

## CLINICAL STUDY

# Age, body mass index, race and other determinants of steroid hormone variability: the HERITAGE Family Study

O Ukkola<sup>1,2</sup>, J Gagnon<sup>3</sup>, T Rankinen<sup>1</sup>, P A Thompson<sup>4</sup>, Y Hong<sup>4</sup>, A S Leon<sup>5</sup>, D C Rao<sup>4</sup>, J S Skinner<sup>6</sup>, J H Wilmore<sup>7</sup> and C Bouchard<sup>1</sup>

<sup>1</sup>Pennington Biomedical Research Center, Baton Rouge, Louisiana 70808, USA, <sup>2</sup>Department of Internal Medicine and Biocenter Oulu, University of Oulu, FIN-90220 Oulu, Finland, <sup>3</sup>Laboratory of Molecular Endocrinology, CHUL Research Center, Laval University, Québec G1K 7P4, Canada, <sup>4</sup>Division of Biostatistics, Washington University School of Medicine, St Louis, Missouri, <sup>5</sup>School of Kinesiology and Leisure Studies, University of Minnesota, Minneapolis, Minnesota 55455, <sup>6</sup>Department of Kinesiology, Indiana University, Bloomington, Indiana 46405 and <sup>7</sup>Department of Health and Kinesiology, Texas A&M University, College Station, Texas 77843, USA

(Correspondence should be addressed to O Ukkola, Pennington Biomedical Research Center, Louisiana State University, 6400 Perkins Road, Baton Rouge, Louisiana 70808, USA; Email: ukkolao@pbrc.edu)

## Abstract

**Objective and methods:** To investigate from the HERITAGE Family Study database, 13 steroid hormones (androstane-3 $\alpha$ , 17 $\beta$ -diol glucuronide, androsterone glucuronide, cortisol, dehydroepiandrosterone (DHEA), DHEA ester (DHEAE), DHEA sulfate (DHEAS), dihydrotestosterone (DHT), estradiol, 17-hydroxyprogesterone, progesterone, pregnenolone ester, sex hormone binding globulin (SHBG) and testosterone in each sex for their relationships with age, body mass index (BMI), race and key lifestyle variables. Sample sizes varied from 676 to 750 per hormone. Incremental regression methods were used to examine the contributions of the variables to steroid hormone variability.

**Results:** Age was a major predictor for most steroid hormones. The greatest contribution of age was a negative relationship with DHEAS ( $R^2 = 0.39$ ). BMI was also associated with the variability of several steroid hormones, being the most important predictor of SHBG ( $R^2 = 0.20$ ) and of testosterone ( $R^2 = 0.12$ ) concentrations. When age and BMI were included, race still contributed significantly to the variations in cortisol ( $R^2 = 0.02$  for men and 0.04 for women), DHT ( $R^2 = 0.02$  for men and 0.03 for women), and progesterone ( $R^2 = 0.03$  for women). Nevertheless, race appeared to be less important than age and BMI. In addition, lifestyle indicators (food and nutrient intakes, smoking and physical activity) influenced steroid hormone variability. Their contributions, however, were minor in most cases once age, BMI and race had been taken into account.

**Conclusions:** We conclude that age was the most important factor, followed by BMI, race and lifestyle factors in explaining steroid hormone variability.

*European Journal of Endocrinology* 145 1–9

## Introduction

The effects of age and sex on steroid hormone concentrations have been studied extensively. In most studies, age has not been associated with a major influence on basal cortisol concentrations (1–3). In contrast, some studies suggested both lower (4, 5) and higher (evening) (6) baseline cortisol concentrations in elderly compared with younger men, in addition to an age-related phase advance of the cortisol rhythm (7) in both men and women. Furthermore, greater total cortisol concentrations in elderly women than in men have been demonstrated (8). It is commonly agreed that the concentrations of dehydroepiandrosterone (DHEA) and of its sulfated metabolite DHEAS, which is the most abundant circulating steroid, decrease with age (9). The ratio of DHEA to DHEAS is greater in

women than in men (9). The decline of free, and a less consistent decline of total, testosterone concentrations with age are well established (10), although research design affects the age–testosterone relationship (11). Conversely, sex hormone-binding globulin (SHBG), the major serum carrier of testosterone, increases with age (10); body mass index (BMI), which correlates negatively with SHBG (12), often increases also.

A few studies have explored the effect of race on steroid hormone variability. Differences in the hypothalamic–pituitary–adrenal axis between black and white individual have been suggested, although they are not reflected in cortisol plasma concentrations (13, 14). In addition, lower plasma aldosterone (15) and renin activity have been reported, possibly attributable to lower potassium intake (16) in black populations compared with white groups. Likewise, there is

evidence that black males have greater testosterone concentrations (17).

In contrast, the relationship of lifestyle factors to steroid hormone variation is unclear, although cigarette smoking has been associated with increased concentrations of testosterone (12, 18, 19) and adrenal androgens (19–22) in some investigations. Dietary factors or physical activity level are not believed to be major determinants of adrenal steroid variability (17, 19), although alcohol intake was associated with increased concentrations of some adrenal steroids in one study (19). Little attention has been paid to this issue.

In the present study, the influences of age, BMI, race and lifestyle indicators (including food and nutrient intake, smoking and physical activity) on steroid hormone variability were examined in the baseline phase of the HERITAGE Family Study for male and female participants.

## Methods

The HERITAGE Family Study is a multicenter study the specific aims, design, inclusion and exclusion criteria and methodology of which have been described elsewhere (23). The study involved a total of 855 healthy individuals. Prescribed medication for a chronic condition was an exclusion criterion. The age range of the participants was from 17 to 65 years in both races and sexes. In the present study, an analysis was performed with baseline steroid data from 750 of the HERITAGE Family Study participants. Reasons for eliminating individuals from the analysis were the following: steroid results missing, menopausal status ambiguous, smoker status or other demographic information unavailable or missing data on food and nutrient intakes or physical activity.

