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Original Research Article

Salivary Estradiol and Testosterone in Filipino Men: Diurnal Patterns and Relationships with Adiposity

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Objectives: We used detailed saliva sampling procedures to test for diurnal changes in men's salivary estradiol (E2) and testosterone (T) and assessed whether greater adiposity predicted higher E2 and T.

Methods: We drew on a subsample of young adults enrolled in a long-running birth cohort study in Metro Cebu, Philippines. Subjects provided saliva samples at four time points during the day (waking, waking +40 min, early evening, and bedtime), which were assayed for E2 and T. Using these detailed hormonal data, we calculated E2 ($n = 29$) and T ($n = 44$) area-under-the-curve values, which provide insights on hormonal production over the study period.

Results: While T declined immediately after waking and reached a nadir in the early evening, E2 did not show significant diurnal change ($P \geq 0.1$) but was positively correlated to T at multiple time points ($P \leq 0.05$). Subjects with higher adiposity (BMI, waist circumference, skinfolds) had elevated E2 secretion throughout the day ($P \leq 0.01$), but adiposity was not related to salivary T.

Conclusions: Consistent with past research, our results indicate that adipose tissue is a significant site of E2 production in males but differ from a limited number of prior studies of young men in that we did not find lower T with increasing adiposity. Given E2's role in male hypothalamic-pituitary-gonadal function and complex interfaces with the immune system, these results have important implications for models of male life history as rates of overweight and obesity rise in populations around the world. Am. J. Hum. Biol. 26:376–383, 2014.

Compared with its critical roles in female reproductive biology, estradiol's (E2) potential importance to male physiological function has been described in much less detail. There are notable sex-based differences in E2 production pathways. In females, the gonads (ovaries) are the primary producers of E2 (Ellison, 2001). By contrast, circulating male E2 appears to be derived less from direct gonadal (testes) production, and more so from local, tissue-specific conversion of testosterone (T) to E2 via the enzyme aromatase (Baird et al., 1969; Labrie et al., 1997). Local aromatization occurs in a range of tissues, including in the brain, in skeletal muscle, and extensively in body fat (de Ronde et al., 2003; Vermeulen et al., 2002). Multiple lines of evidence suggest that E2 might have important implications for male life history. For example, E2 interacts with diverse aspects of the immune system, impacting humoral immunity, autoimmunity, and inflammation pathways (among other effects), to potentially affect maintenance-reproduction trade-offs, day-to-day well being, and longevity (Ahmed et al., 1985; Grossman et al., 1991; Sakiani et al., 2012; Straub, 2007). Animal models indicate that E2 might also influence male fertility via effects on sperm production (de Ronde et al., 2003), and a recent experimental study of reproductive-aged men found that suppression of E2 reduced males' sexual desire and function, independent of T (Finkelstein et al., 2013). Finally, E2 contributes to the negative feedback loop that regulates the hypothalamic-pituitary-testicular (HPT) axis (Bagatell et al., 1994; Raven et al., 2006), which primarily controls T production and is a neurobiological-endocrine mediator of critical male life history trade-offs (Bribiescas, 2001; Ellison, 2001; Hau, 2007). However, to fully integrate E2 into reproductive physiological and ecological models of male life history, we need to expand our knowledge of its mechanistic function and regulation and how they vary across contemporary populations and over the male life course (Bribiescas, 2006a,b; Ellison, 2001; Ellison et al., 2002).

Given E2's negative feedback on the HPT axis and adipose tissue's aromatization capacities, questions of E2's importance to male reproductive physiology, health, and (possibly) behavior are of increasing salience as rates of overweight and obesity rise in populations around the world, highlighting the interface between a common culturally and politically economically influenced niche (i.e., obesogenic environments) and a relatively evolutionarily conserved physiological axis (i.e., the HPT axis). The relationship between body composition, E2, and reproductive physiology in males is complex. E2 influences the production and maintenance of adipose tissue and the pattern of localized fat deposition (Cooke and Naaz, 2004). By regulating an enzyme known as lipoprotein lipase, E2 can inhibit lipid uptake by adipocytes, limiting fat accumulation, though some evidence suggests that these specific effects are less pronounced in males' abdominal fat depots compared to adipose tissue elsewhere on the body (Mayes and Watson, 2004; Ramirez et al., 1997). Moreover, a

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recent experimental trial, in which reproductive-aged men's endogenous T and E2 were suppressed and then systematically replaced in varying doses, showed that men with inhibited E2 gained both subcutaneous and intra-abdominal fat, independent of T (Finkelstein et al., 2013). In contrast, there is also strong evidence that E2 can reduce lipolysis in subcutaneous fat as well as potentially increase adipose precursor cell proliferation (Cooke and Naaz, 2004; Pedersen et al., 2004). Because adipose tissue is a significant site of aromatization (Vermeulen et al., 2002), men becoming overweight and obese often experience increased conversion of T to E2, which likely contributes to a commonly observed obesity-linked phenotype characterized by lower T and elevated E2 (Jensen et al., 2004; Schneider et al., 1979; Tchernof et al., 1995; Van Pottelbergh et al., 2003; Vermeulen et al., 2002).

However, studies in this area frequently draw from clinical studies of HPT axis dysfunction, particularly as connected to pathological adiposity (Schneider et al., 1979; Tchernof et al., 1995), or aging Euro-American samples (Van Pottelbergh et al., 2003; Vermeulen et al., 2002). Consequently, many questions remain about the mutually regulatory interface between adiposity, T, and E2 in early adulthood. In particular, multiple facets of HPT axis physiological dynamics, with implications for E2, vary between older and younger men, for example, T, aromatase, and transport protein (albumin and sex hormone binding globulin) production (Cleland et al., 1985; Mazur, 2009; Van Pottelbergh et al., 2003). Though less explored, hormone receptor quantities, affinities, and sensitivities as well as hormone clearance rates might also vary. Similar cross-population variability could exist based on developmental exposures that likely affect these pathways (e.g., diet, energetic status, pathogenicity, environmental toxicity, etc.).

