	AhR null	5	0.98±0.21	0.59±0.10	0.12±0.11
Female	adult	Wildtype	4	1.08±0.27	0.71±0.20	0.18±0.06
		AhR null	4	0.70±0.13	0.59±0.05	0.06±0.05
	old	Wildtype	4	0.65±0.14	0.56±0.04	0.06±0.01
		AhR null	6	1.39±0.16	0.81±0.10	0.16±0.10
		Wildtype	5	1.27±0.28	0.88±0.14	0.20±0.08
Statistical Comparisons						

Sex	Age	Genotype	Number (n=)	Liver	Lung	Spinal cord
<i>α</i> -Tocopherol (nmol/g)						
Age				P<0.0001	P=0.0007	P=0.0151
Sex				P=0.0123	P=0.0415	
Genotype						
Age × sex				P=0.0399	P=0.0193	
Age × genotype						
Sex × genotype						
Age × sex × genotype						