Pleiotropic Relationships between Cortisol Levels and Adiposity: The HERITAGE Family Study

Mary F. Feitosa, Treva Rice,* Roland Rosmond,†‡ Tuomo Rankinen,‡ Arthur S. Leon,§ James S. Skinner,¶ Jack H. Wilmore,** Claude Bouchard,‡ and D. C. Rao*‡‡††*

Abstract

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Objective: To investigate familial basis for the relationship between cortisol adiposity at baseline and their training responses.

Research Methods and Procedures: Bivariate correlation and segregation analyses were employed between cortisol and several adiposity measures [body mass index, fat mass (FM), fat-free mass, percentage of body fat (% BF), abdominal visceral fat (AVF), abdominal subcutaneous fat (ASF), and abdominal total fat (ATF)] from 99 white families and 105 black families.

Results: In both races, significant inverse phenotypic correlations were generally observed between cortisol and adiposity measures at baseline but not for training responses. Significant cross-trait familial correlations were found for cortisol with abdominal fat (ASF, AVF, ATF) and overall body adiposity (FM, % BF) measures at baseline, which accounted for 14% to 20% of the phenotypic variance in whites. The cross-trait correlations were not significant for baseline phenotypes in blacks, perhaps because of the small sample size. A bivariate segregation analysis showed evidence of polygenic pleiotropy for cortisol with both abdominal fat and overall adiposity measures that accounted for 14% to 17% of the phenotypic covariance, but major gene pleiotropy was not suggested in whites. However, when ASF, AVF, and ATF were additionally adjusted for FM, no familial cross-trait correlations or polygenic pleiotropy between cortisol and the abdominal fat measures remained.

Discussion: Evidence was found for polygenic pleiotropy but not for pleiotropic major gene effects between cortisol and overall adiposity in whites. However, the covariation of cortisol with abdominal fat phenotypes is dependent on concomitant polygenic factors for total-body fat.

Key words: heritability, familial resemblance, bivariate correlation analysis, bivariate segregation analysis

Introduction

Elevated cortisol levels are known to cause insulin resistance and are most likely involved in the pathogenesis of visceral obesity as seen in Cushing's syndrome (1,2). Some cases of abdominal obesity have clinical features of hypercortisolism with dysregulation of the hypothalamic-pituitary-adrenal axis (3). Studies have shown that the stimulated hypothalamic-pituitary-adrenal axis reveals abnormalities in obesity more clearly than measurements of "basal" activity. Such stimulations include food intake (4,5), perceived stress (6), and direct stimulation with corticotropin-releasing hormone or adrenocorticotropin. Adrenocorticotropin tests have shown increased cortisol responses in abdominal obese subjects (7–9).

Despite the vast number of studies concerning cortisol levels and their relationship with obesity, little is known about genetic factors contributing to variation in cortisol levels or about the common familial factors leading to the

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*Division of Biostatistics, Washington University School of Medicine, Saint Louis, Missouri; †Department of Clinical Chemistry, Göteborg University, Göteborg, Sweden; ‡Pennington Biomedical Research Center, Louisiana State University, Baton Rouge, Louisiana; §School of Kinesiology and Leisure Studies, University of Minnesota, Minneapolis, Minnesota; ¶Department of Kinesiology, Indiana University, Bloomington, Indiana; **Department of Health and Kinesiology, Texas A & M University, College Station, Texas; ††Department of Genetics, Washington University School of Medicine, Saint Louis, Missouri; and ‡‡Department of Psychiatry, Washington University School of Medicine, Saint Louis, Missouri.
Address correspondence to Mary Furlan Feitosa, PhD, Division of Biostatistics, Campus Box 8067, Washington University School of Medicine, 660 S. Euclid; St. Louis, MO 63110-1093.
E-mail: maryf@wubios.wustl.edu

covariation between cortisol levels and abdominal adiposity measures. Studies have shown that the heritability of cortisol levels is ~45% in twin samples (10,11). In the Health, Risk Factors, Exercise Training and Genetics (HERITAGE) study, the heritabilities were only slightly lower in nuclear families at baseline (38%) and for cortisol changes in response to 20 weeks of exercise training (32%) (12). Furthermore, this familial effect could be attributed to a major gene rather than polygenic factors. Moreover, both polygenic and major gene effects are known to play important roles in variation of total body fat composition and abdominal adiposity (13,14). Whether the genes that influence cortisol levels are in part the same affecting body fat and abdominal adiposity has not been addressed. In the current study, we will investigate whether genetic and environmental determinants are shared between cortisol levels and body fat measures using bivariate methods that examine both polygenic and major gene factors in HERITAGE white and black families.

Research Methods and Procedures

Study Design and Data Sample

The HERITAGE Family Study was designed to investigate the effects of regular exercise on several cardiovascular disease and diabetes risk factors and to determine the role of genetic factors in cardiovascular, metabolic, and hormonal responses to endurance exercise training. The study design, sample, and protocol have been described in detail elsewhere (15). In summary, nuclear families (i.e., parents and their biological offspring) were recruited and studied in four clinical centers. Subjects were required to be sedentary at baseline, defined as engaging in no regular vigorous physical activity over the previous 6 months. Subjects were required to be between 17 and 65 years old and in good health. The body mass index (BMI) was <40 kg/m^2, although exceptions were made if certified by a physician (blacks: two fathers, three mothers, three sons, and nine daughters; whites: three fathers, three mothers, and two sons). Subjects with blood pressures >159 mm Hg for systolic and/or >99 mm Hg for diastolic or taking antihypertensive or lipid-lowering drugs were excluded. The study was approved by the Institutional Review Board at each center, and written informed consent was obtained from each subject. There were 501 white subjects in 99 families and 277 black subjects in 117 families participating in the HERITAGE Family Study. From these groups, there were 476 white subjects and 247 black subjects with complete data.

