A Genetic Study of Cortisol Measured Before and After Endurance Training: The HERITAGE Family Study

Mary F. Feitosa, Treva Rice, Roland Rosmond, Ingrid B. Borecki, Ping An, Jacques Gagnon, Arthur S. Leon, James S. Skinner, Jack H. Wilmore, Claude Bouchard, and D.C. Rao

The aim of this study was to investigate whether there are familial influences on cortisol levels at baseline and in response to endurance exercise training and, if so, whether there is evidence for a major gene effect. There were 476 white individuals in 99 nuclear families and 247 black individuals in 105 families with valid cortisol data in the HERITAGE Family Study. Data adjustments were carried out separately in each of 8 sex by generation by race groups, using stepwise multiple regression procedures. The familial factors underling the variability in baseline cortisol (log-transformed and adjusted for age and baseline body mass index [BMI]) and its training response (post-training minus baseline, adjusted for age, baseline BMI, and the baseline cortisol value) were assessed by estimating familial correlations and carrying out segregation analysis. In the white sample, significant familial resemblance was detected for both baseline cortisol and the training response, with maximal heritabilities of 38% and 32%, respectively. However, significant familial correlations were not detected for either cortisol phenotype in the black sample, perhaps owing, in part, to the much smaller family sizes. Results of segregation analysis of the white sample provided evidence for Mendelian additive genes influencing baseline cortisol and its training response. The major genes accounted for 33% and 31% of the variance for baseline cortisol and the training response with 48% and 5% of the sample homozygous for the genotype leading to high values, respectively. In conclusion, we found significant familial effects influencing levels of baseline cortisol and its training response in the white sample. The putative major gene effects appear to explain most of the observed familial resemblance, this will motivate further linkage and association studies.

CORTISOL IS ONE of the major secretory product of the adrenal cortex, and in excess causes obesity, insulin resistance, hypertension, and dyslipidemia.[1,2] Adrenocorticotropin (ACTH) released from the pituitary stimulates the adrenals to produce and release cortisol, which in turn is kept within an optimal range through the feedback action of cortisol. This action is mediated via centrally located glucocorticoid receptors interacting with neural control mechanisms.[3,4] Once cortisol is bound to these receptors, the activity of the hypothalamic-pituitary-adrenal (HPA) axis is regulated.[3,4]

Environmental and genetic factors influence the HPA axis, as well as the variation in the cortisol level.[5-7] Environmental challenges, such as stress, endurance training, inactivity, and fasting, transiently increase the plasma concentrations of cortisol.[8-12] Wide variations in cortisol levels, at baseline and in response to different stimuli, have been described in normal individuals, and they seem to be age- and sex-dependent.[8,13-17] Moreover, there is diurnal variation, although there is intraindividual stability when measures are taken early in the morning after an 8-hour fast.[6,12]

Despite reports from several twin studies, the degree of heritability for cortisol secretion is still unclear.[8,18-21] For example, although the heritabilities were similar between the results of Meikle at al[19] (45%) and Inglis at al[20] (46%), the latter estimate was not significant, probably due to small sample size. Kirschbaum et al[8] investigated the genetic effects on cortisol levels at baseline and in response to 3 different stimulation procedures in twin pairs: synthetic human corticotropin-releasing hormone (hCRH), ergometry, and psychological stress. Maximum cortisol responses to these 3 stimuli were significantly intercorrelated in males, but in females only the cortisol responses to hCRH and ergometer exercise showed a significant correlation. However, the heritabilities, based on correlation coefficients of monozygotic and dizygotic pairs, were significant only for variation in cortisol levels at baseline and in response to hCRH, but not for response to exercise and psychological stress.

These twin studies suggest that genetic factors are relevant. However, comparable studies in nuclear families or extended pedigrees have not been reported and major gene hypotheses have not been tested. Thus, the purpose of this study was to investigate the extent of familial resemblance and evidence for major gene effects on cortisol levels both at baseline and in response to endurance training in white and black subjects participating in the HERITAGE Family Study.

MATERIAL AND METHODS

Sample

The HERITAGE Family Study is a multicenter project designed to investigate the role of genetic factors on cardiovascular and diabetes risk factors, and metabolic and hormonal responses to endurance exercise training. The specific aims and study design have been described elsewhere by Bouchard et al.[22] Through extensive publicity and advertisement, 529 white individuals from 99 nuclear families and 326 black

From the Division of Biostatistics, and Departments of Genetics and Psychiatry, Washington University School of Medicine, Saint Louis, MO; Department of Heart and Lung Diseases, Göteborg University, Göteborg, Sweden; Pennington Biomedical Research Center, Louisiana State University, Baton Rouge, LA; Laboratory of Molecular Endocrinology, CHUL Research Center, Ste-Foy, Québec, Canada; School of Kinesiology and Leisure Studies, University of Minnesota, Minneapolis, MN; Department of Kinesiology, Indiana University, Bloomington, IN; and the Department of Health and Kinesiology, Texas A & M University, College Station, TX.

