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Androgen activity and markers of inflammation among men in NHANES III

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Androgen activity and markers of inflammation among men in NHANES III

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Abstract

Objectives: Inflammation contributes to chronic diseases. Lower serum testosterone among men is associated with less inflammation, yet immune defense is thought to trade-off against reproduction with androgens adversely affecting immune function. Anti-androgens are effective at castrate levels of serum testosterone, suggesting serum testosterone may not capture all androgen activity. The association of two androgen biomarkers with key markers of inflammation was examined.

Methods: The adjusted association of serum testosterone and androstanediol glucuronide with C-reactive protein, white blood cell, granulocyte and lymphocyte count, fibrinogen and hemoglobin, as a control outcome because testosterone administration raises hemoglobin, were examined in a nationally representative sample of 1490 US men from NHANES III phase 1 (1988-91) using multivariable linear regression.

Results: Serum testosterone and androstanediol glucuronide were weakly correlated (0.13). Serum testosterone was associated with lower white blood cell count ($-0.26*10^{-9}$ per standard deviation, 95% confidence interval (CI) −0.37 to −0.14) and granulocyte count ($-0.21*10^{-9}$, 95% CI −0.29 to −0.13) but not with hemoglobin (0.02 g/L, 95% CI −0.89 to 0.92), adjusted for age, education, race/ethnicity, smoking and alcohol. Similarly adjusted, androstanediol glucuronide was not associated with white blood cell count ($0.10*10^{-9}$, 95% CI −0.05 to −0.25), granulocyte count ($0.12*10^{-9}$, 95% CI −0.02 to 0.25) or fibrinogen (0.05g/L, 95% CI −0.004 to 0.11), but was with hemoglobin (0.70g/L, 95% CI 0.07 to 1.32).

Conclusions: Different androgen biomarkers had different associations with inflammatory markers, highlighting the need to consider several androgen biomarkers. The possibility remains that androgens may generate inflammatory processes with implications for chronic diseases.

Keywords

testosterone; androgen glucuronide; C-reactive protein; fibrinogen; leukocyte

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INTRODUCTION

Non-communicable chronic diseases are increasingly recognized as inflammatory conditions (Swirski and Nahrendorf, 2013;Tabas and Glass, 2013) potentially reversible by therapies targeting systemic low-grade inflammation (Tabas and Glass, 2013). Since testicular extracts were first developed in the 1920s androgens have been known to affect components of the immune system in animals, such as the size of the thymus (Dougherty, 1952;Korenchevsky et al., 1932). Within a life history framework (Stearns, 2005), fitness is thought to depend on how well resource allocation is optimized between reproductive success and survival, with androgens being a potential mechanism driving this allocation (Folstad and Karter, 1992). Males may gain more fitness (descendants) by concentrating on reproductive success at the expense of a less well functioning immune system and potentially shorter lives whilst females may gain more from longevity (Rolff, 2002;Zuk, 2009). Although empirical support for this hypothesis in animals is not comprehensive (Kotiaho, 2001), it is thought that there are trade-offs between immune defense and reproductive success (Schroderus et al., 2010), with some evidence that androgens suppress immunity in animals (Hepworth et al., 2010;Pinto et al., 2010;Sasaki et al., 2013). The potential relevance of interactions of sex-steroids with the immune system to humans is also increasingly being recognized (Grossman, 1985;Sakiani et al., 2012).

Generally women are more vulnerable to auto-immune diseases, with the difference emerging at puberty, whilst men are more vulnerable to infections (Marriott and Huet-Hudson, 2006); most markedly at life stages when androgens are higher (Guerra-Silveira and Abad-Franch, 2013). However, women may be more vulnerable to infectious diseases where a strong immune response enhances pathogenesis (Guerra-Silveira and Abad-Franch, 2013). These sex-specific patterns of diseases in humans are thought to arise because estrogens promote a more vigorous immune response which results in greater risk of auto-immune disease as well as a stronger response to infections, whilst androgens are thought to be immunosuppressive (Cutolo et al., 2004;Schuurs and Verheul, 1990). Correspondingly androgen deprivation therapy may enhance aspects of immune response (Aragon-Ching et al., 2007;Morse and McNeel, 2010). The causes of the low-grade systematic inflammation which characterizes many non-communicable chronic conditions is not fully understood, but is not thought to be due to autoimmunity (Tabas and Glass, 2013). Instead such inflammation appears to be a state of immune dysregulation or dysfunction, to which factors that potentially affect immune function could be relevant. However, the association of androgens with markers of low-grade systemic inflammation in humans, such as C-reactive protein (CRP), fibrinogen (Tabas and Glass, 2013) or white cell count (Bonaterra et al., 2010;Lowe, 2005) is not well established.