Endurance Training Program

Each subject was exercise-trained under supervision on a cycle ergometer three times per week for 20 weeks using the same standardized training protocol in each of the four clinical centers. The intensity and duration of the training program were adjusted every 2 weeks, beginning at a heart rate corresponding to 55% of their baseline maximal oxygen uptake ($V\mathrm{O}_{2\mathrm{max}}$) for 30 minutes per session and increasing gradually to a training heart rate associated with 75% of the subject's $V\mathrm{O}_{2\mathrm{max}}$ for 50 minutes during the last 6 weeks. The power output of the cycle ergometer was adjusted automatically to maintain the desired heart rate of the subject at all times during all training sessions. All training sessions were supervised onsite (15,16).

Measures

Before (baseline) and after the endurance training program, a battery of measures relevant to cardiovascular disease and diabetes risk factors was obtained on each family member. Training responses were simply computed as the difference between posttraining and baseline values.

Blood samples were collected from an antecubital vein into vacutainer tubes with no anticoagulant in the morning (after a 12-hour fast with participants in a semirecumbent position). Samples were obtained twice at baseline, drawn at least 24 hours apart, and twice after the endurance training program, with one sample drawn 24 hours and the other 72 hours posttraining. This study is based on mean values from these two samples obtained at baseline and two 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 2000*g* for 15 minutes at 4 °C, two aliquots of 2 mL were placed in cryogenic tubes and frozen at −80 °C until shipment (within 1 month). Serum samples from the three 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 Inc., Webster, TX). Reproducibility was studied from cortisol data obtained across 4 days in an Intracenter Quality Control, 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 Intracenter Quality Control samples from each of the four Clinical Centers. For day-to-day variation in baseline cortisol levels, the values were TE = 107, ICC = 0.52, and CV = 26% in 325 men, and TE = 110, ICC = 0.88, and CV = 25% in 420 women. For the intracenter quality control substudy, the values were TE = 108, ICC = 0.55, and CV = 26% in 35 men, and TE = 115, ICC = 0.70, and CV = 21% in 25 women. Intra-assay error rates were 6.6% in men and 8.7% in women (12).

The adiposity phenotypes include both baseline and response to training for BMI (kilograms per square centimeter), as well as body composition components measured by

Table 1. Number of subjects (*N*), means ± SD, minimum (Min) and maximum (Max) values of unadjusted variables for white and black samples

	Father			Mother			Son			Daughter		
Variable	***N***	**Mean ± SD**	**Min–Max**	***N***	**Mean ± SD**	**Min–Max**	***N***	**Mean ± SD**	**Min–Max**	***N***	**Mean ± SD**	**Min–Max**
White sample												
Cortisol baseline	98	371.7 ± 100.6	171–671	93	378.1 ± 130.9	137–780	156	393.8 ± 115.3	121–683	168	531.7 ± 272.7	145–1470
Response	93	11.2 ± 94.7	−187–230	90	49.5 ± 129.4	−247–328	138	20.8 ± 122.2	−275–308	153	59.2 ± 222.9	−605–783
BMI	98	28.4 ± 4.4	21–41	94	27.6 ± 5.0	19–48	160	25.6 ± 4.9	17–44	167	23.7 ± 4.5	17–39
AVF	98	158.6 ± 61.5	55–380	93	120.1 ± 59.0	32–328	157	77.6 ± 43.2	13–242	166	52.7 ± 29.8	13–175
ASF	98	269.3 ± 108.3	76–622	93	363.1 ± 121.5	96–746	157	204.0 ± 145.4	21–718	166	251.3 ± 146.0	40–737
ATF	98	427.9 ± 147.2	140–811	93	483.4 ± 164.4	131–986	157	281.6 ± 183.7	34–925	166	304.5 ± 167.7	63–865
FM	92	24.6 ± 9.0	8–58	85	27.0 ± 10.4	10–62	147	17.1 ± 10.9	0–53	167	18.0 ± 9.8	3–59
FFM	92	62.3 ± 7.5	47–80	85	44.5 ± 5.0	35–57	147	64.3 ± 8.1	45–87	167	46.1 ± 5.2	34–65
%BF	92	27.6 ± 6.4	11–42	85	36.6 ± 7.9	20–54	147	19.7 ± 9.1	1–43	167	26.7 ± 9.0	7–53
Black sample												
Cortisol baseline	28	336.5 ± 92.7	149–566	57	307.9 ± 119.0	144–688	84	366.3 ± 116.4	151–815	139	391.1 ± 196.5	81–1293
Response	24	9.3 ± 10.0	−304–123	48	−5.1 ± 105.5	−286–224	64	2.8 ± 125.3	−230–322	111	2.0 ± 169.7	−823–450
BMI	29	27.5 ± 5.2	19–42	59	29.4 ± 5.2	20–43	86	27.4 ± 5.8	17–44	147	27.9 ± 7.0	18–51
AVF	29	105.9 ± 69.3	33–311	57	95.7 ± 43.8	10–245	83	67.6 ± 50.4	8–231	145	59.7 ± 36.0	10–197
ASF	29	243.9 ± 139.1	30–609	57	388.7 ± 144.3	60–717	83	230.9 ± 186.8	9–693	145	337.6 ± 191.7	50–860
ATF	29	349.7 ± 190.7	64–829	57	484.4 ± 174.7	71–875	83	298.6 ± 228.4	19–911	145	397.3 ± 218.2	63–941
FM	25	21.3 ± 8.7	10–49	39	31.1 ± 10.3	10–56	81	20.7 ± 12.6	2–55	121	27.1 ± 13.4	8–73
FFM	25	60.2 ± 5.9	49–69	39	46.3 ± 5.1	35–61	81	65.5 ± 9.5	41–93	121	46.3 ± 6.6	28–69
%BF	25	25.3 ± 6.0	16–43	39	39.2 ± 7.3	21–51	81	22.3 ± 8.9	3–41	121	35.1 ± 9.1	16–55