Data on E2's diurnal regulation are also relatively sparse (compared with T, for example), with some studies indicating that male E2 is relatively stable across the day (Bribiescas, 2005; Leymarie et al., 1974; Murono et al., 1982) and results elsewhere suggesting that E2 fluctuates diurnally (Berg and Wynne-Edwards, 2001; Juneja et al., 1991). In terms of incorporating E2 more rigorously into studies of male life history, it is imperative to describe the normative physiological mechanics of its production. As an exemplar, psychobiological research on cortisol, which has a diurnal curve characterized by high waking values, a spike shortly thereafter (awakening response), and then declining production over the course of the day, has shown that the steepness of the diurnal curve and the magnitude of the awakening response are affected by day-to-day emotional disruption (Adam et al., 2006) and dysregulated in conditions such as depression and chronic fatigue syndrome (Dienes et al., 2013; Nater et al., 2008). Characterizing E2's diurnal curve will enable us to better test hypotheses regarding alterations in its regulation and its physiological roles as internal conditions and demands shift throughout the life course, such as with energy excess (overweight/obesity), psychosocial stress, active infection, or senescence.

Finally, prior research has shown that correlations between salivary and plasma E2 are less strong among males than females, suggesting that salivary E2 might provide unreliable assessments of circulating levels (among males), for example, compared with plasma-salivary correlations for T (Shirtcliff et al., 2000). As a consequence of these issues, human male psychobiological studies using salivary E2 might be predisposed to Type II errors (Shirtcliff et al., 2000). To date, most studies of E2-body composition relationships have relied on hormonal values assayed from plasma/serum samples, rather than more field-friendly, less invasive saliva sampling techniques commonly used by anthropologists studying human biology (but see Bribiescas, 2005). It is relatively unknown whether salivary E2 might be more valid for body composition studies (compared with male psychobiology-behavioral physiology research) due to the aromatization capacities of fat tissue. This is an important methodological issue for advancing anthropological research in this domain.

Here, using saliva samples collected at four time points over the course of the day, we present diurnal rhythms for both salivary E2 ($n = 29$) and T ($n = 44$) from a subsample of young adult men (all of whom are fathers) enrolled in a well-characterized, one-year birth cohort study in the Philippines (Adair et al., 2011). We first test whether E2 shows a diurnal decline across the day, similar to T, and test for positive correlations between E2 and T. We then test the hypotheses that subjects with greater adiposity will have elevated E2 and lower T secretion over the course of the day. We approach these hypotheses from two directions. First, we test whether adiposity is predictive of E2 at single time points (i.e., waking and bedtime, respectively), as is common in field-based studies of human physiology. We then draw on our detailed (four time point) hormonal data to conduct area under the curve (AUC) analyses (Pruessner et al., 2003), which provide a more rigorous perspective on hormonal secretion/exposure for a given timeframe by integrating repeated measures from each subject into a single variable (Pruessner et al., 2003). Using this AUC approach, we test whether men with greater adiposity have higher E2 and lower T over the course of the study period.

METHODS

Study population

Data collection took place in 2010 as part of the Cebu Longitudinal Health and Nutrition Survey (CLHNS), a population-based birth cohort begun in 1983–1984 (Adair et al., 2011). Men were a mean of age 26.6 ± 0.3 (SD) years at the time of data and sample collection. Cebuano-speaking interviewers collected socioeconomic, demographic, health, and general behavioral data using questionnaire-based, in-home interviews (Adair et al., 2011). We conducted this research under conditions of written informed consent with human subjects' clearance from the Institutional Review Board of Northwestern University.

Sample characteristics

Subjects in this analysis were participants in a separate father-toddler interaction study ($n = 45$; Gettler et al., 2011c, 2013b). We selected fathers for the father-child interaction study based on them living with at least one biological child, older than 1 year of age and <4 years of age, and the mother of that child, having no adopted or step-children, and having full data from prior CLHNS surveys, conducted in 2005 and 2009. We have previously described the full criteria for inclusion in the study (Gettler et al., 2011c, 2013b).

TABLE 1. Sample characteristics

	Full E2 data[a]		Full T data[b]	
	Mean	SD	Mean	SD
Demographic characteristics				
Age (years)	26.7	0.3	26.6	0.3
Education (highest grade)	9.7	3.3	9.6	3.3
Currently employed (%)	96.6	–	93.2	–
Urban barangay (neighborhood; %)	58.6	–	61.4	–
Fatherhood characteristics				
Duration of marriage (years)	5.1	2.2	5.4	2.3
Number of children	2.2	1.1	2.3	1.0
Years as a father	4.2	2.3	4.4	2.2
Anthropometric characteristics				
Height (m)	1.62	0.1	1.62	0.1
Weight (kg)	64.2	15.9	62.2	14.9
BMI (kg/m^2)	24.3	5.4	23.6	5.2
Waist circumference (cm)	79.8	12.7	78.4	11.9
Triceps skinfolds (mm)	18.1	8.8	16.7	8.9
Suprailiac skinfolds (mm)	19.6	9.6	19.0	10.3
% Overweight ($25 \leq$ BMI < 30)	10.3	–	9.1	–
% Overweight (BMI ≥ 30)	17.2	–	15.9	–

[a]Descriptive statistics for subjects with full E2 data ($n = 29$).
[b]Descriptive statistics for subjects with full T data ($n = 44$).

Saliva collection

The day following the father-toddler interaction, subjects provided saliva samples at their homes, at the following time points: immediately upon waking [6:08 AM ± (SD) 1:41], ~40 min after waking (6:46 AM ± 1:40), early in the evening (6:37 PM ± 0:31), and before bedtime (9:22 PM ± 1:23). Our research team retrieved the samples and transported them to the USC-Office of Population Studies Foundation (Philippines), where they were frozen at −35°C. All samples were later shipped on dry ice to Northwestern University, where they were stored at −80°C.

Salivary hormonal analyses

We ran the T and E2 assays at separate time points at Northwestern University's Laboratory for Human Biology Research using enzyme immunoassay kits from Salimetrics (State College, PA): E2 No. 1–4,702; T No. 1–2,402. Interassay coefficients of variation for high and low controls were as follows: E2 = 3.8% and 5.7%; T = 6.4% and 7.2%. When we conducted the T analyses, the full sample ($n = 45$) had sufficient saliva for all four of the sampling time points. We were unable to include one subject because his bedtime T value had a CV outside of the acceptable range, resulting in a sample of 44 subjects with T data. We assayed the samples for E2 secondarily, with 40 subjects having adequate saliva volume for these analyses. Nine subjects had at least one E2 value that fell below the assay's level of detection (see below). We were unable to include one subject because his bedtime E2 value had a CV outside of the acceptable range, and we eliminated another subject because of an E2 value 8 SD above the sample mean. Thus, we had a sample of 29 men with full E2 data.

