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ORIGINAL ARTICLE

Is There a Testosterone Awakening Response in Humans?

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Abstract Circulating testosterone (T) follows a diurnal pattern with high waking levels that decline across the day. The hypothalamic-pituitary-adrenal axis produces cortisol in a similar manner but also undergoes an abrupt increase in hormone secretion immediately upon waking (a cortisol awakening response, CAR). Whether the hypothalamic-pituitary-gonadal axis, and circulating T levels, exhibit a similar post-waking response is unclear. Here we describe post-waking T changes in a sample of 108 young adult males from metropolitan Cebu City, the Philippines. As expected, salivary T was higher at waking than in the evening but, remarkably, 60 % of this diurnal decline occurred within 30 min of awakening. There was a strong inverse linear relationship between waking T and the post-waking T decline, such that men with higher waking T experienced a more rapid decline in the hormone. Even though fathers had lower waking T, they experienced a greater post-waking decline than non-fathers. Men with a larger positive CAR had modestly attenuated post-waking T declines. We speculate that these findings reflect a testosterone awakening response (TAR) that helps partition the target tissue effects of T by time of day. T rises overnight to facilitate muscle anabolism at a time when the hormone's impacts on social behavior are limited. Upon waking, the rapid drop in T helps shift from anabolic to catabolic processes in support of physical activity, while also calibrating T levels in line with the competing social priorities of the

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individual, as determined by the current balance of behavioral investment towards mating and parental effort.

Keywords Psychophysiology · Reproductive strategies · Life history · Cortisol · Mating

Introduction

Testosterone (T) is the primary male sex steroid produced by the testes, and has wide-ranging effects on metabolic and behavioral traits related to intrasexual competition, mating and reproductive effort (Apicella et al. 2015; Archer 2006; Bribiescas 2001; Carré and Olmstead 2015; Flinn et al. 2012; Gettler 2014; Gray 2003; Muller and Wrangham 2001; Peters et al. 2008). In humans T typically follows a diurnal pattern characterized by peak levels after waking and a decline across the day to an evening nadir (Bremner et al. 1983). A steady rise in hormone levels during sleep leads once again to peak levels the following morning (Andersen et al. 2011). The fluctuations in T levels are related to the sleep/wake cycle rather than to the day/night cycle such that when sleep patterns are altered (e.g., sleep during the day) a peak in T is consistently seen at awakening (Axelsson et al. 2005), even when duration of sleep is restricted to 5 h per night (Leproult and Van Cauter 2011).

Although this average pattern of higher T levels in the morning than evening (under a normal sleep schedule) is well-described, the functional significance of the marked diurnal dynamics in the hormone are less well understood. By analogy, the adrenal steroid cortisol (CORT) follows a similar diurnal pattern in which changes in hormone levels are understood as reflecting the shifting function and requirement for CORT's metabolic and behavioral roles across the day. Similar to T, CORT levels are highest in the morning and then drop to a lower pre-bed evening nadir. Although CORT levels rise during sleep, most individuals experience a marked surge in cortisol production, over and above the circadian pattern, immediately after waking (the cortisol awakening response, or CAR), which precedes the gradual, sustained decline in hormone levels across the day (Chida and Steptoe 2009; Clow et al. 2010; Pruessner et al. 1997; Wilhelm et al. 2007). The function of the CAR is still a matter of debate but it has been hypothesized that it may assist with the spontaneous retrieval of persistent memories as neuronal networks shift from a sleeping to an awake state (Wilhelm et al. 2007) while enhancing emotional preparedness for the day (Adam et al. 2006; Fries et al. 2009). Glucocorticoids also have key metabolic functions: in concert with other hormones, they mobilize energy resources by up-regulating glucose secretion into the bloodstream (Vegiopoulos and Herzig 2007). Following an overnight fast, the shift towards catabolism, associated with the CAR, may thus provide important energy as the organism transitions from sleep to wakefulness and activity.

Although T also reaches peak levels upon waking (usually in the morning) and a nadir before sleep (usually in the evening), it is less clear whether there are abrupt changes in T levels upon waking, analogous to the CAR. Given evidence for co-regulation of CORT and T (Gettler et al. 2011a), one possibility is that, like CORT, T rises transiently upon waking in support of the same functions. Alternatively, a shift in metabolic priorities might favor a drop in T as CORT rises.