Submitted May 14, 2001; accepted September 17, 2001.

Address reprint requests to Mary F. Feitosa, PhD, Division of Biostatistics, Campus Box 8067, Washington University School of Medicine, 660 S Euclid, St Louis, MO 63110-1093.

0026-0495/02/5103-0011$35.00/0
doi:10.1053/meta.2002.30519

individuals from 105 family units were studied in 4 clinical centers. The inclusion and exclusion criteria were described previously.[22] In summary, individuals were (1) within 17 to 65 years of age; (2) sedentary at baseline, defined as not having engaged in regular vigorous physical activity over the previous 6 months; (3) in good health; (4) with a body mass index (BMI) less than 40 kg/m^2, unless certified by a physician that the subject was capable of undertaking the testing and training program; and (5) with systolic and diastolic blood pressures not greater than 159 mm Hg and 99 mm Hg, respectively. The study was approved by each of the institutional review boards and written informed consent was obtained from each individual. After accounting for missing data, individuals who did not complete the training protocol and outliers (±4 SD from the mean), the final analysis sample comprised 476 white subjects and 247 black subjects.

Endurance Training Program

Each individual was exercise-trained under supervision on a cycle ergometer 3 times a week for 20 weeks using the same standardized training protocol in each of the 4 clinical centers. The intensity and duration of the training program were adjusted every 2 weeks so that during the last 6 weeks of the training the subject exercised at the heart rate associated with 75% of maximum oxygen uptake (VO_2 max) for 50 minutes. The power output of the cycle ergometer was adjusted by computer to match the subject's actual heart rate with the programmed training heart rate.[23]

Measures

A battery of measures relevant to cardiovascular disease and diabetes risk factors was obtained both prior to (baseline) and after the endurance training program. Blood samples were obtained from an antecubital vein into vacutainer tubes with no anticoagulant in the morning, after a 12-hour fast with participants in a semi-recumbent position. Samples were obtained twice at baseline and drawn at least 24 hours apart, and twice after the endurance training program, with one sample drawn 24 hours and the other 72 hours post-training. The present study is based on mean values from these 2 samples obtained at baseline and 2 samples obtained after the endurance training program. For eumenorrheic women, all samples were obtained in the early follicular phase of the menstrual cycle. Fasting serum was prepared according to a standard protocol. After centrifugation of blood at 2,000 × *g* for 15 minutes at 4°C, 2 aliquots of 2 mL in cryogenic tubes were frozen at −80°C until shipment within a month. Serum samples from the 3 United States 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 Québec City. Serum cortisol levels were assayed directly by radioimmunoassay using a commercially available kit (Diagnostic System Laboratories, Webster, TX). Reproducibility was studied from cortisol data obtained across 4 days in an intracenter quality control (ICQC), using technical errors (TE), coefficients of variation (CV) for repeated measures, and intraclass correlation coefficients (ICC) obtained on the main cohort, as well as on ICQC samples from each of the 4 Clinical Centers (described in more detail elsewhere[24-26]). For day-to-day variation in baseline cortisol levels, the values were TE = 107, ICC = 0.52, and CV = 26% in 325 males, and TE = 110, ICC = 0.88, and CV = 25% in 420 females. For the ICQC substudy, the values were TE = 108, ICC = 0.55, and CV = 26% in 35 males, and TE = 115, ICC = 0.70, and CV = 21% in 25 females. Intra-assay error rates were 6.6% in males and 8.7% in females.

Data Adjustments

Baseline cortisol levels were transformed using natural logarithms to correct for nonnormality. All adjustments prior to genetic analysis were carried out separately in each of 8 sex by generation by race groups, using stepwise multiple regression analysis and retaining terms that were significant at the 5% level. The adjusted phenotypes were finally standardized to a mean of zero and an SD of 1. Baseline cortisol was adjusted for the effects of a polynomial in age (age, age^2, age^3) and baseline BMI. The training response was adjusted for the effects of a polynomial in age, baseline BMI, and the baseline cortisol values.

