Genetic and Environmental Influences of Daily and Intra-individual Variation in Testosterone Levels in Middle-Aged Men

Matthew S. Panizzon[1,2,*], **Richard Hauger**[1,3], **Kristen C. Jacobson**[4], **Lindon J. Eaves**[5], **Timothy P. York**[5], **Elizabeth Prom-Wormley**[5], **Michael D. Grant**[6], **Michael J. Lyons**[6], **Ruth McKenzie**[6], **Sally P. Mendoza**[7], **Hong Xian**[8,9], **Carol E. Franz**[1,2], and **William S. Kremen**[1,2,3]

[1]Department of Psychiatry, University of California, San Diego, La Jolla, CA

[2]Twin Research Laboratory, Center for Behavioral Genomics, University of California, San Diego, La Jolla, CA

[3]Center of Excellence for Stress and Mental Health, VA San Diego Healthcare System, San Diego, CA

[4]Department of Psychiatry, University of Chicago, Chicago, IL

[5]Virginia Institute for Psychiatric and Behavioral Genetics, Virginia Commonwealth University School of Medicine, Richmond, VA

[6]Department of Psychology, Boston University, Boston, MA

[7]Department of Psychology, University of California, Davis, Davis, CA

[8]Department of Medicine, Washington University School of Medicine, St. Louis, MO

[9]VA St. Louis Healthcare System, St. Louis, MO

Abstract

Background—Testosterone regulates numerous physiological processes, and evidence suggests that it plays a critical role in male aging. It has yet to be determined whether the heritability of testosterone varies in accordance with its diurnal rhythm. Similarly, it is unclear whether changes in testosterone level throughout the day are genetically influenced. The aim of the present study was to determine the degree to which genetic and environmental factors contribute to individual differences in testosterone throughout the day in middle-aged men.

*Corresponding Author: Dr. Matthew S. Panizzon, Department of Psychiatry, University of California, San Diego, 9500 Gilman Drive (MC 0738), La Jolla, CA 9293-0738; Tel: 858-534-8269; Fax: 858-822-5856; mspanizz@ucsd.edu.

Methods—Saliva-based measures of free testosterone, sampled at multiple time-points both at-home and in-lab, were collected from 783 male twins (193 monozygotic pairs, 196 dizygotic pairs, 5 unpaired twins) as part of the Vietnam Era Twin Study of Aging (VETSA). The average age of participants was 55.9 years (SD=2.6).

Results—Testosterone levels declined substantially over the course of the day, with 32%–39% of the change occurring in the first 30 minutes after waking. Heritability estimates for specific time-points ranged from .02 to .39. The heritability of the average at-home and in-lab testosterone values were notably higher (.42 and .47 respectively). Daily rates of change showed some evidence of genetic influence, with heritability estimates ranging from .15 to .29, whereas there were no observable genetic influences on coefficients of variation.

Conclusions—Genetic influences account for a significant proportion of the variance in average testosterone levels, while environmental factors account for the majority of intra-individual variability. These results highlight the need to explore both genetic and individual-specific environmental factors as determinants of free testosterone levels in aging men.

Keywords

The primary male androgen testosterone regulates numerous physiological processes, and has been related to a variety of health and behavioral outcomes. Although frequently linked to levels of aggression, physical strength, and sexual functioning in men (Zitzmann and Nieschlag, 2001), increasing evidence suggests that testosterone, as well as the broader hypothalamic-pituitary-gonadal (HPG) axis, plays a critical role in male aging. In adult men, testosterone levels begin to decline as early as the fourth decade of life (Feldman et al., 2002; Ferrini and Barrett-Connor, 1998; Harman et al., 2001), leading to functional changes in androgen receptor-regulated tissues (Stanworth and Jones, 2008). Lower or substantial declines in testosterone levels in aging men have been associated with increased risk for cardiovascular disease (Zmuda et al., 1997), metabolic syndrome (Saad and Gooren, 2009), physical frailty (Hyde et al., 2010), depression (Joshi et al., 2010), and overall mortality (Laughlin et al., 2008). The hormone has also been repeatedly linked to age-related changes in cognitive functioning (Holland et al., 2011), as well as disorders of cognition such as mild cognitive impairment and Alzheimer's disease (Chu et al., 2008; Hogervorst et al., 2004; Moffat et al., 2004). Genetic studies have further shown that testosterone interacts with variants of the apolipoprotein-E (*APOE*) gene to influence cognition and brain structure (Panizzon et al., 2010; Raber, 2008), and that the hormone may regulate the genetic determinants of brain structures such as the hippocampus (Panizzon et al., 2012). Given the apparent wide-ranging effects of testosterone on male physiology and aging, there is a clear need to elucidate the determinants, both biological and environmental, of individual differences in testosterone levels in aging men.

