



Effect of Race/Ethnicity and Smoking on Diurnal Variations in Testosterone Levels

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Abstract

The effect of race/ethnicity and smoking on diurnal variations in total serum testosterone level (T-TST) was evaluated by analyzing data from NHANES for 2011-2012 for adolescent and adult males. For adolescent males, diurnal variability in T-TST levels was substantially smaller among smokers than nonsmokers for non-Hispanic white (NHW) and Hispanics but the reverse was true for non-Hispanic black (NHB). For adult males, morning levels of T-TST were 21-31% higher than the T-TST levels during evening among NHW and there was no diurnal variation in T-TST levels among NHB nonsmokers but NHB smokers had 3%-12% higher T-TST levels during the morning as compared to the evening. Among adolescents, overall, NHW and NHB had statistically significantly higher T-TST levels in the morning as compared to these levels in the evening ($p \leq 0.02$). Adult NHW, NHB, and NHAS nonsmokers had statistically significantly lower T-TST levels than NHW, NHB, and NHAS smokers respectively ($p \leq 0.02$).

Keywords

Physical activity, Smoking, Race/ethnicity, Testosterone

Introduction

In males, testosterone (TST) is the principal male sex hormone and an anabolic steroid. TST plays a key role in the development of male reproductive tissues such as the testis and prostate as well as promoting secondary sexual characteristics such as increased muscle, bone mass, and the growth of body hair [1]. TST is essential for health and well-being [2]. The effect of low TST levels on cardiovascular risk factors, Vitamin D levels, and bone mineral density has been extensively studied. TST levels affect body fat distribution and insulin sensitivity in men [3]. Schooling (2013) [4] analyzed National Health and Nutrition Examination (NHANES) III data to report on the relationships between androgen activity and several cardiovascular risk factors. Mondul et al. [5] using data among NHANES III participants did not find TST levels to differ across four quantiles of cholesterol. An inverse relationship between high-density lipoprotein cholesterol and free TST was observed among black and white boys aged 10 to 15 years [6]. The association between Vitamin D and testosterone levels, among others, has been studied by Tak et al. [7], Wulaningsih et al. [8], Lerchbaum et al. [9], Anagnostis et al. [10], Bellastella et al. [11], and Jorde et al. [12]. The effect of TST levels on bone loss has been studied by Barrett-Conner et al. [13], Yang et al. [14], Van Hemelrijck et al. [15], and Paller et al. [16]. The association between androgen activity and diabetes has been reported by Selvin et al. [17].

Paller et al. [16] used data from NHANES III and showed low free TST levels to be associated with low bone mineral density. Trabert et al. [18] used NHANES 1999-2004 data and showed an inverted relationship between free and total TST with body fat. Shiels et al. [19] used NHANES III for males and showed association between smoking and higher levels of total TST, number of alcohol drinks and total TST, and a positive relationship between physical activity and free and total TST. It has been postulated that alcohol consumption may damage the Leydig cells or impair the hypothalamus-pituitary-gonadal axis [20].

Albertsson-Wikland et al. [20] studied 24-hour nocturnal variations in testosterone levels among boys aged 8.7 to 18.2 years old and concluded that TST levels increase during both day and night in puberty. Mitamura et al. [21] found TST levels to increase during early morning hours among boys aged 4.4 to 19.3 years old. Crofton et al. [22] found increasing levels of TST prior to pubertal onset and into early clinical puberty. Boyar et al. [23] found increased levels of TST during normal nocturnal sleep among 9 pubertal boys but these results were not confirmed among three sexually mature young men. Yong African-American males had 29.4% higher TST levels at 8 AM than at 8 PM and Caucasian males had 23.9% higher TST levels at 8 AM than at 8 PM [24]. In a study of 10 healthy young males (mean age 27.3 years) and 10 healthy elderly males (mean age 70.7 years), mean 24-h levels of non-sex-hormone binding globulin were reported to be 1.91 ± 0.62 nM/L among young males and 0.86 ± 0.01 nM/L among elderly males and circadian rhythm was reported to be blunted with normal aging [25]. Brambilla et al. [26] reported total testosterone levels to be 20-25% lower at 1600 h than at 0800 h among males aged 30-40 years and about 10% lower at 1600 h than at 0800 h among males aged 70 years. The possibility of seasonal variations in TST levels has been reviewed by Smith et al. [27].

Lopez et al. [28] found non-Hispanic black (NHB) 12-15 year adolescent males to have lower total TST levels than non-Hispanic white (NHW) and Mexican Americans (MA) had the higher TST levels as compared to NHB. No racial/ethnic differences were observed between NHW, NHB, and MA among 16-19 year old males for total TST even though MA had the highest levels [28]. No racial/ethnic differences were observed among healthy children aged 6-18 years [29]. In morning session data from NHANES III, NHW and NHB adult males did not differ in TST levels but MA had higher levels than both NHB and NHW [30]. For males aged ≥ 60

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years, adjusted levels of TST were highest among Asian-Americans, intermediate among African-Americans, and lowest among whites [31]. TST levels did not differ among African-American and white males without prostate cancer [32]. Among males aged ≥ 24 years, TST levels did not differ by race after adjustments were made for age, body mass index and waist circumference [33]. In another study which included Singapore-Chinese, African-American, American white, and American Hispanic, and Japanese-American males, TST levels did not differ by race/ethnicity [34]. In a longitudinal study of males aged 30-79 years, [35] also did not find any effect of race/ethnicity on TST levels. Male TST levels did not differ by race/ethnicity among Vietnam era veterans [36]. In a study of college students, black males were found to have 15% higher TST levels than white males [37].