Familial Correlations

The familial correlation model involves 4 types of individuals (*f* = fathers, *m* = mothers, *s* = sons, *d* = daughters) leading to 8 interindividual correlations in 3 classes (1 spouse [*fm*]; 4 parent-offspring [*fs*, *fd*, *ms*, *md*], and 3 sibling [*ss*, *dd*, *sd*]). Familial correlations were estimated by fitting a model directly to data under the assumption of multivariate normality using the maximum likelihood computer program SEGPATH.[27] Reduced models considering sex and generation differences in the correlations were tested using the likelihood ratio test (LRT), which is the difference in minus twice the log-likelihoods (*-2 ln L*) between the reduced model and a more general model. Nonnested models were compared by Akaike's[28] information criterion (*AIC*), which is computed as minus twice the log likelihood of the model plus twice the number of estimated parameters. The model with the lowest AIC indicates the most parsimonious fit to the observed data. Maximal heritability (h^2) was computed from the familial correlations according to the following equation[29]:

$$h^2 = (r_{sibling} + r_{parent\text{-}offspring})\,(1 + r_{spouse})/$$
$$(1 + r_{spouse} + 2\,r_{spouse} \cdot r_{parent\text{-}offspring}).$$

It represents the maximal heritability because both genetic and shared environmental sources of variation are reflected in the familial correlations.

Segregation Model

Segregation analysis was conducted using the unified mixed model[30] as implemented in the computer program POINTER.[31,32] The mixed model assumes that a phenotype is influenced by the independent and additive contributions from a major gene locus, a multifactorial component, and a random environmental component. The major gene effect results from segregation at a single locus having 2 alleles (*A*, *a*), with the genotypes in Hardy-Weinberg equilibrium. There are 7 parameters in the model: the overall variance (*V*); the overall mean (*u*); the allele frequency (*q*), which determines the relative proportion (q^2) of the component distribution with the highest mean; the displacement between the 2 homozygous means (*t*); the relative position of the mean of the heterozygotes, or dominance (*d*); and 2 parameters representing the multifactorial heritabilities in children (*H*) and parents (*HZ*). The transmission pattern from parents to offspring can be tested via transmission probabilities (τ_1, τ_2, and τ_3) to verify if the gene is segregating according to Mendelian expectations. For a single diallelic locus, the 3 τ values denote the probabilities of transmitting allele *A* for genotypes *AA*, *Aa*, and *aa*, with Mendelian expectations of 1, 1/2, and 0, respectively. No transmission of the major effect is obtained when the 3 τ values are equal. Parameters are estimated by maximizing the joint likelihood of the nuclear family data. All analyses are conducted using maximum likelihood methods, and the most parsimonious models are determined using likelihood ratio tests and AIC.

RESULTS

Table 1 gives the sample sizes (N), means, standard deviations (SD), range (minimum and maximum values) of unadjusted baseline cortisol, and its training response, within each of the 4 sex by generation groups for the white and black samples. Based on a comparison of standard errors, these sex and generation differences were significant, with higher mean levels in females than in males, and higher levels in offspring

Table 1. Means, Standard Deviations, and Ranges of Unadjusted Baseline Cortisol and the Training Response for White and Black Samples

Sample/Variable	Fathers N	Fathers Mean ± SD	Fathers Range	Mothers N	Mothers Mean ± SD	Mothers Range
White						
Age	99	53.5 ± 5.3	44.4-64.3	95	52.0 ± 5.1	42.4-65.2
BMI	98	28.4 ± 4.43	20.6-41.5	94	27.6 ± 5.0	18.5-47.5
Baseline cortisol (nmol/L)	98	371.7 ± 100.6	171.5-671.2	93	378.1 ± 130.9†	137.1-779.9
Training response (nmol/L)	97	10.7 ± 92.8	−186.9-230.3	91	48.9 ± 128.9†	−247.6-327.7
Black						
Age	29	50.0 ± 7.2	39.3-65.9	59	46.7 ± 6.6	33.7-64.8
BMI	29	27.5 ± 5.2	19.4-41.9	59	29.4 ± 5.2	20.2-43.5
Baseline cortisol (nmol/L)	28	336.5 ± 92.7	149.0-565.7	57	307.9 ± 119.0†	144.1-688.2
Training response (nmol/L)	25	9.2 ± 97.8	−303.7-123.3	49	−3.3 ± 105.1	−286.4-223.6
	Sons N	Sons Mean ± SD	Sons Range	Daughters N	Daughters Mean ± SD	Daughters Range
White						
Age	163	25.2 ± 6.0	17.0-40.3	171	25.4 ± 6.3	17.2-40.9
BMI	160	25.6 ± 4.9	17.3-44.2	168	23.7 ± 4.5	17.0-39.4
Baseline cortisol (nmol/L)	157	394.3 ± 115.1*	121.3-682.8	169	534.6 ± 274.5*†	145.1-1470.5
Training response (nmol/L)	143	15.7 ± 124.1*	−296.5-307.8	155	55.4 ± 227.2*†	−605.5-783.2
Black						
Age	88	27.0 ± 7.2	15.9-45.8	148	27.7 ± 7.5	16.4-48.1
BMI	84	27.4 ± 5.7	17.4-43.6	147	27.9 ± 7.0	17.5-50.9
Baseline cortisol (nmol/L)	84	366.3 ± 116.4*	150.6-815.0	139	391.1 ± 196.5*†	80.7-1293.0
Training response (nmol/L)	66	−0.13 ± 131.5	−336.6-321.5	117	4.5 ± 166.4	−823.2-450.3