To date, a number of twin and family studies have examined the heritability of male testosterone levels (i.e., the degree to which genetic factors contribute to individual differences in the hormone level). These studies have overwhelmingly examined measures of total testosterone – representing the sum total of hormone that is unbound, bound to sex hormone binding globulin (SHBG), and loosely bound to other proteins like albumin (Stanworth and Jones, 2008) – and with few exceptions have been largely consistent with one another, establishing a moderate level of heritability in the range of .40 to .60 (Bogaert et al., 2008; Harris et al., 1998; Hoekstra et al., 2006; Hong et al., 2001; Kuijper et al., 2007; Meikle et al., 1987; Ring et al., 2005; Sluyter et al., 2000; Storgaard et al., 2006). In contrast, only three studies have examined the heritability of free testosterone, hormone that is not bound to SHBG and is therefore physiologically active. Meikle and colleagues

estimated the heritability of free testosterone at .34 in a sample of men ages 20 to 60 (Meikle et al., 1987), whereas Hoekstra and colleagues observed a substantially larger heritability of .52 in a sample of twelve-year-old boys (Hoekstra et al., 2006). More recently, Caramaschi and colleagues found evidence for no heritability of free testosterone in five-month-old infants; however, their analyses combined results for male and female twin pairs, thus results for male infants alone were not provided (Caramaschi et al., 2012). Clearly, there is limited information as to the degree to which genetic and environmental factors contribute to the level of free testosterone in adult men.

It is also the case that the majority of studies that have examined the heritability of testosterone, regardless of the type, have to date been based on one measurement taken exclusively during the morning, typically between 8 and 11 a.m. Although less pronounced than the well-documented diurnal rhythm of cortisol (Hellhammer et al., 2007; Stone et al., 2001), the HPG axis secretes testosterone with a significant diurnal variation, with levels reaching their highest point early in the morning followed by a gradual decline throughout the day (Diver et al., 2003). This diurnal rhythm has been shown to become blunted with increasing age, suggesting that aging may be associated with reductions in both overall testosterone output and daily variation (Bremner et al., 1983). Within the clinic, it has been recommended that testosterone be assessed only during the morning hours in order to avoid the potential confounding effects of this diurnal variation (Brambilla et al., 2009). Epidemiological studies of testosterone and male hypogonadism have tended to use this same approach. It has yet to be determined whether changes in testosterone level throughout the day are to some extent genetically driven; moreover, it remains to be seen whether the heritability of testosterone varies in accordance with substantial changes in the level of the hormone that are observed throughout the day. Filling these knowledge gaps will clarify whether testosterone level is more or less vulnerable to environmental influences over the course of the day, as well as clarify whether genetically informative studies of testosterone are utilizing the optimal (i.e., most heritable) phenotype.

The goal of the present study was to determine the degree to which genetic and environmental influences contribute to individual differences in testosterone level in a sample of middle-aged male twins (ages 51 to 60). Utilizing multiple time-points of data across multiple collection days, we sought to establish whether the heritability of free testosterone remained constant throughout the day, or whether the degree of genetic and environmental influences differ as function of collection time. In addition, we examined the degree to which two measures of intra-individual variation in free testosterone, the rate of change (slope) and the coefficient of variation, were influenced by genetic and environmental factors. By utilizing a middle-aged sample with a relatively narrow age range, the present study aims to provide estimates of the degree to which both genes and the environment contribute to individual differences in testosterone during what is known to be a critical transition period in the aging process.

Methods

Participants

Data were obtained as part of the Vietnam Era Twin Study of Aging (VETSA), a longitudinal study of aging with baseline in midlife (Kremen et al., 2006). VETSA participants were sampled from the Vietnam Era Twin (VET) Registry, a nationally distributed sample of male-male twin pairs who served in the United States military at some point between 1965 and 1975 (Goldberg et al., 2002). Although all VETSA participants are military veterans, the vast majority did not experience combat situations during their military careers. In total, 1237 men ages 51 to 60 participated in the primary VETSA project. The average age was 55.4 years (SD = 2.5). Participants were predominantly

Caucasian (89.7%), with an average education of 13.8 years (SD = 2.1). In comparison to U.S. census data, VETSA participants are similar in demographic and health characteristics to American men in their age range (Centers for Disease Control and Prevention, 2003). Zygosity for 92% of the sample was determined by analysis of 25 microsatellite markers obtained from blood samples. For the remainder of the sample zygosity was determined through a combination of questionnaire and blood group methods. A comparison of these two approaches in the .VETSA sample has demonstrated an agreement rate of 95%.

As part of the VETSA project, participants traveled to either the University of California San Diego or Boston University for a daylong series of physical, psychosocial, and neurocognitive assessments. To be eligible both members of a twin pair had to agree to participate and be between the ages of 51 and 59 at the time of recruitment. In the third year of the project levels of testosterone, cortisol, and dehyrdoepiandrosterone sulfate (DHEAS) were obtained via saliva samples (N = 783, 193 monozygotic pairs, 196 dizygotic pairs, 5 unpaired twins). Approval from local institutional review boards was obtained for each study site, and all participants provided signed informed consent prior their participation.

Testosterone Collection and Assay

All hormone measurements were obtained on two days at home during a participant's typical week, as well as on the assessment day. The at-home samples were collected approximately two weeks prior to the assessment day in order to avoid disruption of normal schedules that could be caused by travel to the testing site. Saliva was collected at waking, 30 minutes after waking (wake +30), 10:00 a.m., 3:00 p.m., and evening/bedtime on all days. These time-points were selected primarily to capture diurnal changes in cortisol levels. Participants were mailed a saliva collection kit that included individualized instructions, labeled 4.5 ml Cryotube vials, Trident original sugarless gum, straws, tissues, a daily log, pen, reminder watch, and a storage container with an electronic track cap for determining compliance with the protocol. Precise times of sample collection were recorded by the participant, and were later confirmed against data from the electronic track cap. Once collected, samples were sent via overnight mail to the University of California, Davis for assay.