While diurnal variations among TST levels have been studied in small experimental studies as quoted above, I do not know if diurnal variations in a nationally representative sample of males have been presented before in spite of the fact that NHANES does provide data on serum TST for samples drawn on three different times of the day. In the Mobile Examination Center used in NHANES, participants are assigned to report for examination in one of the three sessions, namely, in the morning, afternoon, and evening. A comparison between the TST levels among these three sets of samples could provide for the magnitude and variability among TST levels by race/ethnicity, smoking, and other factors. Consequently, this study was undertaken with the aim to (i) delineate diurnal variability among total TST levels across three testing sessions, namely, morning, afternoon, and evening, (ii) study the variations in TST levels by age, race/ethnicity, level of physical activity, and smoking status, and (iii) study how age used as a continuous variable, body mass index, waist circumference, fasting time, and alcohol consumption affect TST levels. For 2011-2012 cycle of NHANES, data on only total TST (T-TST) levels have been released. In the interest of providing the analysis of the most up-to-date data, a decision was made to use NHANES 2011-2012 data for males of all ages. It is hypothesized that irrespective of the age, gender, and race/ethnicity, the levels of T-TST will not vary with the time of the testing or the time when blood is drawn.

Materials and Methods

Data source and study population

In addition to providing data on androgens for NHANES III (CDC, 2015a) [38] which was conducted between 1988 and 1994, data on the levels of androgens were also publically made available for NHANES cycles 1999-2000 (CDC, 2015b), 2001-2002 (CDC, 2015c) [39,40], and 2003-2004 (CDC, 2015d) [41]. However, these data for NHANES cycles 1999-2000, 2001-2002, and 2003-2004 for sex hormones were made available from surplus samples only or only for those NHANES

participants for whom serum samples in sufficient volume were still available after all other analyses were completed. Sampling weights for these data were not available and as such these data cannot be used for making generalizations about U.S. population. Consequently, these data for 1999-2004 were not analyzed for this study. No further data on androgen levels were released until 2011-2012 cycle of NHANES (CDC, 2015e) [42].

Thus, publicly available data from NHANES (CDC, 2015f) [43] for 2011-2012 from demographic, testosterone, body measure, serum cotinine, and physical activity questionnaires were downloaded and match merged. The sampling plan for NHANES was a complex, stratified, multistage, probability cluster designed to be representative of the civilian, non-institutionalized U.S. population. Sampling weights were created in NHANES to account for the complex survey design, including oversampling, survey non-response, and post-stratification.

After deleting those for whom T-TST levels were not available, a total of 456 male children aged 6-11 years, 554 adolescents males aged 12-19 years, and 2409 adult males aged ≥ 20 years were available for analysis. Detailed weighted and un-weighted sample sizes are given in table 1.

Outcome variable

Because of the highly skewed distribution for T-TST, log₁₀ transformed levels of T-TST were used as outcome variable in each of the two regression models fitted for adolescents males aged 12-19 years, and adult males aged ≥ 20 years.

Covariates

Categorical variables used as covariates in the regression models were: race/ethnicity (non-Hispanic white or NHW, non-Hispanic black or NHB, Hispanics or HISP, and non-Hispanic Asians or NHAS, other unclassified race/ethnicities or OTH), smoking status (nonsmoker, smoker), physical activity level (vigorous, moderate, or neither), and testing session (morning, afternoon, evening). Smokers were defined as those having serum cotinine levels of ≥ 10 ng/mL and nonsmokers were defined as those having serum cotinine levels of < 10 ng/mL. Continuous variables used as independent variables/covariates were: age, body mass index, waist circumference, alcohol consumption in grams, and fasting time in hours. A consideration was given to use alcohol consumption as an independent variable. In some of the previous NHANES studies [19], data from food frequency questionnaire available in NHANES were used. However, food frequency questionnaire data were not available for NHANES 2011-2012. Instead, data on total alcohol consumption in grams were available from the two total food frequency questionnaires (CDC,

Table 1: Sample sizes by age, race/ethnicity, and blood draw session. Data from National Health and Nutrition Examination Survey 2011-2012.

