Int J Mol Epidemiol Genet 2012;3(2):134-143
www.ijmeg.org /ISSN1948-1756/IJMEG1203003

Original Article

Female reproductive status and circulating blood leukocyte expression of selected metabolic or signaling genes involved in sex steroid metabolism

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Received March 30, 2012; accepted April 15, 2012; Epub May 15, 2012; Published May 30, 2012

Abstract: Objective: To examine the blood leukocyte expression of 22 sex steroid metabolic/signaling genes according to female reproductive status. Methods: Michigan Fisheaters' Cohort participants underwent blood collection during the luteal phase of the menstrual cycle or randomly in non-menstruating participants. Gene expression (GE) was measured using Taqman hydrolysis probes and quantitative RT-PCR. Repeatability of four genes was determined in a subgroup. Results: Five premenstrual, 57 premenopausal (20 users of systemic hormonal contraception), and 43 postmenopausal females participated. After Bonferroni correction for multiple comparisons of median GE between groups, three findings remained significant: greater GE of *AhR* in postmenopausal women than in premenopausal non-users of systemic hormonal contraception; and greater GE of *ESR2* and *HSD17B7* in premenstrual girls compared to postmenopausal women. Modest intra-class correlations were identified for *CYP 19, ESR1,* and *ESR2* GE measured both in 2007 and 2010, but no intra-class correlation over the same time period was found for *CYP17*. Conclusions: There was little differential variation of blood leukocyte sex steroid GE between premenopausal women in the luteal phase of the menstrual cycle and postmenopausal women for most genes analyzed, but it will be necessary to make statistical adjustments in future epidemiologic studies in two circumstances: 1) when comparing *AhR* GE in premenopausal women non-users of systemic hormone contraception with postmenopausal women and 2) when comparing *ESR2* and *HSD17B7* GE in studies that include premenstrual girls. Developmental differences may explain the differential GE found in *ESR2* and *HSD17B7* in premenstrual girls compared with postmenopausal women.

Keywords: Steroid gene expression, blood leukocyte gene expression, female reproductive status

Introduction

Although multiple studies in laboratory animals have advanced insights into molecular biological mechanisms controlling determinants of reproduction, translation into non-diseased human subjects on a large scale has been difficult, owing to the unavailability of normal human tissue for analysis. Because of the ease of procurement, use of peripheral blood as a surrogate for organ-specific sex steroid gene expression offers a distinct advantage over invasive methods required to obtain tissue specimens in normal human populations. However, it is well known that activation of genes to which RNA polymerase binds is tissue-specific, leading to varying gene expression (GE), depending on the tissue analyzed [1]. Little is known about the expression of the genes of sex steroid metabolism in the peripheral blood of humans and whether or not this expression reflects the physiological status of the individual from a reproductive standpoint. The only literature available on this topic is from Vottero et al, who in 2006 demonstrated the differential expression of aromatase (*CYP19*) in the peripheral blood of a small number of children and female and male adults and its correlation with blood levels of sex steroid hormones [2].

We sought to expand on this knowledge by analyzing the peripheral blood leukocyte expression

of 22 metabolic or signaling genes involved in sex steroid metabolism in females categorized by reproductive status. These genes are listed and described in **Table 1**.

Materials and methods

Population

Study participants were taken from a cohort of families participating in the Michigan Fish-eaters' Cohort, which was originally established by the Michigan Department of Community Health to study the effects of organochlorine (OC) exposure on human health [3]. Anglers and their spouses who fished in Lake Michigan or its tributaries and lived in western Michigan were recruited between 1973 and 1991. The living members of this cohort who had previously agreed to be re-contacted were called in 2000 and asked to identify the addresses of their offspring. In 2005, we recruited female offspring into a new study related to in-utero OC exposure and associated gene function of a select group of genes involved in sex steroid metabolism. This study was expanded in 2007 to include the analysis of the expression of several more genes involved in sex steroid signaling or metabolism. Each phase of the study was approved by the institutional review board of Michigan State University (MSU) and renewed according to university guidelines.