Prior to assay, saliva samples were centrifuged at 3000 rpm for 20 minutes to separate the aqueous component from mucins and other suspended particles. Salivary concentrations of free testosterone were determined in duplicate using commercial radioimmunoassay kits (Beckman Coulter Inc., formerly Diagnostics Systems Laboratories, Webster, TX). Assay procedures were identical to those described by Granger and colleagues (Granger et al., 1999). Intra-assay and inter-assay coefficients of variation were 3.141 pg/ml and 4.878 pg/ml, respectively. The least detectable concentration for the assay was 1.3697 pg/ml. All samples from each participant were assayed together; and data from one to three individuals were included in each assay batch. Assays were always performed without knowledge of the zygosity of the twin pairs. Values greater than three standard deviations above the mean waking testosterone level, the highest value of the day, were set to missing in order to eliminate outlying data points. Scores for missing values were imputed only if the participant had no more than one missing value on a day. In order to impute missing data, we calculated the full samples' mean change in testosterone level between the time-point with the missing value and the adjacent time-point. We then added or subtracted the mean change in testosterone for those two points from the participant's non-missing time-point. This was done for less than 1% of all available hormone samples. Data from participants who reported taking testosterone supplements or other medications known to alter testosterone levels were excluded (N = 4).

For the present analyses, at-home testosterone values were averaged at corresponding times in order to create a single value for analysis at each time-point. In addition to individual time-points, we also examined the average testosterone levels across all time-points for the at-home and in-lab collection days separately. Variation within the day was measured by the rate of change in testosterone level (i.e., the slope). Due to a notable differences in the rate of change in testosterone over the course of the day (see Figure 1), we utilized three slope measures: 1) wake to evening, 2) wake to wake +30, and 3) wake +30 to evening. In addition, we calculated a coefficient of variation for each individual, defined as the standard deviation of each individual's data divided by his average testosterone level. This provided an indicator of daily intra-individual variability that might not be reflected in a measure of simple linear decline, as well as a measure of variability that was independent of the individual's average hormone level. Since testosterone levels possessed marginal positive skewness all measures were square-root transformed prior to analysis in order to normalize the distributions. Average testosterone values, rates of change, and coefficients of variation were calculated based on the raw variables and were then later transformed if necessary.

Data Analysis

In the classical twin design the variance of any phenotype can be decomposed into the proportion attributed to additive genetic *(A)* influences, common or shared environmental (*C*) influences (environmental factors that make both members of a twin pair similar to one another), and unique environmental *(E)* influences (environmental factors that make members of a twin pair different from one another, including measurement error) (Eaves et al., 1978). Monozygotic (MZ) twins are assumed to correlate perfectly with respect to their additive genetic influences, as they share 100% of their DNA. Dizygotic (DZ) twins, in contrast, share on average 50% of their segregating DNA, and are therefore assumed to correlate .50 for additive genetic influences. The common environment is assumed to correlate perfectly between members of a twin pair regardless of zygosity.

In order to account for the partial nesting of twin pairs within assay batches, as well as the nesting of batches within twin pairs, estimates of relative genetic and environmental influence for each testosterone measure were determined within a Bayesian framework using Markov Chain Monte Carlo methods with the publically available WinBUGS software package (Spiegelhatlter et al., 2004). Detailed descriptions of our analytic approach and requisite assumptions have been published elsewhere (Franz et al., 2010; Prom-Wormley et al., 2011). Briefly, the estimated testosterone measure of the jth twin of the ith pair is denoted as Y_{ijk}, where *k* indicates the batch in which the value was assayed.

We then let:

$$Y_{ijk} = \mu + {}_{ij} + {}_{k}$$

where μ represents the measured testosterone level, ${}_{ij}$ represents the random differences between twins, conceivably correlated between pairs, and ${}_{k}$ the random differences between batches. For each type of twin (MZ and DZ) we assume that the twin effects (${}_{ij}$) are bivariate normal with standard deviation and intraclass correlations of ${}_{MZ}$ for MZ and ${}_{DZ}$ for DZ pairs, respectively. The batch effects are assumed to have a normal distribution. Samples from the posterior distribution of variance components allowed for the estimation of their 95% confidence intervals.

The contribution of additive genetic influences to the observed variance (i.e., the heritability) was primarily calculated as twice the difference between the MZ and DZ intraclass correlations, $A=2({}_{MZ} - {}_{DZ})$. The contribution of the common environment was estimated as twice the DZ correlation minus the MZ correlation, $C=2 {}_{DZ} - {}_{MZ}$. Unique environmental influences were calculated as 1 minus the MZ correlation, $E=1 - {}_{MZ}$. Note

that estimates of C may be negative if there are large non-additive genetic effects, as would be indicated by an MZ correlation that is more than twice the DZ correlation. In such a scenario estimates of *A* can be dramatically and erroneously inflated (e.g., an MZ correlation of .75 and a DZ correlation of .20 would result in a heritability estimate of 110%). To avoid this bias, negative estimates of C were constrained to zero, and *A* was instead allowed to be equal to the MZ correlation. Similarly, in cases where the DZ correlation exceeded the MZ correlation, *A* was constrained to zero, and *C* was allowed to be equal to the MZ correlation. This ensured that the combined total of all variance components did not exceed 1.0.

