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Genetic and environmental influences of daily and intra-individual variation in testosterone levels in middle-aged men

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Summary
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.
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).

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Results: Testosterone levels declined substantially over the course of the day, with 32–39% of the change occurring in the first 30 min 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.

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–.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 et al. (1987) estimated the heritability of free testosterone at .34 in a sample of men ages 20–60, whereas Hoekstra et al. (2006) observed a substantially larger heritability of .52 in a sample of twelve-year-old boys. More recently, Caramaschi et al. (2012) 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. 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 51–60). 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

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