Recruitment

Subjects were eligible if they were six and older and female. An introductory letter with a study description and a newsletter describing results of the 2005 study was mailed to participants of that study. Two weeks later, interviewers began contacting the women and current contact information on family members was collected. Those who agreed to participate consented to a blood donation and a telephone interview. Those who were custodial parents and who allowed their dependent children to participate provided informed consent, and assent was obtained from the child at the time of the interview. Participants were compensated $10 for the telephone interview and $25 for the blood draw.

Telephone interview

Information collected comprised demographic data, medical history including medication, alcohol, tobacco and illicit drug use, reproductive history (for adults only), developmental history (children only) and an informal diet history. Parents of dependent children responded to their child's medical and developmental history. For the 6-12 year age group, parents completed the entire interview for their child. For children aged 13-17, questions were first asked of the parent, the parent was then asked to leave the room, and a separate interview was conducted with the adolescent obtaining similar information about medical and developmental history and medication usage, as well use of tobacco, alcohol and illicit drugs.

Trained personnel conducted the interview and entered responses directly into a database. A second research assistant was present for each interview, whose role was to record responses onto a hard copy of the questionnaire. Following the interview, responses were compared. Discrepancies were resolved by re-contacting the participant.

Procedure for blood specimen collection

Following the telephone interview, arrangements were made for non-fasting blood to be collected. Because gene expression varies throughout the ovulatory cycle [2], menstruating premenopausal participants had blood collected during days 3-12 of the cycle. A laboratory of the participant's choosing was called and arrangements made. An anesthetic cream was offered to participants 6-12 years to decrease the phlebotomy discomfort. Participants were mailed a box containing the necessary equipment for returning the blood specimens. Plasma for gene expression analysis was collected in two PAXgene Blood RNA Tubes (PreAnalitiX, Plymouth, UK), and mailed via FedEx on ice to the MSU Genomics Laboratory where it was processed and stored at -20°C prior to analysis.

Gene expression analyzed

The expression of selected metabolic or signaling genes involved in sex steroid metabolism were chosen for analysis and categorized as follows: 1) Anabolic Genes (involved in Sex Steroid Biosynthesis): a) *CYP 11A1*; b) *HSD3B2*; c) *CYP17*; d) *HSD17B1*; e) *HSD17B3*; f) *HSD17B5* (also involved in sex steroid catabolism); g) *HSD17B7* (also involved in sex steroid catabolism); and h) *CYP19*; 2) Catabolic Genes

Table 1. ABI Assay ID, Definition and Major Function of Genes Measured in Current Study

