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Genetics of Serum Dehydroepiandrosterone Sulfate and Its Relationship to Insulin in a Population-Based Cohort of Twin Subjects

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Previous studies have shown a significant effect of insulin administration on serum dehydroepiandrosterone sulfate (DHEA-S) concentration and its metabolic rate, with evidence for the effect in men, but not in women. This could lead to differences in the sources of variation in serum DHEA-S between men and women and in its covariation with insulin concentration. This study aimed to test whether these hypotheses were supported in a sample of healthy adult twins.

Serum DHEA-S (n = 2287) and plasma insulin (n = 2436) were measured in samples from adult male and female twins recruited through the Australian Twin Registry. Models of genetic and environmental sources of variation and covariation were tested against the data.

DHEA-S showed substantial genetic effects in both men and women after adjustment for covariates, including sex, age, body mass index, and time since the last meal. There was no significant phenotypic or genetic correlation between DHEA-S and insulin in either men or women.

Despite the experimental evidence for insulin infusion producing a reduction in serum DHEA-S and some effect of meals on the observed DHEA-S concentration, there were no associations between insulin and DHEA-S at the population level. Variations in DHEA-S are due to age, sex, obesity, and substantial polygenic genetic influences. (*J Clin Endocrinol Metab* 87: 682–686, 2002)

DEHYDROEPIANDROSTERONE sulfate (DHEA-S) is the most abundant circulating steroid hormone in humans. It can be readily converted to unconjugated DHEA by ubiquitous tissue steroid sulfatases, and thus presumably serves as a reservoir for DHEA. DHEA exhibits a very high turnover, which is characteristic of a biologically active hormone (1). Low circulating DHEA-S is associated with heart disease in men (2–8). Paradoxically, serum DHEA-S may be positively correlated with blood pressure (9, 10), although an inverse relationship has also been reported (11).

Regulation of DHEA-S metabolism is incompletely understood. ACTH acutely stimulates adrenal DHEA release, but not that of DHEA-S. Moreover, the dissociation of serum DHEA and DHEA-S from that of glucocorticoids at various times in human life and during certain conditions of stress (12) makes it clear that other factor(s), probably of nonpituitary origin, must also regulate DHEA-S metabolism.

It has been proposed that one factor regulating DHEA-S metabolism may be insulin. Insulin appears to reduce circulating DHEA-S both acutely and chronically in men (13–17). However, evidence also suggests that insulin's regulation of DHEA-S metabolism may be sex specific and germane to men only (15, 18, 19). This idea is compatible with evidence that differences in DHEA-S metabolism exist between men and women.

For example, some studies, but not all, suggest that serum DHEA-S levels are lower and unconjugated DHEA levels are higher in women than in men (20) despite the fact that DHEA-S is virtually exclusively of adrenal origin in both sexes. *In vivo* hydrolysis of isotopically labeled DHEA-S to DHEA is greater in women than in men (21), as is the serum ratio of DHEA to DHEA-S (20). A genetic study suggested that heritable factors were the primary determinants of circulating DHEA-S concentrations in women, whereas in men heredity played a minor role, and other unidentified factors appeared to dominate (22). In that study sex-specific familiality (combined polygenic and familial environmental effects) for circulating DHEA-S was estimated to be 74% in women compared with only 29% in men.

In addition to genetic factors, gonadal steroids may regulate adrenal androgen metabolism. One study (23) showed that DHEA-S levels declined in male to female transsexuals when E2 was administered, whereas they rose in female to male transsexuals during T administration.

In view of the above observations, we hypothesized that familial inheritance for circulating DHEA-S concentrations would be greater in women than in men. We also hypothesized that in men serum DHEA-S would correlate strongly with serum insulin, reflecting chronic regulation of DHEA-S metabolism by insulin in men, whereas in women no correlation between serum DHEA-S and insulin would be observed. To test these hypotheses, serum DHEA-S and plasma

Abbreviations: BMI, Body mass index; DHEA, dehydroepiandrosterone; DHEA-S, dehydroepiandrosterone sulfate.

insulin concentrations were determined in stored serum and plasma samples from over 2000 adult twin subjects.

