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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), wild-type and AhR-null mice. Plasma α-tocopherol concentrations in AhR-null mice (2.3±1.2 μmol/L, n=19) were lower than those of wild-type mice (3.2±1.2, n=17, P=.0131); those in old mice (3.2±1.2, n=20) were higher than those of adults (2.2±1.0, n=16, P=.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<.0001). Hepatic Cyp3a concentrations in AhR-null mice were greater than those in wild-type mice (P=.0011). Genotype (P=.0047), sex (P<.0001) and age (P<.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<.05) and α-CEHC (r=0.4260, P<.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 wild-type mice.

Keywords: Alpha tocopherol; Gamma tocopherol; Carboxyethyl hydroxychroman (CEHC); Xenobiotic metabolism

1. 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 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 β-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 protein, although shown to ω-hydroxylate the tocopherol side chain [6,7], 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 upstream regulator of various xenobiotic pathways [12], including regulation of CYP3A [13]. Initially, non-α-tocopherol forms of vitamin E were thought to

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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 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 wild-type 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.

2. Materials and methods

2.1. 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, USA, maintained by Laboratory Animal Research Center, Oregon State University, Corvallis, OR, USA) were maintained on a 12-h 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; adult mice ages were between 2 and 5 months. Mice were not fasted. They were sedated with CO_2 gas, followed by exsanguination. Blood samples were collected with ethylenediaminetetraacetic acid and placed on ice. Plasma was obtained by centrifugation of blood samples for 10 min at 1500×*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.

2.2. 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 of 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, USA) and detected using an Applied Biosystems Cytofluor multiwell plate reader, series 4000 (Foster City, CA, USA).

2.3. 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 μl) 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 N_2 and the residue resuspended in 1:1 H_2O/methanol. Extracted α- and γ-CEHCs were analyzed by a reverse-phase liquid chromatography method using a gradient of (A) methanol or (B) H_2O, 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 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.

2.4. 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, USA) using a Potter-Elvehjem-type homogenizer. Homogenates were centrifuged for 15 min at 1000×*g* (4°C) and supernatants were stored at −80°C for Western blot analysis.

Total protein concentrations were determined using Coomasie Plus reagent, according to manufacturer's instructions (Pierce, Rockford, IL, USA). Equal amounts of protein were added to lauryl dodecyl sulfate loading buffer (Invitrogen, Carlsbad, CA, USA), denatured at 70°C for 10 min 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% nonfat milk for 2 h at room temperature. Fresh TBST with 3% nonfat milk and primary antibody was incubated on membranes overnight at 4°C at the following dilutions: Cyp3A2 (Chemicon, Temecula, CA, USA), 1:4000; Cyp4F2 (a generous gift from Jerome M. Lasker, Institute for Biomedical Research, Hackensack University Medical Center); and actin (Santa Cruz Biotechnology), 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, USA) 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.

2.5. Statistical analysis

Data are expressed as mean±S.D. ANOVA was performed using JMP Statistical Discovery Software (SAS Institute, Cary, NC, USA) 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 honestly significant differences (HSD) test was performed. Where no overall significant interaction was found, main effects and associated means±S.D. are reported in the text. The results were considered to be statistically significant at $P<.05$.

3. Results

3.1. Body and liver weights

Body weights of old AhR-null (33±8 g, $n=11$) and old wild-type (38±10, $n=9$) mice were greater than those of adult AhR-null (25±3, $n=8$) and adult wild-type (21±5, $n=8$) mice [Age×Genotype interaction, $P=.038$; Tukey HSD, $P<.05$]. Males (35±10, $n=17$) weighed more than females (26±7, $n=19$) (age effect, $P<.0001$; Table 1).

Table 1
Body and liver weights; liver cholesterol concentrations

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
		Wild type	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
		Wild type	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
		Wild type	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
		Wild type	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<.0001	P=.0002		P=.0004	P<.0001
Sex				P<.0001	P=.0025		P=.0038	P=.0284
Genotype						P=.035		
Age×Sex							P=.0001	P=.0016
Age×Genotype				P=.0175				
Sex×Genotype								
Age×Sex×Genotype								

For body weights, old AhR null and wild-type mice were greater than those of adult AhR null and wild-type mice (Tukey HSD, P<.05); male mouse body weights were greater than those of females (Tukey HSD, P<.05). Adult males had lower liver cholesterol concentrations (μmol/g liver) than did old males or either adult or old females (Tukey HSD, P<.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<.05).