The following steroid hormones were assayed: androstane-3 $\alpha$ -, 17 $\beta$ -diol glucuronide (3 $\alpha$ DIOL-G), androsterone glucuronide (ADT-G), cortisol, DHEA, DHEA ester (DHEAE), DHEAS, dihydrotestosterone (DHT), estradiol (E<sub>2</sub>), 17-hydroxyprogesterone (OH-PROG), progesterone, pregnenolone ester (PREG-E), SHBG and testosterone. A total of 750 individuals were available for all the hormones, except for DHEA, DHEAE, progesterone and PREG-E, for which the sample size was 676.

## Lifestyle measurements

At baseline, the participants completed a health habit questionnaire, the Willett Food Frequency (FFQ) inventory (24) (to assess smoking habits, the usual alcohol, food and nutrient intakes) and the ARIC-Baecke Physical Activity Questionnaire (to assess physical activity levels) (25).

## Steroid hormone assays

In the morning, after a 12-h fast, blood samples were obtained from an antecubital vein into vacutainer tubes with no anticoagulant, with participants in a semi-recumbent position. Samples were obtained twice at baseline and drawn at least 24 h apart. The present study was based on mean values from these two samples. For eumenorrheic women, all samples were obtained in the early follicular phase of the menstrual cycle. None of the women in the reproductive age had dramatically irregular menstrual cycles. Fasting serum was prepared according to a standard procedure. After centrifugation of blood at 2000  $\times g$  for 15 min at 4 °C, two aliquots of 2 ml in cryogenic tubes were frozen at –80 °C until shipment within 1 month. Serum samples from the three USA HERITAGE Clinical Centers were shipped in the frozen state to the HERITAGE Steroid Core Laboratory in the Molecular Endocrinology Laboratory at the Laval University Medical Center in Quebec City.

For non-conjugated steroids, DHEA and testosterone were differentially extracted with hexane ethyl acetate, and DHT with petroleum ether (35–65 °C), respectively. In-house RIA was performed to measure these three steroids. Progesterone, OH-PROG, cortisol, E<sub>2</sub> and DHEAS were assayed directly using a commercially available kit (Diagnostic System Laboratories Inc., Webster, TX, USA). For glucuronide (ADT-G and 3 $\alpha$ DIOL-G) and ester (DHEAE and PREG-E) conjugated steroids, ethanol extraction was performed, followed by C18 column chromatography (26). Glucuronide conjugates were submitted to hydrolysis with  $\beta$ -glucuronidase (Sigma Co., St Louis, MO, USA). Fatty acid derivatives were submitted to saponification. Steroids from each fraction were further separated by elution on LH-20 columns. Steroid concentrations were measured by RIA (27). SHBG was determined with an IRMA-count solid phase assay using iodine-125 (Diagnostic System Laboratories Inc., Webster, TX, USA).

## Reproducibility of steroid hormone assays

The reliability of the steroid hormone assays was tested using the HERITAGE Family Study Intercenter Quality Control (ICC) samples (28, 29). The steroid assays were repeated in 5% of the samples. Reliability coefficients for thirteen steroid hormones derived from these repeated assays on 35 participants are as follow: eight of the 13 steroid hormones had excellent assay reliabilities (0.81–0.98), in the remaining five (DHEA, DHEAE, DHT, PREG-E and progesterone), reliability values were only moderate (0.51–0.76).

## Statistical methods

All independent variables used in the present study, along with their abbreviations, are listed in Table 1.

**Table 1** Independent variables used to examine steroid hormone variability.

Variable	Definition
Race	A binary indicator, with 0 = white individual, 1 = black individual
Age	Age as a continuous variable
BMI	Body mass index as a continuous variable
B-SI	Baecke Sports Index as a continuous variable
B-WI	Baecke Work Index as a continuous variable
B-LTI	Baecke Leisure Time Index as a continuous variable
W-Cal	Willett FFQ total calories as a continuous variable
W-Cho	Willett FFQ % carbohydrates as a continuous variable
W-Fat <sub>a</sub>	Willett FFQ % animal-source fat as a continuous variable
W-Fat <sub>v</sub>	Willett FFQ % vegetable-source fat as a continuous variable
W-Prot <sub>a</sub>	Willett FFQ % animal-source protein as a continuous variable
W-Alc	Willett FFQ alcohol consumed per day as a continuous variable
W-Caf	Willett FFQ caffeine use, mg/day as a continuous variable
Cig	Current cigarette smoker as a binary indicator with 0 = non-smoker, 1 = smoker

Because the distribution of steroid hormones is generally considered to be logarithmically normal, all regression analyses were performed on log-transformed values. Data were analyzed separately for men and women. Pearson correlation coefficients between dependent and independent variables were also calculated. Statistical significance was set at three Bonferroni-corrected levels:  $P \leq 0.003$ ,  $0.033 < P \leq 0.010$  and  $0.010 < P \leq 0.050$ . These values were chosen to correct for multiplicity of analysis on correlated variables.

### Incremental $R^2$ evaluation

Models were tested by incremental  $R^2$  methods (30, 31). Using these methods, sets of nested models (models that contain increasingly larger sets of variables) can be easily compared. Rather than focusing on the coefficients and the significance of individual variables, emphasis is on the importance of the group of variables taken as a set. For instance, when the importance of variable  $A$  in explaining a dependent measure is examined,  $R_A^2$  measures variance accounted for by the variable. When another variable  $B$  is also of interest,  $R_{AB}^2$  for the combined regression indicates overall fit due to both variables. The incremental fit of  $A$  given  $B$  measures the amount of variance contributed by  $A$  and not  $B$ , and can be computed as  $R_{A/B}^2 = R_{AB}^2 - R_B^2$ ; it may be interpreted as the contribution of  $A$  above and beyond what  $B$  can account for. Similarly,  $R_{B/A}^2$  measures the unique contribution of variable  $B$ . The regression coefficients in the models for  $A$  and  $B$  are directly related to the incremental fit values.