Gene	ABI Assay ID*	Definition/Product	Function
		Genes of Sex Steroid Anabolism	
CYP 11A1	Hs00167984_m1	Cytochrome P450, family 11, subfamily A, polypeptide 1	Product converts cholesterol to pregnenolone
HSD3B2 (AKR1C2)	Hs00605123_m1	Hydroxy-D-5-steroid dehydrogenase, 3-β and steroid Δ-isomerase 2	Product oxidizes 3-beta hydroxy steroids and ketosteroids in several key steps in steroid biosynthesis.
CYP17	Hs00164375_m1	Cytochrome P450, family 17, subfamily A, polypeptide 1 (17-α-hydroxylase)	Product hydroxylates progesterone and pregnenolone and lyases these to dehydroepiandrosterone and androstenedione, respectively.
CYP19	Hs00240671_m1	Cytochrome P450, family 19, subfamily A, polypeptide 1 (aromatase)	Product aromatizes androstenedione to estrone and testosterone to estradiol. Present in many tissues.
HSD17B1	Hs00166219_g1	Hydroxysteroid (17-β) dehydrogenase type 1	Product converts estrone to estradiol.
HSD17B3	Hs00609319_m1	Hydroxysteroid (17-β) dehydrogenase type 3	Product converts androstenedione to testosterone.
HSD17B5 (AKR1C3)	Hs00366267_m1	Hydroxysteroid (17-β) dehydrogenase type 5	Product converts androstenedione to testosterone
HSD17B7	Hs00996127_m1	Hydroxysteroid (17-β) dehydrogenase type 7	Product converts estrone to estradiol.
		Genes of Sex Steroid Catabolism	
CYP1A1	Hs00153120_m1	Cytochrome P450, family 1, subfamily A, polypeptide 1, (aryl hydrocarbon hydroxylase)	Product hydroxylates estradiol to 2-hydroxyestone. Regulated by the Aromatic Hydrocarbon (Ah) Receptor. Also metabolize drugs and aromatic hydrocarbons.
CYP1A2	Hs00167927_m1	Cytochrome P450, family 1, subfamily A, polypeptide 2	
CYP3A4	Hs00604506_m1	Human cytochrome P450, family 3, subfamily A, polypeptide 4	
CYP1B1	Hs00164383_m1	Cytochrome P450, family 1, subfamily B, polypeptide 1	Product hydroxylates estradiol to 4-hydroxyestrone. Regulated by the Aromatic Hydrocarbon Receptor (AhR).
SRD5A2	Hs00165843_m1	Steroid 5-alpha reductase type 2	Product reduces testosterone to dihydrotestosterone
COMT	Hs00241349_m1	Catechol-O- methyltransferase	Product methylates the hydroxyestrones, making them water soluble and ready for excretion.
HSD17B2	Hs00157993_m1	Hydroxysteroid (17-β) dehydrogenase type 2	Product converts dihydrotestosterone to 5-α-androstenedione in the prostate gland; converts estradiol and testosterone to their estrone derivatives.
HSD17B5 (AKR1C3)	As above, Hs00366267_m1	Hydroxysteroid (17-β) dehydrogenase type 5	Product in the prostate gland converts dihydrotestosterone to 5-α-androstane-3-α,17-β-diol and then to androsterone; converts 5-α-androstanedione to androsterone
HSD17B7	As above, Hs00996127_m1	Hydroxysteroid (17-β) dehydrogenase type 7	Product converts dihydrotestosterone to 5-α-androstane-3-α,17-β-diol and to 3-α-androstane-3-β,17-β-diol in the prostate
		Steroid Receptors/ Other Important Sex-Steroid-Related Genes	
ESR1	Hs00174860_m1	Estrogen Receptor α; Estrogen Receptor 1	Products act as a transcription factors through estrogen and non-estrogen dependant binding mechanisms.
ESR2	Hs00230957_m1	Estrogen Receptor β; Estrogen Receptor 2	
AR	Hs00171172_m1	Androgen Receptor; Nuclear Receptor subfamily 3, group C, member 4); NR3C4	Product acts as a transcription factor through androgen-dependant binding mechanisms.
AhR	Hs00169233_m1	Aromatic Hydrocarbon Receptor	Product acts as a transcription factor through natural and toxic substance-dependant binding mechanisms; endogenous ligand unknown.
LEPR	Hs00174497_m1	Leptin Receptor ; member of class 1 cytokine family	Product acts as a transcription factor through leptin binding mechanisms.
LEP	Hs00174877_m1	Leptin; obesity factor	Product binds to the leptin receptor and controls appetite as well as other endocrine functions.
AP-1/JUN	Hs00277190_s1	Activator Protein 1/ Protooncogene c-JUN	Product acts as a transcription factor; regulates both cellular proliferation and apoptosis.

*ABI Assay ID = Applied Biosystems Taqman assay identification

(involved in Sex Steroid Breakdown); a) *CYP1A1*; b) *CYP1A2*; c) *CYP1B1*; d) *CYP3A*; e) *SRD5A2*; f) *HSD17B2*; and g) *COMT*. *HSD17B5* and *HSD17B7* are involved in both sex steroid synthesis and breakdown. 3) Steroid Receptors/ Other Important Sex-Steroid-Related Genes: a) *ESR1*; b) ESR2; c) *AR*; d) *AhR*; e) *LEPR*; f) *LEP* g) *AP-1/JUN*. The respective Applied Biosystems Taqman assay identification (ABI Assay ID) numbers are listed in Table 1.