NOTE. Significant ($P < .05$) mean differences for *father-son or *mother-daughter (within sex), and also for †father-mother or †son-daughter (within generation) comparisons.

than parents, for both baseline cortisol and its training response in the white sample. By contrast, for the black sample, there were also significant sex and generation differences in baseline cortisol, with lower mean levels in mothers than fathers, but higher levels in daughters than sons and also higher levels in offspring. However, there were no significant sex or generation differences in the training response in the black families.

BMI for mothers, and age^3 and BMI for daughters were significant predictors of the phenotypic variance of baseline cortisol in the white sample, accounting for 8.7% and 13.8% of the variance, respectively. For the training response in the white sample, significant terms accounted for 10.9% (baseline cortisol), 13.9% (baseline cortisol), 35.7% (baseline cortisol and BMI), and 29.5% (baseline cortisol and BMI) of the variance, in fathers, mothers, sons, and daughters, respectively. For baseline cortisol in the black sample, BMI accounted for 3.9% in daughters, while for training response the significant terms accounted for 23.4% (baseline cortisol), 27.3% (baseline cortisol), and 46% (age, age^2, and baseline cortisol) of the variance, in mothers, sons, and daughters, respectively.

Hypothesis tests regarding familial correlation models for baseline cortisol and the training response in the white and black samples are given in Table 2. The significance tests suggested that sex and generation differences in the correlations were significant for baseline cortisol in the white sample. Both hypotheses of no sex differences in the offspring correlations (model 2: $\chi^2_4 = 13.10$, $P = .011$) and no sex differences in offspring or parents (model 3: $\chi^2_5 = 18.99$, $P = .002$) were rejected. The hypothesis of no sex or generation differences was also rejected (model 4: $\chi^2_6 = 19.11$, $P = .004$). The significance tests suggest that each of the sibling (model 5: $\chi^2_3 = 21.69$, $P < .001$), parent-offspring (model 6: $\chi^2_4 = 22.80$, $P < .001$), and spouse correlations (model 7: $\chi^2_1 = 7.25$, $P = .007$) were greater than zero. The test of no familial resemblance (model 8: $\chi^2_8 = 48.40$, $P < .001$) was rejected, and the correlations could not be equated (model 9: $\chi^2_7 = 19.69$, $P = .006$). Thus, the general model provided the best fit to the data according to the LRT and AIC. The familial correlations were: $fm = 0.28$, $fs = 0.18$, $fd = 0.00$, $ms = 0.39$, $md = 0.21$, $ss = 0.18$, $sd = 0.40$, and $dd = 0.11$. Even though the restricted hypothesis with equal parent-offspring and equal sibling correlations (model 3) was rejected, we can use this model to obtain an approximate estimate of maximal heritability, which was 38%.

For the training response in the white sample, there were parent-offspring (model 6: $\chi^2_4 = 14.27$, $P = .006$) and spouse (model 7: $\chi^2_1 = 13.69$, $P < .001$) correlations. However, the sibling correlations (model 5: $\chi^2_3 = 7.19$, $P = .066$) were not significant, nor were there any sex or generation differences (model 4: $\chi^2_6 = 2.61$, $P = .856$). Thus, the LRT suggested a model with no sex or generation differences and no sibling correlation (ie, $fs = fd = ms = md$, $ss = dd = sd = 0$). Although this model fit by LRT ($\chi^2_6 = 8.08$, $P = .23$), the AIC (10.08) was larger than that for model 4 (AIC = 6.61). Thus, the most parsimonious model was for no sex or generation differences, ie, $fm = 0.38$, $fs = fd = ms = md = ss = sd = dd = 0.18$ (model 4). The maximal heritability was 32%.