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<.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<.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 wild-type mice (56±9, n=17, P=.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 (μmol/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×Gender interaction, P=.0001; Tukey HSD, P<.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×Gender interaction, P=.0016; Tukey HSD, P<.05].

Taken together, these data show that old female AhR mice were smaller than old wild-type mice. Relative to body size, livers from AhR-null mice were smaller than wild-type 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.

3.2. Plasma vitamin E and lipid concentrations

Plasma α- and γ-tocopherol concentrations in AhR-null and wild-type, 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 μmol/L, n=19) were less than those of wild-type mice (3.2±1.2, n=17, genotype effect, P=.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=.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=.0015).

Table 2
Plasma vitamin E and lipid concentrations

Sex	Age	Genotype	Number (n)	α-Tocopherol (μmol/L)	γ-Tocopherol (μmol/L)	Lipids (mmol/L)	α-Tocopherol/lipids (mmol/mol)	γ-Tocopherol/lipids (mmol/mol)
Male	Adult	AhR null	4	2.2±0.9	0.07±0.06	1.53±0.34	1.4±0.4	0.04±0.03
		Wild type	4	2.4±0.8	0.10±0.04	2.10±0.66	1.2±0.3	0.05±0.02
	Old	AhR null	5	3.1±0.8	0.12±0.04	1.49±0.21	2.0±0.3	0.08±0.03
		Wild type	4	3.9±1.9	0.15±0.11	1.93±0.34	2.1±1.3	0.08±0.08
Female	Adult	AhR null	4	1.1±0.3	0.08±0.00	1.89±0.12	0.6±0.1	0.04±0.00
		Wild type	4	3.2±1.0	0.07±0.06	2.14±0.36	1.6±0.6	0.03±0.02
	Old	AhR null	6	2.7±1.4	0.13±0.04	2.17±0.43	1.3±0.7	0.06±0.02
		Wild type	5	3.4±0.9	0.16±0.04	3.70±1.18	1.0±0.3	0.05±0.01
Statistical comparisons								
Age				P=.0075	P=.0015	P=.0406		P=.0114
Sex						P=.0009	P=.0021	
Genotype				P=.0131		P=.0011		
Age×Sex						P=.0121		
Age×Genotype								
Sex×Genotype								
Age×Sex×Genotype							P=.0461	

Plasma lipids are the sum of triglycerides and total cholesterol; old females>old males or adult males or adult females (Tukey HSD, P<.05). Plasma α-tocopherol/lipids: groups were not significantly different.

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=.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<.0001, Table 2). Triglyceride concentrations (mmol/L) were approximately double in old wild-type (2.1±1.0, n=9) compared with adult wild-type (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<.05; Fig. 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 wild-type (3.5±1.2, n=17, genotype effect, P=.0003); lower in adult (2.6±0.7, n=16) compared with old (3.3±1.2, n=19, age effect, P=.0003); and lower in female (2.5±1.0, n=19) compared with male mice (3.5±0.9, n=17, sex effect, P<.0001).

When normalized to plasma lipid concentrations, plasma α-tocopherol per lipids showed a significant Age×Sex×Genotype interaction (P<.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 wild-type mice, but only α-tocopherol increases in the AhR-null mice (Fig. 1). Thus, the old AhR mice compared with their adult counterparts (Tukey HSD, P<.05) have nearly double the plasma α-tocopherol when expressed per triglycerides (Age×Genotype, P=.019).

3.3. Tissue vitamin E concentrations

3.3.1. 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<.0001, main effect) and were greater in females (28±10, n=19) than in males (22±9, n=17, P=.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<.0001, main effect) mice, as well as being higher in male (49±45, n=17) than in female (36±22, n=19, P=.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<.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<.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=.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).

3.3.2. 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=.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