Few of the randomized controlled trials (RCTs) of testosterone therapy among men have reported effects on inflammatory markers. The available evidence from these RCTs generally shows little effect of testosterone therapy on CRP (Aversa et al., 2010;Frederiksen et al., 2013;Kapoor et al., 2007;Nakhai-Pour et al., 2007;Ng et al., 2002), white blood cell count (Kalinchenko et al., 2010) or fibrinogen (Smith et al., 2005), although one RCT reported testosterone decreased CRP (Kalinchenko et al., 2010) among a subset of men. Larger observational studies among men usually, but not always, report serum testosterone

inversely associated with CRP (Gannage-Yared et al., 2011;Haring et al., 2012;Kupelian et al., 2010;Laaksonen et al., 2003;Nakhai Pour et al., 2007;Zhang et al., 2013), white blood cell counts and/or its differentials (Brand et al., 2012;Haring et al., 2012;Tang et al., 2007) and fibrinogen (Bonithon-Kopp et al., 1988;Haring et al., 2012;Yang et al., 1993). Experimental and observational evidence may differ for a number of reasons: the small size of most RCTs of testosterone therapy, differences in the action of exogenous and endogenous testosterone, serum testosterone acting as a marker of health status or serum testosterone not capturing all androgen activity (Labrie et al., 2009).

Serum testosterone largely originates from the gonads, and then may act directly via the androgen receptor, may be metabolized to dihydrotestosterone, which also acts via the androgen receptor, but with greater potency, or may be metabolized to estrogen (Auchus and Auchus, 2012). Androgen precursors may also be produced in other parts of the body, such as the adrenals (Auchus and Auchus, 2012), or androgens may be produced and used locally without entering the circulation (Labrie, 1991). In contrast to testosterone, androgen glucuronides, and specifically androstanediol glucuronide (3α-diol-G), is a measure of the final breakdown product of all sources of androgens (Mauvais-Jarvis et al., 1970;Moghissi et al., 1984) and as such may be a measure of total androgen activity in the body. As much as 40% of total androgens are thought to be produced and used locally without circulating as serum testosterone (Labrie et al., 2009). Until recently most interest in androgens has focused on serum testosterone. However, successful treatment of ‘hormone-resistant’ prostate cancer (Fizazi et al., 2012;Scher et al., 2012) with anti-androgens at castrate levels of serum testosterone (Labrie et al., 2009) has changed thinking about testosterone and re-focused attention on the role of total androgen activity in prostate cancer (Auchus and Auchus, 2012). However, these new insights, suggesting testosterone is more a measure of gonadal production and androstanediol glucuronide (3α-diol-G) is more a measure of androgen activity may have general relevance to understanding the role of androgens in health. No previous studies examining the association of androstanediol glucuronide (3α-diol-G) with markers of the low-grade systemic inflammation, which characterizes many chronic diseases, in humans could be identified. To clarify the role of androgens in inflammation among men, this study takes advantage of a nationally representative US sample, where these androgen biomarkers were assayed for a subset of men, to assess the relation of these two different androgen biomarkers with markers of low-grade systemic inflammation..

MATERIALS AND METHODS

Sources of data

The National Health and Nutrition Examination Survey (NHANES) III was conducted from 1988 through 1994 by the National Center for Health Statistics of the Centers for Disease Control and Prevention (CDC). The NHANES III survey used complex, multi-stage, stratified, clustered samples of civilian, non-institutionalized populations of age 2 months or older to collect information about the health of people residing in the US. The participants were randomly assigned to a physical examination in the morning, afternoon or evening. During the physical examination blood was taken and analyzed for a range of inflammatory

markers, including CRP, white blood cell count and its differentials and fibrinogen. Of the 2,205 males aged at least 12 years who participated in NHANES III phase 1 (1988 to 1991) and attended a morning examination session, 1,637 had surplus sera, previously stored at −70°C, assayed for sex-steroids using competitive electrochemiluminescence immunoassays on the 2010 Elecsys autoanalyzer (Roche Diagnostics, Indianapolis, IN) for serum testosterone, estradiol, and sex hormone binding globulin and an enzyme immunoassay (Diagnostic Systems Laboratories, Webster, TX) for androstanediol glucuronide (3-alpha-diol-G) (AAG), as previously described (Selvin et al., 2007). NHANES III was approved by the CDC Institutional Review Board and all participants provided written informed consent.