Determination of gene expression

Blood leukocytes collected in RNALater were isolated and purified using the PAXgene Blood RNA Kit (Qiagen, Hilden, Germany) and assessed for quality using an Agilent Bioanalyzer (Agilent Technologies, Inc., Santa Clara, CA). The High Capacity cDNA Reverse Transcription Kit (Applied Biosystems, Foster City, CA) was used to convert purified RNA to cDNA, which was then profiled for the expression of the individual genes utilizing the appropriate Taqman assay, which is based on a fluorescent emission assay utilizing an ABI 7900ht Sequence Detection System (Applied Biosystems, Foster City, CA). Each specimen was run in triplicate. Gene expression (GE) assay results were then averaged and normalized to GE results of RNA Polymerase II, a housekeeping gene (ABI Assay ID Hs00172187_m1). The number of cycles at which fluorescence (and therefore cDNA) became exponential, and the point at which it crossed the threshold was named Ct (cycle count). The relative expression of the target and housekeeping gene was expressed as ΔCt, representing the difference between the GE of the housekeeping and the target gene [4]. Housekeeping genes are ubiquitously expressed and have a low Ct value; the lower the value of the ΔCt, the higher the expression of the target gene.

Definitions of reproductive status

Participants were divided into 3 reproductive phases: 1) premenstrual, 2) premenopausal, and 3) postmenopausal. The premenopausal group was further categorized into users or nonusers of systemic hormonal contraception. Premenstrual girls were defined as those in whom menarche had not yet been initiated, whether or not they had entered puberty. The premenopausal group was defined as participants who a) had experienced at least one menstrual period within the previous 12 months; b) had had a uterine ablation and were 50 years old or less, or c) previous a hysterectomy but had at least one ovary and were 50 years of age or less. Systemic hormonal contraception included those using oral contraceptives or depro-provera injections. The postmenopausal group was defined as those who a) had a uterus, had not had uterine ablation, and had not had a menstrual period in the previous 12 months; b) had had a uterine ablation and were more than 50 years old, c) previous a hysterectomy but had at least one ovary and were more than 50 years of age; or d) previous both ovaries removed. Of the 43 participants classified as postmenopausal, one used estrogen replacement therapy; one used an estrogen receptor blocker; and two used an aromatase inhibitor. Because of the low numbers of participants using medications that could influence steroid GE, this group was not categorized further.

Definitions of other variables

Age was reported in years to the first decimal according to the age at interview. Participants self-reported age at menarche in years. Those who could not remember their age at menarche were asked to report the grade in school at its onset. Six years were added to the grade level to obtain an estimate of the age of onset of menarche. Parity was defined as the number of live and still births. Height was self-reported in feet and inches and converted to centimeters (cm). Weight was self-reported in pounds and converted to kilograms (kg). Height and weight were used to calculate body mass index (BMI) using the formula kg/m^2. In a subgroup (n = 46), height and weight were directly measured: two investigators visited participants, one of whom performed the measurements and the other who recorded results. Standing height was measured with a calibrated portable stadiometer. Subjects were asked to face forward and stand as tall as possible against a wall in their bare feet. Measurements were taken three times to the nearest tenth of a centimeter and averaged. Weight was measured three times with a portable calibrated electronic scale to the nearest tenth of a pound and averaged. The correlations for measured and reported height and weight was estimated using standard formulas.