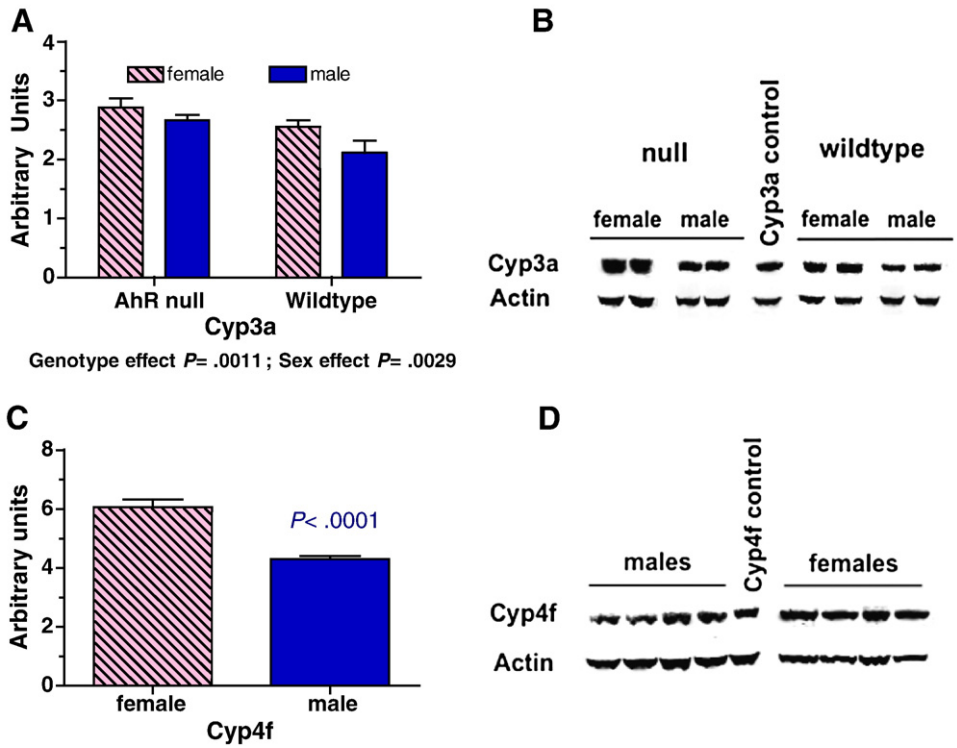

Fig. 1. Plasma vitamin E and triglyceride concentrations. Plasma a-tocopherol (μmol/L, A), triglyceride (mmol/L, B) and α-tocopherol per triglycerides (mmol/mol, C) are shown for adult (solid bars) and old (striped bars) AhR-null and wild-type mice. Plasma α-tocopherol concentrations were higher in AhR-null compared with wild-type mice (P<.01, genotype effect) and higher in young than in old mice (P<.01, age effect). Plasma triglycerides were higher in old wild-type mice (A) than in any other group (B; Age×Genotype, P=.0389; Tukey HSD, P<.05). Plasma α-tocopherol per triglycerides were nearly double in old AhR mice compared with their adult counterparts (Age×Genotype, P=.019; Tukey HSD, P<.05). In each graph, bars not bearing the same letter are significantly different.

Table 3
Liver, lung and spinal cord α- and γ-tocopherols (nmol/g)

Sex	Age	Genotype	Number (*n*)	α-Tocopherol (nmol/g)		
				Liver	Lung	Spinal cord
Male	Adult	AhR null	4	15.7±1.3	16.3±2.1	2.4±0.5
		Wild type	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
		Wild type	4	30.9±9.1	16.0±2.6	5.1±1.5
Female	Adult	AhR null	4	18.5±2.3	18.8±0.5	3.1±1.4
		Wild type	4	18.2±1.9	18.0±2.4	2.6±0.2
	Old	AhR null	6	36.4±5.1	17.7±2.5	6.8±1.9
		Wild type	5	33.8±10.0	21.1±5.8	7.6±2.7
Statistical comparisons						
Age				*P*<.0001		*P*<.0001
Sex				*P*=.0291	*P*=.0022	
Genotype						
Age×Sex						
Age×Genotype						
Sex×Genotype						
Age×Sex×Genotype						

Sex	Age	Genotype	Number (*n*)	α-Tocopherol (nmol/g)		
				Liver	Lung	Spinal cord
Male	Adult	AhR null	4	0.66±0.08	0.62±0.09	0.11±0.03
		Wild type	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
		Wild type	4	1.08±0.27	0.71±0.20	0.18±0.06
Female	Adult	AhR null	4	0.70±0.13	0.59±0.05	0.06±0.05
		Wild type	4	0.65±0.14	0.56±0.04	0.06±0.01
	Old	AhR null	6	1.39±0.16	0.81±0.10	0.16±0.10
		Wild type	5	1.27±0.28	0.88±0.14	0.20±0.08
Statistical comparisons						
Age				*P*<.0001	*P*=.0007	*P*=.0151
Sex				*P*=.0123	*P*=.0415	
Genotype						
Age×Sex				*P*=.0399	*P*=.0193	
Age×Genotype						
Sex×Genotype						
Age×Sex×Genotype						

Liver γ-tocopherol: old females>old males>adult males or females (Tukey HSD, *P*<.05). Lung γ-tocopherol: old females>old males or adult males or adult females (Tukey HSD, *P*<.05).