Note: For total white sample, skewness of 0.96, 1.30, and 1.88 and kurtosis of 1.03, 2.08, and 5.06 for BMI, AVF, and ATF before log transformation, respectively; and after log transformation, skewness of 0.45, −0.12, and 0.31 and kurtosis of −0.07, −0.64, and 0.52, respectively. For total black sample, skewness of 0.74, 1.31, and 1.91 and kurtosis of 0.27, 2.53, and 6.00 for BMI, AVF, and ATF before log transformation, respectively; and after log transformation, skewness of 0.25, −0.40, and 0.24 and kurtosis of −0.47, −0.20, and 0.70, respectively.

BMI, body mass index; AVF, abdominal visceral fat; ASF, abdominal subcutaneous fat; ATF, abdominal total fat; FM, fat mass; FFM, fat-free mass; %BF, percentage of body fat.

the hydrostatic weighing technique (17). The mean of the highest 3 of 10 measurements was used to calculate percentage of body fat (%BF) from body density (18). Total-body fat mass (FM; in kilograms) and fat-free mass (FFM; in kilograms) were calculated from %BF and body weight. Abdominal fat area (in square centimeters) was assessed by computerized tomography scan (19). Subjects were examined in a supine position with their arms stretched above the head. The abdominal scan was obtained between the fourth and fifth lumbar vertebrae. Abdominal total fat (ATF) was calculated by delineating the abdominal scan with a graph pen and computing the adipose tissue surface with an attenuation range from −190 to −30 Hounsfield units. The abdominal visceral fat (AVF) was defined by drawing a line within the muscle wall surrounding the abdominal cavity. The abdominal subcutaneous fat (ASF) was calculated by subtracting the AVF from the ATF. A reliability study (20) of the HERITAGE subjects, as well as a traveling crew of representative subjects who were measured at each of the clinical sites, found the anthropometric and body composition measures to be highly reproducible in terms of day-to-day variability and measurement errors. For example, intraclass correlations for the total sample ranged from 0.95 to 0.99 for the anthropometric measures and from 0.97 to 1.00 for the body composition measures. Menopausal status and hormonal intake were assessed as dichotomous covariates (scored as yes/no).

Data Adjustments

Baseline cortisol levels, BMI and AVF were transformed using natural logarithms to improve distributional properties (see Table 1). All adjustments before genetic analysis were carried out separately within each of eight sex × generation × race groups, using stepwise multiple regression and retaining the terms that were significant at the 5% level. During model development, individuals with extreme

scores (>4 SDs from the mean) were temporarily set aside so that they would not unduly influence the regressions. While baseline cortisol was adjusted for the effects of a polynomial in age (age, age^2, age^3), menopausal status, and hormonal intake, the adiposity phenotypes (BMI, ASF, AVF, ATF, FM, FFM, and %BF) were individually adjusted for the effects of age (age, age^2, age^3). The residual variances were also examined for age effects (heteroscedasticity) by regressing the squared residual from the mean age regression on another cubic polynomial in age in a stepwise manner and retaining significant terms. For cortisol levels, menopausal status and hormonal intake together with age effects were examined in the residual variances. A similar set of stepwise regressions (by sex and generation groups) was also performed on ASF, AVF, and ATF phenotypes using polynomials in age (age, age^2, age^3) and FM. The final phenotypes were computed using the best regression models and standardized to a mean of 0 and an SD of 1. Similar procedures were applied to the training responses, although the responses were also adjusted for the respective baseline values. After these models were developed, the extreme outliers were added back to the data, and adjusted scores were computed for the entire sample. A final check of the adjusted data revealed two values for baseline cortisol, four for response cortisol, one for AVF, and four for FM with extreme values (~5 SD or greater from the mean). These outlying values involved a total of four individuals, who were excluded from further analysis.