Anthropometrics

We measured body weight (kg), height (cm), waist circumference (cm), and skinfold thicknesses (triceps, suprailiac; mm) using standard anthropometric techniques (Lohman et al., 1988). We calculated the body mass index (BMI) as the ratio of weight (kg)/height (m^2).

Statistical analyses

We conducted our analyses using Stata 12.1 software. Drawing on the sample of men with full hormonal data (T: $n = 44$; E2: $n = 29$), we employed paired, two tailed t-tests to assess diurnal change in E2 and T and correlated E2 and T at each time point. We then applied multiple statistical approaches to test for relationships between E2, T, and three measures of body composition (BMI, waist circumference, and skinfold thickness). As is common in many studies of human steroidal physiology (van Anders et al., 2014), including multiple previous behavioral (Gettler et al., 2011a, 2012a, 2013a; Kuzawa et al., 2009) and anthropometric-related (Gettler et al., 2010, 2011b) studies in this field setting, we predicted men's hormonal values at single time points (E2 and T assayed from waking samples and bedtime samples, respectively) from our independent variables (BMI, waist circumference, and skinfold thickness) using linear regression. We then used an AUC technique, which provides a more robust perspective (than single measures, such as waking or bedtime) on total hormonal secretion and exposure throughout the day, to assess hormone-adiposity relationships (Pruessner et al., 2003). We calculated subjects' E2 and T diurnal secretion using the "AUC with respect to ground" trapezoid formula (Le Floch et al., 1990; Pruessner et al., 2003). For E2, we first calculated AUC values using the subsample of men with fully detectable E2 at all time points ($n = 29$). Because the assay that we employed to examine male E2 is designed for a normative range of female E2 (Salimetrics LLC), a number of subjects ($n = 9$) had at least one E2 value that fell below the assay's level of detection. We ascribed these undetectable samples values of 0.1 pg/ml, which is the lower limit of assay sensitivity, according to the assay manufacturer (Salimetrics LLC). We calculated a second set of E2 AUC values that combined the subsample of men with fully detectable E2 at all time points ($n = 29$) and the subsample for whom at least one undetectable value was ascribed ($n = 9$), resulting in a subject pool of 38 men. We then predicted men's E2 AUC (with separate models for men with full E2 data versus the sample that includes subjects with ascribed values) and T AUC from body composition using linear regression. We adjusted our hormonal measures for wake time. We used G*Power3, a freely available statistical software package, to conduct a power analysis (Faul et al., 2007).

RESULTS

Table 1 provides descriptive statistics for the sample. As predicted, T decreased over the course of the day, with a decline shortly after waking and an evening nadir. In contrast, we detected no significant diurnal pattern for E2 (Fig. 1), though it did tend to decrease immediately after waking ($P = 0.1$). We conducted a post-hoc power analysis for the comparison between waking E2 and wake + 40 min E2, finding that a sample of 81 subjects would be necessary to document a significant ($P < 0.05$) decline in E2 after waking, given our effect size and with statistical power set to 0.8. As predicted, E2 and T were positively correlated at multiple time points: wake $r = 0.36$, $P = 0.057$; wake +40 min $r = 0.25$, $P = 0.2$; early evening $r = 0.36$, $P = 0.054$; bedtime $r = 0.62$, $P = 0.0003$.

Using linear regression ($n = 29$), we tested whether men with greater adiposity had elevated E2 at waking or at bedtime, finding no significant associations between E2

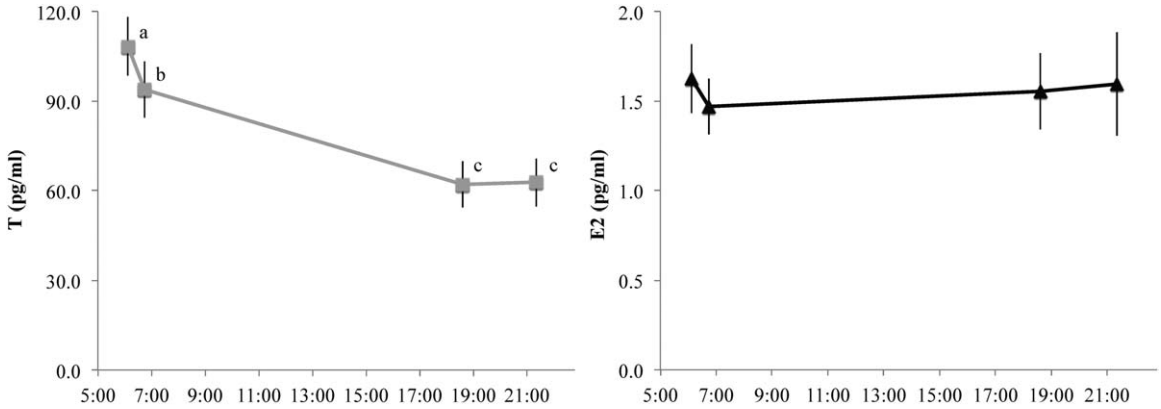

Fig. 1. Patterns of diurnal change in salivary E2 and T, measured at four time points: waking (6:08 AM), ~40 min after waking (6:46 AM), early in the evening (6:37 PM), and before bedtime (9:22 PM). Values for E2 did not significantly vary across the day (all $P \geq 0.1$). T values with varying letters differ significantly from one another (all $P < 0.001$; paired t-tests). Error bars indicate 95% CI.