Unlike studies of HPA function and CORT, few past studies of T have used protocols capable of characterizing the nature of post-waking T change. Measuring the CAR typically involves CORT measurement in a sample collected immediately upon waking, which is compared to levels measured in samples collected 30 to 45 min after waking (Adam and Kumari 2009). The increasingly-common collection of waking and post-waking samples in HPA research reflects the growing awareness of the biological and health importance of the CAR. In contrast, most studies of diurnal variation in T levels have collected samples according to clock time, as their primary aim has been to establish the existence of circadian rhythmicity in T secretion. Because individuals wake at different times, grouping individuals' hormone levels based upon clock time pools individuals who are still sleeping, others who have just woken, and others who have been up for a while. This will tend to underestimate peak waking levels while also obscuring any rapid post-waking changes. Thus, even studies that employ frequent catheter sampling of circulating T, but which collect and plot samples based upon clock time (often reporting mean hourly values) rather than wake time (e.g., every 30 min, Diver et al. 2003; every 10 min, Tenover et al. 1988), are incapable of evaluating the nature of post-waking T change.

We are aware of two prior studies that used a protocol capable of measuring post-waking changes in T in adult males. Among a small sample of 45 young fathers from the Philippines (mean age 26.6 years), Gettler et al. (2014) recently reported that salivary T declines during the first 40 min after awakening accounted for 31.7 % of the total decline in T over the course of the day. Because fathers, especially those who invest greatly in childcare, have been shown to have lower waking and evening T than non-fathers in this sample (Gettler et al. 2011b), it is not clear whether findings might differ among non-fathers. Similarly, a large study of middle-aged male Vietnam veterans (mean age ca. 56 years; N=783) also documented a clear post-waking decline in salivary T levels, with the hormonal decline 30 min following awaking accounting for 32–39 % of the overall decline in T between waking and bedtime (Panizzon et al. 2013). The authors did not examine if these diurnal patterns varied by partner or fatherhood status. Thus, although past studies point to a relatively abrupt post-waking decline in salivary T, many questions remain unresolved, including whether the magnitude of this change varies by age or due to factors like social-behavioral regulation, as exemplified by fatherhood or pairbonding status (Bribiescas and Hill 2010; Ellison et al. 2002; Gettler 2014; Mazur 2009).

Because there is evidence for co-regulation and also cross-talk between the hypothalamic-pituitary-adrenal (HPA) and hypothalamic-pituitary-gonadal (HPG) axes (Gettler et al. 2011a; Mehta and Josephs 2010; Viau 2002), it is similarly unclear whether any awakening changes in T might relate to, or be moderated by, the concurrent changes in cortisol production (CAR). Although acute CORT production in response to severe stress can suppress T function (Cumming et al. 1983; Doerr and Pirke 1976), concentrations of T and CORT tend to be positively correlated across the day, pointing to the likely complementary roles of these hormones in coordinating metabolism and behavior (Gettler et al. 2011a). The only study that we are aware of that has evaluated the relationship between post-waking T and CORT changes (Panizzon et al. 2013) found that men who had a greater post-waking increase in CORT showed a smaller post-waking decline in T.

Our aim in this paper is to characterize the nature of post-waking T change in a sample of young adult men living in metropolitan Cebu City, Philippines, who are all of a similar age but vary in partner and fatherhood status. To that end, we measure T and CORT in

saliva samples collected before bed, and then immediately after and again 30 min after waking. We characterize the nature of post-waking T change and evaluate whether the magnitude of this change varies in relation to the CAR, or among men who are partnered or fathers. Based upon our findings, we suggest that the changes that we document reflect a biological response to waking, which we label the testosterone awakening response (TAR), and speculate on its possible functional basis and implications for the study of hormone-behavior relationships in human and non-human primate research.

Materials and Methods

Study Population

Data come from the Cebu Longitudinal Health and Nutrition Survey (CLHNS), a population-based birth cohort study of mothers and their infants born in 1983–84 (Adair et al. 2011). Men were a mean of 21.5 years old (range 20.9–22.1 years) at the time of data and sample collection in 2005. We selected a random sample of men for this analysis, with oversampling of fathers to allow adequate power to evaluate any differences in post-waking T dynamics by parenthood status. After exclusion of one outlier with implausibly high morning T (likely indicating blood contamination), the final sample for this analysis was 108 men. For comparative purposes, T was also measured in a smaller sample of women from the cohort (age range 21.0–22.0 years; $N=18$). Information on socioeconomic status, parenthood and other factors were collected during in-home interviews (Adair et al. 1993, 2001; Kuzawa and Adair 2003). We defined pairbonded as currently living with a partner and/or being legally married, while fathers were defined as men who were living with one or more son or daughter (Kuzawa et al. 2009). Because hormone profiles may be modified by abnormal sleep cycles (i.e., shift work), we excluded individuals with abnormal sleep cycles following criteria described previously (Desantis et al. 2015; Lee et al. 2014). This research was conducted under conditions of informed consent with human subjects clearance from the Institutional Review Boards of the University of North Carolina, Chapel Hill and Northwestern University.