Correlation Analysis

Sex-specific familial correlation models were used to investigate the extent of familial variation in each trait and the covariation between two traits in a bivariate analysis. The model accounts for four types of subjects (f = fathers, m = mothers, s = sons, d = daughters) leading to eight interindividual correlations (fm, fs, fd, ms, md, ss, dd, sd). In the bivariate extension, in addition to estimating the interindividual correlations for each trait, the inter- and intraindividual cross-trait correlations also are estimated, leading to a total of 34 estimated correlations (21). Thus, for the general bivariate model, the estimated interindividual cross-trait correlations include 4 sibling (s_1s_2, d_1d_2, s_1d_2, s_2d_1), 8 parent-offspring (f_1s_2, f_1d_2, m_1s_2, m_1d_2, f_2s_1, f_2d_1, m_2s_1, m_2d_1), and 2 spouse correlations (f_1m_2, f_2m_1); 4 intraindividual cross-trait correlations (s_{12}, d_{12}, f_{12}, m_{12}); and 16 interindividual correlations within trait (trait 1: f_1m_1, f_1s_1, f_1d_1, m_1s_1, m_1d_1, s_1s_1, d_1d_1, s_1d_1; trait 2: f_2m_2, f_2s_2, f_2d_2, m_2s_2, m_2d_2, s_2s_2, d_2d_2, s_2d_2). In all cases, trait 1 refers to cortisol levels and trait 2 refers to an adiposity measure. Familial correlations were estimated by maximum likelihood under the assumption of multivariate normality of all data within each family, using the computer program SEGPATH (22). Reduced models (hypotheses) were evaluated using the likelihood ratio test, which is the difference minus twice the log-likelihoods ($-2\ln L$) between a reduced model and a more general model. The likelihood ratio test is asymptotically distributed as a χ^2, with degrees of freedom being given by the difference in the number of parameters estimated in the two models. Non-nested models were compared by Akaike's (23) information criterion (AIC), which is computed as minus twice the log likelihood of the model plus twice the number of parameters estimated. The model with the lowest AIC indicates the most parsimonious fit to the observed data. The maximal heritability (h^2) was computed using the following equation (24):

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

where r represents the respective correlations. This represents the maximal heritability because both genetic and shared environmental sources of variation are reflected in the familial correlations.

Segregation Model

Bivariate segregation analysis was performed using the Pedigree Analysis Package (PAP), version 4.0 (25). The bivariate model is an extension of the univariate case, where the phenotype is influenced by the independent and additive contributions from a major gene, a polygenic/multifactorial background, and a nontransmitted environmental component. The major gene is modeled to have two alleles (*A*, a), where the upper case allele, with frequency p, is associated with lower phenotypic values and the allele. The other parameters in the model are the mean values for the three genotypes (μAA, μAa, μaa, where the order of the means is constrained to be $\mu_{AA} \leq \mu_{Aa} \leq \mu_{aa}$); the common SD within major locus genotypes (σ); the polygenic heritability (H), after accounting for the major gene effect; and parent-to-offspring transmission probabilities for the three genotypes (τ_{AA}, τ_{Aa}, and τ_{aa}). For a single diallelic locus, the three τ's denote the probability of transmitting allele *A* for genotypes *AA*, *Aa*, and *aa*, with Mendelian expectations of 1, 1/2, and 0, respectively; whereas under an environmental (no transmission) model, $p = \tau_{AA} = \tau_{Aa} = \tau_{aa}$. Recessive ($\mu_{AA} = \mu_{Aa}$) and dominant ($\mu_{Aa} = \mu_{aa}$) modes of transmission were tested. The bivariate segregation model assumes that the single locus potentially influences both of the quantitative traits. The major locus is parameterized identically to the univariate model; however, distinct genotypic means and residual variances are estimated for each quantitative trait (e.g., FM and cortisol), with a single allele frequency and a single set of transmission probabilities. Thus, the correlation between the two phenotypes can be attributed to three potential sources: a pleiotropic major gene, a correlation between the respective polygenic components for each trait (r_G), and a correlation between the nontransmitted environmental components (r_E).

Table 2. Pearson correlation coefficients at baseline and in response to exercise training between cortisol levels and adiposity measures in white and black samples

	White sample			Black sample		
Adiposity	***N***	**Correlation**	***p* Value**	***N***	**Correlation**	***p* Value**
			Baseline			
BMI	511	−0.12	0.006	308	−0.14	0.015
ASF	511	−0.13	0.003	302	−0.13	0.021
AVF	511	−0.13	0.003	301	−0.10	0.079
ATF	511	−0.13	0.002	302	−0.13	0.024
FM	486	−0.14	0.001	258	−0.17	0.008
FFM	488	−0.08	0.097	258	−0.10	0.101
%BF	488	−0.13	0.003	258	−0.16	0.011
			Response to exercise training			
BMI	473	−0.04	0.414	246	0.04	0.518
ASF	463	0.05	0.274	244	−0.04	0.524
AVF	463	0.02	0.744	243	−0.08	0.214
ATF	463	0.03	0.499	244	−0.05	0.465
FM	446	0.03	0.576	203	0.04	0.565
FFM	446	−0.08	0.073	203	0.02	0.830
%BF	446	0.05	0.336	203	0.06	0.397

BMI, body mass index; AVF, abdominal visceral fat; ASF, abdominal subcutaneous fat; ATF, abdominal total fat; FM, fat mass; FFM, fat-free mass; %BF, percentage of body fat.

In summary, we test whether the major gene and/or the polygenic factor for the adiposity trait is/are influencing the variation of cortisol levels. We assume a major gene with distinct genotypic means (μ_{AA}, μ_{Aa}, μ_{aa}) and residual variances (σ) for each of trait 1 and trait 2 but a single (common) allele frequency (p) and a single set of transmission probabilities (τ_{AA}, τ_{Aa}, and τ_{aa}). In addition, two polygenic heritabilities (H) are estimated for trait 1 and trait 2, whereas the genetic pleiotropy (either from major gene or polygenic) between trait 1 and trait 2 is ascertained by r_G and the environmental pleiotropy is ascertained by r_E.

Results

Table 1 presents the sample sizes, means, SDs, and minimum and maximum values for unadjusted baseline phenotypes, as well as cortisol levels in response to training, within each of the four sex × generation groups in white and black subjects (12,24,26). In summary, based on a comparison of means, there were significant differences across sex, generation, and race for all baseline and training responses phenotypes. Age was a significant predictor for all phenotypes at baseline, and FM together with age were significant predictors for ASF, AVF, and ATF. For training responses, baseline values were significant predictor for all phenotypes, and in some cases, age was also included as predictor. Particularly for cortisol levels, menopausal status and hormonal intake were significant predictors of baseline values in white and black daughters, accounting for 32% and 36% of the variance, respectively. For the cortisol training response, baseline cortisol levels accounted for 11% to 26% of the variance in both races.