	Age Group											
	6-11 Years				12-19 Years				≥ 20 Years			
	Un-weighted		Weighted		Un-weighted		Weighted		Un-weighted		Weighted	
	N	%	N	%	N	%	N	%	N	%	N	%
Total	456	100.0	8915143	100.0	554	100.0	15238105	100.0	2409	100.0	100673365	100.0
Race/Ethnicity												
Non-Hispanic White	114	25.0	4888867	54.8	128	23.1	8220734	53.9	922	38.3	68308850	67.9
Non-Hispanic Black	122	26.8	1251732	14.0	167	30.1	2276602	14.9	585	24.3	9795640	9.7
Hispanic	157	34.4	2119202	23.8	153	27.6	3356034	22.0	490	20.3	14952756	14.9
Non-Hispanic Asian	37	8.1	312789	3.5	77	13.9	685044	4.5	336	13.9	4916685	4.9
Other Race/Ethnicities	26	5.7	342554	3.8	29	5.2	699691	4.6	76	3.2	2699434	2.7
Blood Draw Session												
Morning	184	40.4	3845195	43.1	272	49.1	7397651	48.5	1193	49.5	51184273	50.8
Afternoon	166	36.4	3053377	34.2	195	35.2	51113274	33.5	875	36.3	33156508	32.9
Evening	106	23.2	2016571	22.6	87	15.7	2727179	17.9	341	14.2	16332584	16.2
Smoking Status												
Nonsmoker*	N/A		N/A		486	87.7	13228890	86.8	1685	69.9	71225701	70.7
Smoker*	N/A		N/A		67	12.1	1982102	13.0	722	30.0	29287753	29.1

*Total may not sum to 100% because of missing values

2015g, 2015h) [44,45] and they were used in the analyses. However, there were only 38 adolescents who reported consuming > 0 grams of alcohol. The numbers of person using alcohol was not considered large enough to use alcohol as a variable for adolescents and as such these 38 persons were deleted from the database used for adolescents for this study. Possible biological mechanism between the association of alcohol abuse and plasma testosterone has been provided by [46].

Statistical analysis

SAS version 9.3 was used to do all statistical analyses. All analyses incorporated appropriate sampling weights and sampling design characteristics. SAS Proc SURVEYMEANS was used to do all univariate analyses including computing unadjusted geometric means by age, race/ethnicity, testing session, and smoking status. Multivariate regression analyses were done using SAS Proc SURVEYREG. Log10 transformed values of T-TST were used as dependent variables in the regression models. Separate regression models were fitted for adolescents and adults. Categorical and continuous variables were used as defined above. Two way interactions between race/ethnicity, smoking status, testing session, and physical activity levels were assessed but were included in the final models only if they were found to be statistically significant at $\alpha = 0.05$.

Results

Based on the un-weighted data, about 25% of children and adolescents were NHW. Among adults, 38.3% were NHW (Table 1). Approximately half of the children, adolescents, and adults were tested in the morning session. Only about 15% the adolescents and adults were tested in the evening session. Among adolescents, 12.1% were smokers and among adults, 30% were smokers (Table 1).

Effect of race/ethnicity on diurnal variations in T-TST levels based on unadjusted data

Irrespective of age, T-TST levels during morning session were statistically significantly higher than during the afternoon session and/or the evening session (Table 2, $p \leq 0.01$) for the total population. The same was true for NHW (Table 2, $p \leq 0.05$). While for NHB children aged 6-11 years old, T-TST levels for the morning session

were statistically significantly higher than for the evening session (Table 2, $p < 0.03$), no statistically significant differences were found for T-TST levels between morning, afternoon, and evening sessions for adolescents and adults. Neither HISP nor NHAS children nor adolescents had any statistically significant different T-TST levels between morning, afternoon, and evening sessions. However, both HISP and NHAS adult males aged ≥ 20 years had statistically significantly higher T-TST levels for the morning session than both afternoon and evening sessions (Table 2, $p \leq 0.01$). The only instance where T-TST levels were statistically significantly higher in the afternoon session than in the evening session was for all children (Table 2, $p = 0.01$) or for NHW children (Table 2, $p = 0.03$).

Effect of smoking on diurnal variations in T-TST levels based on unadjusted data

Among nonsmoking male adolescents, except for NHB, T-TST levels were statistically significantly higher for the morning session than the afternoon and/or the evening sessions (Table 3, $p \leq 0.01$). There were no statistically significant differences for T-TST levels by testing session among nonsmoking NHB male adolescents. While no statistically significant differences were observed for T-TST levels by testing session for NHW, HISP, and NHAS adolescent smokers, for NHB T-TST levels by testing session were found to be in the order afternoon > morning > evening (Table 3, $p \leq 0.02$).

For nonsmoking adults aged ≥ 20 years, T-TST levels were found to be statistically significantly higher (Table 3, $p \leq 0.03$) during the morning session than afternoon and/or evening session for the total population, NHW, HISP, and NHAS but no statistically significant differences were found for NHB nonsmoking adults. For adult smokers aged ≥ 20 years, T-TST levels were found to be statistically significantly higher (Table 3, $p \leq 0.04$) during the morning session than afternoon and/or evening session for total population, NHW, NHB, and NHAS but no statistically significant differences were found for HISP adult smoker.

Variability in total T-TST levels among adolescent males based on adjusted data analysis

Sample size for the model fitted for adolescents was 498 and R^2

Table 2: Unadjusted geometric means with 95% confidence interval in ng/dL by blood draw session for males by age and race/ethnicity. Data from National Health and Nutrition Examination Survey 2011-2012.