Salivary T and CORT Measurement

Participants were provided with instructions and three tubes for saliva collection. The first sample was collected immediately prior to bed. After collection, they sealed the tube and kept it at room temperature. They were instructed to place the second tube next to their bed and to collect the second sample immediately upon waking the following morning. Participants were also supplied with a timer, which they were instructed to set for 30 min immediately after waking. Thus 30 min following the collection of the first morning saliva samples, participants provided a second one using the third tube. Respondents reported time of saliva collection, wake time, and usual wake time. Saliva tubes were collected later the second day by an interviewer, who placed the tubes on ice packs in a cooler while in transit to freezer storage at −35 C. They were shipped on dry ice to Northwestern University, where they were stored at −80 C. They were thawed, centrifuged, supernatant separated, and aliquoted into smaller tubes for analysis of individual analytes.

Salivary T

Salivary testosterone was determined at the Laboratory for Human Biology Research at Northwestern University, using a commercially available enzyme immunoassay protocol (Salimetrics, State College, PA; Kit No. 1–2402). The inter-assay coefficients of variation were 13.7 and 11.5 % for high and low controls, respectively. Salimetrics reports intra-assay coefficients of variation of 2.5 and 6.7 % for high and low controls.

Salivary CORT

Cortisol was assayed in saliva samples sent to a laboratory in Trier, Germany. Samples were assayed in duplicate using a time-resolved immunoassay with fluorometric detection (DELFIA). The intra-assay coefficients of variation were between 4.0 and 6.7 %, and the corresponding inter-assay coefficients of variation ranged from 7.1 to 9.0 %. Samples with coefficients of variance over 12 % were rerun.

Female Hormone Data

All three T measures were also available for a small sample of female cohort members (n=18, 10 mothers and 8 non-mothers). Although the sample was not large enough for multivariate regression analysis, for comparative purposes we plot mean T values for these women alongside the male data in Fig. 1. These data, which have been described previously (Kuzawa et al. 2010) were collected in the same survey round as the male data and used the same protocols.

Statistical Analysis

Statistical analyses were conducted using Stata 13.1 (Stata Corporation, College Station, TX). The cortisol awakening response (CAR), a measure that is often related

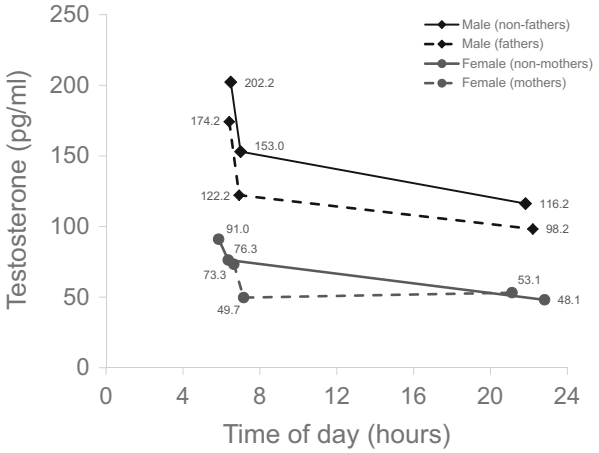

Fig. 1 Circadian testosterone profiles stratified by parenthood status (available data from 18 females also plotted for comparison). The pre-bed measure was collected the night before the waking and wake+30 measures, but is plotted here on the same day to approximate a typical diurnal pattern of T change

to perceived stress and fatigue (Pruessner et al. 1999; Roberts et al. 2004; Schlotz et al. 2004), was calculated by subtracting the waking CORT from the wake+30 CORT. The testosterone awakening response (TAR) was calculated from the same samples using the same approach. After calculating descriptive statistics, we ran a series of models aimed at exploring the predictors of the testosterone awakening response, focusing on partner and fatherhood status before and after adjusting for other hormone measures (waking and bedtime T, CAR). Measures of socioeconomic status, including household income, assets score (reflecting ownership of high status items) and the individual's highest educational grade completed, were all not-significant as predictors of the post-waking change in testosterone (all $p>0.35$) and were thus not included in the models reported here. Significance level was set at alpha <0.05.

Results

Roughly two thirds of the men in our sample were partnered, and about one third were fathers (Table 1). Cortisol values followed the expected pattern of high waking levels, a post-waking rise (CAR) followed by much lower levels prior to bed. Testosterone followed a distinct post-waking pattern, with levels 30 min after waking 26 % lower than waking values. Notably, viewing all men pooled, 61.2 % of the diurnal decline between waking and pre-bed T measures (i.e., 50.3 pg/ml of the 82.2 pg/ml decline) had already occurred within 30 min of waking, a pattern that is qualitatively similar among the 18 available women in our dataset (Fig. 1).