Results

Participants who provided saliva-based hormone samples were not statistically different from the rest of the VETSA sample (p>.05) in terms of years of education, body mass index, smoking status, self-reported symptoms of depression, or self-reported classification of overall health status. A small but nevertheless significant difference in age was observed (Present Sample: Age=55.9; Remaining VETSA Sample: Age=54.6: p<.0001). We observed no significant differences in testosterone levels between participants who described themselves as Caucasian versus non-Caucasian. As anticipated, levels of testosterone both at home and on the day of testing were highest at waking, and declined substantially over the course of the day (see Figure 1). The average waking testosterone level was significantly higher for at-home measures in comparison to the assessment day (p<.0001); however, for all other time-points testosterone level on the day of testing was significantly greater than the corresponding at-home measurement (p values ranged from .048 to <.0001). A pronounced decrease in testosterone level was noted from waking to wake +30, accounting for roughly 39% of the total daily change for the at-home measures and 32% of the change on the day of testing. The rate of change between waking and wake +30 was significantly greater than the two other rate of change measures (all p values <.0001).

Genetic and environmental variance components for each collection time-point, as well as the daily averages for the at-home and day of testing measurements are presented in Table 1. These values are also presented in Figure 2 relative to the overall phenotypic variance of each measurement. As can be seen in Figure 2, across both the at-home and in-lab assessment days, the phenotypic variance of the testosterone measurements declined dramatically from waking to evening, corresponding to the decline in observed hormone level. With the exception of the waking measurement, the variance of in-lab measurements was consistently higher than at-home measurements. At-home heritability estimates ranged from a low of .02 in the evening to a high of .37 for both 10 a.m. and 3 p.m. Genetic factors on the day of testing were at their weakest in the evening (Heritability= .05), and had their strongest effect at waking (Heritability=.39). Midday heritability estimates on the day of testing, those taken at 10 a.m. and 3 p.m., were nearly half the magnitude of the at-home measures, and substantial but non-significant effects of the shared environment were observed. Adjusting for variability in waking time, as well as variability in the time of all other sample collections, did not alter the contributions of the genetic or environmental variance components at the specific time-points. The heritability of the average at-home testosterone value was .42, notably higher than the estimates for the individual time-points. Similar to the at-home results, the heritability of the average day-of-testing testosterone level was .47, and showed no evidence of shared environmental influences.

Heritability estimates for the daily rates of change and coefficients of variation in testosterone level are presented in Table 2. For the at-home measures, a small but nevertheless significant genetic influence was observed for the wake to evening slope (Heritability =.16), while the wake to wake +30 slope was not heritable. The rate of change

between wake +30 and evening, however, was significantly heritable (Heritability = .23). On the day of testing, all rate of change measures were significantly heritable, with estimates ranging from .15 to .29. Both the at-home and in-lab assessment day coefficients of variation demonstrated no genetic influences, suggesting that nearly all variability in this measure is due to unique environmental influences.

Secondary Analyses

In order to determine whether factors that have been associated with, or shown to alter, testosterone levels influenced the observed heritability estimates, all analyses were repeated after adjusting for the following covariates: age, body mass index (BMI), alcohol consumption (number of drinks consumed over the past two weeks), smoking status (current smoker versus non-smoker), sleep quality, and self-reported health. Results from these analyses are presented in Supplementary Tables 1 and 2. Sleep quality was assessed with the total score from the Pittsburgh Sleep Quality Index, a self-report instrument that examines numerous dimensions of sleep (e.g., quality, duration, efficiency) over the past month (Buysse et al., 1989). Self-reported health was assessed with the Charlson Comorbidity Index, a composite score reflecting the total number of diagnosed medical conditions known to negatively influence mortality (Charlson et al., 1994; Charlson et al., 1987).

Heritability estimates for the adjusted at-home testosterone levels declined slightly, but maintained the same overall pattern observed for the unadjusted measures. For the individual time-points, decreases in the heritability ranged from .01 to .04 in magnitude. The most prominent change in heritability was for the average at-home testosterone level, which declined from .42 to .33. None of these adjusted heritability estimates significantly differed from the unadjusted estimates based on the overlapping 95% confidence intervals. Similarly, small decreases ranging from .00 to .04 were observed for the heritability estimates of the at-home rate of change measures and the at-home coefficient of variation. More noticeable differences were observed for the in-lab heritability estimates. The wake +30, 3 p.m., and daily average heritability estimates all decreased by .10 or more; however, they did not significantly differ from the unadjusted estimates based on the 95% confidence intervals. Heritability estimates for the other in-lab time points showed little or no changes, with the exception of the evening estimate which increased from .05 to .14, but was still not significantly different from zero. The heritability estimate did not change for the wake to evening slope; however, for the wake to wake +30 slope, and the wake +30 to evening slope, genetic influences dropped to zero after accounting for the covariates.

Given the potential opposing actions of testosterone and cortisol (Viau, 2002), additional analyses were preformed in which corresponding measures of cortisol were included in addition to the other covariates. These results are presented in Supplementary Tables 3 and 4. Organizing the analyses in this way allowed us to determine the degree to which cortisol influenced the heritability of testosterone above and beyond the other potential covariates. At-home and in-lab cortisol levels as well as measures of intra-individual variability in cortisol were calculated in the identical fashion as the corresponding testosterone measures. Detailed descriptions of the cortisol data cleaning procedures, as well as the heritability estimates for these measures have been previously published (Franz et al., 2010). Correlations between the corresponding testosterone and cortisol measures ranged from .07 (at-home coefficients of variation) to .36 (in-lab evening sample).