### Results

General characteristics and lifestyle factors are shown in Table 2, by sex and ethnic origin. Men consumed more calories and alcohol, had higher Baecke Work and Sports Indexes and were heavier than women. Black women were heavier than white women. In addition, black participants were younger than white participants. White individuals consumed more alcohol and caffeine and were more physically active than black individuals. Moreover, the proportion of calories from vegetable fat was greater in white individuals.

Table 3 presents the mean concentrations of the steroid hormones by race and sex. Sex differences were

**Table 2** General characteristics and lifestyle factors by race and sex. Values are means (s.d.).

	Black persons		White persons		$P \leq 0.05^\dagger$
	Men	Women	Men	Women	
<i>n</i>	93–97	160–181	202–227	221–245	
Weight (kg)	85.1 (18.3)	75.0 (17.9)	85.1 (16.6)	66.5 (13.5)	a,b,d
Age (yr)	33.9 (12.1)	32.7 (11.5)	36.3 (15.0)	34.8 (14.1)	b
BMI (kg/m <sup>2</sup> )	27.5 (5.3)	28.4 (6.6)	26.9 (5.0)	24.8 (4.8)	b,d
Cal (kcal/day)	2330.2 (1157.5)	2222.6 (1369.8)	2444.2 (1001.5)	2082.9 (811.8)	a
Cal:Cho (%)	52.6 (8.9)	53.3 (8.8)	51.8 (7.0)	52.9 (7.7)	
Cal:Prot <sub>a</sub> (%)	12.1 (3.8)	12.3 (3.6)	11.1 (2.9)	11.7 (3.4)	b,c,d
Cal:Other protein (%)	4.4 (1.3)	4.5 (1.3)	4.7 (1.3)	5.1 (1.3)	a,b,d
Cal:Fat <sub>a</sub> (%)	19.0 (5.8)	18.5 (6.1)	18.4 (4.9)	17.7 (5.6)	
Cal:Fat <sub>v</sub> (%)	12.0 (3.8)	12.5 (3.8)	13.5 (4.5)	12.9 (3.5)	b,c
Alcohol (g/day)	4.7 (9.0)	2.0 (5.4)	8.2 (13.1)	4.2 (6.4)	a,b,c,d
Cig. Smoking (no=0, yes=1)	0.12 (0.33)	0.12 (0.32)	0.15 (0.36)	0.18 (0.38)	
Caffeine (mg/day)	104.6 (166.6)	98.7 (144.6)	213.3 (236.5)	165.2 (202.5)	b,c,d
B-WI	2.44 (0.91)	2.06 (0.89)	2.32 (1.00)	2.11 (0.97)	a
B-SI	1.81 (0.92)	1.53 (0.77)	1.96 (0.93)	1.78 (0.88)	a,b,d
B-LTI	1.94 (0.52)	1.98 (0.52)	2.21 (0.49)	2.28 (0.46)	b,c,d

† Significant differences between: a, sexes; b, the two races; c, in men between the two races; d, in women between the two races. *n* = Number of individuals. Abbreviations as in Table 1.

**Table 3** Mean concentrations of steroid hormones by race and sex. Values are means (s.d.); units are nmol/l, except for E<sub>2</sub> (pmol/l).

	Black persons		White persons		<i>P</i> ≤ 0.05†
	Men	Women	Men	Women	
ADT-G	158.2 (65.1)	93.2 (55.9)	160.6 (9.7)	91.0 (56.7)	a
Cortisol	351.1 (104.0)	369.2 (184.6)	386.9 (111.3)	485.4 (247.6)	a,b,c,d
DHEA	13.9 (6.5)	12.2 (7.6)	16.1 (10.8)	15.0 (10.7)	a,d
DHEAE	9.3 (4.5)	7.4 (4.3)	8.9 (5.8)	7.7 (5.9)	a
DHEAS	5796.0 (2805.5)	4019.1 (2973.3)	5971.9 (3481.4)	3739.8 (2298.8)	a
DHT	2.9 (1.2)	0.6 (0.2)	2.5 (1.1)	0.5 (0.2)	a,b,c,d
3αDIOL-G	28.8 (11.4)	14.0 (7.5)	30.3 (21.6)	14.2 (8.3)	a
E <sub>2</sub>	79.3 (40.1)	141.8 (164.8)	67.9 (43.7)	134.8 (180.1)	a,b,d
OH-PROG	4.2 (2.1)	1.9 (1.6)	5.9 (2.9)	2.3 (1.9)	a,b,c,d
PREG-E	17.4 (8.8)	14.2 (8.1)	15.9 (9.7)	13.7 (9.5)	a,b
Progesterone	1.8 (0.9)	1.9 (3.8)	2.0 (3.3)	1.6 (1.5)	a
SHBG	36.7 (16.0)	68.3 (41.5)	38.8 (16.9)	89.6 (50.5)	a,b,d
Testosterone	15.1 (5.6)	1.4 (0.6)	14.6 (5.9)	1.4 (0.7)	a

† Significant differences between: a, sexes; b, the two races; c, men between the two races; d, women between the two races.

found for all hormones. Concentrations of some of the hormones (cortisol, DHT and OH-PROG) were consistently different between sexes and races: cortisol and OH-PROG concentrations were lower and DHT concentrations higher in black individuals than in white individuals. Black women had higher E<sub>2</sub> and lower SHBG and DHEA concentrations than white women. A number of hormones (ADT-G, DHEAE, DHEAS, 3αDIOL-G, progesterone and testosterone) did not show any differences between races.