Table 1 Characteristics of Cebu males ($n=108$)

	mean	SD	range	
Age (years)	21.5	0.3	20.9	22.1
Father (%)	38.0 %			
Partnered (%)	63.0 %			
Waking T (pg/ml)	191.6	61.1	70.7	357.5
Wake + 30 T (pg/ml)	141.3	48.1	45.8	321.9
Pre-bed T (pg/ml)	109.4	53.6	36.6	368.0
TAR[a] (pg/ml)	−50.3	56.2	−213.4	90.2
Waking CORT (nmol/l)	6.4	3.1	0.2	19.2
Wake + 30 CORT (nmol/l)	8.2	3.9	0.3	20.5
Pre-bed CORT (nmol/l)	2.4	3.3	0.1	22.7
CAR[b] (nmol/l)	1.7	3.5	−5.6	11.4
Waking collection time (hours)	6:27		4:00	10:57
Wake + 30 collection time (hours)	6:58		4:30	11:27
Pre-bed collection time (hours)	21:58		18:00	3:10

[a] testosterone awakening response

[b] cortisol awakening response

In this sample, 81.7 % of males experienced a post-waking decline in T (Fig. 2a), while, as expected, most individuals (62.7 %) experienced a post-waking rise in cortisol (Fig. 2b). Of the 108 males, 11 (10.2 %) had pre-bed T levels that were higher than waking T levels (Fig. 2c).

We next evaluated the predictors of the post-waking T change in multivariate models (Table 2). Adjusting for waking time only (not shown), neither partner or fatherhood status were significant predictors of the post-waking T change (Model 1, $F_{3,\ 104}=0.69$, $p<0.5601$). Adding waking and pre-bed T to the model strengthened both coefficients, and with fathers now having borderline significantly larger post-waking T drops ($p<0.059$). In a full model, the CAR was a significant independent and positive predictor of T change (Model 3, $F_{6,\ 101}=25.42$, $p<0.00001$). Thus, men's post-waking T decreased less when their post-waking cortisol increase was larger, on average (Fig. 3c). Pre-bed T was weakly but significantly associated with the magnitude of T change the next morning (Fig. 3b), while waking T was strongly and negatively associated with T change (Fig. 3a). In the full model, fatherhood status was a significant independent predictor of a larger than expected post-waking T drop.

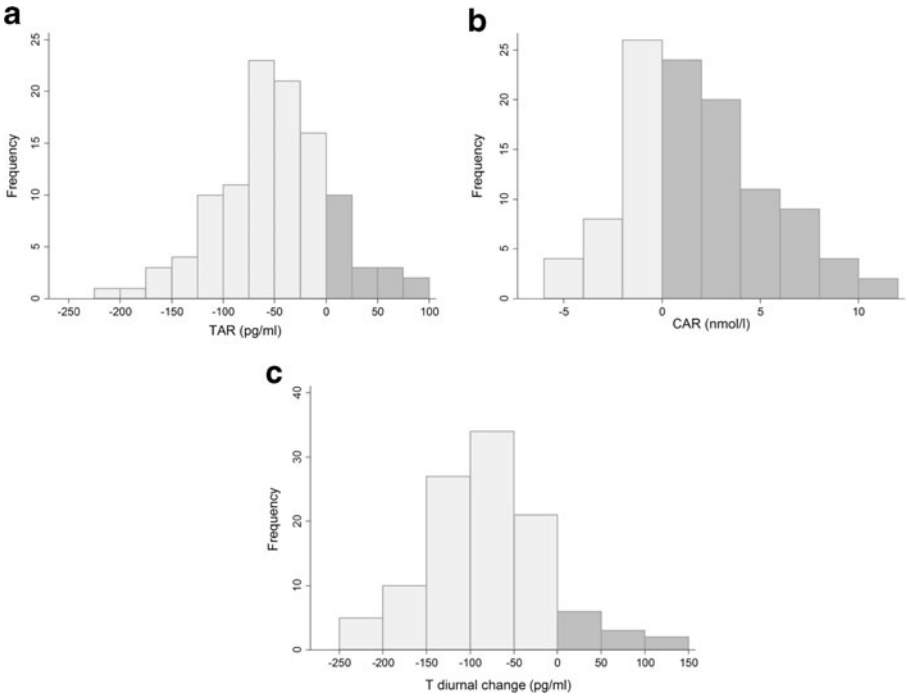

Fig. 2 Frequency distribution of **a** testosterone awakening response (TAR), **b** cortisol awakening response (CAR), and **c** the magnitude of diurnal change in T (from waking to pre-bed). *Light and dark bars* represent individuals with decreasing and increasing hormone concentrations, respectively, after awakening

Table 2 Regression models predicting the testosterone awakening response (pg/ml)