Subjects and Methods

Participants in this study were recruited through the Australian National Health and Medical Research Council Twin Registry. From 1993–1995, a telephone-based semistructured interview designed to assess the physical, psychological, and social manifestations of alcoholism and related disorders (SSAGA-OZ) was administered to 2456 twin pairs and 771 unpaired twins. Characteristics of the sample have been described previously (24, 25). Blood samples were collected from 3377 of these subjects, including 1405 complete twin pairs, between 1993 and 1996. Time since last meal at the time of blood collection (fasting time) was also recorded, along with the time of blood collection, the interval between blood collection and processing, and the time interval each sample spent stored at −70 C before assay (3–6 yr).

DHEA-S and insulin measurements were obtained from the serum and EDTA plasma components of the blood samples for 2287 and 2436 individuals, respectively. Serum DHEA-S (Diagnostics Systems Laboratories, Inc., Webster, TX) and plasma insulin (Diagnostic Products, Los Angeles, CA) were determined by RIA. The intraassay coefficients of variation were 6.3–9.4% and 3.1–9.3% for DHEA-S and insulin, respectively, whereas the interassay coefficients of variation were 9.6–10.0% and 4.9–10.0%, respectively. Cross-reactivity of the insulin assay for C peptide and glucagon was low, but was 40% for proinsulin at midcurve for the assay.

Zygosity of twins was decided on the basis of their responses to standard questions about similarity and the degree to which others confused them. Pairs giving inconsistent responses were contacted for clarification. Such procedures have been shown to give at least 95% agreement with diagnosis based on extensive blood typing (26, 27). This has been confirmed in a subsample of 329 same-sex twin pairs in the current study. Of 131 pairs reporting themselves to be dizygotic, 5 pairs were concordant at 11 highly polymorphic loci (probability of monozygosity, >0.9999), whereas none of the 198 self-reported MZ twin pairs were found to be dizygotic (28). This small rate of misdiagnosis of zygosity (1.5%) will bias heritability estimates downward.

Maximum likelihood analysis methods for continuous raw data were used to implement tests for equivalence and structural equation modeling in Mx (29). This method allows covariates such as age and sex to be included as fixed effects on mean values while simultaneously estimating twin correlations or components of the residual variance (30).

Structural equation modeling was used to determine which combination of additive genetic effects A, shared environment C, and nonshared environment E provides the simplest explanation for the observed data. The most parsimonious model is determined by comparing the relative goodness of fit of models as assessed by the likelihood ratio χ^2 (31).

Extension to bivariate analysis allows the determination not only of the sources of covariation for the individual measures of interest, but also the pattern or structure in which these differentially influence the covarying measures. For this purpose, we used the Cholesky decomposition, where each source of covariance (A, C, or E) among n variables is decomposed into a series of n independent factors. In bivariate analysis, the first factor loads on both observed variables and demonstrates how much of the genetic and environmental influences on the first variable are also shared with the second variable. The second factor loads only on the second variable and shows how much of its variance is accounted for by genetic and/or environmental influences unique to that variable.

Results

DHEA-S measurements were obtained for 1563 women and 724 men, and insulin levels were measured in 1611 women and 825 men. Frequency distributions for both variables exhibited departures from normality. As a result, logarithmic transformations ($\log_{10}$) were used for further analyses of both measures.

Both fasting time and (fasting time)^2 were highly significantly associated with insulin levels ($P < 0.001$), but only marginally with DHEA-S ($P = 0.036$ and $P = 0.030$). There was no association between the time of day when the blood was collected and the DHEA-S values, but there was a significant effect on insulin ($P = 0.005$), and this was independent of the effect of time since the last meal.

The time interval between blood collection and processing and the time spent in storage before the assay were not significantly associated with levels of DHEA-S in the assayed samples. The interval between blood collection and processing was significant for insulin levels ($P = 0.001$), but the storage time was not.