TABLE 2. Bivariate linear regression models predicting waking and bedtime E2 from measures of adiposity (n = 29)[a]

	Waking E2		*P* value	Model R2	Bedtime E2		*P* value	Model R2
BMI	0.01	(−0.03, 0.05)	0.6	0.010	0.02	(−0.04, 0.07)	0.5	0.020
Waist circumference	0.004	(−0.01, 0.02)	0.6	0.012	0.01	(−0.01, 0.03)	0.4	0.024
Skinfold thicknesses	0.003	(−0.01, 0.01)	0.5	0.014	0.01	(−0.01, 0.03)	0.2	0.049

[a]Values are β (95% CI) of E2.

and our three anthropometric measures of adiposity (all $P > 0.2$; Table 2). Similarly, men did not differ for body fatness based on their waking or bedtime T ($n = 44$; all $P > 0.3$).

However, consistent with our predictions, men with greater levels of adiposity (BMI, waist circumference, skinfold thickness) had higher diurnal E2 secretion over the course of the day in models limited to males with detectable E2 at all time points ($n = 29$; Fig. 2; Table 3; all $P \leq 0.01$). To aid with visualization, we present a comparison of E2 AUC for healthy BMI and overweight/obese BMI subjects in Figure 3. Although the effect sizes tended to mildly decrease, we found similar results when we expanded the analytical sample ($n = 38$; Table 3; all $P < 0.05$) to include subjects with undetectable E2, by ascribing them values for undetectable samples (see Methods). Contrary to our predictions that high adiposity males would have lower T, we found no significant relationships between T AUC and any measure of body composition (all $P > 0.4$).

DISCUSSION

Data on the diurnal pattern of E2 in males are relatively sparse (Bribiescas, 2005; Juneja et al., 1991; Leymarie et al., 1974; Murono et al., 1982), and studies testing for relationships between E2 and body composition among young, reproductively healthy males have been few when compared to the literature on aging males and clinical samples. Here, we showed that T declined significantly over the course of the day, reaching a nadir in the early evening, whereas E2 was relatively unchanged. E2 and T were moderately positively related at multiple time points, which is unsurprising given that the E2 is derived from T in the testes and peripheral tissues (in part; Baird et al., 1969). Finally, consistent with our predictions, men with greater adiposity had elevated E2 secretion across the day. In contrast, when we restricted our E2-adiposity analyses to single sampling time points (i.e., waking and evening E2), as is commonly done in studies of human biology, we did not find that men with greater body fat had higher E2. These discrepancies highlight the comparatively enhanced reliability (relative to single samples) and greater statistical power of the area-under-the-curve approach for indexing individual hormonal profiles and correlates thereof (Pruessner et al., 2003; Wust et al., 2000).

Although E2 may prevent fat accumulation in some depots (Cooke and Naaz, 2004), men with greater adipose tissue commonly have elevated E2 (Ferrini and Barrett-Connor, 1998; Jensen et al., 2004; Schneider et al., 1979; Tchernof et al., 1995; Van Pottelbergh et al., 2003; Vermeulen et al., 2002), similar to our results here. This positive relationship results from secretion of E2 by adipose tissue, which is a prominent site of T-to-E2 aromatization. Because E2 contributes to negative feedback regulation of the HPT axis (Bagatell et al., 1994; Raven et al., 2006), conversion of T to E2 (and thus lower circulating T) in high adiposity men might not be compensated for by greater testicular T production (Zumoff et al., 2003). Thus, our finding that men's T secretion was not lower as their adiposity increased ran counter to our predictions. In addition to heightened E2, obese men also commonly have other physiological characteristics, such as higher insulin and lower sex hormone binding globulin, that are known to reduce circulating free T (Haffner et al., 1994; Pasquali, 2006; Pasquali et al., 1991). With a larger sample size, we could have tested for either nonlinear

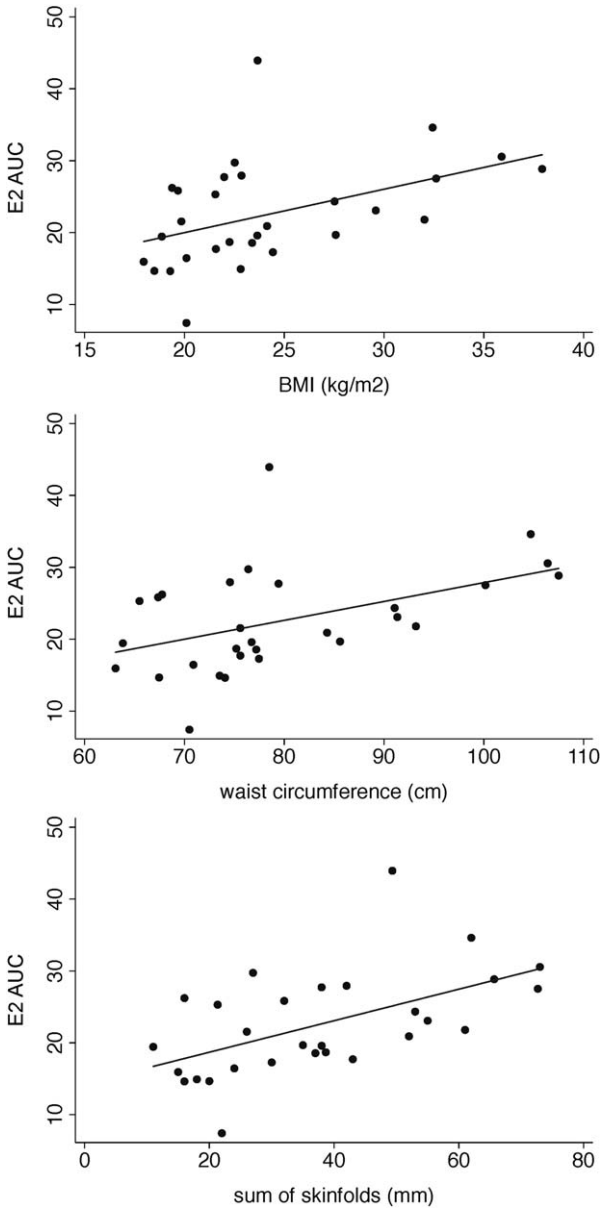

Fig. 2. Scatter plot of diurnal E2 AUC regressed on measures of adiposity. Dark line = best fit linear relationship between E2 AUC and each adiposity measure. BMI, waist circumference, and sum of skinfolds significantly predicted E2 AUC (all $P \leq 0.01$; see Results for full model details). We calculated E2 AUC using a formula from Pruessner et al., 2003 (see Methods): E2 AUC = [0.5 × (t2 − t1) × (wake E2 + wake 40 E2)] + [0.5 × (t3 − t2) × (wake 40 E2 + early PM E2)] + [0.5 × (t4 − t3) × (early PM E2 + bedtime E2)]. Sample times in the E2 AUC equation: t1 = wake E2, t2 = wake + 40 E2, t3 = early evening E2, and t4 = bedtime E2.

relationships between T and adiposity (with low T being restricted to the obese) or E2-T relationships being moderated by obesity.