	Model 1			Model 2			Model 3		
	β	(SE)	p	β	(SE)	p	β	(SE)	p
Partnered	4.86	11.63	0.677	−13.46	8.14	0.101	−11.27	7.95	0.159
Father	−3.52	11.56	0.762	−14.92	7.82	0.059	−15.06	7.60	0.05
Waking T (pg/ml)				−0.74	0.06	0.0001	−0.75	0.06	0.0001
Pre-bed T (pg/ml)				0.27	0.07	0.0001	0.26	0.07	0.0001
CAR[a] (nmol/l)							2.75	1.03	0.009
Adj R^2	−0.01			0.55			0.58		

All models adjusted for waking time (not shown). Age and socioeconomic measures were not significant and thus not included (see methods)

[a] Cortisol awakening response

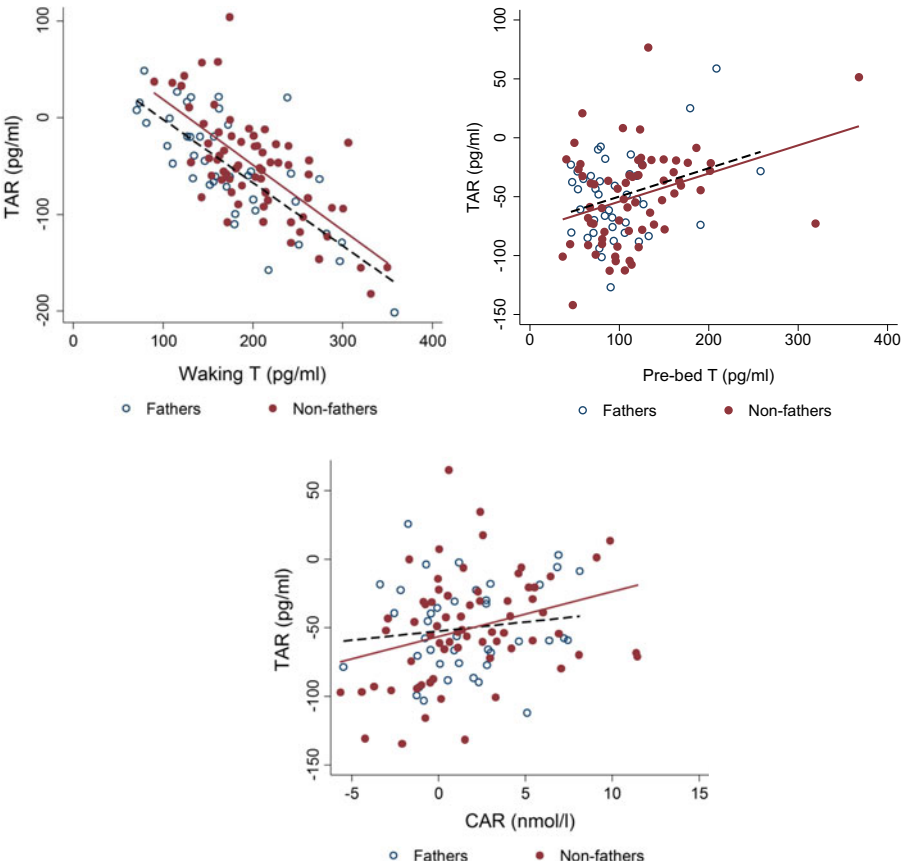

Fig 3 Relationships in fathers (*open circles*, *dashed line*) and non-fathers (*solid circles*, *solid line*) between the testosterone awakening response (TAR) and **a** waking T adjusted for partner and fatherhood status, wake time, pre-bed T and CAR, **b** pre-bed T adjusted for partner and fatherhood status, wake time, waking T and CAR, and **c** the cortisol awakening response (CAR) adjusted for partner and fatherhood status, wake time, pre-bed T and waking T

Discussion

In this sample of healthy young adult men from Cebu, in the Philippines, a drop in T equivalent to roughly 60 % of the diurnal decline in salivary T occurred within 30 min of waking. Men with higher waking T had greater post-waking T declines, which was characterized as a tight inverse linear relationship between the two measures. Although fathers had significantly lower waking T, they had a larger post-waking T drop despite this, thus pointing to a more rapid pace of post-waking hormonal decline among fathers. Although the available sample was small, the post-waking decline in T also appears to follow a similar pattern in same-aged females from this cohort. These findings point to a rapid and sizeable decline in the levels of bioactive T soon after waking.