Both DHEA-S and insulin measurements were significantly associated with age ($P < 0.001$). The effects of age on DHEA-S in men and women are shown in Fig. 1. Body mass index (BMI) was significantly associated with variations in both DHEA-S and insulin ($P < 0.001$ in each case), with higher BMI being associated with higher DHEA-S and insulin concentrations. The mean values of log-transformed serum DHEA-S and insulin by sex, age, and BMI groups are shown in Table 1.

The correlations between DHEA-S and insulin measurements for men and women were also examined. Both correlation values were extremely small (r = 0.026 in women; r = −0.041 in men) and were not significantly different from each other or from zero. However, a significant difference

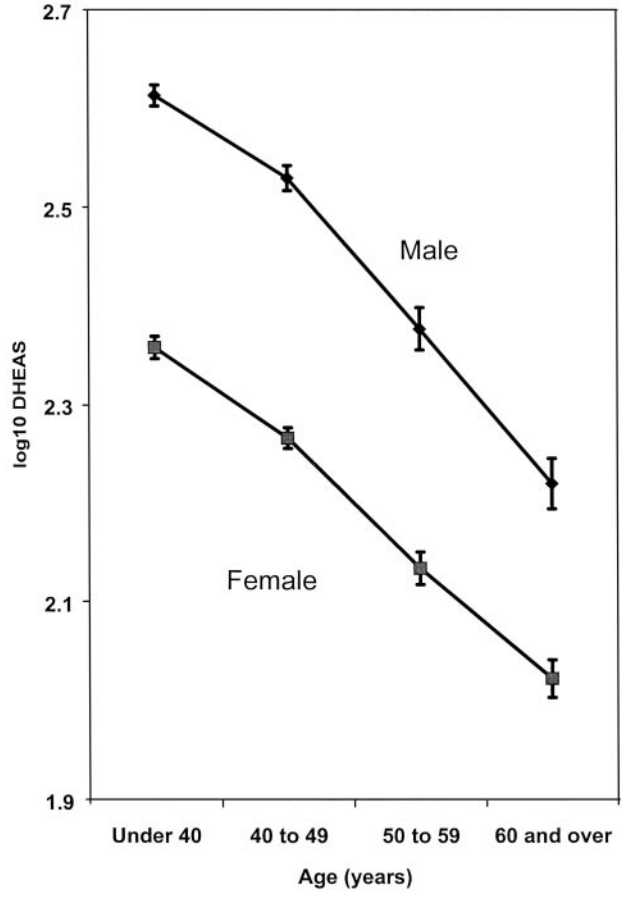

FIG. 1. Changes in mean serum DHEA-S concentration with age in men and women. Note the logarithmic scale for DHEA-S. *Bars* represent SEs.

TABLE 1. Descriptive statistics for log-transformed DHEA-S and insulin results, adjusted for fasting time, time of collection, time from collection to processing, and storage time

	Age <50 yr			Age 50 yr and over		
	DHEA-S	Insulin	Correlation	DHEA-S	Insulin	Correlation
Men						
BMI <25 kg/m^2	2.543 ± 0.012 (249)	1.096 ± 0.011 (271)	−0.05[a]	2.297 ± 0.014 (70)	1.143 ± 0.015 (72)	0.05[a]
BMI 25–26.9 kg/m^2	2.560 ± 0.015 (117)	1.199 ± 0.015 (142)	0.02[a]	2.314 ± 0.016 (52)	1.246 ± 0.016 (62)	−0.08[a]
BMI ≥27 kg/m^2	2.579 ± 0.013 (157)	1.285 ± 0.013 (188)	−0.06[a]	2.334 ± 0.015 (69)	1.332 ± 0.015 (79)	−0.18[a]
Women						
BMI <25 kg/m^2	2.305 ± 0.008 (649)	1.015 ± 0.009 (676)	0.01[a]	2.059 ± 0.012 (257)	1.062 ± 0.012 (249)	−0.05[a]
BMI 25–26.9 kg/m^2	2.322 ± 0.014 (134)	1.119 ± 0.014 (134)	0.21[b]	2.076 ± 0.015 (91)	1.165 ± 0.015 (104)	−0.12[a]
BMI ≥27 kg/m^2	2.341 ± 0.047 (257)	1.204 ± 0.012 (258)	0.18[b]	2.096 ± 0.013 (149)	1.251 ± 0.013 (163)	0.04[a]

Values shown are the means, SEMs, and number of subjects, and the correlation coefficients between adjusted log DHEAS and adjusted log insulin, by groups.