Overall, there is evidence of significant familial resemblance for both baseline cortisol and its training response in the white sample. Given the significant spouse correlation with additional parent-offspring and sibling correlations, the heritability is likely to reflect both genetic and familial environmental deter-

Table 2. Summary of Familial Correlation Hypotheses for Baseline Cortisol and Its Training Response in White and Black Samples

		White						Black					
		Baseline Cortisol			Training Response			Baseline Cortisol			Training Response		
Model	*df*	χ^2	*P*	AIC	χ^2	*P*	AIC	χ^2	*P*	AIC	χ^2	*P*	AIC
1. General (*fm, fs, fd, ms, md, ss, dd, sd*)				16.00*			16.00			16.00			16.00
2. No sex offspring (*fm, fs = fd, ms = md, ss = dd = sd*)	4	13.10	.011	21.10	2.04	.729	10.04	3.86	.426	11.86	2.87	.580	10.87
3. No sex difference in offspring and parents (*fm, fs = fd = ms = md, ss = dd = sd*)	5	18.99	.002	24.99	2.05	.843	8.05	4.75	.447	10.75	3.99	.551	9.99
4. No sex or generation differences (*fm, fs = fd = ms = md = ss = dd = sd*)	6	19.11	.004	23.11	2.61	.856	6.61†	6.34	.386	10.34	4.27	.640	8.27
5. No sibling correlation (*fm, fs, fd, ms, md, ss = dd = sd* = 0)	3	21.69	<.001	31.69	7.19	.066	17.19	1.08	.783	11.08	0.79	.852	10.79
6. No parent-offspring correlations (*fm, fs = fd = ms = md* = 0, *ss, dd, sd*)	4	22.80	<.001	30.80	14.27	.006	22.27	9.75	.045	17.75	5.98	.201	13.98
7. No spouse correlation (*fm* = 0, *fs, fd, ms, md, ss, dd, sd*)	1	7.25	.007	21.25	13.69	<.001	27.69	0.00	.987	14.00	2.89	.089	16.89
8. No familial correlation (*fm = fs = fd = ms = md = ss = sd = dd* = 0)	8	48.40	<.001	48.40	34.83	<.001	34.83	10.64	.223	10.64	8.97	.345	8.97
9. No difference in the correlations (*fm = fs = fd = ms = md = ss = sd = dd*)	7	19.69	.006	21.69	6.64	.467	8.64	6.94	.435	8.94	4.69	.697	6.69

NOTE. Correlations values for the parsimonious model: **fm* = 0.28, *fs* = 0.18, *fd* = 0.00, *ms* = 0.39, *md* = 0.21, *ss* = 0.18, *sd* = 0.40, and *dd* = 0.11; †*fm* = 0.38, *fs = fd = ms = md = ss = sd = dd* = 0.18.

minants. On the other hand, for both baseline cortisol and training response in the black sample, the familial correlations generally were not significantly greater than zero (model 8: $\chi^2_8 = 10.64$, $P = .223$; $\chi^2_8 = 8.97$, $P = .345$, respectively), perhaps owing in part to the much smaller sample size. However, there was some borderline evidence of parent-offspring resemblance for baseline levels ($\chi^2_4 = 9.75$, $P = .045$). Thus, the major gene hypothesis was only investigated in the white sample.

The results of segregation analysis for baseline cortisol and training response are presented in Table 3. For baseline cortisol, there was no generation difference ($Z = 1$) in the multifactorial component (model 2 *v* 1: $\chi^2_1 = 0.22$, $P = .64$). There was a significant familial component ($d = t = q = H = 0$) (model 3 *v* 2: $\chi^2_4 = 27.39$, $P < .001$), although neither of the hypotheses of no major gene ($d = t = q = 0$) (model 4 *v* 2: $\chi^2_3 = 4.76$, $P = .19$) or no multifactorial effect ($H = 0$) (model 5 *v* 2: $\chi^2_1 = 0.25$, $P = .62$) could be rejected. In the absence of a multifactorial component, the recessive ($d = 0$) mode of inheritance (model 6 *v* 5: $\chi^2_1 = 5.59$, $P = .02$) was rejected, whereas the dominant mode was borderline (model 8 *v* 5: $\chi^2_1 = 3.18$, $P = .07$), and the additive mode best fit the data (model 7 *v* 5: $\chi^2_1 = 1.59$, $P = .21$). The transmission probabilities were tested under the additive mode. The Mendelian hypothesis (model 7 *v* 9: $\chi^2_3 = 0.07$, $P > .99$) was not rejected, and the no-transmission hypothesis (model 10 *v* 9: $\chi^2_3 = 18.78$, $P < .001$) was rejected. Thus, while either the major gene (model 7) or the multifactorial model (model 4) fit the data, the additive locus hypothesis (model 7) was the most parsimonious as suggested by the AIC. The additive major gene accounted for 33% of the overall phenotypic variance, and approximately 48% (q^2) of the sample were homozygous for the genotype, leading to high values.