Relative to the overall diurnal decline, the post-waking decline in T documented here is about two times greater than that reported in two studies using an identical sampling protocol in adult men: one focused on middle-aged (mean age 56 years) American men (Panizzon et al. 2013), and the second on a sample of fathers from the same Philippines cohort measured 4.5 years after the survey reported here (Gettler et al. 2014). One possible explanation for this variation across studies is that the magnitude of the TAR declines with age, given that both other studies focused on older males than our current sample. Indeed, the nocturnal rise in T among middle-aged men, as compared to young men, is lower (Diver et al. 2003), which our present findings suggest might lead to an attenuated post-waking T decline. Differences in age seem less likely to fully explain the discrepancy between our present findings and those reported from slightly older fathers in the same cohort (Gettler et al. 2014). It is notable that the men in the 2014 study were not only older at the time of T measurement, but they were a group of fathers recruited for a study of father-child interaction, and had substantially lower waking T than the fathers described here. Although speculative, the older age, and likely greater average fatherhood experience of these men, could contribute to their lower waking T and relatively attenuated post-waking T decline. More work is needed to clarify the extent of variation in the post-waking T decline across populations, including how it relates to social relationships, aging and other factors that might modify its magnitude.

We measured T in salivary samples, which reflects the bioavailable fraction of the hormone. Although our data do not allow us to evaluate the biological basis of the post-waking T decline, the rapid change in the hormone could reflect some combination of reduced testicular T production along with rapid clearance or a decrease in the fraction of free (bioavailable) T due to increased binding by binding factors. Estimates of the half-life of bioactive T in humans vary by study but generally are in the range of 10–60 min (Rommerts 2004; Veldhuis et al. 2010). There is a lag between changes in LH production and testicular T output of 40–70 (median: 50) minutes, suggesting that the abrupt decline in T after awakening is not likely secondary to reduced LH secretion (Spratt et al. 1988; Veldhuis and Iranmanesh 2004). Circulating concentrations of sex hormone binding globulin (SHBG) generally rise at the times of day when free T is declining, and vice versa, pointing to a likely role of SHBG binding to the circadian changes in free T (Diver et al. 2003). Alternatively, an increase in T clearance rate could contribute to the observed pattern. Short-term fasting has been shown to increase the clearance rate of T, as indicated by a decreased concentration of salivary T but

increased urinary T the morning following a fast (Trumble et al. 2010). Finally, although speculative, the increase in peripheral circulation that follows waking could increase blood flow, and hormone binding/clearance, in peripheral tissues. Clearly, future research is needed to identify the mechanistic basis for the rapid post-waking decline in salivary T that we document.

While the underlying biology remains to be clarified, we propose that the rapid post-waking drop in T is an active process, which we label the testosterone awakening response (TAR). We speculate that the TAR's function is to help partition T's primary functions and target tissues to muscle anabolism while asleep, and to catabolism, behavior and social interaction while awake. Overnight, T has been shown to rise progressively, reaching highest levels close to the time of waking (Evans et al. 1971). It has been hypothesized that high T during sleep may facilitate the hormone's role in anabolic processes, such as the building and maintenance of skeletal muscle. The anabolic effects of the hormone are well-documented, and show that T contributes to skeletal muscle synthesis and maintenance, while T supplementation increases basal metabolic rate (BMR) in animal models (Buchanan et al. 2001; Oppliger et al. 2004; Ros et al. 2004). Similarly, in humans T supplementation or replacement can increase BMR and lean body mass (Welle et al. 1992), specifically by stimulating muscle growth via protein synthesis (Bhasin et al. 1996, 2001; Griggs et al. 1989; Herbst and Bhasin 2004; Tsai and Sapolsky 1996).

Even though some studies have not found an effect of evening weight training on the nocturnal pattern of T secretion (Hackney et al. 1989; Kern et al. 1995), at least one found that individuals undergoing resistance weight training experienced a greater overnight rise in T, suggestive of an anabolic effect of the hormone during early morning sleep (McMurray et al. 1995). Another study found that the nocturnal T response correlated positively with a measure of muscle synthesis, the mixed-muscle protein fractional synthetic rate (Betts et al. 2011). Conversely, sleep deprivation and disruption have been linked to muscle atrophy in rats (Dattilo et al. 2012). Sleep debt in humans is associated with increased cortisol production and reductions in T and Insulin-Like Growth Factor 1 (IGF1) secretion, creating a proteolytic environment that has been hypothesized to lead to muscle atrophy (Dattilo et al. 2011). For example, in overweight human subjects on a diet, sleep restriction decreased the fraction of weight lost from fat by 55 %, while losses of fat-free body mass (i.e., muscle) increased by 60 % (Nedeltcheva et al. 2010). It has been hypothesized that diurnal changes in the ratio of T to CORT reflects a shift towards anabolic processes in the evening when the T-CORT ratio is maximal (Hayes et al. 2012). By this logic, the combination of the pronounced post-waking rise in CORT (the CAR), paralleled by a rapid decline in T (the TAR), could work together to rapidly shift the body to a catabolic state, which will help mobilize resources and prepare for the day (Hucklebridge et al. 2000; Pruessner et al. 1997).