Statistical analyses

Since the distributions of gene expression data

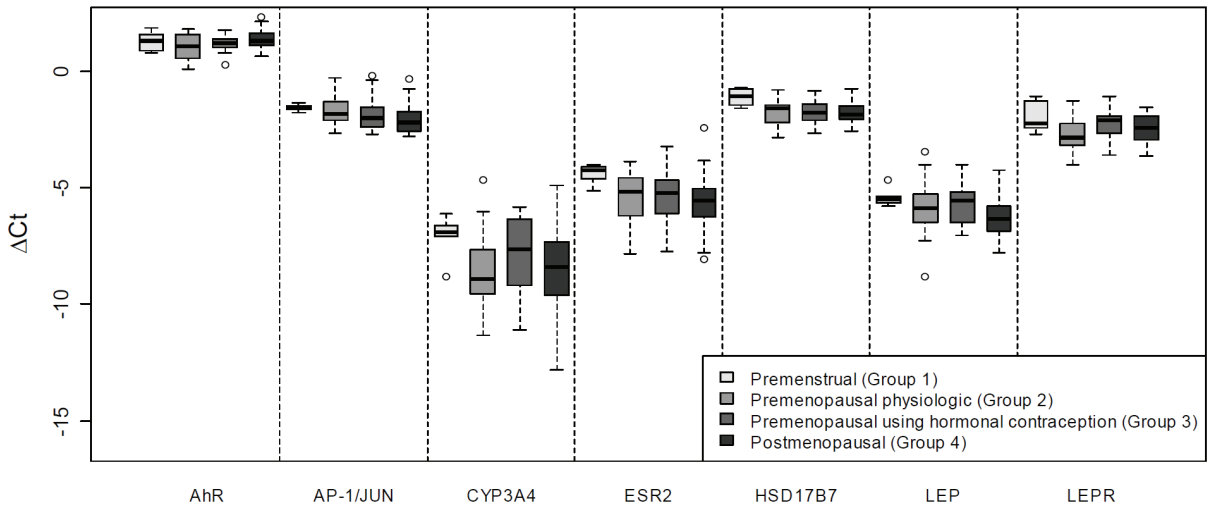

Figure 1. Box plots illustrating Blood Leukocyte mRNA counts for Seven Metabolic or Signaling Genes Involved in Sex Steroid Metabolism according to Female Reproductive Status in 105 Participants of the Michigan Fisheaters' Cohort.

are skewed, medians, fifth and ninety-fifth percentiles were computed. Non-parametric tests were used to evaluate the unadjusted effects of endocrine status of the four groups on GE and boxplots constructed. Linear mixed effects models for correlated data were used to account for the familial association of some participants [5]. A Blom transformation was used to achieve normally-distributed data [6]. All comparisons with and without the Bonferroni correction are presented. All statistical analyses were conducted using the SAS software (version 9.2).

Results

Blood from one hundred five female participants was analyzed; Group 1 representing premenstrual girls (n=5); Group 2 representing premenopausal women who were not using systemic hormonal contraception, (termed "premenstrual, physiologic"; n=37); Group 3 representing premenopausal women using systemic hormonal contraception (n=20; 19 using oral contraceptives and one using depo-provera injections); and Group 4 representing postmenopausal women (n=43).

Characteristics for participants, including reproductive status, parity, age in years, height, weight, BMI, and age at menarche are described in **Table 2**. The median ages were as follows: 11.0 years for the premenstrual girls, 24.0 years for the premenopausal group using systemic hormonal contraception, 39.5 years for the premenopausal physiologic group, and 62.9 years for the postmenopausal women. The median age at menarche was 13 for all groups that had reached puberty. Reported median BMIs for Groups 1-4 were 18.0, 24.2, 23.0, and 27.7, respectively. In a subgroup (n=46) of this cohort in whom height and weight were both self-reported and directly measured, the correlation of self-report to actual measurement was high: for height, $r_{Spearman}$=0.969, and for weight, $r_{Spearman}$=0.990. The small degree of disagreement was accounted for by participants underestimating their weight and overestimating their height.

The median, 5th and 95th percentiles of blood leukocyte mRNA counts for each of the analyzed genes in the four groups is shown in **Table 3**. (*SRD5A2* was not expressed in any of the groups and was therefore dropped from further consideration). The expression of seven of 21 measured genes (*AhR*; *AP-1/JUN*; *CYP3A4; ESR2; HSD17β7; LEP;* and *LEPR)* demonstrated significant differences between at least two of the groups. Multiple comparisons of median GE between groups are also shown in **Table 3** and illustrated in **Figure 1** using box plots. Based on the analyses without Bonferroni correction, of 42 possible group differences in the statistically significant findings, 15 were found. Ten of these differences were demonstrated between premenstrual girls and other groups (GE for *CYP3A4* and *LEPR* greater in premenstrual girls than in the premenopausal physiologic group;