Alternatively, the men in our analysis were young, compared to the subjects in most other studies in this area, thus it is possible that any potential negative implications of high adiposity and elevated E2 for reproductive health (such as dysregulated HPT axis function, or reduced T) only emerge after more chronic, longer-term exposure, particularly in conjunction with senescence. Although not well-characterized in human males, the extent to which increasing E2 inhibits the HPT axis might depend on between-subject variation, physiological changes across the life course, and differences across populations based on developmental milieus (Bribiescas, 2006a,b; Ellison et al., 2002; Veldhuis and Iranmanesh, 2005; Veldhuis et al., 2009). For example, comparable research elsewhere on males in early adulthood has yielded variable results, with a large Danish study finding low T and elevated E2 among overweight men, with modest effect sizes, and a Polish study showing low T among overweight/obese males, with no differences for E2 (Jankowska et al., 2000; Jensen et al., 2004). Pathways for cross-population and life course variation in HPT axis function in response to E2 include the quantity and sensitivity of E2 receptors expressed by pituitary gonadotropes (Shupnik, 2002; Shupnik and Schreihofer, 1997). While our analysis has the advantage of drawing on a sample from a long-running, one-year birth cohort study, thus removing the confounding effects of age that must be carefully controlled in studies of body composition and reproductive steroids with more diverse age demographics (e.g., Ferrini and Barrett-Connor, 1998), the age range of our subjects also prevents us from testing whether senescence moderates the hormonal-adiposity results we observed here. Lastly, adrenal androgens likely also provide precursors for aromatization and could be involved in the E2-adiposity patterns here (i.e., helping to explain how E2 could increase without decreasing the reservoir of circulating T; Labrie et al., 1997; Tchernof et al., 1995).

In line with our predictions, T declined across the daytime period, with a nadir in the early evening and low levels thereafter. We found no diurnal pattern for salivary E2, although our sample was underpowered to detect a potential decline in E2 shortly after waking. The flatness of the E2 diurnal curve between the morning and the evening ran counter to our hypothesis that it would mimic the decrease in T over the course of the day. While consistent with a small number of prior studies (Bribiescas, 2005; Leymarie et al., 1974; Murono et al., 1982), the lack of diurnal curve for E2 is somewhat surprising given its derivation from testicular-produced T and adrenal androgens, which also appear to decline diurnally (Laudenslager et al., 2013). It is possible that aspects of E2 physiology (e.g., aromatase production/activity, particularly in adipose tissue) are regulated in a circadian fashion, contributing to a decoupling of E2's rhythm from that of T. For example, circadian clock genes are active in fat depots, and genes that regulate steroid metabolism in human adipose tissue exhibit circadian profiles (Garaulet et al., 2011; Hernandez-Morante et al., 2009; Zvonic et al., 2006). While our study lacks the statistical power to specifically to test for moderating effects of overweight/obesity on E2's diurnal pattern, this might merit consideration in future research.

Prior psychobiological research questioned the appropriateness of measuring male E2 via saliva, suggesting that salivary values under-represented associations between E2 and behavioral measures (i.e., prone to Type II error), compared with E2 assayed from serum (Shirtcliff et al., 2000). Because adipose tissue directly contributes to circulating E2, it is possible that relationships between male salivary E2 and body composition are more reliable than E2-behavioral correlations. However, little

TABLE 3. Bivariate linear regression models predicting E2 AUC from measures of adiposity[a]

	Subsample with fully detectable E2[b]		*P* value	Model R2	Subsample that includes subjects with undetectable E2[c]		*P* value	Model R2
BMI	0.60	(0.13, 1.08)	0.014	0.204	0.57	(0.04, 1.10)	0.037	0.116
Waist circumference	0.26	(0.06, 0.46)	0.012	0.212	0.28	(0.05, 0.51)	0.019	0.144
Skinfold thicknesses	0.22	(0.09, 0.35)	0.002	0.300	0.18	(0.03, 0.33)	0.023	0.136

[a]Values are β (95% CI) of E2 AUC.
[b]$n = 29$. Limited to subjects with detectable E2 at all four sampling time points.
[c]$n = 38$. We ascribed subjects with undetectable E2 at any sampling point a value of 0.1 pg/ml. See Methods.

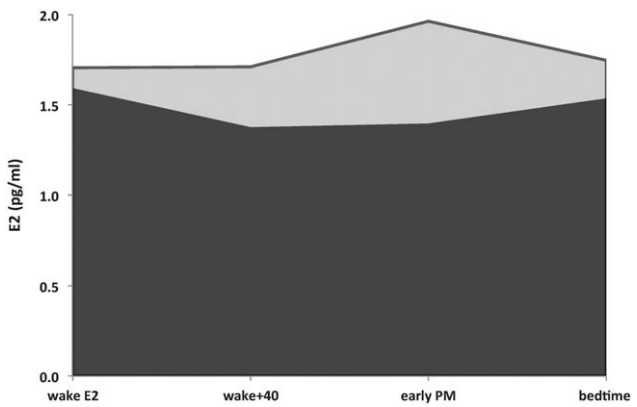

Fig. 3. For visual purposes, we present E2 AUC for men with healthy BMI compared with men with overweight/obese BMI. The dark shaded area indicates E2 AUC for men with healthy BMI (< 25 kg/m^2; $n = 21$). The light shaded area indicates the extent to which overweight/obese men (25 kg/m$^2 \leq$ BMI; $n = 8$) have E2 AUC that exceeds that of healthy BMI subjects. In a one-tailed, unpaired t-test subjects with healthy BMI had lower E2 AUC than overweight/obese men ($P = 0.045$).