The importance of normal, physiological-range inter-individual variability in T levels as an explanation for differences in muscle mass and strength has recently been questioned on the grounds of weak, and at times inconsistent, evidence across studies (Alvarado et al. 2015). Some of the studies that fail to find relationships between T and strength or lean mass are likely under-powered, and may not have measured T at a biologically-interpretable or consistent time point. As one example, one study that failed to detect a relationship between T and muscle strength or lean mass relied on a

cross-sectional sample of 26 college football players and weight lifters and used blood samples 'drawn at approximately the same time in the afternoon' (Fahey et al. 1976). Our model proposes that it is specifically T during sleep, as best approximated by waking samples, that will predict muscle and strength. Conversely, a longitudinal study of young male soccer players tracked 98 11-year-olds for 2 years, with repeat measures of T and strength (Hansen et al. 1999). Both elite and non-elite players increased strength over the 2-year study period, and the increase was greater among males with higher T levels. Positive associations between T and measures of muscularity and strength have been reported in other samples of peri-pubescent boys (Ramos et al. 1998), among men over 70 years old (Perry et al. 2000; van den Beld et al. 2000), and in cross-sectional samples of men ranging from 15 to 90 years old (Araujo et al. 2008; Ellison and Panter-Brick 1996; Lassek and Gaulin 2009; Lukas et al. 2004; Roy et al. 2002). Furthermore, other studies point to additional biological factors that could mediate and/or obscure the relationship between T and somatic development. For example, in a study of 152 Kenyan men, Campbell et al. (2007) found relationships between salivary T and body composition measures that were contingent upon androgen receptor repeat polymorphism (CAG repeat length), which is known to moderate the effect of T on target tissues.

Perhaps of greatest relevance to the current study, our previous published work at Cebu, which used hormone and body composition data from the same 2005 survey round, reported evidence consistent with the model that we propose here (Gettler et al. 2010). In a study of the full sample of males (n=872), we found that waking T, but not pre-bed T, predicted arm muscle area, grip strength and fat free mass, but only among males who reported higher levels of physical activity. Waking T was strongest as a predictor of these traits among the most physically active men, but did not predict somatic traits among less active men. The finding that only waking T predicted strength and muscle in this sample supports the hypothesis that the overnight rise in T, rather than T levels across the waking hours, is what predicts muscle and strength. The fact that these relationships were only found in interaction with activity may also shed light on the failure of some past studies, which do not consider activity, to identify similar relationships. Based upon our findings, we hypothesize that relationships between T and somatic traits will be strongest, and easiest to detect, if T is measured immediately at waking, and when interacted with measures of habitual muscle use and activity.

After awakening, in addition to its metabolic and somatic effects, T might take on a more prominent role in social functions, such as through its influence on behavior and social interaction throughout the day. Testosterone acts directly on the brain (Höfer et al. 2013) and a range of human studies have shown that it can modulate not just aggressive interactions (Cote et al. 2013), but also social cognitive performance, including empathy and cooperative tendencies, risk-taking and mate-seeking behaviors (Bos et al. 2012; Carré and Olmstead 2015; Crespi 2015; Eisenegger et al. 2011). Conversely, fathers with lower T have been shown to be more responsive to cues of infant need, such as crying (Fleming et al. 2002; Storey et al. 2000). Consistent with greater involvement of daytime T in social interactions, past work in human populations has shown evidence for stronger relationships between behavioral and social factors and T levels in the evening compared to the morning (Muller et al. 2009). Similarly, among male chimpanzees dominance rank was found to correlate with afternoon, but not morning, urinary T concentrations (Muller and Wrangham 2004).

Although basal T levels across the day may relate most strongly to current social and relationship context, T production is also acutely responsive to physically demanding activities, such as felling trees and clearing fields (Trumble et al. 2013), suggesting that activities across the day might also augment T production in service of the hormone's somatic functions.

Alvarado et al. (2015) recently proposed that the evolution of human biparental care involved selection for a decrease in the need for T for anabolic functions, which they hypothesize would allow men to maintain and even increase muscle and workload as they transition to fatherhood and lower T (Gettler et al. 2011b). According to this logic, the strong behavioral benefits (e.g., to dependent offspring) of lowering T among human fathers would be expected to pleiotropically decrease muscle anabolism, unless muscle metabolism was decoupled from requiring T's anabolic effects. Although not mutually exclusive, the partitioning of some of T's target tissue effects by time of day, as we speculate occurs, might work towards a similar end. Relatively high overnight T levels could provide benefits to muscle anabolism at a time of day when social interactions are minimized, while the dramatic post-waking drop in T would allow calibration of daytime T to that individual's current social and relationship context. The fact that we find large post-waking T declines among the fathers in our sample, despite their relatively lower (than non-father) waking T levels, points to a larger contrast between overnight and daytime T levels among fathers, at least at the young age represented by our current sample. Future work is needed to establish the role of circadian T changes, including the abrupt post-waking decline in the hormone, in coordinating T's various somatic and behavioral effects across the day.