All steroid hormones showed significant negative correlations with age, except E<sub>2</sub> and SHBG (Table 4). The strongest negative correlations for age were observed for DHEA, DHEAE and DHEAS. Some of the hormones (cortisol, DHEA, progesterone and SHBG) correlated negatively with BMI. Correlations between lifestyle factors and steroid hormones were also measured, but they were not generally high. Nevertheless, cigarette smoking correlated positively with DHEAS (Table 4) in both white and black individuals and in both sexes. This correlation persisted after adjustment for age and BMI. Menopause status correlated non-significantly with E<sub>2</sub> concentrations but, after adjustment for age and BMI, negative correlations ( $P < 0.05$ ) between menopause status and E<sub>2</sub> concentrations were observed among both black and white women ( $r = -0.23$  and  $-0.08$  respectively).

### Regression models for steroid hormones

Regression models for the 13 steroid hormones are presented in Table 5. When the full model with all independent variables was considered (column 'All' in Table 5), all were significant ( $P \leq 0.003$ ), except for cortisol in men. When the individual effects of independent variables were considered, age was an important predictor for many of the steroid hormone variables (Table 5). Age was significant ( $P \leq 0.003$ ) for 22 of the 26 cases. Race was significant in five and BMI

in nine of the 26 cases. The set of lifestyle variables (food and nutrient intakes, smoking and physical activity) (column 'Lifestyle factors') was significant ( $P \leq 0.003$ ) in 13 of the 26 cases. Various combinations of these variables were also examined (Table 5, columns 'Age+BMI', 'Age+BMI+Race' and 'All'). The  $R^2$  values of these combinations were sometimes larger than individual  $R^2$  values, but in many cases the increase resulting from the inclusion of the additional variables was negligible.

The models containing increasingly larger sets of variables and incremental  $R^2$  values are shown in Table 6. Column 'Age+BMI|Race' describes the additional information accounted for by age and BMI when race was already included in the model. In 24 of 26 cases, this variance was significant at the Bonferroni-corrected level of  $P \leq 0.003$ . However, the variance accounted for by race that was not accounted for by age and BMI (column 'Race|Age+BMI') was significant ( $P \leq 0.003$ ) in only four of 26 cases. When the incremental contributions of race to the other sets of variables (age, BMI and lifestyle variables including food and nutrient intakes, smoking and physical activity) were examined, race remained significant ( $P \leq 0.003$ ) in two (DHT in women and progesterone in men) of the 26 cases. Thus, of the original five cases (cortisol, DHT and SHBG in women and progesterone in men and women) in which race was significant, other variables accounted for the race differences in three (cortisol, SHBG and progesterone in women) of these cases. There were only three hormones (cortisol, DHEAS and OH-PROG) in women for which the lifestyle variables accounted for important portions of variance not accounted for by age, BMI or race.

### Discussion

The results of this study suggest that age accounts for a significant fraction of the variability of most plasma

**Table 4** Correlation coefficients of the relationships between dependent and independent variables. Only significant ( $P \leq 0.05$ ) correlation values are shown.

Dependent variable	Independent variables												
	Menopause	Age	BMI	B-LTI	B-WI	B-SI	W-Cal	W-Cho	W-Prot <sub>a</sub>	W-Fat <sub>a</sub>	W-Fat <sub>v</sub>	W-Alc	Cig
ADT-G	—, —	-0.39 <sup>a</sup> , -0.46 <sup>b</sup>	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	0.02, -0.11
Cortisol	-0.24, -0.19	-0.38 <sup>c</sup> , -0.17 <sup>d</sup>	-0.08, -0.14	0.09, -0.13	NS	NS	NS	NS	NS	NS	NS	NS	0.08, 0.12
	NS	-0.09, -0.11	-0.34, -0.21	0.15, 0.12	NS	NS	NS	NS	NS	NS	NS	NS	NS
DHEA	—, —	-0.61, -0.49	-0.19, -0.17	NS	0.00, -0.04	NS	NS	NS	NS	NS	NS	NS	NS
DHEAE	-0.37, -0.22	-0.56, -0.40	-0.20, -0.15	NS	-0.13, -0.18	NS	NS	NS	NS	NS	NS	NS	NS
	—, —	-0.35, -0.42	NS	NS	-0.01, -0.13	NS	NS	NS	NS	NS	-0.21, -0.16	NS	NS
DHEAS	-0.28, -0.24	-0.36, -0.21	NS	NS	-0.13, -0.13	NS	NS	NS	NS	NS	-0.09, -0.14	NS	NS
	—, —	-0.57, -0.58	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	0.12, 0.13
DHT	-0.44, -0.30	-0.55, -0.34	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	0.09, 0.11
	NS	-0.32, -0.27	NS	0.08, -0.02	NS	NS	NS	NS	NS	NS	NS	NS	0.03, -0.21
3 $\alpha$ DIOL-G	—, —	-0.15, -0.26	NS	-0.00, -0.17	NS	NS	NS	NS	NS	NS	NS	NS	0.00, 0.13
	-0.19, -0.18	-0.34, -0.28	NS	NS	NS	NS	NS	0.03, -0.11	NS	NS	NS	NS	0.03, -0.18
E <sub>2</sub>	NS	-0.24, -0.17	NS	NS	NS	NS	NS	0.05, -0.13	NS	NS	NS	NS	0.09, 0.14
	—, —	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
OH-PROG	-0.43, -0.29	-0.40, -0.45	NS	NS	-0.07, -0.09	NS	0.14, 0.06	NS	NS	NS	NS	NS	0.12, -0.02
	—, —	-0.38, -0.17	NS	NS	-0.13, -0.12	NS	0.07, -0.1	NS	NS	NS	NS	NS	0.16, 0.12
PREG-E	-0.25, -0.18	-0.25, -0.34	NS	NS	-0.07, -0.03	NS	NS	NS	NS	NS	NS	NS	NS
	—, —	-0.35, -0.18	NS	NS	-0.14, -0.17	NS	NS	NS	NS	NS	NS	NS	NS
Progesterone	-0.25, -0.24	-0.33, -0.31	-0.32, -0.34	NS	NS	NS	0.11, 0.08	0.07, -0.03	NS	0.04, 0.05	NS	NS	0.12, -0.13
	—, —	-0.31, -0.22	-0.09, -0.14	NS	NS	NS	0.06, -0.10	0.01, -0.12	NS	0.01, 0.12	NS	NS	0.10, 0.10
SHBG	-0.01, 0.21	NS	-0.25, -0.29	-0.07, -0.01	NS	-0.03, 0.07	NS	NS	NS	NS	NS	-0.01, -0.15	NS
	—, —	-0.25, -0.24	-0.34, -0.44	-0.14, 0.21	NS	0.01, 0.14	NS	NS	NS	NS	NS	0.08, -0.11	NS
Testosterone	-0.10, -0.23	-0.20, -0.31	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
	—, —	-0.25, -0.24	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS

<sup>a</sup> White male, <sup>b</sup> Black male, <sup>c</sup> White female, <sup>d</sup> Black female, for entire table. NS, not significant. Abbreviations for independent variables as in Table 1.

**Table 5** Regression models ( $R^2$  values) for the steroid hormones.

	Variables and their combinations							
	Sex	Age	BMI	Age+BMI	Race	Age + BMI + Race	Lifestyle factors	All
ADT-G	M	0.17*	0.01#	0.17*	0.00	0.17*	0.03	0.21*
	F	0.10*	0.00	0.11*	0.00	0.11*	0.07*	0.13*
Cortisol	M	0.01	0.01	0.02	0.02**	0.04#	0.06#	0.10#
	F	0.09*	0.11*	0.16*	0.07*	0.20*	0.14*	0.28*
DHEA	M	0.36*	0.03*	0.36*	0.00	0.37*	0.13*	0.39*
	F	0.25*	0.04*	0.25*	0.01#	0.27*	0.15*	0.32*
DHEAE	M	0.16*	0.00	0.17*	0.01	0.17*	0.08#	0.20*
	F	0.10*	0.00	0.11*	0.00	0.12*	0.13*	0.18*
DHEAS	M	0.39*	0.01#	0.39*	0.00	0.39*	0.13*	0.43*
	F	0.23*	0.00	0.25*	0.00	0.25*	0.20*	0.31*
DHT	M	0.10*	0.06*	0.13*	0.02**	0.14*	0.04	0.16*
	F	0.03**	0.01#	0.06*	0.05*	0.09*	0.05	0.11*
3 $\alpha$ DIOL-G	M	0.11*	0.02**	0.11*	0.00	0.11*	0.04	0.14*
	F	0.05*	0.00	0.06*	0.00	0.06*	0.07**	0.09*
E <sub>2</sub>	M	0.04**	0.00	0.04**	0.02#	0.05*	0.06	0.11*
	F	0.08*	0.00	0.08*	0.01#	0.09*	0.05	0.11*
OH-PROG	M	0.18*	0.02**	0.18*	0.00	0.18*	0.09*	0.22*
	F	0.13*	0.00	0.13*	0.00	0.13*	0.19*	0.20*
PREG-E	M	0.10*	0.02#	0.11*	0.01	0.11*	0.06	0.15*
	F	0.10*	0.00	0.10*	0.01	0.11*	0.12*	0.17*
Progesterone	M	0.10*	0.10*	0.14*	0.09*	0.24*	0.09**	0.28*
	F	0.07*	0.02*	0.07*	0.03*	0.11*	0.10*	0.15*
SHBG	M	0.09*	0.07*	0.22*	0.00	0.22*	0.05	0.26*
	F	0.01	0.20*	0.21*	0.06*	0.22*	0.07*	0.25*
Testosterone	M	0.07*	0.12*	0.16*	0.00	0.16*	0.05	0.20*
	F	0.07*	0.00	0.08*	0.00	0.08*	0.08*	0.15*

M, male; F, female. Significance levels: \* $P \leq 0.003$ ; \*\* $0.033 < P \leq 0.010$ ; #  $0.010 < P \leq 0.050$ .

steroid hormone concentrations. It was a strong predictor in 22 of 26 relationships, and a less significant predictor in two of the other four hormone concentrations. The greatest  $R^2$  value (0.39) was for DHEAS, which showed a strong negative correlation with age, consistent with the findings of earlier studies (9). It has been suggested that adrenal delta 5 steroid (DHEAS, OH-PREG, PREG) secretory capacity is significantly decreased in elderly persons (32), perhaps caused by alterations in the zona reticularis of the adrenal cortex (33). Similarly, the decline of testosterone with age observed in the present study is well established (10). In contrast, aging has not been consistently associated with a major change in plasma cortisol concentrations, as age-related changes in cortisol metabolism (including a decrease in secretion rate coupled with a decrease in metabolic clearance) may compensate for each other (34). Nonetheless, some earlier reports found decreasing cortisol concentrations with age in both sexes (35) and in men (4, 5). In the current study, age was a significant predictor of cortisol concentrations only in women, in whom it correlated negatively with age.