[a] $P > 0.05$.

[b] $P < 0.05$.

was observed between the results obtained for subjects less than 50 yr of age and those 50 yr or older. In women less than 50 yr old, the correlation between DHEA-S and insulin levels was 0.082, whereas the correlation between these measurements in men less than 50 yr of age was −0.029. This difference in correlation was marginally significant ($P = 0.052$), and the female correlation was significantly different ($P = 0.024$) from zero. In the older age group (≥50 yr), DHEA-S and insulin levels were negatively correlated in both men and women ($r = -0.066$ and $r = -0.072$, respectively), and these correlations were not significantly different from each other ($P = 0.877$) or from zero ($P = 0.307$). Details of the phenotypic DHEA-S/insulin correlations by subgroup of subjects are provided in Table 1.

Table 2 presents the proportions of variance in DHEA-S and insulin attributable to additive genetic and nonshared environmental effects, stratified by age group (<50 yr *vs.* ≥50 yr). In both age groups, approximately 60% of the variance in DHEA-S was attributable to additive genetic influences, the remainder is due to nonshared environmental effects. In the younger age group, approximately 0.6% of the variance in insulin levels was attributable to the same genes influencing DHEA-S, and a further 28% of the variance was due to genetic effects separate from those relating to DHEA-S. The genetic correlation between DHEA-S and insulin was estimated to be 0.14, and setting the value at zero produced a marginally significant change in the goodness of fit of the model ($P = 0.053$). This effect was not seen in the older age group, in whom the genes influencing insulin levels and DHEA-S were entirely distinct. Nonshared environmental effects acting on each measure were also unique to that measure; environmental influences on DHEA-S did not contribute to variance in insulin levels. In each age group, shared environmental effects had no significant influence on either DHEA-S or insulin (change in $\chi^2_3 = 0.00$).

Discussion

This study was prompted by previous work that led to two predictions: firstly, that there would be differences between men and women in the balance between genetic and nongenetic sources of variation in DHEA-S, and secondly, that the relationship between DHEA-S and insulin would be stronger in men than in women.

TABLE 2. Twin correlations for DHEA-S and insulin levels for five zygosity groups, with number of complete twin pairs on which the correlations are based

Zygosity	r_{DHEA-S}	No. of pairs	$r_{Insulin}$	No. of pairs
MZ female	0.58	438	0.3	357
MZ male	0.61	160	0.36	138
DZ female	0.34	224	0.27	167
DZ male	0.33	84	0.03	61
DZ opposite sex	0.24	223	0.19	174

Results are corrected for mean effects of sex, age, BMI, time of blood collection, fasting time, (fasting time)^2, time interval between blood collection and processing, storage time before assay, and (storage time)^2.

There is no doubt that substantial differences exist between men and women in the mean circulating concentration of DHEA-S. The concentrations decline with age in a very similar way for men and women; this is illustrated in Fig. 1. The question of whether the sources of interindividual variation are different in men and women requires comparison of the within-pair correlations for the dizygotic same-sex and opposite-sex pairs, which are shown in Table 3. The correlation coefficient for the opposite-sex pairs is not significantly less than those for the female and male same-sex dizygotic pairs. This suggests that the sources of variation are not different in men and women.

Similarly, the hypothesis that the relationship between insulin and DHEA-S is stronger in men than in women was not supported by our data. A minor sex difference in correlation coefficients was found in subjects less than 50 yr of age, but the effect was just significant in women and nonsignificant in men, which is the opposite of the postulated difference. Division of the subjects into multiple groups, by sex, age, and BMI and inspection of the correlations between DHEA-S and insulin in each group did not suggest any strong relationship within any particular group.