Comparative work will provide important opportunities to test the functional significance of the TAR proposed here. Documenting the diurnal changes of T production in non-human primates, which display a broad variety of life history and mating strategies, can provide context for the role of diurnal shifts in T levels in relation to the social and somatic priorities of individual males. Studies focusing on species that differ in the temporal distribution of mating and paternal effort would be useful in this respect. Three predictions that could be tested at the interspecific level would provide particularly valuable insights into the functional significance of the TAR, and the overall diurnal patterns of T secretion: (1) Males are predicted to exhibit a greater TAR and overall diurnal fluctuations (both in comparison to females from the same species and to males from other species) in primates with greater sexual dimorphism in body size and skeletal muscle development (e.g., highly polygynous species); and (2) Species with aseasonal breeding are expected to show a more consistent TAR throughout the year, while a distinct TAR would be seen only during the breeding season for species that do not breed continuously; finally, (3) studies of diurnal variation in T levels among the few primates in which males engage in paternal care (Fernandez-Duque et al. 2009) would help clarify whether the magnitude of the TAR is related to the increased demands of offspring care.

Most studies of primate endocrine profiles, especially in the wild, have examined diurnal variation in T production merely to account for a potential confounder in their analyses (Marshall and Hohmann 2005; Muller and Lipson 2003; Robbins and Czekala 1997; Stoinski et al. 2002; Surbeck et al. 2012). Nevertheless, some studies have found evidence that social factors (e.g., whether individuals are housed socially or in groups, with or without sexually receptive females, and whether they receive aggression from

other males) can affect the diurnal variation in T excretion (Martensz et al. 1987; Stoinski et al. 2002). Reproductive seasonality has also been shown to affect diurnal changes: a distinct difference between T levels between resting (highest T) and active (lowest T) periods was only present during the mating season in the mouse lemur, *Microcebus murinus* (Perret 1985), suggesting that maintaining high T levels during the resting period may be particularly critical during periods of increased mating effort. The variation in mating systems of non-human primates provide important opportunities to clarify the social and ecological correlates of diurnal T dynamics, including the presence and function of a rapid post-waking T decline as observed here.

Our findings highlight the need to pay careful attention to the time of sample collection in the morning, regardless of the species under investigation. For instance, many studies have used protocols based upon a uniform time of day (clock time), an approach that our findings suggest could capture distinct components of physiology depending on when in the post-waking decline the samples are collected. Between-study variation in sample collection times, relative to wake time, could also bias calculations of the AM to PM ratio in T levels. Standardizing sample collection times, with morning T always measured immediately upon waking, would improve comparability of findings between studies.

Several limitations of this study warrant mention. First, we only had a single hormonal measure at each time of day, while averaging across several days would yield more reliable estimates and an increase in statistical power (Dabbs 1990). Despite the limited sample size for which we had funds to measure wake + 30 T, we document large post-waking T changes, and significant predictors of these dynamics, suggesting that we are able to identify biologically important relationships. Although we asked participants to collect their waking sample immediately after waking, we have no independent means of verifying the lag between waking and first sample collection; given the opposing post-waking changes in CORT and T, any delays in waking sample collection will tend to over- and under-estimate waking CORT and T level, respectively, thus leading to an underestimation of the true magnitudes of the CAR and TAR. Although we have previously published analyses of longitudinal change in waking and pre-bed T between 2005 and 2009 (Gettler et al. 2011b), we do not have wake+30 T measures, or any cortisol measures, in 2009, which ruled out a longitudinal design here. We also did not collect information on co-sleeping in 2005, which our analyses from the 2009 survey suggest is an important influence on men's circadian T dynamics (Gettler et al. 2012). Because the overwhelming majority of families at Cebu do co-sleep, the general similarities that we document in the TAR among fathers and non-fathers is not likely due to low co-sleeping status among the fathers.

In sum, in this sample of young adult Filipino men living in Cebu, we find evidence that salivary T, reflecting the bioavailable fraction of the hormone, declines rapidly immediately after awakening, accounting for roughly 60 % of the diurnal decline. We propose that this rapid decline in T is an active biological response, which we call the testosterone awakening response (TAR). We speculate that the TAR facilitates an abrupt shift in the functions and target tissues of the hormone during sleep and the waking state.

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