	Blood Draw Session			Statistically Significant Differences
	Morning (M)	Afternoon (A)	Evening (E)	
Age: 6-11 Years old				
Total	4.5 (3.4-5.9)	3.9 (3.2-4.8)	2.5 (1.9-3.2)	M > E ($p \leq 0.01$), A > E ($p = 0.01$)
Non-Hispanic White	4.4 (3-6.6)	4.2 (2.8-6.3)	2.6 (1.8-3.7)	M > E ($p = 0.046$), A > E ($p = 0.034$)
Non-Hispanic Black	4.5 (2.7-7.4)	3.3 (2.5-4)	2.1 (1.4-3.3)	M > E ($p = 0.026$)
Hispanic	4.5 (2.7-7.3)	3.5 (2.7-4.6)	2.6 (1.8-3.8)	
Non-Hispanic Asian	4.8 (2.3-10.4)	4.4 (2.3-8.3)	8.4 (3.5-19.8)	
Other Race/Ethnicities	4 (2.3-6.9)	6.9 (2-23.4)	1.1 (0.6-1.8)	M > E ($p < 0.01$), A > E ($p = 0.01$)
Age: 12-19 Years old				
Total	336.7 (292.4-387.7)	214 (161.5-283.6)	180.3 (118-275.4)	M > A ($p < 0.01$), M > E ($p < 0.01$)
Non-Hispanic White	335.8 (261.1-431.9)	179.6 (99.2-325)	154.8 (77.4-309.3)	M > A ($p = 0.03$)
Non-Hispanic Black	363.7 (316.9-417.4)	277.5 (184.7-416.9)	265.1 (194.8-360.6)	
Hispanic	326.4 (249-427.9)	241.3 (200.9-289.8)	228.7 (166.5-314.1)	
Non-Hispanic Asian	379.1 (287.4-500.1)	247.1 (168.5-362.4)	260.2 (155.1-436.6)	
Other Race/Ethnicities	231.1 (165.1-323.6)	294.1 (210.3-411.4)	150.2 (92.7-243.4)	A < E ($p = 0.049$)
Age: ≥ 20 Years old				
Total	391.3 (373.7-409.6)	347.5 (327.1-369.1)	323.5 (297-352.5)	M > A ($p < 0.01$), M > E ($p < 0.01$)
Non-Hispanic White	387.7 (366.6-410)	346 (320.9-373)	315.6 (285.9-348.3)	M > A ($p = 0.01$), M > E ($p < 0.01$)
Non-Hispanic Black	374.1 (339.2-412.6)	364.7 (348.9-381.3)	370 (313.8-436.2)	
Hispanic	406.4 (372.7-443.2)	337.3 (317.6-358.1)	336.1 (299.8-376.9)	M > A ($p < 0.01$), M > E ($p = 0.01$)
Non-Hispanic Asian	415.5 (388.5-444.4)	343.2 (319.9-368.2)	352 (314.5-393.9)	M > A ($p < 0.01$), M > E ($p < 0.01$)
Other Race/Ethnicities	411.2 (358.4-471.9)	359.4 (260.5-496)	288.7 (209.8-397.4)	M > E ($p = 0.046$)

Table 3: Unadjusted geometric means with 95% confidence interval in ng/dL by blood draw session for males by age, smoking status, and race/ethnicity. Data from National Health and Nutrition Examination Survey 2011-2012.

Smoking Status		Blood Draw Session			Statistically Significant Differences
		Morning (M)	Afternoon (A)	Evening (E)	
Nonsmokers	Age: 12-19 Years old				
	Total	313.5 (269.4-364.9)	195.5 (143.8-265.9)	167.5 (107.1-262)	M > A (p < 0.01), M > E (p < 0.01)
	Non-Hispanic White	303.1 (226.2-406.1)	155.4 (80-301.9)	139.5 (69.8-278.9)	M > E (p < 0.01)
	Non-Hispanic Black	338.1 (285.9-399.9)	260.5 (177.1-383.2)	269.6 (192.3-377.8)	
	Hispanic	323.6 (246.6-424.6)	232.6 (191.2-282.9)	213.2 (138.7-327.8)	M > E (p = 0.055)
	Other Race/Ethnicities	399.8 (335-477.3)	244.5 (164.5-363.2)	260.2 (155.9-434.4)	M > A (p = 0.04)
Smokers	Age: 12-19 Years old				
	Total	492 (421.8-573.9)	440 (327.7-590.8)	341.3 (226.3-514.8)	
	Non-Hispanic White	508.1 (417.7-618.1)	499.3 (344.1-724.5)	330.9 (169.6-645.8)	
	Non-Hispanic Black	499.5 (434.8-573.9)	743.7 (679.8-813.6)	247.1 (150.5-405.7)	A > M > E (p < = 0.02)
	Hispanic	397.8 (140.6-1125.7)	273.1 (161.9-460.8)	553.3 (374.7-817)	
	Other Race/Ethnicities	209.4 (2.9-15141.3)	365.8 (365.8-365.8)	No data	
Nonsmokers	Age: ≥ 20 Years old				
	Total	382 (360.9-404.3)	325.7 (301.1-352.2)	317 (288.2-348.6)	M > A (p < 0.01), M > E (p < 0.01)
	Non-Hispanic White	383.5 (356.5-412.5)	321.2 (290-355.8)	313.3 (281.8-348.5)	M > A (p < 0.01), M > E (p < 0.01)
	Non-Hispanic Black	323 (280.8-371.4)	342.7 (317.4-370.1)	344 (282.1-419.5)	
	Hispanic	393.5 (368.8-419.8)	327 (302.5-353.5)	324.5 (275.7-381.8)	M > A (p < 0.01), M > E (p = 0.03)
	Other Race/Ethnicities	402.4 (378.5-427.8)	339.3 (314.4-366.2)	332.2 (297-371.6)	M > A (p < 0.01), M > E (p < 0.01)
Smokers	Age: ≥ 20 Years old				
	Total	412.7 (392.7-433.6)	408.7 (385.1-433.7)	343.1 (299.4-393.1)	M > E (p < 0.01), A > E (p = 0.03)
	Non-Hispanic White	396.7 (375-419.8)	415.3 (380.8-453)	323.4 (264.6-395.4)	M > E (p = 0.03), A > E (p = 0.02)
	Non-Hispanic Black	457.5 (422.4-495.4)	403.8 (352.9-462.1)	407.8 (339.3-490)	M > A (p = 0.04)
	Hispanic	458.5 (360.3-583.4)	379.8 (320.4-450.3)	370.8 (314.2-437.6)	
	Other Race/Ethnicities	490.5 (430.2-559.2)	364 (300.4-441.2)	406.2 (316.3-521.6)	M > E (p < 0.01)
		440.4 (345.4-561.6)	430.5 (334.8-553.4)	293.2 (192.2-447.1)	A > E (p = 0.01)