After adding cortisol as a covariate no clear pattern of change in the resulting heritability estimates was observed. For 15 of the 20 measures we observed no or small changes in the heritability estimates ranging from −.05 to +.04 in magnitude. The at-home wake +30 estimate fell from .28 to .16. The in-lab evening sample and the at-home wake to evening slope heritability estimates declined from .14 to .05 and .12 to .04, respectively. These

estimates were not significantly different from the previous adjusted or unadjusted estimates based on the 95% confidence intervals. The wake to wake +30 and the wake +30 to evening slopes both showed increases in their heritability estimates from .00 to .14 and from .00 to .11, respectively. Of these two measures, only the heritability estimate for the wake to wake +30 slope was significantly different from zero.

Discussion

The results of the present study demonstrate that while a substantial portion of the variability in average daily free testosterone level is determined by genetic influences, at distinct time-points throughout the day the magnitude of these genetic influences can vary dramatically. Both at home and on the assessment day, the heritability of testosterone level was moderate in the early morning (ranging from .30 to .39), and declined to near zero by the evening. These heritability estimates for morning free testosterone levels are consistent with the estimate provide by Meikle and colleagues (Meikle et al., 1987), the only other twin study to examine free testosterone in adult men of which we are aware. During the in-lab assessment heritability estimates were noticeably lower at mid-day, while contributions of the common environment were higher than at other time-points. The smaller standard deviations for the in-lab collection times clearly indicate that there was less variability in the timing of sample collections when participants were in the lab; thus, the observed differences may be the result of the structure and coordination of the participants' experiences over the course of the assessment day.

Heritability estimates for the average testosterone levels both at home and on the assessment day were greater than all individual estimates. The discrepancy between the average testosterone levels and those from specific time-points was likely due to the reduction in measurement error, and therefore reduction in unique environmental variance, brought about by averaging across multiple data points. This suggests that repeated measurement of free testosterone over the course of a day may be necessary in order to establish reliable and robust estimates of genetic influence, as well as more reliable estimates of the overall hormone level.

After controlling for age, BMI, alcohol consumption, smoking status, sleep quality, self-reported health, as well as corresponding measures of cortisol we observed generally small changes in heritability estimates for individual time-points and overall daily averages. These changes, however, were not consistent in their magnitude or direction, and the resulting heritability estimates did not significantly differ from the unadjusted estimates.

The importance of diurnal variation in hormone levels has primarily been the topic of cortisol research (Hellhammer et al., 2007; Stone et al., 2001). In contrast, little attention has been given to the patterns of daily variation in testosterone. We found evidence of significant genetic factors accounting for approximately one-fifth of the variance in all of the rate-of-change (slope) measures from early morning to evening. There were, however, no observable genetic influences on the coefficient of variation for testosterone levels, a measure of intra-individual variability that is independent of the hormone level. This might suggest that testosterone is much more reactive to environmental factors than has previously been considered. It remains to be seen whether measures of intra-individual variability in total testosterone, which when sampled in the morning provides heritability estimates that are larger than those reported here, would demonstrate the same pattern of effects as free testosterone.

Intriguingly, we observed a substantial drop in testosterone level between awakening and 30 minutes after awakening, a drop which accounted for 32%–39% of the overall daily change.

This finding has been observed in past studies examining the effects of sleep on testosterone level (Axelsson et al., 2005; Diver et al., 2003), although its potential meaning has not been explored further. There has been substantial attention paid to the cortisol awakening response, another aspect of diurnal variation. Cortisol and testosterone are thought to have opposing actions (Viau, 2002); thus, it is of interest that this substantial decline in free testosterone coincides with a period that is typically characterized by a sharp increase in cortisol (Hellhammer et al., 2007). In the present study, corresponding measurements of testosterone and cortisol were all positively correlated with one another; moreover, the rates of change from wake to wake +30 for both hormones were correlated .35 for the at-home assessments and .14 for the in-lab assessment. Although no negative associations were observed, due to the opposing signs of the testosterone and cortisol wake to wake +30 slopes, these results indicate that a greater decline in testosterone after waking is associated with a smaller cortisol awakening response. Despite the somewhat larger correlation, there was no observable effect of cortisol on the heritability of the wake to wake +30 slope for the at-home assessments. In contrast, after adding cortisol to the other covariates the heritability for the slope from the in-lab assessment day went from .00 to a significant heritability of .14. Associations such as these suggest that while the two awakening responses are somewhat related, other contributing factors are likely at work. These differences might be due to differences in sleep quality, individual difference in metabolism, factors that differ between at-home and day-of-testing, or they could simply reflect error variance. However, in the absence of supporting data such hypotheses are purely speculative. In any case, the clinical significance of the testosterone awakening response remains unclear.

It remains to be seen what specific genetic factors may be contributing to free testosterone level in late middle age. A recent genome-wide association study of serum-based testosterone levels identified two single nucleotide polymorphisms associated with the sex hormone binding globulin (*SHBG*) gene, associations that proved significant for both overall level of testosterone and the classification of low-testosterone (Ohlsson et al., 2011). An association has also been found between the nuclear receptor coactivator-3 (*NCOA3*) gene with free testosterone but not total testosterone level in late middle-aged men (Sheu et al., 2006). The *APOE* gene has been associated with free testosterone levels, specifically men with the 4 allele have been found to have higher levels of free testosterone relative to non-carriers (Berteau-Pavy et al., 2007). Finally, variation in the androgen receptor (*AR*) gene, specifically the number of trinucleotide repeats on exon 1, may also influence testosterone level, suggesting that genetically determined androgen sensitivity contributes in part to the degree of hormone production (Huhtaniemi et al., 2010).