Significant negative Pearson correlations between cortisol levels and BMI, ASF, ATF, FM, and %BF at baseline were found for both races. Also AVF for whites (Table 2) was inversely correlated with cortisol levels. For training response, no significant correlations were observed between cortisol levels and the adiposity phenotypes. Bivariate familial correlation analysis was performed to investigate whether there was a familial basis for these observed phenotypic correlations. Table 3 shows the summary of *p* values for various models testing cross-trait correlations between cortisol levels and body-fat measures at baseline in white and black families. There was evidence of common familial factors at baseline between cortisol levels and AVF, ASF, ATF, FM, and %BF in white families. In the black sample, both

Table 3. Summary of *p* value and Akaike's information criterion (AIC) for cross-trait hypothesis tests model between cortisol levels and body-fat measures at baseline in white and black families

	BMI		ASF		AVF		ATF		FM		%BF	
Hypothesis/covariate	***p* Value**	**AIC-c**	***p* Value**	**AIC-c**	***p* Value**	**AIC-c**	***p* Value**	**AIC-c**	***p* Value**	**AIC-c**	***p* Value**	**AIC-c**
White sample												
General model		4.47		1.39		3.20		0.00		8.90		7.06
No cross-trait—sibling	0.171	2.87	0.107	0.00	0.133	2.26	0.073	0.57	0.401	4.94	0.530	2.23
No cross-trait—parent-offspring	0.076	2.70	0.017	2.94	0.009	7.58	0.014	3.26	0.036	9.36	0.018	9.53
No cross-trait—spouse	0.040	6.93	0.045	2.60	0.336	1.38	0.087	0.89	0.046	11.05	0.037	9.66
No cross-trait—intraindividual	0.013	9.00	0.008	6.26	0.003	11.43	0.003	8.39	0.008	14.75	0.041	9.00
No cross-trait—interindividual	0.049	0.00	0.010	2.50	0.031	2.62	0.010	2.00	0.048	4.08	0.010	8.35
Black sample												
General model		9.37		8.81				8.79		7.12		5.68
No cross-trait—sibling	0.729	7.49	0.920	6.82			0.888	6.81	0.281	5.28	0.133	5.94
No cross-trait—parent-offspring	0.436	7.03	0.694	5.54			0.861	5.09	0.348	5.23	0.235	4.58
No cross-trait—spouse	0.432	6.38	0.730	5.44			0.730	5.42	0.891	3.35	0.795	2.14
No cross-trait—intraindividual	0.024	12.82	0.030	11.83			0.042	11.12	0.029	10.18	0.034	8.44
No cross-trait—interindividual	0.757	0.00	0.946	0.00			0.944	0.00	0.718	0.00	0.504	0.00

Whites: c (BMI) = 2849.43; c (ASF) = 2899.37; c (AVF) = 2804.69; c (ATF) = 2868.75; c (FM) = 2727.73; c (%BF) = 2673.23.
Blacks: c (BMI) = 1718.65; c (ASF) = 1673.74; c (ATF) = 1675.81; c (FM) = 1461.06; c (%BF) = 1451.25.
BMI, body mass index; AVF, abdominal visceral fat; ASF, abdominal subcutaneous fat; ATF, abdominal total fat; FM, fat mass; FFM, fat-free mass; %BF, percentage of body fat.

for baseline (Table 3) and training response (results not shown), the cross-trait correlations were not significantly different from zero. These results need to be viewed with caution, because the lack of familial resemblance in the blacks could be caused by the smaller sample size.

For cortisol levels and ASF (Table 3), the hypothesis of no sibling cross-trait correlations was not rejected ($p = 0.107$), and it was the most parsimonious model by AIC. The estimates of interindividual cross-trait correlations were: $f_1m_2 = 0.07$, $f_2m_1 = -0.21$, $f_1s_2 = -0.06$, $f_1d_2 = -0.16$, $m_1s_2 = -0.23$, $m_1d_2 = -0.03$, $f_2s_1 = -0.05$, $f_2d_1 = -0.06$, $m_2s_1 = -0.12$, and $m_2d_1 = -0.17$. There was no suggestion of either sibling cross-trait correlations or spouse cross-trait correlations between cortisol and AVF ($p = 0.133$ and $p = 0.336$, respectively). The combined hypothesis of no sibling and no spouse cross-trait correlations was the most parsimonious model (result not shown), with estimates of $f_1s_2 = 0.09$, $f_1d_2 = -0.07$, $m_1s_2 = -0.12$, $m_1d_2 = -0.04$, $f_2s_1 = 0.13$, $f_2d_1 = -0.11$, $m_2s_1 = -0.09$, and $m_2d_1 = -0.24$. For cortisol and ATF, the most parsimonious model was the general model, and the cross-trait correlations were $f_1m_2 = 0.03$, $f_2m_1 = -0.21$, $f_1s_2 = 0.06$, $f_1d_2 = -0.16$, $m_1s_2 = -0.20$, $m_1d_2 = -0.14$, $f_2s_1 = -0.03$, $f_2d_1 = -0.14$, $m_2s_1 = -0.16$, and $m_2d_1 = -0.28$. For cortisol levels with FM as well as with %BF, there was a suggestion of significant cross-trait correlations in parent-offspring but not in siblings or in spouses (Table 3). The hypothesis of no sex difference in cross-trait sibling correlations was the most parsimonious model for cortisol levels and FM ($f_1m_2 = 0.09$, $f_2m_1 = -0.24$, $f_1s_2 = f_1d_2 = -0.03$, $m_1s_2 = m_1d_2 = -0.06$, $f_2s_1 = f_2d_1 = -0.14$, $m_2s_1m_2d_1 = -0.17$, and $s_1s_2 = d_1d_2 = s_1d_2 = s_2d_1 = -0.06$), whereas the no cross-trait in sibling correlations was the most parsimonious model for cortisol and %BF ($f_1m_2 = 0.12$, $f_2m_1 = -0.22$, $f_1s_2 = -0.01$, $f_1d_2 = -0.20$, $m_1s_2 = -0.01$, $m_1d_2 = -0.07$, $f_2s_1 = -0.05$, $f_2d_1 = -0.16$, $m_2s_1 = -0.08$, and $m_2d_1 = -0.23$). Assuming no sex differences in the cross-trait correlations in parents and offspring, we computed approximate estimates of maximal cross-trait heritabilities of 16%, 19%, 20%, 14%, and 16% for ASF, AVF, ATF, FM, and %BF, respectively. These proportions reflect shared genetic and/or environmental factors between cortisol levels and adiposity phenotypes. However, when the abdominal fat measures (ASF, AVF, and ATF) were additionally adjusted for FM, the familial cross-trait correlations were not significant (results not shown). We also note that complete analyses were carried out in the black families. However, given the smaller sample sizes (i.e., often as few as two members per family), the model was simplified to assume no sex differences (i.e., father = son and mother =