BMI, which often increases with age, appears to account for a significant amount of the variance for most steroid hormones. This is not surprising, because the age-related increase in BMI is primarily related to body fat accretion and adipose tissue is an important

site of steroid metabolism (36). BMI was the most important predictor of both SHBG and testosterone and, in keeping with earlier data (12, 19), SHBG correlated negatively with BMI. Indeed, excessive adiposity in men seems to have a key role in determining abnormalities of sex hormone metabolism (37). The age-dependent increase in SHBG reported by others (10) was not observed in the present study. As BMI increases with aging, it may attenuate the expected increase in SHBG, resulting in a lack of correlation. For other steroid hormones, the  $R^2$  values for the combination of age and BMI did not generally differ from those for age alone, indicating that BMI added very little to the variance accounted for by age.

Race represented only a minor component in the variability of most steroid hormones, with a few exceptions. For instance, race contributed significantly to the variances of progesterone and DHT, and marginally to the variance of cortisol, when the other independent variables were already taken into account. Consistent with earlier data (17), DHT concentrations were found to be greater in black individuals in the present study. In contrast to recent work documenting greater testosterone concentrations in African-American men 40 years of age or younger (38), total testosterone concentrations did not differ between the two races in our series. Cortisol concentrations were lower in our black participants than in the white ones;

**Table 6** R<sup>2</sup> differences for combinations of variables.

Dependent variable	Sex	Combinations of variables and R <sup>2</sup> differences						
		Age   Race (1)	Race   Age (2)	Age + BMI   Race (3)	Race   Age + BMI (4)	Age + BMI + Race   Lifestyle (5)	Lifestyle   Age + BMI + Race (6)	Race   Age + BMI + Lifestyle (7)
ADT-G	M	0.00*	0.17	0.17*	0.00	0.19*	0.04	0.00
	F	0.00*	0.10	0.11*	0.00	0.06*	0.02	0.00
Cortisol	M	0.02	0.01**	0.02	0.02**	0.03#	0.06	0.01#
	F	0.08*	0.10*	0.13*	0.04*	0.14*	0.08*	0.01**
DHEA	M	0.01*	0.37#	0.37*	0.01#	0.27*	0.03	0.01
	F	0.02*	0.26*	0.26*	0.02**	0.17*	0.05**	0.01
DHEAE	M	0.00*	0.16	0.16*	0.00	0.12*	0.04	0.00
	F	0.00*	0.10	0.12*	0.00	0.05*	0.06#	0.00
DHEAS	M	0.00*	0.39	0.39*	0.00	0.30*	0.04	0.00
	F	0.00*	0.23	0.25*	0.00	0.11*	0.05*	0.00
DHT	M	0.01*	0.09#	0.12*	0.02#	0.12*	0.02	0.02**
	F	0.05**	0.03*	0.04*	0.03*	0.06*	0.02	0.03*
3α-DIOL-G	M	0.00*	0.11	0.11*	0.00	0.10*	0.03	0.00
	F	0.00*	0.05	0.06*	0.00	0.02	0.04	0.00
E <sub>2</sub>	M	0.01#	0.03#	0.04#	0.01#	0.05	0.06	0.02**
	F	0.01*	0.08	0.08*	0.00	0.06*	0.02	0.00
OH-PROG	M	0.00*	0.18	0.18*	0.00	0.13*	0.04	0.00
	F	0.00*	0.13	0.13*	0.00	0.01	0.07*	0.00
PREG-E	M	0.00*	0.10	0.10*	0.00	0.09*	0.03	0.00
	F	0.00*	0.09	0.10*	0.00	0.05*	0.06#	0.00
Progesterone	M	0.10*	0.11*	0.14*	0.09*	0.18*	0.04	0.06*
	F	0.04*	0.08*	0.08*	0.03*	0.05*	0.04	0.02**
SHBG	M	0.00*	0.09	0.21*	0.00	0.20*	0.04	0.00
	F	0.06	0.01*	0.16*	0.01#	0.17*	0.03	0.00
Testosterone	M	0.00*	0.07	0.16*	0.00	0.14*	0.04	0.01#
	F	0.00*	0.07	0.08*	0.00	0.07*	0.07**	0.00

(1) The additional information accounted for by age when race was already included in the model. (2) The additional information accounted for by race when age was already included in the model. (3) The additional information accounted for by age and BMI when race was already included in the model. (4) The additional information accounted for by race when age and BMI were already included in the model. (5) The additional information accounted for by age, BMI and race when lifestyle factors were already included in the model. (6) The additional information accounted for by lifestyle factors when age, BMI and race were already included in the model. (7) The additional information accounted for by race when age, BMI and lifestyle factors were already included in the model. Significance levels: \* $P \leq 0.003$ , \*\* $0.033 < P \leq 0.010$ , #  $0.010 < P \leq 0.050$ .

this is in line with results from a recent study (39). However, when the other independent variables (age, BMI and lifestyle factors) were considered, evidence for a racial contribution to cortisol concentrations was less clear. Even though differences in the hypothalamic–pituitary–adrenal axis between black and white populations have been suggested (13, 14), these differences have commonly not been reflected as alterations in cortisol secretion. It should be noted that the assessment of cortisol in the present study was performed at only one time point on two mornings, when the secretion of cortisol is usually greatest. Therefore, further research is needed, preferably based on repeated samplings over 24 h, to establish whether differences between white and black populations with respect to cortisol concentrations do indeed exist.

Lifestyle factors, including smoking, diet and activity measures, were only weak predictors of steroid hormone variability. When lifestyle indicators were examined after age, BMI and race had been taken into account, they continued to add significantly to three (cortisol, DHEAS and OH-PROG in women) of the 26 relationships. However, the impact of lifestyle indicators

diminished after age, BMI and race were taken into account. In previous studies, cigarette smoking has been associated with greater testosterone (12, 18, 19) and adrenal androgen concentrations (18–22). We found that, after adjustment for age and BMI, smoking correlated positively with DHEAS in both races and in both sexes. Even though data on the topic are limited, the other lifestyle factors have not been suggested to be major determinants of steroid hormone variability (17, 19), although relationships between some dietary factors and steroid hormones have been suggested (19, 40–42).