The present results should be considered in the context of the following limitations. The primarily Caucasian composition of the VETSA sample and the narrow age range, limits our ability to generalize these results to other populations. It is also the case that the cross-sectional design of the present study does not allow us to determine whether the observed genetic and environmental influences of free testosterone are long-standing, or represent changes from earlier periods of life. It is entirely plausible that the factors that lead to age-related declines in testosterone, whether genetic or environmental, are independent from those that influence the hormone earlier in life. Thus, although heritability estimates may be comparable, the specific genetic and environmental factors that influence testosterone may be very different in a younger cohort of men. It is also well established that testosterone levels rebound during sleep, and that disruption of the sleep cycle can impact testosterone levels in otherwise healthy individuals (Luboshitzky et al., 2001). It remains to be seen if testosterone levels during sleep may be more genetically influenced than daytime samples or if the rate of change between bedtime and waking testosterone levels is itself heritable. It is important to note that measures of testosterone which are based off of a single assessment day are likely to be less reliable than estimates which are based off of multiple assessment

days. Therefore, it is possible that heritability estimates from the in-lab assessment day are fundamentally less reliable, than those based off of multiple at-home assessment days. In the present study the in-lab assessment had to be limited to one day, thus, we are unable to test this hypothesis.

Finally, the present study is limited by the fact that we cannot elucidate what role SHBG may play in the present results. Free-testosterone by definition represents hormone that is not bound to SHBG; therefore, one would anticipate that they would be correlated with one another. Like measures of total and free testosterone, SHBG has been found to be heritable in adult men (Kuijper et al., 2007; Ring et al., 2005). Whether the genetic determinants of SHBG and free-testosterone are the same or different remains to be seen. We are aware of only two studies that have examined the genetic and environmental overlap between free testosterone and SHBG. Bogaert et al (2008) observed a week phenotypic correlation between the two hormones (r_p=. −06) and a correspondingly week genetic correlation (r_g=. 14) in young adult men (age range 25 to 45). Coviello et al (2011) found a significant genetic correlation between the two hormones (r_g=−.60) in adult women, however, the findings also suggest that both hormones are influenced by unshared genetic factors. It should be noted that both of these studies were based on extended family designs, and were not twin studies; thus, their estimates of heritability and genetic correlation are likely to be less precise than those derived from a twin sample. Additional studies are needed in order to determine whether free testosterone levels, and changes in free testosterone levels with increasing age, are influenced by the same or difference genetic determinants as those influencing SHBG.

In summary, the present study demonstrates that while measures of average daily free testosterone provide robust heritability estimates, the magnitude of the genetic influences of the hormone are not constant throughout the day. Moreover, while daily rates of change in testosterone demonstrated some genetic influence, the overall intra-individual variability in the hormone appears to be primarily due to individual specific environmental factors. These results highlight the importance of both genetic and environmental factors in regulating the functioning of the hypothalamic-pituitary-gonadal axis, as well as the need to explore both genetic and individual-specific environmental factors as potential determinants of age-related testosterone changes.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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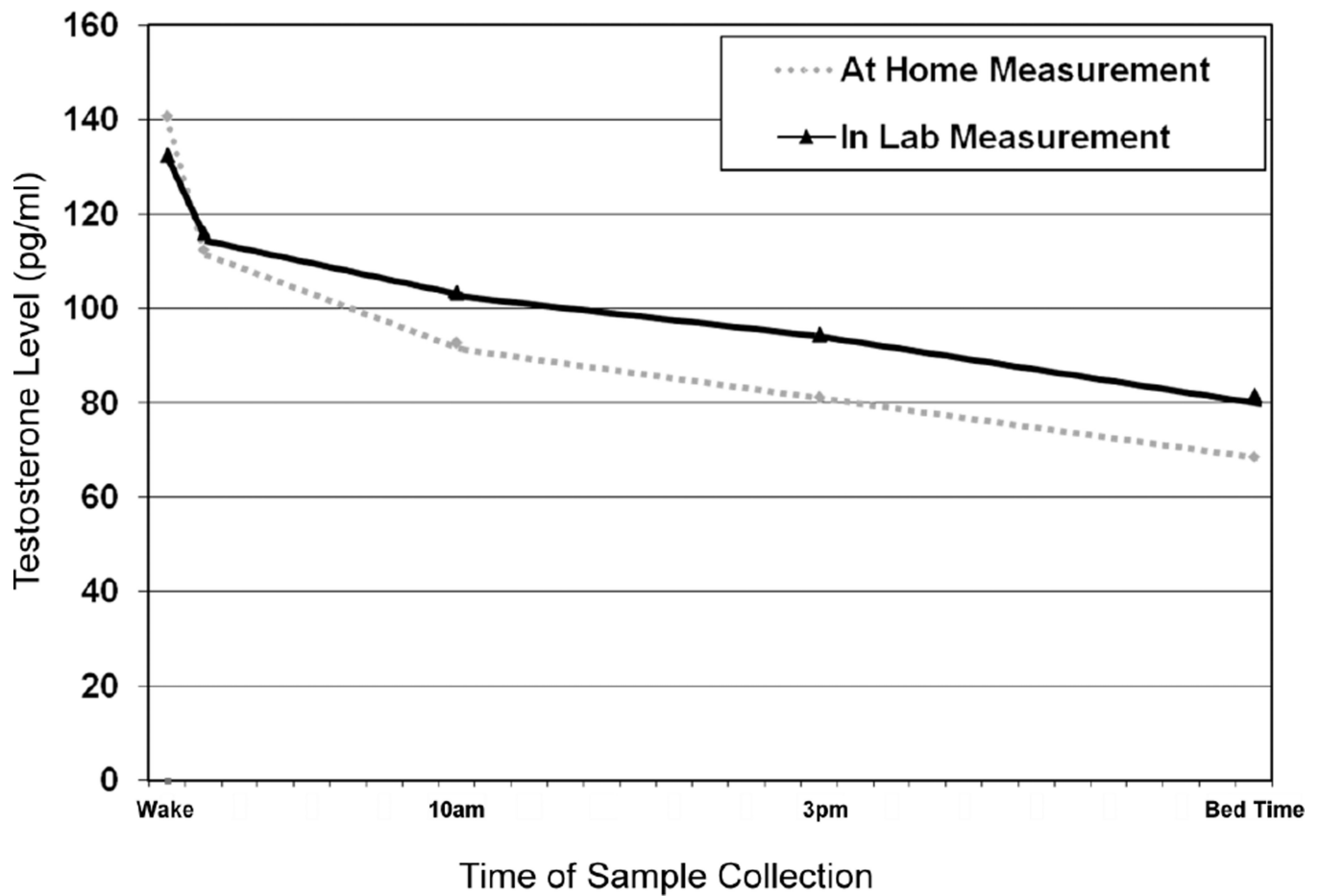
Figure 1.
Average testosterone levels across at-home and in-lab assessment days. Standard errors for the points of measurement ranged from 0.99 to 1.9, and not presented in the figure due to the overall scale. All in-lab testosterone measurements are significantly different from the corresponding at-home level at the p < .05 level.