Table 4: Regression coefficients with p-values for continuous variables used in the model for log10 transformed values of total serum testosterone Data from National Health and Nutrition Examination Survey 2011-2012.

Variable	Age: 12-19 Years		Age: ≥ 20 Years	
	Slope	P-value	Slope	P-value
Age	0.10979562	< 0.01	-0.001028629	0.047
Body Mass Index	0.009342005	0.56	-0.007095311	0.09
Waist Circumference	-0.007102172	0.28	-0.002863613	0.07
Fasting Time in hours	-0.006733424	0.65	0.004088971	< 0.01
Alcohol Consumption in grams	Not in Model		0.000115362	0.56
R ²	40.0%		18.2%	
N	498		2156	

was 40% (Table 4). Interaction between race/ethnicity and testing session was found to be statistically significant (p < 0.01). Age was positively associated with T-TST levels ($\beta = 0.1098$, p < 0.01). Body mass index, waist circumference, and fasting time did not affect the levels of T-TST (Table 4). Adjusted T-TST levels for NHW were statistically significantly smaller than for HISP (203.6 vs. 283.2 ng/dL, p = 0.03, Table 5) and NHAS (203.6 vs. 318.8 ng/dL, p < 0.01, Table 5). Physical activity level was not found to be associated with the levels of T-TST (Table 5). T-TST levels were statistically significantly higher during the morning session than during the evening session (338.5 vs. 200.3 ng/dL, p = 0.02, Table 5). However, when interaction between race/ethnicity and testing session was taken into account, only NHW (348.8 vs. 140.8 ng/dL, p = 0.02, Table 6) and NHB (382.0 vs. 196.5 ng/dL, p = 0.01, Table 6) had statistically significantly higher adjusted T-TST levels during the morning session as compared to the evening session (Table 6). For NHAS and HISP, statistically significant differences for T-TST levels by testing sessions were not observed (Table 6).

Variability in total T-TST levels among adult males based on adjusted analysis

Sample size for the model fitted for adolescents was 2156 and R² was 18.2% (Table 4). Statistically significant differences between

race/ethnicity and testing session (p < 0.01) and race/ethnicity and smoking status were observed (p = 0.03). Age was negatively associated with T-TST levels ($\beta = -0.0010$, p = 0.047). Body mass index and waist circumference were not found to be associated with T-TST levels. Alcohol consumption did not affect the levels of T-TST (Table 4). Fasting time was positively associated with the adjusted levels of T-TST ($\beta = 0.0041$, p < 0.01, Table 4).

NHW had statistically significantly lower adjusted levels of T-TST than NHB (361.0 vs. 382.5 ng/dL, p = 0.02, Table 5) and NHB had higher adjusted T-TST levels than NHAS (382.5 vs. 341.9 ng/dL, p = 0.02, Table 5). Smokers were found to have statistically significantly higher levels of T-TST than nonsmokers (384.8 vs. 346.8 ng/dL, Table 5, p < 0.01). Level of physical activity and session during which T-TST serum samples were drawn did not affect the levels of T-TST (Table 5). However, when interaction between race/ethnicity and testing session was considered, morning and afternoon T-TST adjusted levels for NHW were found to be statistically significantly higher than the evening session levels (386.0 and 369.9 vs. 329.5 ng/dL, p ≤ 0.02, Table 6).