daughter). In most cases the correlations were similar in magnitude to those in the white families. However, because of smaller sample sizes, the SEs were larger, and these low-order of magnitude correlations (i.e., −0.05 to −0.2 range) were not significantly different from zero. Thus, we conjecture that the effect may be similar in both races, but there is not enough power to detect these low-order effects in the black families.

Complete univariate segregation analyses were conducted separately for cortisol levels and ASF, AVF, ATF, FM, and %BF, as well as joint phenotypes. The results for the univariate analyses confirmed the previously published results already described (12,27), with evidence either of a major gene and/or multifactorial component (results not shown). Based on the univariate segregation results for each phenotype, we employed the bivariate segregation analysis. To illustrate, a major gene acting in a dominant fashion on cortisol and recessively on ASF, with additional multifactorial effects were modeled, and we tested whether the putative major gene and/or the multifactorial components were the same for both traits.

Table 4 gives the model-fitting results of the bivariate segregation analysis. For cortisol levels and ASF, the major locus for ASF had no effect on cortisol levels ($\mu_{AA} = \mu_{Aa} = \mu_{aa}$) (model 2 vs. model 1, $p = 0.689$), i.e., no major gene pleiotropy. The environmental correlation was not significant (model 3 vs. model 2, $p = 0.689$), but the polygenic correlation between ASF and cortisol was significant (model 4 vs. model 2, $p = 0.001$). Therefore, there was a suggestion of polygenic pleiotropy with respect to cortisol levels and ASF, i.e., the phenotypic covariance of 15% could be explained by (or associated with) shared familial factors.

The bivariate segregation results between cortisol levels and ATF were similar to ASF. For cortisol levels and AVF (Table 4), the allele frequency parameter bounded to −1.0 in the general model (model 1) as well as in the model of no major gene for cortisol levels (model 2), suggesting that there was not a common major gene effect. Moreover, the hypotheses of no major gene for cortisol levels (model 2 vs. model 1, $p = 0.920$) and for AVF (model 3 vs. model 2, $p > 0.99$) were not rejected. The hypothesis of no environmental correlation (model 4) was not rejected ($p = 0.680$), but the hypothesis of no polygenic correlation was rejected (model 4 vs. model 2, $p = 0.001$). The latter suggested polygenic pleiotropy between cortisol levels and AVF, accounting for 14% of the covariance.

As FM was a significant predictor of abdominal fat measures, we also performed bivariate segregation analysis between cortisol levels and FM-age-adjusted ASF, AVF, and ATF. After the FM adjustment, the evidence of polygenic pleiotropy between cortisol levels and abdominal fat measures disappeared (results not shown), suggesting that the polygenic pleiotropy is caused by the covariation between cortisol levels and FM instead of abdominal fat. Furthermore, the bivariate segregation analysis between cortisol levels and FM suggested polygenic pleiotropy accounting for 17% of the covariance. Suggestion of polygenic pleiotropy was also observed between cortisol levels and %BF accounting for 14% of the covariance (Table 4).

Discussion

Insulin resistance and hyperinsulinemia are well-recognized consequences of elevated cortisol. Endocrine perturbations can lead to obesity and storage of an elevated proportion of fat in visceral depots, followed by metabolic and hemodynamic abnormalities (28).

Similar to earlier reports (29,30), this study showed significant negative correlations between cortisol levels and total-body fatness and abdominal adiposity at baseline in white and black subjects. However, correlations between training-induced changes in cortisol levels and the changes in adiposity phenotypes were not significant in either race.

Variation in adiposity is known to be partly heritable, arising from the additive and interactive effects of a large number of genes (31). In addition, several studies using segregation analysis have inferred the presence of major genes influencing body-fat phenotypes, including AVF (27,32,33) and FM (33–35). Both a major gene effect accounting for 54% of the variance and a multifactorial gene effect accounting for 17% of the variance were significant for baseline AVF in white families from the HERITAGE study (27). However, the major gene evidence disappeared after AVF was adjusted for the effects of total level of body fat (FM). On the other hand, for FM, there was evidence of a putative major gene accounting for 64% of the phenotypic variance (27). Additionally, linkages with several body-fat phenotypes have been reported in distinct chromosomal regions (14,36).