research has been conducted on the utility of salivary E2 to studies of body composition and its health correlates, with most relevant studies focusing on E2 assayed from blood samples. In contrast to our findings here, a prior field-based, anthropological study by Bribiescas did not show that Ache Amerindian men with greater adiposity had higher salivary E2, but this probably reflects the leanness (mean BMI: 23.8 kg/m2) of the sample (Bribiescas, 2005). Indeed, in that study, Ache men with higher E2 also had greater T, with comparable effect sizes to our results and those for studies examining E2 and T from blood (Van Pottelbergh et al., 2003; Vermeulen et al., 2002), suggesting that the salivary E2 measures were reliable (Bribiescas, 2005). Given the strength of the relationships that we documented here between salivary E2 and multiple measures of adiposity, we suggest that field-based studies of body composition and health can draw on noninvasive salivary measures to evaluate male E2, although thorough diurnal sampling (allowing for AUC calculation) is potentially critical in studies with sample sizes comparable to ours versus those in larger clinical or epidemiological studies (e.g., Jensen et al., 2004; Van Pottelbergh et al., 2003; Vermeulen et al., 2002).

One potential limitation of our study is that this subsample of men was composed entirely of fathers residing with their children, a demographic-behavioral profile we have previously linked to reduced T (Gettler et al., 2011a, 2012a) and elevated prolactin (Gettler et al., 2012b). In the only study of its kind, to date, expectant Canadian fathers had higher E2 than non-fathers (Berg and Wynne-Edwards, 2001). However, the subjects' body composition was not measured, and preliminary US and European-based research suggests that fathers are more likely to be overweight/obese than nonfathers, possibly due to being less physically active or consuming poorer diets (Laroche et al., 2007; Nielsen et al., 2006; Weng et al., 2004). While there is some precedent for upregulated E2 production among nonhuman primate fathers (Ziegler et al., 2004) and greater brain aromatase activity among fathers of one rodent species (Trainor et al., 2003), we think it unlikely that fatherhood directly affects the aromatization of E2 by adipose tissue, specifically. If true, our results, linking E2 secretion and body composition, are relevant to broader questions of male reproductive physiology, rather than being solely germane to fatherhood. It is also plausible that in cultural contexts in which men commonly invest in their children, such as Cebu, fathers are especially prone to low T (downregulation via parenthood/childcare) and high E2 (upregulated in conjunction with overweight/obesity; Gettler et al., 2011a, 2012a), which has important implications for conceptualizing fatherhood's effects on men's health (Garfield et al., 2006; Gettler et al., 2011a).

We also analyzed males' salivary E2 using a commercially available kit optimized for a normative range of female salivary E2 (Salimetrics LLC). The mean E2 values (Fig. 1) for our male sample approach the value of the lowest calibrator (1.0 pg/ml). Thus, our data may be less precise than results from samples with higher average E2, which could increase the likelihood of type II error due to reduced statistical power. Moreover, nine of the 40 eligible subjects in this study did not have complete E2 data for analysis because a value fell below the assay detection limit. Such subjects did not differ from included men for body composition variables and were not more (or less) likely to be overweight/obese (not shown), suggesting that our results were not biased by their exclusion. When we ascribed these men E2 values based on the assay kit's lower limit of sensitivity (see Methods) and incorporated them in our E2 AUC-adiposity analyses, the results were comparable, though with lower effect sizes, which is unsurprising, given reduced reliability of ascribed values. Future research in this area would benefit from the development and validation (against values assayed from serum) of a high sensitivity assay oriented towards normative ranges of male salivary E2 (Gettler et al., 2013b).

In sum, drawing on a sample of early adult Filipino men, we found that salivary E2 follows a relatively flat diurnal curve. While T and E2 were modestly correlated at multiple time points during the day, men with greater adiposity did not differ from other subjects for T but had higher E2 secretion, indicating increased systemic

exposure to the hormone. The impacts this may have on reproduction, behavior and health for reproductive-aged adult males are poorly characterized, but could be significant, given the increasing incidence of overweight/obesity in many populations around the globe.

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LITERATURE CITED

Adair LS, Popkin BM, Akin JS, Guilkey DK, Gultiano S, Borja J, Perez L, Kuzawa CW, McDade T, Hindin MJ. 2011. Cohort profile: The Cebu Longitudinal Health and Nutrition Survey. Int J Epidemiol 40:619–625.

Adam EK, Hawkley LC, Kudielka BM, Cacioppo JT. 2006. Day-to-day dynamics of experience–cortisol associations in a population-based sample of older adults. PNAS 103:17058–17063.

Ahmed SA, Penhale W, Talal N. 1985. Sex hormones, immune responses, and autoimmune diseases mechanisms of sex hormone action. Am J Pathol 121:531.

Bagatell CJ, Dahl KD, Bremner WJ. 1994. The direct pituitary effect of testosterone to inhibit gonadotropin secretion in men is partially mediated by aromatization to estradiol. J Androl 15:15–21.

Baird D, Horton R, Longcope C, Tait J. 1969. Steroid dynamics under steady-state conditions. Recent Prog Horm Res 25:611.

Berg SJ, Wynne-Edwards KE. 2001. Changes in testosterone, cortisol, and estradiol levels in men becoming fathers. Mayo Clin Proc 76:582–592.

Bribiescas RG. 2001. Reproductive ecology and life history of the human male. Year Book Phys Anthropol 44:148–176.

Bribiescas RG. 2005. Age-related differences in serum gonadotropin (FSH and LH), salivary testosterone, and 17-β estradiol levels among Ache Amerindian males of Paraguay. Am J Phys Anthropol 127:114–121.

Bribiescas RG. 2006a. On the evolution, life history, and proximate mechanisms of human male reproductive senescence. Evol Anthropol 15: 132–141.

Bribiescas RG. 2006b. Men-evolutionary and life history. Cambridge, MA: Harvard University Press. p 306.

Cleland WH, Mendelson CR, Simpson ER. 1985. Effects of aging and obesity on aromatase activity of human adipose cells. J Clin Endocrinol Metab 60:174–177.

Cooke PS, Naaz A. 2004. Role of estrogens in adipocyte development and function. Exp Biol Med 229:1127–1135.

de Ronde W, Pols HA, Van Leeuwen JP, De Jong FH. 2003. The importance of oestrogens in males. Clin Endocrinol 58:529–542.