Table 2. Characteristics of 105 Participants of the Michigan Fisheaters' Cohort

Variable	Number	Median	5th Percentile	95th Percentile
Premenstrual Girls				
Age at interview (years)	5	11.0	6.4	14.0
Height (cm)	5	152.4	124.5	167.6
Weight (kg)	5	41.7	25.0	54.9
Body Mass Index (kg/m^2)	5	18.0	14.5	20.8
Premenopausal, Physiologic				
Age at interview (years)	37	39.5	15.3	52.3
Height (cm)	37	170.2	160.0	180.3
Weight (kg)	37	70.3	56.7	98.9
Body Mass Index (kg/m^2)	37	24.2	19.2	36.0
Age at menarche (years)	37	13	10	15
Parity	37	2	0	3
Premenopausal, Using Systemic Hormonal Contraception				
Age at interview (years)	20	24.0	15.0	52.0
Height (cm)	20	161.3	157.5	179.1
Weight (kg)	20	60.8	50.8	106.6
Body Mass Index (kg/m^2)	20	23.0	18.3	39.8
Age at menarche (years)	20	13	10	16
Parity	20	0	0	2
Postmenopausal				
Age at interview (years)	43	62.9	50.8	83.4
Height (cm)	43	162.6	154.9	172.7
Weight (kg)	43	74.8	58.1	111.1
Body Mass Index (kg/m^2)	43	27.7	20.8	38.4
Age at menarche (years)	43	13	10	15
Parity	43	3	1	5

GE for *AP-1/JUN* and *LEP* greater in premenstrual girls than in the postmenopausal group; GE for *ESR2* and *HSD17β7*greater in the premenstrual girls than in all three of the other groups). The other five differences between groups were as follows: 1) greater GE of *AP-1/JUN* in the premenopausal physiologic group than in the postmenopausal group; 2) greater GE of *CYP3A4* and *LEPR* in users vs. non-users of systemic hormonal contraception; 3) greater GE of LEP in the premenopausal group using systemic hormonal contraception than in the postmenopausal group; and 4) greater GE of *AhR* in the postmenopausal group compared to the premenopausal physiologic group.

For analyses that involve a large number of comparisons, further statistical adjustments become necessary to account for spurious results that might have been found simply by chance. The most common method uses the Bonferroni correction, which is used here. Using this correction three findings remain significant: 1) greater GE of *AhR* in the postmenopausal group than in the premenopausal physiologic group; 2) greater GE of *ESR2* in premenstrual girls than in the postmenopausal group; and 3) greater GE of *HSD17B7* in premenstrual girls than in the postmenopausal group.

Forty-six women had blood drawn for GE in both 2007 and 2010. In 2007, analysis was limited to four genes: *CYP17, CYP19, ESR1,* and *ESR2*. Intraclass correlation coefficients with 95% confidence intervals for temporal expression of these four genes are shown in **Table 4**. Modest correlations were found for three of the four genes, while almost no correlation for *CYP17*

Table 3. Comparison of Blood Leukocyte mRNA counts for 21 Metabolic or Signaling Genes Involved in Sex Steroid Metabolism according to Female Reproductive Status in 105 Participants of the Michigan Fisheaters' Cohort.