In the current study, two different statistical methods (familial cross-trait correlations and bivariate segregation analysis) gave consistent results, with both suggesting common familial components underlying cortisol and body-fat covariation. This familial effect accounted for 16% to 20% of the phenotypic variances in white families and seems to be caused by common polygenic (but not major gene) determinants. However, the underlying genetic components could be oligogenic (i.e., a few genes each with moderate effects). These genes could be involved in the regulation of energy balance, adipogenesis, nutrient partitioning, and other pathways.

Thus, the evidence of genetic pleiotropy between cortisol levels and overall adiposity in the HERITAGE study can be usefully exploited for gene discovery and mapping. In fact, several studies have shown that sequence variations in candidate genes were associated with cortisol levels and obesity-related phenotypes. For instance, some studies have

Table 4. Bivariate segregation analysis results for cortisol levels (trait 1) and adiposity phenotypes (trait 2) at baseline in white families

Model/ parameter	1	2	3	4	1	2	5	6	7	1	2	3	4	1	2	3	4	1	2	3	4
p	0.661	0.663	0.663	0.664	1.0*	1.0*	[1]	[1]	[1]	0.687	0.688	0.689	0.685	0.648	0.648	0.648	0.651	0.707	0.587	0.590	0.611
r_G	−0.573	−0.547	−0.513	[0]	−0.390	−0.390	−0.390	−0.355	[0]	−0.612	−0.583	−0.529	[0]	−0.374	−0.376	−0.421	[0]	−0.477	−0.465	−0.412	[0]
r_E	0.044	0.036	[0]	−0.146	0.032	0.032	0.032	[0]	−0.114	0.060	0.053	[0]	−0.128	−0.051	−0.050	[0]	−0.200	−0.001	0.049	[0]	−0.112
Trait 1																					
μ_{AA}	−0.063	−0.004	−0.003	−0.001	−0.002	−0.002	−0.002	−0.002	−0.001	−0.060	−0.002	−0.002	−0.001	0.001	−0.002	−0.002	−0.000	−0.041	−0.003	−0.003	−0.001
μ_{Aa}	0.042	[μ_{AA}]	[μ_{AA}]	[μ_{AA}]	−1.58	[μ_{AA}]	[μ_{AA}]	[μ_{AA}]	[μ_{AA}]	0.049	[μ_{AA}]	[μ_{AA}]	[μ_{AA}]	−0.007	[μ_{AA}]	[μ_{AA}]	[μ_{AA}]	0.413	[μ_{AA}]	[μ_{AA}]	[μ_{AA}]
μ_{aa}	[μ_{Aa}]	[μ_{AA}]	[μ_{AA}]	[μ_{AA}]	[μ_{Aa}]	[μ_{AA}]	[μ_{AA}]	[μ_{AA}]	[μ_{AA}]	[μ_{Aa}]	[μ_{AA}]	[μ_{AA}]	[μ_{AA}]	[μ_{Aa}]	[μ_{AA}]	[μ_{AA}]	[μ_{AA}]	[μ_{Aa}]	[μ_{AA}]	[μ_{AA}]	[μ_{AA}]
σ	1.001	1.002	1.002	1.001	1.002	1.002	1.002	1.002	1.003	1.001	1.002	1.001	1.001	1.003	1.003	1.004	1.001	0.994	1.003	1.002	1.001
H	0.403	0.402	0.397	0.388	0.401	0.401	0.401	0.398	0.404	0.404	0.402	0.395	0.390	0.407	0.407	0.414	0.384	0.401	0.407	0.402	0.401
Trait 2	ASF				AVF					ATF				FM				%BF			
μ_{AA}	−0.199	−0.199	−0.199	−0.195	−0.005	−0.005	−0.005	−0.004	−0.003	−0.184	−0.184	−0.183	−0.184	−0.212	−0.212	−0.213	−0.208	−0.125	−0.238	−0.235	−0.213
μ_{Aa}	[μ_{AA}]	[μ_{AA}]	[μ_{AA}]	[μ_{AA}]	[μ_{AA}]	[μ_{AA}]	[μ_{AA}]	[μ_{AA}]	[μ_{AA}]	[μ_{AA}]	[μ_{AA}]	[μ_{AA}]	[μ_{AA}]	[μ_{AA}]	[μ_{AA}]	[μ_{AA}]	[μ_{AA}]	[μ_{AA}]	[μ_{AA}]	[μ_{AA}]	[μ_{AA}]
μ_{aa}	2.282	2.295	2.298	2.297	2.286	2.286	[μ_{AA}]	[μ_{AA}]	[μ_{AA}]	2.286	2.298	2.304	2.279	2.125	2.124	2.118	2.156	1.443	1.205	1.215	1.276
σ	0.783	0.783	0.782	0.779	0.986	0.986	0.986	0.986	0.987	0.787	0.787	0.787	0.782	0.744	0.744	0.745	0.741	0.889	0.832	0.832	0.837
H	0.419	0.417	0.410	0.380	0.336	0.336	0.336	0.333	0.339	0.360	0.360	0.350	0.344	0.482	0.481	0.489	0.488	0.469	0.412	0.408	0.409
−2lnL + c	0.00	0.16	0.32	10.55	0.00	0.01	0.01	0.18	8.86	0.00	0.90	1.26	11.59	0.00	0.01	0.26	5.92	0.00	0.52	0.77	7.51
Test		2–1	3–2	4–2		2–1	3–2	4–3	5–3		2–1	3–2	4–2		2–1	3–2	4–2		2–1	3–2	4–2
df		1	1	1		1	2	1	1		1	1	1		1	1	1		1	1	1
χ^2		0.16	0.16	10.39		0.01	0.00	0.17	8.85		0.90	0.36	10.69		0.01	0.25	5.91		0.52	0.25	6.99
p		0.689	0.689	0.001		0.920	>0.99	0.680	0.003		0.343	0.549	0.001		0.920	0.617	0.015		0.471	0.617	0.008
AIC	22.00	20.16	18.32†	28.55	22.00	20.01	16.01†	14.18	22.86	22.00	20.90	19.26†	29.59	22.00	20.01	18.25†	23.91	22.00	20.52	18.25†	24.99