For the training response (Table 3), there was a major effect

Table 3. Segregation Results for Baseline Cortisol and Training Response in White Sample

		Baseline Cortisol					Training Response			
.Model	*df*	$-2\ln L + c^1$	χ^2	*P*	AIC	Test	$-2\ln L + c^2$	χ^2	*P*	AIC
1. Mixed		0.00			14.00		0.00			14.00
2. Mixed ($Z = 1$)	1	0.22	0.22	.64	12.22	2 vs. 1	0.10	0.10	.75	12.10
3. Sporadic ($d = t = q = H = 0$)	4	27.61	27.39	<.001	31.61	3 vs. 2	29.97	29.87	<.001	33.97
4. No-major effect ($d = t = q = 0$)	3	4.98	4.76	.19	10.98	4 vs. 2	11.46	11.36	.01	17.46
5. No-multifactorial ($H = 0$)	1	0.47	0.25	.62	10.47	5 vs. 2	3.05	2.95	.09	13.05
6. Recessive ($d = 0$)	1	6.06	5.59	.02	14.06	6 vs. 5	6.06	3.01	.08	14.06
7. Additive ($d = 0.5$)*	1	2.06	1.59	.21	10.06	7 vs. 5	5.01	1.96	.16	13.01
8. Dominant ($d = 1$)	1	3.65	3.18	.07	11.65	8 vs. 5	8.23	5.18	.02	16.23
9. Free τ_S ($d = 0.5$)	3	1.99	0.07	>.99	15.99	7 vs. 9	1.29	3.72	.29	15.29
10. Equal τ_S ($d = 0.5$; $\tau_S = 1 - q$)	3	20.77	18.78	<.001	28.77	10 vs. 9	18.53	17.24	<.001	26.53
*Parsimonious Model ($d = 0.5$)	*V*	*u*		*t*	*q*	*V*	*u*		*t*	*q*
	1.01 ± 0.05	−.01 ± 0.04		1.77 ± 0.15	0.69 ± 0.07	1.03 ± 0.05	0.02 ± 0.04		1.91 ± 0.19	0.23 ± 0.06

NOTE. $-2lnL + c$ = minus twice the log likelihood plus constant; $c^1 = 1332.09$; $c^2 = 1337.79$; *V* = overall variance; *u* = overall mean; *d* = degree of dominance; *t* = displacement between the two extreme component means; *q* = allele frequency.

(model 4 *v* 2: $\chi^2_3 = 11.36$, $P = .01$), but no multifactorial effect (model 5 *v* 2: $\chi^2_1 = 2.95$, $P = .09$). The mode of inheritance appeared to be additive (model 7 *v* 5: $\chi^2_1 = 1.96$, $P = .16$), the Mendelian model was not rejected (model 7 *v* 9: $\chi^2_3 = 3.72$, $P = .29$), and the no-transmission hypothesis was rejected (model 10 *v* 9: $\chi^2_3 = 17.24$, $P < .001$). The additive major gene accounted for 31% of the overall phenotypic variance, with approximately 5% of the sample homozygous for the allele, leading to high values. Thus, the segregation analysis results suggested the presence of a major locus controlling cortisol levels at baseline, as well as in response to endurance training.

DISCUSSION

Cortisol has a strong circadian variation as well episodic secretion. In order to minimize some of these variations, blood samples were collected in the morning based on mean values from 2 samples obtained at baseline and 2 samples obtained after the endurance training program. The HERITAGE data showed a wide interindividual variability in both raw baseline cortisol levels and the training response in white and black samples (Table 1). However, the day-to-day baseline cortisol levels were moderately reproducible with coefficient of variations of 25% to 26% and intraclass correlation coefficients for repeated measurements from different days of 52% to 88%. Interindividual variation as well as intraindividual stability of cortisol levels at baseline and also in response to some stimulation procedures have been described previously.[6,8] For example, Huizenga et al[6] showed intraindividual stability of serum cortisol levels at baseline and 2.5 years later in response to suppression reaction using a low dose of dexamethasone in both sexes. Furthermore, the variation in cortisol levels can be age and sex dependent, as indicated in Table 1 in the present study, which is in accordance with other investigations.[8,13-17]

Despite some twin reports[5-7] of possible genetic factors influencing the variation in cortisol levels, investigations applying other genetic epidemiology methods are scarce. In the present study, evidence of familial resemblance was found both for baseline cortisol and for its training response, in a sample of white nuclear families, with significant maximal heritabilities of 38% and 32%, respectively. However, the familial correlations for baseline cortisol showed considerable variability by sex and generation, and were not consistent with a simple genetic model. Most perplexing was the absence of a significant father-daughter correlation. In regard to the black sample, both for baseline cortisol and its training response, the familial correlations generally were not significantly different from zero. These results need to be viewed with caution, since the lack of familial resemblance in blacks could be due to smaller sample and/or sampling variability.