		Group 1 (n=5) Premenstrual Girls			Group 2 (n=37) Premenopausal physiologic#			Group 3 (n=20) Premenopausal using hormonal contraception#			Group 4 (n=43) Postmenopausal			Kruskal-Wallis test	Multiple comparison	
Response Variable	Gene Type†	Median	5th PCTL	95th PCTL	Median	5th PCTL	95th PCTL	Median	5th PCTL	95th PCTL	Median	5th PCTL	95th PCTL	p-value	Type I error Unadjusted	Type I error adjusted
AhR	R	1.2654	0.7592	1.8452	1.0601	0.1739	1.7857	1.1971	0.5278	1.7157	1.3024	0.7551	1.8903	0.0469*	4>2	4>2
AP-1/JUN	R	-1.5399	-1.7817	-1.3506	-1.8441	-2.6187	-0.4887	-2.0139	-2.6480	-0.3098	-2.2050	-2.7539	-0.7962	0.0057*	1>4; 2>4	
AR	R	-5.4467	-6.6046	-4.2801	-6.0320	-7.3933	-4.9123	-5.6384	-6.7383	-4.7006	-5.7403	-7.1566	-4.6865	0.0925		
COMT	C	1.0911	0.4088	1.5284	0.6477	-0.1359	1.3418	0.8088	-0.3692	1.3908	0.8168	-0.0285	1.4963	0.1461		
CYP1A1	C	-8.1372	-9.1982	-6.8893	-7.5861	-10.9370	-6.1107	-8.1517	-10.7557	-5.7129	-7.3274	-10.2287	-5.8938	0.3534		
CYP1A2	C	-9.3858	-11.2495	-7.9592	-10.3464	-12.4374	-8.4077	-10.4646	-12.0781	-7.8025	-10.6473	-12.1724	-8.4009	0.2577		
CYP1B1	C	-1.2681	-2.2279	-0.1499	-1.0277	-2.2140	0.0226	-0.7314	-1.9900	-0.0690	-0.7221	-1.5402	0.4358	0.1210		
CYP3A4	C	-6.9295	-8.8216	-6.0998	-8.9284	-10.8429	-6.0487	-7.6659	-10.9023	-5.9987	-8.3803	-10.8003	-5.8133	0.0416*	1>2; 3>2	
CYP11A1	A	-6.7618	-8.7791	-4.4515	-5.9636	-8.1767	-4.4198	-5.8229	-8.7416	-3.9338	-6.2963	-9.4556	-4.1746	0.7226		
CYP17	A	-9.5339	-9.8446	-7.6637	-9.9217	-11.9439	-7.8881	-9.6152	-11.3792	-8.0345	-10.0712	-11.6794	-7.5353	0.5018		
CYP19	A	-6.9498	-10.4334	-4.7229	-7.1488	-10.3029	-5.1864	-6.9513	-9.8061	-4.1889	-6.6359	-10.5577	-4.9297	0.8710		
ESR1	R	-3.1893	-3.9407	-2.4335	-3.6700	-4.8805	-2.9351	-3.8299	-4.8367	-2.6356	-3.7119	-4.8480	-2.6790	0.2567		
ESR2	R	-4.2649	-5.1569	-4.0048	-5.1774	-7.5901	-4.1470	-5.2141	-7.5243	-3.5196	-5.5809	-7.7742	-3.9233	0.0496*	1>2; 1>3; 1>4	1>4
HSD3B2	A	-11.1629	-11.9063	-9.9061	-11.0465	-13.0367	-9.2585	-11.1973	-13.0122	-8.7922	-11.3317	-12.4861	-8.6574	0.8542		
HSD17B1	A	-7.0404	-9.2263	-5.8948	-7.3929	-11.1520	-4.8545	-7.5541	-9.9428	-4.6640	-7.4276	-9.1811	-5.0968	0.8497		
HSD17B2	C	-11.2495	-11.9063	-9.3926	-11.3278	-12.8790	-8.5741	-11.2056	-13.0122	-9.4698	-11.4776	-12.5631	-9.1782	0.7653		
HSD17B3	A	-5.9820	-6.7085	-4.9669	-6.2406	-7.2604	-5.1886	-6.0257	-7.2720	-5.1611	-6.3076	-7.0509	-5.1845	0.6155		
HSD17B5	A/C	-2.4887	-2.6736	-2.1858	-2.3633	-3.4237	-0.9476	-2.6446	-3.8889	-1.7242	-2.3038	-3.3213	-1.1176	0.4609		
HSD17B7	A/C	-1.0940	-1.5830	-0.7366	-1.6041	-2.8395	-1.0393	-1.7969	-2.5888	-1.0065	-1.8772	-2.4115	-1.1101	0.0445*	1>2; 1>3; 1>4	1>4
LEP	R	-5.4924	-5.8030	-4.6818	-5.8971	-7.2716	-4.0383	-5.5535	-7.0362	-4.1371	-6.3441	-7.3764	-4.8236	0.0286*	1>4; 3>4	
LEPR	R	-2.2620	-2.7241	-1.0964	-2.8441	-3.6966	-1.3017	-2.1303	-3.6004	-1.2215	-2.4487	-3.2657	-1.6820	0.0298*	1>2; 3>2	

†Gene type: A=Anabolism; C=Catabolism; R=Receptor; #Measured during days 3-10 of the menstrual cycle; PCTL = Percentile

Table 4. Intraclass Corrlation Coefficient for temporal gene expression data (2007-2010)

Gene	Number of subjects	Number of observations	Intraclass Correlation Coefficient (ICC) (95% C.I.)
CYP19	46	92	0.2939 (0.1031, 0.6010)
CYP17	46	90	0.0357 (0.0000, 0.9945)
ESR1	46	92	0.3297 (0.1315, 0.6151)
ESR2	46	92	0.3895 (0.1836, 0.6440)

between the two examinations (three years apart) was found.