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*<.05; main effects of age (*P*=.0007) and of sex, *P*=.0415].

3.3.3. 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*<.0001; γ-tocopherol, *P*=.0151).

3.4. 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 (Fig. 2).

Hepatic Cyp3a concentrations in AhR-null (2.7±0.4 arbitrary units relative to actin, *n*=15) were higher than those in wild-type (2.3±0.4, *n*=15, *P*=.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*=.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*<.0001, sex effect). Previously, female rats have been reported to have higher CYP4F2 concentrations [29].

3.5. Liver α-CEHC concentrations

Genotype (*P*=.0047), sex (*P*<.0001) and age (*P*<.0001) were significant modifiers of liver α-CEHC concentrations. AhR-null mice (153±179 pmol/g liver, *n*=18) had greater α-CEHC concentrations than wild-type 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.

4. 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 wild-type mice, as were their plasma lipid concentrations. Indeed, genotype showed no statistically significant effects on liver α- or γ-tocopherol

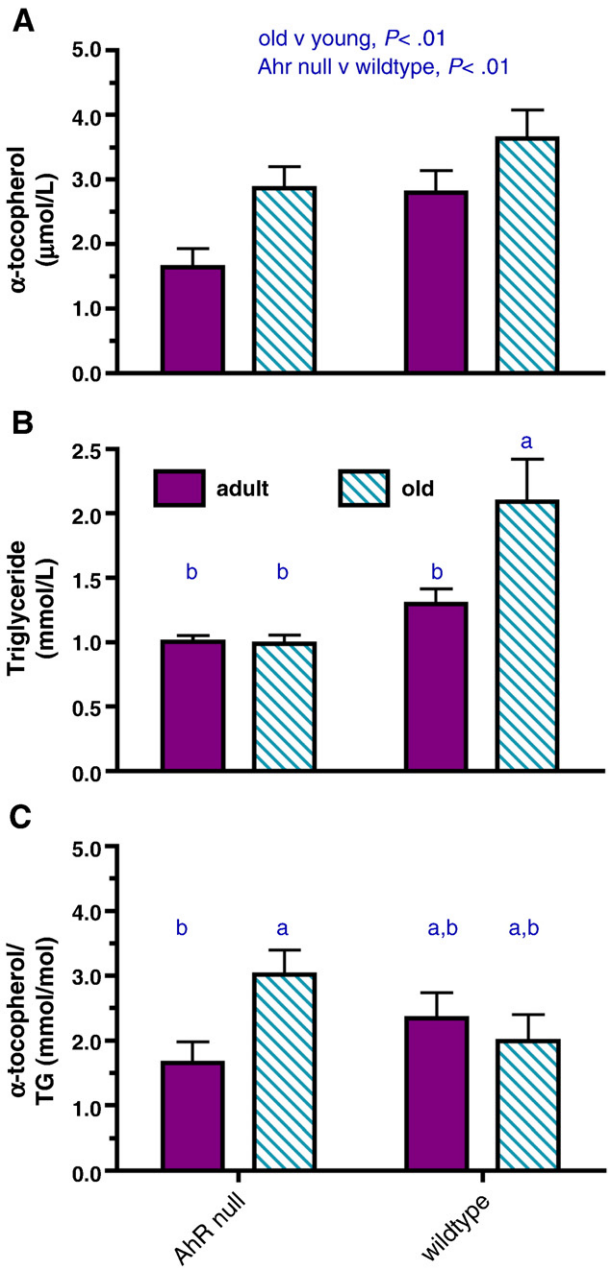

Fig. 2. Hepatic Cyp3a and Cyp4f concentrations. Hepatic Cyp3a concentrations in AhR-null (arbitrary units relative to actin, n=15) and wild-type (n=15) mice, in female (striped bars, n=13) and male mice (solid bars, n=17) are shown in (A); representative Western blots from individual animals are shown in (B). Hepatic Cyp4f concentrations in female (arbitrary units relative to actin, n=14) and in male mice (n=17) are shown in (C); representative Western blots from individual animals are shown in (D).

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, but 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 in 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 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 wild-type mice (P=.0011, genotype effect). Cyp3a concentrations were also correlated with hepatic α-tocopherol (r=0.3957, P<.05) and α-CEHC (r=0.4260, P<.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 is 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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