Dienes KA, Hazel NA, Hammen CL. 2013. Cortisol secretion in depressed, and at-risk adults. Psychoneuroendocrinology 38:927–940.

Faul F, Erdfelder E, Lang A, Buchner A. 2007. G* power 3: A flexible statistical power analysis program for the social, behavioral, and biomedical sciences. Behavior Res Methods 39:175–191.

Ellison PT. 2001. On fertile ground: A natural history of human reproduction. Cambridge, MA: Harvard University Press. p 346.

Ellison PT, Bribiescas RG, Bentley GR, Campbell BC, Lipson SF, Panter-Brick C, Hill K. 2002. Population variation in age-related decline in male salivary testosterone. Hum Reprod 17:3251–3253.

Ferrini RL, Barrett-Connor E. 1998. Sex hormones and age: a cross-sectional study of testosterone and estradiol and their bioavailable fractions in community-dwelling men. Am J Epidemiol 147:750–754.

Finkelstein JS, Lee H, Burnett-Bowie SM, Pallais JC, Yu EW, Borges LF, Jones BF, Barry CV, Wulczyn KE, Thomas BJ. 2013. Gonadal steroids and body composition, strength, and sexual function in men. N Engl J Med 369:1011–1022.

Garaulet M, Ordovás JM, Gómez-Abellán P, Martínez JA, Madrid JA. 2011. An approximation to the temporal order in endogenous circadian rhythms of genes implicated in human adipose tissue metabolism. J Cell Physiol 226:2075–2080.

Garfield CF, Clark-Kauffman E, Davis MM. 2006. Fatherhood as a component of men's health. JAMA 296:2365–2368.

Gettler LT, Agustin SS, Kuzawa CW. 2010. Testosterone, physical activity, and somatic outcomes among Filipino males. Am J Phys Anthropol 142: 590–599.

Gettler LT, Agustin SS, McDade TW, Kuzawa CW. 2011c. Short-term changes in fathers' hormones during father-child play: Impacts of paternal attitudes and experience. Horm Behav 60:599–606.

Gettler LT, McDade TW, Agustin SS, Feranil AB, Kuzawa CW. 2013a. Do testosterone declines during the transition to marriage and fatherhood relate to men's sexual behavior? Evidence from the Philippines. Horm Behav 64:755–763.

Gettler LT, Mcdade TW, Agustin SS, Kuzawa CW. 2013b. Progesterone and estrogen responsiveness to father-toddler interaction. Am J Hum Biol 25:491–498.

Gettler LT, McDade TW, Feranil AB, Kuzawa CW. 2011a. Longitudinal evidence that fatherhood decreases testosterone in human males. PNAS 108:16194–16199.

Gettler LT, McDade TW, Feranil AB, Kuzawa CW. 2012b. Prolactin, fatherhood, and reproductive behavior in human males. Am J Phys Anthropol 148:362–370.

Gettler LT, McDade TW, Kuzawa CW. 2011b. Cortisol and testosterone in Filipino young adult men: evidence for co-regulation of both hormones by fatherhood and relationship status. Am J Hum Biol 23:609–620.

Gettler LT, McKenna JJ, Agustin SS, McDade TW, Kuzawa CW. 2012a. Does cosleeping contribute to lower testosterone levels in fathers? Evidence from the Philippines. PLoS One 7:e41559.

Grossman CJ, Roselle GA, Mendenhall CL. 1991. Sex steroid regulation of autoimmunity. J Steroid Biochem Mol Biol 40:649–IN8.

Haffner SM, Karhapaa P, Mykkanen L, Laakso M. 1994. Insulin resistance, body fat distribution, and sex hormones in men. Diabetes 43:212–219.

Hau M. 2007. Regulation of male life history traits by testosterone: implications for the evolution of vertebrate life histories. Bioessays 29:133–144.

Hernandez-Morante J, Gomez-Santos C, Milagro F, Campión J, Martínez J, Zamora S, Garaulet M. 2009. Expression of cortisol metabolism-related genes shows circadian rhythmic patterns in human adipose tissue. Int J Obes 33:473–480.

Jankowska E, Medras M, Rogucka E. 2000. Body mass index, waist/hip ratio and androgen-estrogen activity in younger versus older Polish men. Aging Male 3:177–184.

Jensen TK, Andersson A, Jorgensen N, Andersen A, Carlsen E, Petersen J, Skakkebaek NE. 2004. Body mass index in relation to semen quality and reproductive hormones among 1,558 Danish men. Fertil Steril 82: 863–870.

Juneja H, Karanth S, Dutt A, Parte P, Meherjee P. 1991. Diurnal variations and temporal coupling of bioactive and immunoactive luteinizing hormone, prolactin, testosterone and 17-beta-estradiol in adult men. Horm Res Paediat 35:89–94.

Kuzawa CW, Gettler LT, Muller MN, McDade TW, Feranil AB. 2009. Fatherhood, pairbonding, and testosterone in the Philippines. Horm Behav 56:429–435.

Labrie F, Belanger A, Cusan L, Candas B. 1997. Physiological changes in dehydroepiandrosterone are not reflected by serum levels of active androgens and estrogens but of their metabolites: intracrinology. J Clin Endocrinol Metab 82:2403–2409.

Laroche HH, Hofer TP, Davis MM. 2007. Adult fat intake associated with the presence of children in households: Findings from NHANES III. J Am Board Fam Med 20:9–15.

Laudenslager ML, Calderone J, Philips S, Natvig C, Carlson NE. 2013. Diurnal patterns of salivary cortisol and DHEA using a novel collection device: electronic monitoring confirms accurate recording of collection time using this device. Psychoneuroendocrinology 38:1596–1606.

Le Floch J, Escuyer P, Baudin E, Baudon D, Perlemuter L. 1990. Blood glucose area under the curve: methodological aspects. Diabetes Care 13: 172–175.

Leymarie P, Roger M, Castanier M, Scholler R. 1974. Circadian variations of plasma testosterone and estrogens in normal men: A study by frequent sampling. J Steroid Biochem 5:167–171.

Lohman TG, Roche AF, Martorell R. 1988. Anthropometric standardization reference manual. Champaign, IL: Human Kinetics Books.