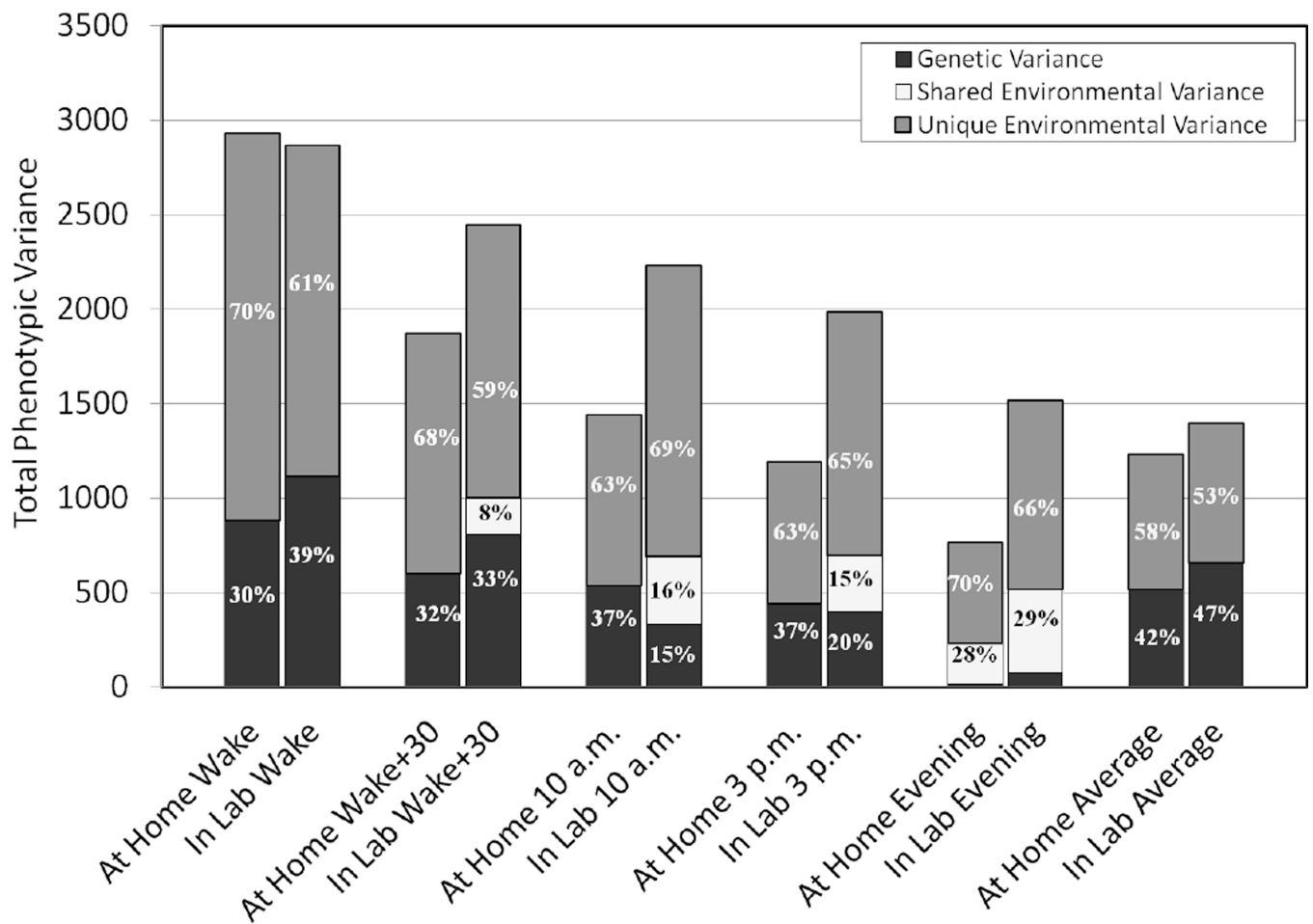**Figure 2.**
Genetic and environmental contributions to the phenotypic variance of free testosterone level. The vertical axis represents the total phenotypic variance for the raw (untransformed) free testosterone measures.