TABLE 3. Proportions of variance attributable to additive genetic effects (A_1 and A_2) and nonshared environmental effects (E_1 and E_2)

	A_1	A_2	E_1	E_2
Age <50 yr				
DHEA-S	0.57		0.43	
Insulin	0.006	0.28	0.00	0.71
Age ≥50 yr				
DHEA-S	0.6		0.4	
Insulin	0.00	0.41	0.00	0.59

A_1 and E_1 potentially affect both DHEA-S and insulin, whereas A_2 and E_2 represent effects on insulin only.

The sources of variation in serum DHEA-S were approximately 60% genetic and 40% nongenetic. The nongenetic component will, of course, include variation due to measurement error or to intraindividual day to day variation, so it is likely that most of the variation (other than that due to sex and age) depends on variation in genetic makeup between subjects. There are few other published twin studies of variation in DHEA-S for comparison.

Meikle *et al.* (32) reported heritability, adjusted for age, smoking, and obesity, of 0.58 in a small number of male twin pairs. Subsequently, this group found intraclass correlations of 0.80 for 63 male MZ pairs and 0.67 for 44 male DZ pairs (33). At first sight, this appears to be evidence for a substantial shared environment effect, but it must be remembered that serum DHEA-S varies greatly with age, and that age is a characteristic shared within twin pairs regardless of zygosity. As there is no mention of age correction in the 1997 paper, and the age range of their subjects was 25–75 yr, it is possible that they overestimated the shared environmental effect and underestimated the heritability.

Three family studies have reported DHEA-S data from parent-offspring or sibling comparisons (22, 34, 35). Rotter *et al.* (34) gave a heritability estimate of 0.65 after adjusting for the substantial effects of age. The results presented by Rice *et al.* (22) are more difficult to interpret, because they recruited two groups of families, one based on probands who had survived myocardial infarction and a control group. There was evidence of familial resemblance in serum DHEA-S, but it was difficult to decide whether it was due to genes or shared environments; the family groups were selected for cohabitation. These researchers concluded that under some assumptions (and if only the control families were considered) there was evidence of sex-specific familiality (0.74 in females, but 0.29 in males). A subsequent report on familial resemblance in black subjects suggested a heritability for serum DHEA-S of 66%, similar to the estimate of 58% for white families (35).

We can be confident, given the substantial number of subjects in our study, of genetic effects on serum DHEA-S. There is no evidence of sex-specific genetic effects. The nature of the relevant genes cannot, of course, be inferred by the twin method, except to say that there is little overlap with genes affecting insulin concentration.

Despite the lack of meaningful phenotypic or genetic correlations between DHEA-S and insulin concentrations (after adjustment for sex, age, BMI, and meal effects), there is a change in DHEA-S after meals that is probably a metabolic response to changes in glucose and/or insulin levels. Examination of the data showed that a time since the last meal of less than 30 min or more than 360 min was associated with a higher DHEA-S concentration, with the lowest values observed between 120–150 min after a meal. These observations are consistent with the reported acute decreases in serum DHEA-S concentrations during a hyperinsulinemic-euglycemic clamp (13–15).

However, there is an element of paradox in the lack of overall correlation between the corrected DHEA-S and insulin concentrations in the population studied. It can reasonably be assumed that the subjects who had high values of insulin also had some degree of insulin resistance. If so, they might be relatively resistant to the effect of insulin on the serum DHEA-S concentration and its metabolic rate. Further investigation of the relationships between DHEA-S, insulin, and insulin resistance, and obesity or the metabolic syndrome would be of interest.

In conclusion, experimental evidence has suggested a strong effect of insulin on DHEA-S and a differential effect between men and women. In this genetic epidemiological study we found no evidence to support the predicted relationships, but there was evidence of substantial heritability of serum DHEA-S levels once factors such as age and sex were incorporated into the model.

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