About 45% of the variation in cortisol levels in twins is heritable,[19,20] and the intraclass correlations in monozygotes (~0.55 to 0.95) are higher than in dizygotes (~0.24 to 0.50)[8,19] both at baseline and in response to different stimuli.[8] However, correlations among members of nuclear families or extended pedigrees have not been described yet. The present analysis shows a clear suggestion of familial aggregation, as reflected by significant sibling and parent-offspring correlations. However, environmental effects also are likely to have an important role in the variability of cortisol levels given the magnitude of the spouse correlation as compared to the parent-offspring and siblings correlations.

This study is also unique in that we investigated a major gene hypothesis controlling cortisol levels for both the baseline and the response to endurance training. For the white sample, evidence of a major gene controlling the baseline cortisol levels and its response to endurance training was obtained, accounting for 33% and 31% of the phenotypic variance, respectively. It is interesting that there was no additional multifactorial effect, ie, the familial effect appears to be due entirely to a major gene (33% and 31%) for each trait. In fact, the percentage of variance accounted for by the major gene is similar to the accountable variance from the familial correlation model (38% and 32%). Since the mode of inheritance of the major gene was additive, these findings are consistent across the 2 types of methods (familial correlations *v* segregation analysis). In fact, the perplexing finding was the suggestion of an environmental component in the familial correlations (as evidenced by significant spouse correlation), which was not found in the segregation models (multifactorial component not significant).

In summary, the present study suggests that despite the complex pattern of familial resemblance for cortisol levels, segregation analysis supports a major gene effect influencing baseline cortisol and the response to training. However, circulating levels of cortisol are influenced by a feedback loop involving the HPA axis, in addition to mediaton by glucocorticoid receptors. Thus, further genetic studies using linkage and association analyses particularly with regard to genes involved in the HPA axis are warranted.

ACKNOWLEDGMENT

The HERITAGE Family Study is supported by the National Heart, Lung, and Blood Institute through the following grants: HL45670 (C. Bouchard, PI), HL47323 (A.S. Leon, PI), HL47317 (D.C. Rao, PI), HL47327 (J.S. Skinner, PI), and HL47321 (J.H. Wilmore, PI). It is also supported by a NIH grant to the University of Minnesota Clinical Research Center. Thanks are expressed to Dr Alain Belanger and his collaborators in the Molecular Endocrinology Laboratory at Laval University where the steroids were assayed. A.S. Leon is also supported in part by the Henry L. Taylor Professorship in Exercise Science and Health Enhancement. C. Bouchard also is partially supported by the George A. Bray Chair in Nutrition.

REFERENCES

1. Rosmond R, Björntorp P: The interactions between hypothalamic-pituitary-adrenal axis activity, testosterone, insulin-like growth factor I and abdominal obesity with metabolism and blood pressure in men. Int J Obes Relat Metab Disord 22:1184-1196, 1998

2. Fraser R, Ingram MC, Anderson NH, et al: Cortisol effects on body mass, blood pressure, and cholesterol in the general population. Hypertension 33:1364-1368, 1999

3. Jacobson L, Sapolsky R: The role of the hippocampus in feedback regulation of the hypothalamic-pituitary-adrenocortical axis. Endocr Rev 12:118-134, 1991

4. De Kloet ER, Vreugdenhil E, Oitzl MS, et al: Brain corticosteroid receptor balance in health and disease. Endocr Rev 19:269-301, 1998

5. Linkowski P, Van Onderbergen A, Kerkhofs M, et al: Twin study of the 24-h cortisol profile: Evidence for genetic control of the human circadian clock. Am J Physiol 264:E173-181, 1993

6. Huizenga NA, Koper JW, de Lange P, et al: Interperson variability but intraperson stability of baseline plasma cortisol levels, and its relation to feedback sensitivity of the hypothalamo-pituitary-adrenal axis to a low dose of dexamethasone in elderly individuals. J Clin Endocrinol Metab 83:47-54, 1998

7. Rosmond R, Chagnon YC, Holm G, et al: A glucocorticoid receptor gene marker is associated with abdominal obesity, leptin, and dysregulation of the hypothalamic-pituitary-adrenal axis. Obes Res 8:211-218, 2000