In conclusion, age accounted for a significant component of the variance for most steroid hormones. The greatest contribution of age was for DHEAS, with which it was negatively correlated. BMI was the most important predictor of SHBG and testosterone concentrations. Race contributed significantly to the variances of progesterone and DHT, and marginally to the variance of cortisol, when the other independent variables were taken into account. However, race appeared to be less important than age and BMI. Lifestyle indicators contributed only weakly to steroid hormone variability.

## Acknowledgements

The HERITAGE Family Study is supported by the National Heart, Lung, and Blood Institute through Grants HL-45670 (to C Bouchard), HL-47323 (to A S Leon), HL-47317 (to D C Rao), HL-47327 (to J S Skinner) and HL-47321 (to J H Wilmore). Thanks are expressed to Dr Alain Belanger and his collaborators from the Molecular Endocrinology Laboratory at Laval University for the steroid hormone assays. A S Leon is partially supported by the Henry L Taylor endowed Professorship in Exercise Science and Health Enhancement. C Bouchard is supported in part by the George A Bray Chair in Nutrition. O Ukkola is supported by the Finnish Heart Foundation.

## References

- Seeman TE & Robbins RJ. Aging and hypothalamic–pituitary–adrenal response to challenge in humans. *Endocrine Reviews* 1994 **15** 233–260.
- Huizenga NA, Koper JW, de Lange P, Pols HA, Stolk RP, Grobbee DE *et al.* Interperson variability but intraperson stability of baseline plasma cortisol concentrations, and its relation to feedback sensitivity of the hypothalamo–pituitary–adrenal axis to a low dose of dexamethasone in elderly individuals. *Journal of Clinical Endocrinology and Metabolism* 1998 **83** 47–54.
- Knutsson U, Dahlgren J, Marcus C, Rosberg S, Bronnegard M, Stierna P *et al.* Circadian cortisol rhythms in healthy boys and girls: relationship with age, growth, body composition, and pubertal development. *Journal of Clinical Endocrinology and Metabolism* 1997 **82** 536–540.
- Drafta D, Schindler AE, Stroe E & Neascu E. Age-related changes of plasma steroids in normal adult males. *Journal of Steroid Biochemistry* 1982 **17** 683–687.
- Greenspan SL, Rowe JW, Maitland LA, McAloon-Dyke M & Elahi D. The pituitary–adrenal glucocorticoid response is altered by gender and disease. *Journal of Gerontology* 1993 **48** M72–M77.
- Pavlov EP, Harman SM, Chrousos GP, Loriaux DL & Blackman MR. Responses of plasma adrenocorticotropin, cortisol, and dehydroepiandrosterone to ovine corticotropin-releasing hormone in healthy aging men. *Journal of Clinical Endocrinology and Metabolism* 1986 **62** 767–772.
- Sherman B, Wysman C & Pfohl B. Age-related changes in the circadian rhythm of plasma cortisol in man. *Journal of Clinical Endocrinology and Metabolism* 1985 **61** 439–443.
- Touitou Y, Sulon J, Bogdan A, Touitou C, Reinberg A, Beck H *et al.* Adrenal circadian system in young and elderly human subjects: a comparative study. *Journal of Endocrinology* 1982 **93** 201–210.
- Kroboth PD, Salek FS, Pittenger AL, Fabian TJ & Frye RE. DHEA and DHEA-S: a review. *Journal of Clinical Pharmacology* 1999 **39** 327–348.
- Gray A, Feldman HA, McKinlay JB & Longcope C. Age, disease, and changing sex hormone levels in middle-aged men: results of the Massachusetts male aging study. *Journal of Clinical Endocrinology and Metabolism* 1991 **73** 1016–1025.
- Gray A, Berlin JA, McKinlay JB & Longcope C. An examination of research design effects on the association of testosterone and male aging: results of a meta-analysis. *Journal of Clinical Epidemiology* 1991 **44** 671–684.
- Vermeulen A, Kaufman JM & Giagulli VA. Influence of some biological indexes on sex hormone-binding globulin and androgen levels in aging or obese males. *Journal of Clinical Endocrinology and Metabolism* 1996 **81** 1821–1826.
- Yanovski JA, Yanovski SZ, Harrington L, Gold PW & Chrousos GP. Differences in the hypothalamic–pituitary–adrenal axis of black and white men. *Hormone Research* 1995 **44** 208–212.
- Yanovski JA, Yanovski SZ, Gold PW & Chrousos GP. Differences in the hypothalamic–pituitary–adrenal axis of black and white women. *Journal of Clinical Endocrinology and Metabolism* 1993 **77** 536–541.
- Pratt JH, Jones JJ, Miller JZ, Wagner MA & Fineberg NS. Racial differences in aldosterone excretion and plasma aldosterone concentrations in children. *New England Journal of Medicine* 1989 **321** 1152–1157.
- Langford HG, Cushman WC & Hsu H. Chronic effect of KCl on black–white differences in plasma renin activity, aldosterone, and urinary electrolytes. *American Journal of Hypertension* 1991 **4** 399–403.
- Wu AH, Whittemore AS, Kolonel LN, John EM, Gallagher RP, West DW *et al.* Serum androgens and sex hormone-binding globulins in relation to lifestyle factors in older African-Americans, white, and Asian men in the United States and Canada. *Cancer Epidemiology, Biomarkers and Prevention* 1995 **4** 735–741.
- Dai WS, Gutai JP, Kuller LH & Cauley JA. Cigarette smoking and serum sex hormones in men. *American Journal of Epidemiology* 1988 **128** 796–805.
- Field AE, Goldsmith GA, Willett WC, Longcope C & McKinlay JB. The relation of smoking, age, relative weight, and dietary intake to serum adrenal steroids, sex hormones, and sex hormone-binding globulin in middle-aged men. *Journal of Clinical Endocrinology and Metabolism* 1994 **79** 1310–1316.
- Salvini S, Stampfer MJ, Barbieri RL & Hennekens CH. Effects of age, smoking and vitamins on plasma DHEAS levels: a cross-sectional study in men. *Journal of Clinical Endocrinology and Metabolism* 1992 **74** 139–143.
- Longcope C & Johnston CC. Androgen and estrogen dynamics in pre- and postmenopausal women: a comparison between smokers and nonsmokers. *Journal of Clinical Endocrinology and Metabolism* 1988 **67** 379–383.
- Khaw KT, Tazuke S & Barrett-Connor E. Cigarette smoking and levels of adrenal androgens in postmenopausal women. *New England Journal of Medicine* 1988 **318** 1705–1709.
- Bouchard C, Leon AS, Rao DC, Skinner JS, Wilmore JH & Gagnon J. The HERITAGE Family Study: aims, design, and measurement protocol. *Medicine and Science in Sports and Exercise* 1995 **27** 721–729.
- Willett WC, Sampson L, Stampfer MJ, Rosner B, Bain C, Witschi J *et al.* Reproducibility and validity of a semi-quantitative food frequency questionnaire. *American Journal of Epidemiology* 1985 **122** 51–65.
- Baecke JA, Burema J & Frijters JE. A short questionnaire for the measurement of habitual physical activity in epidemiological studies. *American Journal of Clinical Nutrition* 1982 **36** 936–942.
- Bélanger A, Brochu M & Cliché J. Plasma levels of steroid glucuronides in prepubertal, adult and elderly men. *Journal of Steroid Biochemistry* 1986 **24** 1069–1072.
- Brochu M, Belanger A & Tremblay RR. Plasma levels of C-19 steroids and 5 alpha-reduced steroid glucuronides in hyperandrogenic and idiopathic hirsute women. *Fertility and Sterility* 1987 **48** 948–953.
- El-Moalem HE, Gagnon J, Province MA, Bouchard C, Leon AS, Skinner JS *et al.* Race differences in reproducibilities: the HERITAGE Family Study. *American Journal of Human Biology* 1997 **9** 415–424.
- Gagnon J, Province MA, Bouchard C, Leon AS, Skinner JS, Wilmore JH *et al.* The HERITAGE Family Study: quality assurance and quality control. *Annals of Epidemiology* 1996 **6** 520–529.
- Neter J, Wasserman W & Kutner MH. *Applied Linear Statistical Models*. New York: Wiley, 1985.
- Draper NR & Smith H. *Applied Regression Analysis*. New York: Wiley, 1981.