Mayes J, Watson G. 2004. Direct effects of sex steroid hormones on adipose tissues and obesity. Obes Rev 5:197–216.

Mazur A. 2009. The age-testosterone relationship in Black, White, and Mexican-American men, and reasons for ethnic differences. Aging Male 12:66–76.

Murono E, Nankin H, Lin T, Osterman J. 1982. The aging leydig cell V. Diurnal rhythms in aged men. Acta Endocrinol 99:619–623.

Nater UM, Youngblood LS, Jones JF, Unger ER, Miller AH, Reeves WC, Heim C. 2008. Alterations in diurnal salivary cortisol rhythm in a population-based sample of cases with chronic fatigue syndrome. Psychosom Med 70:298–305.

Nielsen TL, Wraae K, Brixen K, Hermann AP, Andersen M, Hagen C. 2006. Prevalence of overweight, obesity and physical inactivity in 20-to

29-year-old, Danish men. Relation to sociodemography, physical dysfunction and low socioeconomic status: the Odense Androgen Study. Int J Obes 30:805–815.
Pasquali R. 2006. Obesity and androgens: facts and perspectives. Fertil Steril 85:1319–1340.
Pasquali R, Casimirri F, Cantobelli S, Melchionda N, Maria Morselli Labate A, Fabbri R, Capelli M, Bortoluzzi L. 1991. Effect of obesity and body fat distribution on sex hormones and insulin in men. Metabolism 40:101–104.
Pedersen SB, Kristensen K, Hermann PA, Katzenellenbogen JA, Richelsen B. 2004. Estrogen controls lipolysis by up-regulating α2A-adrenergic receptors directly in human adipose tissue through the estrogen receptor α. implications for the female fat distribution. J Clin Endocrinol Metab 89:1869–1878.
Pruessner JC, Kirschbaum C, Meinlschmid G, Hellhammer DH. 2003. Two formulas for computation of the area under the curve represent measures of total hormone concentration versus time-dependent change. Psychoneuroendocrinology 28:916–931.
Ramirez ME, McMurry MP, Wiebke GA, Felten KJ, Ren K, Meikle AW, Iverius P. 1997. Evidence for sex steroid inhibition of lipoprotein lipase in men: comparison of abdominal and femoral adipose tissue. Metab Clin Exp 46:179–185.
Raven G, de Jong FH, Kaufman J, de Ronde W. 2006. In men, peripheral estradiol levels directly reflect the action of estrogens at the hypothalamo-pituitary level to inhibit gonadotropin secretion. J Clin Endocrinol Metab 91:3324–3328.
Sakiani S, Olsen NJ, Kovacs WJ. 2012. Gonadal steroids and humoral immunity. Nat Rev Endocrinol 9:56–62.
Schneider G, Kirschner MA, Berkowitz R, Ertel NH. 1979. Increased estrogen production in obese men. J Clin Endocrinol Metab 48:633–638.
Shirtcliff EA, Granger DA, Schwartz EB, Curran MJ, Booth A, Overman WH. 2000. Assessing estradiol in biobehavioral studies using saliva and blood spots: simple radioimmunoassay protocols, reliability, and comparative validity. Horm Behav 38:137–147.
Shupnik M. 2002. Oestrogen receptors, receptor variants and oestrogen actions in the hypothalamic-pituitary axis. J Neuroendocrinol 14:85–94.
Shupnik MA, Schreihofer DA. 1997. Molecular aspects of steroid hormone action in the male reproductive axis. J Androl 18:341–344.
Straub RH. 2007. The complex role of estrogens in inflammation. Endocr Rev 28:521–574.
Tchernof A, Despres J, Belanger A, Dupont A, Prud'homme D, Moorjani S, Lupien PJ, Labrie F. 1995. Reduced testosterone and adrenal C19 steroid levels in obese men. Metab Clin Exp 44:513–519.
Trainor BC, Bird IM, Alday NA, Schlinger BA, Marler CA. 2003. Variation in aromatase activity in the medial preoptic area and plasma progesterone is associated with the onset of paternal behavior. Neuroendocrinology 78:36–44.
van Anders SM, Goldey KL, Bell SN. 2014. Measurement of testosterone in human sexuality research: methodological considerations. Arch Sex Behav 43:231–250.
Van Pottelbergh I, Braeckman L, De Bacquer D, De Backer G, Kaufman J. 2003. Differential contribution of testosterone and estradiol in the determination of cholesterol and lipoprotein profile in healthy middle-aged men. Atherosclerosis 166:95–102.
Veldhuis JD, Iranmanesh A. 2005. Short-term aromatase-enzyme blockade unmasks impaired feedback adaptations in luteinizing hormone and testosterone secretion in older men. J Clin Endocrinol Metab 90:211–218.
Veldhuis JD, Keenan DM, Liu PY, Iranmanesh A, Takahashi PY, Nehra AX. 2009. The aging male hypothalamic–pituitary–gonadal axis: pulsatility and feedback. Mol Cell Endocrinol 299:14–22.
Vermeulen A, Kaufman J, Goemaere S, Van Pottelberg I. 2002. Estradiol in elderly men. Aging Male 5:98–102.
Weng HH, Bastian LA, Taylor Jr DH, Moser BK, Ostbye T. 2004. Number of children associated with obesity in middle-aged women and men: results from the health and retirement study. J Womens Health 13:85–91.
Wust S, Wolf J, Hellhammer DH, Federenko I, Schommer N, Kirschbaum C. 2000. The cortisol awakening response-normal values and confounds. Noise Health 2:79–88.
Ziegler TE, Washabaugh KF, Snowdon CT. 2004. Responsiveness of expectant male cotton-top tamarins, *Saguinus oedipus*, to mate's pregnancy. Horm Behav 45:84–92.
Zumoff B, Miller LK, Strain GW. 2003. Reversal of the hypogonadotropic hypogonadism of obese men by administration of the aromatase inhibitor testolactone. Metabolism 52:1126–1128.
Zvonic S, Ptitsyn AA, Conrad SA, Scott LK, Floyd ZE, Kilroy G, Wu X, Goh BC, Mynatt RL, Gimble JM. 2006. Characterization of peripheral circadian clocks in adipose tissues. Diabetes 55:962–970.