8. Kirschbaum C, Wust S, Faig HG, et al: Heritability of cortisol responses to human corticotropin-releasing hormone, ergometry, and psychological stress in humans. J Clin Endocrinol Metab 75:1526-1530, 1992

9. Rosmond R, Dallman MF, Björntorp P: Stress-related cortisol secretion in men: relationships with abdominal obesity and endocrine, metabolic and hemodynamic abnormalities. J Clin Endocrinol Metab 83:1853-1859, 1998

10. Ferrando AA, Stuart CA, Sheffield-Moore M, et al: Inactivity amplifies the catabolic response of skeletal muscle to cortisol. J Clin Endocrinol Metab 84:3515-3521, 1999

11. Bell GJ, Syrotuik D, Martin TP, et al: Effect of concurrent strength and endurance training on skeletal muscle properties and hormone levels in humans. Eur J Appl Physiol 81:418-427, 2000

12. Wust S, Federenko I, Hellhammer DH, et al: Genetic factors, perceived chronic stress, and the free cortisol response to awakening. Psychoneuroendocrinology 25:707-720, 2000

13. Lamb EJ, Noonan KA, Burrin JM: Urine-free cortisol excretion: Evidence of sex-dependence. Ann Clin Biochem 31:455-458, 1994

14. Van Cauter E, Leproult R, Kupfer DJ: Effects of gender and age on the levels and circadian rhythmicity of plasma cortisol. J Clin Endocrinol Metab 81:2468-2473, 1996

15. Andrew R, Phillips DI, Walker BR: Obesity and gender influence cortisol secretion and metabolism in man. Clin Endocrinol Metab 83:1806-1809, 1998

16. Bergendahl M, Iranmanesh A, Mulligan T, et al: Impact of age on cortisol secretory dynamics basally and as driven by nutrient-withdrawal stress. J Clin Endocrinol Metab 85:2203-2214, 2000

17. Drew PD, Chavis JA: Inhibition of microglial cell activation by cortisol. Brain Res Bull 52:391-396, 2000

18. Maxwell JD, Boyle JA, Greig WR, et al: Plasma corticosteroids in healthy twin pairs. J Med Genet 6:294-297, 1969

19. Meikle AW, Stringham JD, Woodward MG, et al: Heritability of variation of plasma cortisol levels. Metabolism 37:514-517, 1988

20. Inglis GC, Ingram MC, Holloway CD, et al: Familial pattern of corticosteroids and their metabolism in adult human subjects—The Scottish Adult Twin Study. J Clin Endocrinol Metab 84:4132-4137, 1999

21. Young EA, Aggen SH, Prescott CA, et al: Similarity in saliva cortisol measures in monozygotic twins and the influence of past major depression. Biol Psychiatry 48:70-74, 2000

22. Bouchard C, Leon AS, Rao DC, et al: The HERITAGE Family Study. Aims, design, and measurement protocol. Med Sci Sports Exerc 27:721-729, 1995

23. Skinner JS, Wilmore KM, Krasnoff JB, et al: Adaptation to a standardized training program and changes in fitness in a large, heterogeneous population: The HERITAGE Family Study. Med Sci Sports Exerc 32:157-161, 2000

24. Gagnon J, Province MA, Bouchard C, et al: The HERITAGE Family Study: Quality assurance and quality control. Ann Epidemiol 6:520-529, 1996

25. Skinner JS, Wilmore KM, Jaskolska A, et al: Reproducibility of maximal exercise test data in the HERITAGE Family Study. Med Sci Sports Exerc 31:1623-1628, 1999

26. Ukkola O, Gagnon J, Rankinen T, et al: Age, body mass index, race and other determinants of steroid hormone variability: The HERITAGE Family Study. Eur J Endocrinol 145:1-9, 2001

27. Province MA, Rao DC: A general purpose model and a computer program for combined segregation and path analysis (SEGPATH): Automatically creating computer programs from symbolic language model specifications. Genet Epidemiol 12:203-219, 1995

28. Akaike H: A new look at the statistical model identification. IEEE Trans Autom Control 19:716-723, 1974

29. Rice T, Despres JP, Daw EW, et al: Familial resemblance for abdominal visceral fat: The HERITAGE Family Study. Int J Obes Relat Metab Disord 21:1024-1031, 1997

30. Lalouel JM, Rao DC, Morton NE, et al: A unified model for complex segregation analysis. Am J Hum Genet 35:816-826, 1983

31. Morton NE, Rao DC, Lalouel JM: Methods in Genetic Epidemiology. New York, NY, Karger, 1983

32. Lalouel JM, Morton NE: Complex segregation analysis with pointers. Hum Hered 31:312-321, 1981