- 32 Vermeulen A, Deslypere JP, Schelfhout W, Verdonck L & Rubens R. Adrenocortical function in old age: response to acute adrenocorticotropin stimulation. *Journal of Clinical Endocrinology and Metabolism* 1982 **54** 187–191.
- 33 Parker CR, Slayden SM, Azziz R, Crabbe SL, Hines GA, Boots LR *et al.* Effects of aging on adrenal function in the human: responsiveness and sensitivity of adrenal androgens and cortisol to adrenocorticotropin in premenopausal women. *Journal of Clinical Endocrinology and Metabolism* 2000 **85** 48–54.
- 34 Romanoff LP, Morris P, Welch RM, Rodriguez RM & Pincus G. The metabolism of cortisol-4-C14 in young and elderly men. *Journal of Clinical Endocrinology and Metabolism* 1961 **21** 1413–1435.
- 35 Sharma M, Palacios-Bois J, Schwartz G, Iskandar H, Thakur M, Quirion R *et al.* Circadian rhythms of melatonin and cortisol in aging. *Biological Psychiatry* 1989 **25** 305–319.
- 36 Longcope C & Baker S. Androgen and estrogen dynamics: relationships with age, weight, and menopausal status. *Journal of Clinical Endocrinology and Metabolism* 1993 **76** 601–604.
- 37 Pasquali R, Casimirri F, Cantobelli S, Melchionda N, Morselli Labate AM, Fabbri R *et al.* Effect of obesity and body fat distribution on sex hormones and insulin in men. *Metabolism* 1991 **40** 101–104.
- 38 Kubricht WS 3rd, Williams BJ, Whatley T, Pinckard P & Eastham JA. Serum testosterone levels in African-Americans and white men undergoing prostate biopsy. *Urology* 1999 **54** 1035–1038.
- 39 Pratt JH, Rebhun JF, Zhou L, Ambrosius WT, Newman SA, Gomez-Sanchez CE *et al.* Levels of mineralocorticoids in whites and blacks. *Hypertension* 1999 **34** 315–319.
- 40 London S, Willett W, Longcope C & McKinlay S. Alcohol and other dietary factors in relation to serum hormone concentrations in women at climacteric. *American Journal of Clinical Nutrition* 1991 **53** 166–171.
- 41 Reed MJ, Cheng RW, Simmonds M, Richmond W & James VHT. Dietary lipids: an additional regulator of plasma levels of sex hormone binding globulin. *Journal of Clinical Endocrinology and Metabolism* 1987 **64** 1083–1085.
- 42 Bélanger A, Locong A, Noel C, Cusan L, Dupont A, Prevost J *et al.* Influence of diet on plasma steroid and sex plasma binding globulin levels in adult men. *Journal of Steroid Biochemistry* 1989 **32** 829–833.

---

Received 11 October 2000

Accepted 30 March 2001