Discussion

This study found differential blood leukocyte expression of only one of 21 genes, *AhR,* between premenopausal women in the luteal phase of the menstrual cycle and postmenopausal women. In premenstrual girls, there was greater expression of two of 21 genes compared to postmenopausal women: *ESR2* and *HSD17β7.*

The statistically significant increase in GE of *AhR* in the postmenopausal compared to the premenopausal physiologic group is possibly related to its known role as a mediator of sex-steroid related activity. Although the endogenous ligand for the receptor is not known, when ligand-bound, it interacts with the estrogen or androgen receptor to regulate transcription. A variety of substances can act as ligands to either increase or suppress transcription, including certain drugs, foods, and toxic substances. The biological effect depends on whether the ligand is an agonist or antagonist to the Ah receptor [7]. It is reasonable to speculate that the differences between the premenopausal physiologic group and the postmenopausal group could be due to its role in regulating the estrogen receptor. Given that differences were not found in women using hormonal contraception, it is intriguing to further hypothesize that if menstrual differences do account for our finding, then hormonal contraception may interfere with this regulatory function.

Developmental differences may also explain the differential GE found in the premenstrual girls as compared with the postmenopausal women.

Gene expression is dependent on many factors, many of which fluctuate in an individual. It was somewhat surprising, although reassuring, that there was as much correlation in temporal measurements of GE in three of the four genes measured three years apart.

One of the strengths of this study is the meticulous categorization of the reproductive status of its participants. Another is the inclusion of selected genes along a common pathway.

Although this is a comparatively large study in humans to date reporting on GE of sex steroid metabolizing or signaling genes, the number of participants is still relatively small in each category, which may have influenced the results.

The study's greatest weakness is the measurement of GE in circulating white cells. The obvious strength of easy specimen procurement is also its greatest limitation, as white cell GE may not reflect organ-specific GE. As this field advances, measurement of tissue-specific GE is likely to become necessary.

Voterro *et. al.* conducted the only other similar study in humans to date and examined the differential expression of aromatase in children and adult females. They found similar mRNA levels of aromatase in blood leukocytes in children, premenopausal women in the luteal phase of the menstrual cycle, and postmenopausal women [2]. Our measurements of GE of *CYP19,* the product of which is aromatase, corroborate these findings.

It is worth noting that analyses with Bonferroni correction for multiple comparisons tend to be conservative, because the Bonferroni correction uses a smaller significance level for each comparison to declare significance. In our study, when the type I error is adjusted using the Bonferroni correction, only 3 comparisons were

found to be statistically significant (GE for *ESR2* and *HSD17β7*greater in the premenstrual girls than in the postmenopausal group; GE for *AhR* greater in postmenopausal group than in the premenopausal physiologic group).

Conclusions

There are modest correlations between 2007 and 2010 measurements in GE for *CYP 19, ESR1,* and *ESR2,* while almost no correlation for *CYP17* was found. There was little differential variation of blood leukocyte sex steroid GE between premenopausal women in the luteal phase of the menstrual cycle and postmenopausal women for most genes analyzed, but it will be necessary to make statistical adjustments for *AhR* when comparing premenopausal women non-users of systemic hormone contraception and postmenopausal women, and when comparing *ESR2* and *HSD17B7* GE in studies that include premenstrual girls. Developmental differences may explain the differential GE found in *ESR2* and *HSD17β7 in* premenstrual girls as compared with the postmenopausal women.

Acknowledgements

This research was supported by grants from the Agency for Toxic Substances and Disease Registry (R01 TS000007 and R01 TS000069).

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