Diurnal variability among adolescent males

For the purpose of this study, diurnal variability was defined as

Table 5: Adjusted geometric means (AGM) with 95% confidence intervals for serum total testosterone in ng/dL by age, race/ethnicity, smoking stats, and physical activity status. Data from National Health and Nutrition Examination Survey 2011-2012.

	Age: 12-19 Years		Age: ≥ 20 Years	
	AGM (95% CI)	Statistically Significantly Differences	AGM (95% CI)	Statistically Significantly Differences
Race/Ethnicity				
Non-Hispanic White (NHW)	203.6 (151.1-274.5)	NHW < HISP (p = 0.03), NHW < NHAS (p < 0.01)	361 (341.7-381.3)	NHW < NHB (p = 0.04)
Non-Hispanic Black (NHB)	267.7 (223.1-321.4)		382.5 (363.5-402.5)	
Hispanic (HSP)	283.2 (219.5-365.6)		371.7 (344.2-401.4)	
Non-Hispanic Asian (NHAS)	318.8 (256.1-396.9)	NHAS > OTH (p = 0.02)	341.9 (317.4-368.1)	
Others (OTH)	214.2 (158.1-290.1)		370.7 (286.6-479.6)	
Smoking Status				
Nonsmoker (NSM)	235.9 (199.5-279)		346.8 (321.5-374)	NSM < SM (p < 0.01)
Smoker (SM)	273.2 (216-345.4)		384.8 (363.5-407.4)	
Physical Activity				
Vigorous (V)	249 (181.6-341.4)		379.2 (357-402.7)	
Moderate (M)	243.3 (184.9-320.3)		342.8 (295.9-397.1)	
Neither (N)	270 (237.9-306.5)		375.1 (354-397.4)	
Testing Session				
Morning (M)	338.5 (270.7-423.4)	M > E (p = 0.02)	378.4 (355.3-403.1)	
Afternoon (A)	241.3 (172.1-338.3)		369 (334.7-406.7)	
Evening (E)	200.3 (142.5-281.4)		349.1 (328.5-371)	

Table 6: Adjusted geometric means (AGM) with 95% confidence intervals in ng/dL for total testosterone by race/ethnicity by testing session for those aged 12-19 and ≥ 20 years and by race/ethnicity by smoking status or those aged > = 20 years. Data from National Health and Nutrition Examination Survey 2011-2012.

Demographic Group	Age 12-19 Years		Age > = 20 Years	
	AGM (95% CI)	Statistically Significantly Differences	AGM (95% CI)	Statistically Significantly Differences
Non-Hispanic White Morning Session (NHW_M)	348.8 (233.8-520.3)	NHW_M > NHW_E (p = 0.02)	386 (361.5-412.2)	NHW_M > NHW_E (p < 0.01)
Non-Hispanic White Afternoon Session (NHW_A)	171.9 (84.3-350.7)	NHW_A < NHAS_A (p < 0.01)	369.9 (343.8-398)	NHW_A > NHW_E (p = 0.02)
Non-Hispanic White Evening Session (NHW_E)	140.8 (86.1-230.2)		329.5 (298.9-363.1)	
Non-Hispanic Black Morning Session (NHB_M)	382 (297.4-490.7)	NHB_M > NHB_E (p = 0.01), NHB_M > OTH_M (p = 0.02)	374.5 (342.3-409.7)	
Non-Hispanic Black Afternoon Session (NHB_A)	255.7 (187.6-348.5)		387.2 (372.3-402.7)	NHB_A > NHAS_A (p < 0.01)
Non-Hispanic Black Evening Session (NHB_E)	196.5 (123.3-313.1)		386 (342.1-435.7)	
Hispanics Morning Session (HISP_M)	379.9 (250.4-576.4)		393.5 (360.1-429.9)	HISP_M > NHAS_M (p = 0.02)
Hispanics Afternoon Session (HISP_A)	264.1 (186.3-374.3)		379.8 (353.4-408.3)	HISP_A > NHAS_A (p = 0.02)
Hispanics Evening Session (HISP_E)	226.5 (137.6-372.8)		343.5 (296.9-397.3)	
Non-Hispanic Asian Morning Session (NHAS_M)	464.9 (283.9-761.5)	NHAS_M > NHAS_A (p = 0.04), NHAS_M > OTH_M (p = 0.02)	353.3 (323.3-386)	
Non-Hispanic Asian Afternoon Session (NHAS_A)	248.3 (190.8-323.3)		329.9 (298.5-364.6)	
Non-Hispanic Asian Evening Session (NHAS_E)	280.7 (188.7-417.5)	NHAS_E > OTH_E (p = 0.01)	342.8 (301.6-389.6)	
Other Race/Ethnicities Morning Session (OTH_M)	188.9 (129-276.7)		386.3 (292.8-509.8)	
Other Race/Ethnicities Afternoon Session (OTH_A)	283.8 (168.6-477.6)	OTH_A > OTH_E (p = 0.047)	381 (237.2-611.9)	
Other Race/Ethnicities Evening Session (OTH_E)	183.2 (123.3-272.2)		346.2 (283.5-422.8)	
Non-Hispanic White Nonsmokers (NHW_NSM)			351.3 (332.1-371.7)	NHW_NSM < NHW_SM (p = 0.03)
Non-Hispanic White Smokers (NHW_SM)			370.9 (348.1-395.3)	
Non-Hispanic Black Nonsmokers (NHB_NSM)			363 (339.2-388.6)	NHB_NSM < NHB_SM (p = 0.02), NHB_NSM > NHAS_NSM (p = 0.01)
Non-Hispanic Black Smokers (NHB_SM)			403 (378.1-429.7)	
Hispanics Nonsmokers (HISP_NSM)			354.8 (336-374.6)	HISP_NSM > NHAS_NSM (p = 0.02)
Hispanics Smokers (HISP_SM)			389.3 (343.8-440.9)	
Non-Hispanic Asian Nonsmokers (NHAS_NSM)			319.9 (298.9-342.5)	NHAS_NSM < NHAS_SM (p = 0.02)
Non-Hispanic Asian Smokers (NHAS_SM)			365.3 (326.9-408.1)	
Other Race/Ethnicity Nonsmokers (OTH_NSM)			346.4 (251.6-477)	
Other Race/Ethnicity Smokers (OTH_SM)			396.8 (300.2-524.5)	