Table 1

Summary statistics and variance component estimates for At-Home and Day-of-Testing testosterone levels

Measure	Testosterone level pg/ml (SD)	Collection Time (SD)	Correlations		Variance Components		
			r_{mz} (95% CI)	r_{dz} (95% CI)	A (95% CI)	C (95% CI)	E (95% CI)
At Home (Days 1 & 2)							
Wake	140.65 (54.15)	0631 h (2.25)	.30 (.15; .44)	.13 (.01; .29)	.30 (.15; .44)	--	.70 (.56; .85)
Wake +30*	112.47 (43.27)	0684 h (2.26)	.32 (.17; .47)	.18 (.04; .32)	.32 (.17; .47)	--	.68 (.53; .83)
10 a.m.	92.74 (38.0)	1019 h (1.56)	.37 (.23; .50)	.14 (.04; .27)	.37 (.23; .50)	--	.63 (.50; .77)
3 p.m.	81.12 (34.52)	1502 h (1.66)	.37 (.23; .49)	.12 (.03; .26)	.37 (.23; .49)	--	.63 (.51; .77)
Evening	68.51 (27.71)	2040 h (3.36)	.30 (.15; .44)	.29 (.15; .43)	.02 (−.36; .39)	.28 (−.02; .59)	.70 (.57; .85)
Overall Mean	100.58 (35.10)	-	.42 (.27; .55)	.17 (.03; .32)	.42 (.27; .55)	--	.58 (.45; 73)
Day of Testing							
Wake	132.43 (53.53)	0589 h (0.78)	.39 (.26; .51)	.16 (.04; .31)	.39 (.26; .40)	--	.61 (.49; .74)
Wake +30	115.90 (49.44)	0644 h (0.87)	.41 (.27; .52)	.25 (.10; .38)	.33 (−.02; .67)	.08 (−.21; .37)	.59(.48; .73)
10 a.m.	103.24 (47.23)	0973 h (0.58)	.31 (.15; .45)	.23 (.08; .38)	.15 (−.24; .52)	.16 (−.16; .46)	.69 (.55; .85)
3 p.m.	94.39 (44.59)	1493 h (0.98)	.35 (.20; .48)	.25 (.09; .40)	.20 (−.18; .58)	.15 (−.17; .46)	.65 (.52; .80)
Evening	81.48 (38.94)	2058 h (4.67)	.34 (.20; .46)	.31 (.15; .46)	.05 (−.32; .42)	.29 (−.04; .59)	.66 (.54; .80)
Overall Mean	107.45 (37.35)	--	.47 (.33; .58)	.23 (.06; .38)	.47 (.33; .58)	--	.53 (.42; .67)

*
Results from AE models are presented in cases where the shared environment (C) was positive, but nevertheless accounted for less than 5 percent of the phenotypic variance. r_{mz}= Monozygotic twin correlation; r_{dz}= Dizygotic twin correlation; A = Additive genetic influences; C = Shared environmental influences; E = Unique environmental influences.

Table 2

Standardized variance components for rates of change and coefficients of variation

| Measure | Correlation | | Variance Components | | |
	r_{mz} (95% CI)	r_{dz} (95% CI)	A (95% CI)	C (95% CI)	E (95% CI)
At Home (Days 1 & 2)					
Wake to Evening*	.16 (.01; .30)	.09 (.02; .25)	.16 (.01; .49)	--	.84 (.70; .99)
Wake to Wake +30	.04 (.00; .12)	.10 (.02; .24)	--	.04 (.00; .12)	.96 (.88; 1.0)
Wake +30 to Evening	.23 (.08; .37)	.07 (.02; .19)	.23 (.08; .37)	--	.77 (.63; .92)
Coefficient of Variation	.17 (.02; .31)	.23 (.09; .38)	--	.17 (.02; .31)	.83 (.69; .98)
Day of Testing					
Wake to Evening	.29 (.15; .42)	.06 (.01; .19)	.29 (.15; .42)	--	.71 (.58; .85)
Wake to Wake +30	.15 (.04; .28)	.07 (.01; .19)	.15 (.04; .28)	--	.85 (.72; .96)
Wake +30 to Evening *	.28 (.14; .41)	.15 (.02; .31)	.28 (.14; .41)	--	.72 (.59; .86)
Coefficient of Variation	.08 (.01; .20)	.12 (.02; .25)	--	.08 (.01; .20)	.92 (.80; .99)

*
Results from AE models are presented in cases where the shared environment (C) was positive, but nevertheless accounted for less than 5 percent of the phenotypic variance. r_{mz} = Monozygotic twin correlation; r_{dz} = Dizygotic correlation; A = Additive genetic influences; C = Shared environmental influences; E = Unique environmental influences.