c (FM) = 2726.88; c (%BM) = 2754.01.

c (ASF) = 2837.32; c (AVF) = 2844.37; c (ATF) = 2821.20.

* The parameter reached the boundary values (1.0); [ ] The parameter was constrained to the value shown.

† For the parsimonious models, the variance accounted for by the major gene was 45%, 40%, 44%, and 26% to ASF, ATF, FM, and %BF, respectively, with additional 23%, 21%, 27%, and 30% caused by the polygenic component, respectively.

Models:

(1) $\tau_{AA} = 1$, $\tau_{Aa} = 0.5$, $\tau_{aa} = 0$; trait 1: dominant ($\mu_{Aa} = \mu_{aa}$); trait 2: recessive ($\mu_{AA} = \mu_{Aa}$).
(2) $\tau_{AA} = 1$, $\tau_{Aa} = 0.5$, $\tau_{aa} = 0$; trait 1: no major gene ($\mu_{AA} = \mu_{Aa} = \mu_{aa}$); trait 2: recessive ($\mu_{AA} = \mu_{Aa}$).
(3) $\tau_{AA} = 1$, $\tau_{Aa} = 0.5$, $\tau_{aa} = 0$; trait 1: no major gene ($\mu_{AA} = \mu_{Aa} = \mu_{aa}$); trait 2: recessive ($\mu_{AA} = \mu_{Aa}$), $r_E = 0$.
(4) $\tau_{AA} = 1$, $\tau_{Aa} = 0.5$, $\tau_{aa} = 0$; trait 1: no major gene ($\mu_{AA} = \mu_{Aa} = \mu_{aa}$); trait 2: recessive ($\mu_{AA} = \mu_{Aa}$), $r_G = 0$.
(5) trait 1: no major gene ($\mu_{AA} = \mu_{Aa} = \mu_{aa}$); trait 2: no major gene ($\mu_{AA} = \mu_{Aa} = \mu_{aa}$); $p = 1$.
(6) trait 1: no major gene ($\mu_{AA} = \mu_{Aa} = \mu_{aa}$); trait 2: no major gene ($\mu_{AA} = \mu_{Aa} = \mu_{aa}$); $p = 1$, $r_E = 0$.
(7) trait 1: no major gene ($\mu_{AA} = \mu_{Aa} = \mu_{aa}$); trait 2: no major gene ($\mu_{AA} = \mu_{Aa} = \mu_{aa}$); $p = 1$, $r_G = 0$.

ASF, abdominal subcutaneous fat; AVF, abdominal visceral fat; ATF, abdominal total fat; AIC, Akaike's information criterion.

shown that *Bcl*I restriction fragment length polymorphism in intron 1 of the glucocorticoid receptor (*GR*) gene was associated with abdominal fat (37) and insulin resistance (38), as well as decreased sensitivity to raised postprandial secretion of cortisol (39). In contrast, the TSp509I polymorphism in exon 2 of the *GR* gene did not show any association with an altered sensitivity to glucocorticoids or with obesity and related metabolic and hemodynamic phenotypes (40). Four *GR* gene polymorphisms have been associated with obesity and subtle alterations in the regulation of cortisol levels (40).

The corticotropin-releasing hormone (*CRH*) gene could play a role in the covariation between cortisol levels and adiposity because it is the hypothalamic neuropeptide involved in the control of adrenal production of cortisol. The *Xmn*I polymorphism in the *CRH* gene was not associated with an altered cortisol-secretory pattern or sensitivity to glucocorticoids or with obesity and related metabolic and circulatory perturbations (40). However, when interaction effects between *Tthlll*I and *Xmn*I *CRH* polymorphisms were investigated, the cortisol levels before and during a physiological stress and the total diurnal cortisol secretion were significantly increased among subjects who were carriers of both variants. A polymorphism in the 5′ untranslated region of the tumor necrosis factor-α gene was associated with elevated morning cortisol levels, as well as elevated postprandial cortisol secretion (41).

Another candidate is the melanocortin-4 receptor gene, which is involved in the regulation of food intake. A missense mutation in the melanocortin-4 receptor gene contributed to the variability in BMI and was also associated with diurnal cortisol levels (42).

In summary, evidence was found in this study for a pleiotropic polygenic effect, but not for a pleiotropic major gene effect between cortisol levels and overall adiposity phenotypes. Evidence for polygenic pleiotropy between cortisol levels and abdominal fat was also obtained, but it disappeared when the abdominal phenotypes were fat-adjusted for FM. Thus, a common genetic background may be influencing the variation of cortisol levels and total adiposity, but not abdominal fat specifically.

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