the ratio of the geometric mean for T-TST for the morning session divided by the geometric mean for T-TST for the evening session. Ratios of both unadjusted (RU) as well as adjusted geometric means (RA) were computed and are given in table 7. While RA and RU for adolescent nonsmokers were similar or 1.87 and 1.85 respectively, RU for smokers was 1.44 and RA for smokers was 1.84. Consequently, morning T-TST levels may be more than 80% higher than evening T-TST levels. For nonsmoker adolescents, morning T-TST levels for NHW may be double of what they are for the evening session (RU = 2.17, RA = 2.06, Table 7). The same was found to be true for NHW smoker adolescents since RA = 2.02. For NHB smokers also, morning

T-TST levels could be double of what they are for the evening testing (RA = 2.02) but the morning-evening differences for NHB nonsmokers were only about 25% (Table 7). Similar patterns were observed for NHAS smokers and nonsmokers.

Diurnal variability among adult males

Smoking did not affect diurnal variability to the same degree for adults than it did for adolescents irrespective of race/ethnicity since both RA and RU were about 1.2. Winters et al. [24] found young Caucasians to have 23.9% higher TST levels at 8 AM as compared to the levels at 8 PM. In this study, NHW were found to have 20-23% higher T-TST

Table 7: The ratios of the unadjusted geometric means (RU) and adjusted geometric means (RA) for total TST levels for morning to evening session by age, race/ethnicity, and smoking status. Data from National Health and Nutrition Examination Survey 2011-2012.

Smoking Status		N			RU	RA
		Morning	Afternoon	Evening		
Nonsmokers	Age: 12-19 Years old					
	Total	232	117	77	1.87	1.85
	Non-Hispanic White	52	33	18	2.17	2.06
	Non-Hispanic Black	77	53	13	1.25	1.34
	Hispanic	63	54	25	1.52	1.45
	Non-Hispanic Asian	32	26	15	1.54	1.3
	Other Race/Ethnicities	8	11	6	1.42	2.14
Smokers	Age: 12-19 Years old					
	Total	40	17	10	1.44	1.84
	Non-Hispanic White	14	7	4	1.54	2.02
	Non-Hispanic Black	18	3	3	2.02	1.82
	Hispanic	3	5	2	0.72	1.07
	Non-Hispanic Asian	3	1	0	0.57	2.47
	Other Race/Ethnicities	2	1	1	1.67	0.81
Nonsmokers	Age: ≥ 20 Years old					
	Total	844	610	231	1.21	1.2
	Non-Hispanic White	317	204	95	1.22	1.21
	Non-Hispanic Black	152	161	47	0.94	1
	Hispanic	205	125	52	1.21	1.2
	Non-Hispanic Asian	150	98	29	1.21	1.17
	Other Race/Ethnicities	20	22	8	1.33	1.62
Smokers	Age: ≥ 20 Years old					
	Total	348	264	110	1.20	1.23
	Non-Hispanic White	161	105	39	1.23	1.31
	Non-Hispanic Black	94	96	34	1.12	1.03
	Hispanic	56	32	20	1.21	1.17
	Non-Hispanic Asian	27	21	11	1.07	1.08
	Other Race/Ethnicities	10	10	6	1.50	1.22

levels during morning session than during the evening session which is in confirmation with the results of Winters et al. [24] meaning NHW do have certain amount of diurnal variability in their T-TST levels.

There was almost no diurnal variation in T-TST levels for NHB nonsmokers (diurnal RU = 0.94, RA = 1.0) but NHB smokers did have about 12% higher T-TST levels based on RU but only 3% based on RA during the morning session than during the evening session (Table 7). This means that NHB nonsmokers do not have diurnal variability in their T-TST levels but NHB smokers do exhibit some diurnal variability in their T-TST levels. Thus, finding by Winters et al. [24] that found young African-Americans had 29.4% higher TST levels at 8 AM as compared to the levels at 8 PM is only partially confirmed and that too among smokers. The differences in the results by the two studies may be due to ages covered by these two studies.

Among NHAS, while diurnal variability ratio for nonsmokers was 1.21, it was 1.07 among smokers.

Discussion

Effect of race/ethnicity, smoking, physical activity, testing session and other factors on T-TST levels among male adolescents

Using NHANES III data, Lopez et al. [28] found racial/ethnic differences in the levels of T-TST among 12-15 years old but not so among 16-19 years old. For the combined data for 12-19 years old for this study, NHW were found to have lower adjusted T-TST levels than both HISP and NHAS but not when compared with NHB (Table 5). However, when interaction between race/ethnicity and testing session was taken into account, racial/ethnic differences in T-TST levels almost disappeared (Table 6). However, morning T-TST levels were found to be statistically significantly higher for at least NHW and NHB when compared with evening T-TST levels (Table 6). In fact, morning T-TST levels may be double of what they are during evening testing for both smoker and nonsmoker NHW and 80% higher for NHB smokers (Table 7). Thus, role of race/ethnicity in determining

T-TST levels among male adolescents seems to be limited but both NHW and NHB adolescents do have diurnal variability in their T-TST levels.

Effect of race/ethnicity, smoking, physical activity, testing session, and other factors on T-TST levels among male adults

The order of T-TST levels by race/ethnicity was NHAS < NHW < NHB. These results are not comparable with the results provided by Rohrmann et al. [30] because while Rohrmann et al. [30] used only morning session data, this study used data for all three sessions in the adjusted models. For this study, NHW were found to have the lowest T-TST levels when compared with NHB and Hispanics as was observed by Wu et al. [31] but Asian-Americans were not found to have the highest levels as was reported by Wu et al. [31] which may be because while Wu et al. [31] study was done among those aged ≥ 60 years, this study was done among all those aged ≥ 20 years. Also, while Gapstur et al. [33] did not find any racial/ethnic differences among ≥ 24 years old males, in this study, NHW were found to have lower T-TST levels than NHB.

Limitations of the Study

This study was based on cross-sectional data and as such, diurnal variations reported in this study need to be interpreted with caution. True diurnal variability can only be determined if TST levels are determined for the same persons over time. There were many pair-wise comparisons that were made, for example, between NHW and NHB, NHB and HISP etc. each with its own p-value. The possibility of inflated type I error with so many comparisons should not be overlooked. There are many corrective methods that are available to adjust for multiple comparisons so as to have overall p-value below the nominal level which generally is 0.05. Some of these corrective methods include Bonferoni correction, Newman-Keuls method, Scheffe method, and Tukey's least significant different method. However, as could be expected, different correction methods may lead to different pair-wise comparisons being labeled as statistically

significant. Consequently, none of the correction methods were used to generate results for this study. Instead, actual p-values for each pair-wise comparison as well as regression slopes were presented. In the opinion of this author, a decision should be made by the clinicians about which of the possible and how many comparisons are of clinical interest/significance. Once this has been decided, a decision should be made about what is the total level of type I error that can be allowed for "all" comparisons of interest which usually could be 0.05. Following this, the clinician may want to decide of the total allowable type I error, how much type I error should be allocated to which comparison. Once this allocation has been made, the clinician should look at what is the computed type I error (or p-value) attached with the comparisons of interest. If the computed type I error or p-value for a specific comparison is higher than the allocated type I error, the clinician may want to designate that comparison as "clinically insignificant". If the computed type I error or p-value for a specific comparison is lower than the allocated type I error, the clinician may want to designate that comparison as "clinically significant". The possibility of a result that is "statistically insignificant" being "clinically significant" should also not be ruled out. It should be remembered that there is an inverse association between type I and type II errors. It may be tempting to be too conservative in applying type I error but that also means type II error is inflated accordingly. A clinician may decide what is of greater importance/significance, namely, making a conclusion about the hypothesized differences being statistically/clinically significant when, in fact, they are not (type I error) or making a conclusion about the hypothesized differences not being statistically/clinically significant when, in fact, they are (type II error). Different clinicians may attach different values to type I and type II errors.

Summary and Conclusion

Among adolescent males, (i) diurnal variability in T-TST levels was substantially smaller among smokers than nonsmokers except for NHB for whom the reverse was true, (ii) the order of diurnal variability for T-TST levels by race/ethnicity was NHW > NHAS > HISP > NHB, (iii) NHW had lower levels of T-TST than HISP and NHAS, (iv) smoking and physical activity did not affect the levels of T-TST, (v) morning T-TST levels among NHW and NHB were higher than evening T-TST levels, and (vi) T-TST levels increased with age.

Among adult males, (i) NHW had 21-31% higher T-TST levels during morning than evening for both smokers and nonsmokers, (ii) there was no diurnal variation in T-TST levels among NHB nonsmokers but NHB smokers had 3%-12% higher T-TST levels during morning as compared to evening, (iii) NHW had higher morning and afternoon T-TST levels than evening T-TST levels, (iv) NHW, NHB, and NHAS smokers had higher T-TST levels than NHW, NHB, and NHAS nonsmokers, respectively (iii) T-TST levels decreased with increase in age.

Declaration of Interest

The author declares that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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