Exposures

The primary exposures were the androgen biomarkers serum testosterone and AAG. Initial analysis revealed that considering these exposures log transformed or in their original units gave a similar interpretation, so for ease of interpretability and comparability the exposures were considered as z-scores (i.e., standard deviations) of their original units. To allow for any potentially non-linear associations the exposures were also considered in tertiles, as low (≤4.34 ng/mL), medium (4.34ng/mL-≤6.04ng/mL) and high (>6.04ng/mL) testosterone and low (≤8.33 ng/mL), medium (8.33ng/mL-≤13.82ng/mL) and high (>13.82ng/mL) AAG.

Outcomes

The markers of low-grade systemic inflammation considered were CRP, white blood cell count and its differentials (lymphocytes and granulocytes) and fibrinogen (Lowe, 2005). Fibrinogen was only assayed for those aged 40 years or older.

Control outcome

Hemoglobin was included as a control outcome because hemoglobin is raised by testosterone therapy (Fernandez-Balsells et al., 2010), and hemoglobin rises at puberty among boys in response to rising androgens (Hero et al., 2005). So, a biomarker capturing androgen activity would be expected to be associated with higher hemoglobin.

Exclusions

Men taking testosterone therapy, GnRH inhibitors or 5alpha reductase inhibitors were not excluded, because most of these medications were not available or were only just being introduced into the US at the time of NHANES III phase 1 in 1988-91. Moreover, these medications may influence androgens but the resulting androgens should have similar effects on markers of systemic inflammation. However, the study was restricted to adult men (18+ years) because the relation of androgens with markers of inflammation might be different during the hormonal changes of puberty.

Statistical analysis

Multivariable linear regression was used to assess the adjusted association of the androgen biomarkers with markers of systemic inflammation and hemoglobin. Androgens may vary with age, race/ethnicity, alcohol use and smoking (Allen et al., 2002;Field et al.,1994;Shiels et al., 2009;Suzuki et al., 2009) and adiposity (Derby et al., 2006). Model 1 adjusted for age

(continuous) and race/ethnicity, smoking, alcohol use and education, as categorized in Table 1. Androgens may partially operate via altering body composition. Experimental evidence, to date, indicates that testosterone therapy reduces body fat (Isidori et al., 2005), although under the influence of androgens during puberty central fat may increase (Brufani et al., 2009). Conversely, body fat may reduce androgens through aromatization to estrogens (Kley et al., 1980). So, to assess these pathways model 2 additionally adjusted for body mass index and waist-hip ratio. All statistical analyses were performed using SAS, allowing for the effects of the complex sample design on variance estimation in NHANES III and to weight the sample back to the US population using sample weights for phase 1 participants who had a morning examination.

This study is an analysis of de-identified publically available data, which does not require ethics committee approval.

RESULTS

Of the 1,637 males aged at least 12 years who participated in NHANES III phase 1 (1988 to 1991), attended a morning examination session and had surplus sera, 1,490 were aged at least 18 years and had values for one or other androgen biomarker. Potential confounders were available for all men, although inflammatory markers and measures of adiposity were missing for some men. All available observations were included in the relevant analysis.

Serum testosterone was only weakly positively correlated with AAG (0.13). Table 1 shows serum testosterone was lower among men who were older, least educated, former smokers, ex-drinkers and non-Hispanic white. AAG was lower among older men and non-Hispanic blacks.

Serum testosterone was negatively associated with white blood cell count, and granulocyte count (Table 2). High (compared to low) serum testosterone tertile was also associated with lower CRP (−0.16 mg/dL, 95% confidence interval (CI) −0.29 to −0.02, lower white blood cell count ($-0.55\ 10^{-9}$, 95% CI −0.79 to −0.31), lower lymphocyte count ($-0.08\ 10^{-9}$, 95% CI −0.16 to −0.002) and lower granulocyte count ($-0.47\ 10^{-9}$, 95% CI −0.65 to −0.29). All these associations were attenuated almost to the null by adjustment for adiposity in model 2. Serum testosterone was not associated with hemoglobin in model 1. In contrast, AAG was positively associated with fibrinogen after adjustment for adiposity in model 2 (0.06 g/L per standard deviation AAG, 95% CI 0.002 to 0.11). AAG had no clear association with CRP or lymphocyte count, and was weakly positively associated with white blood cell count and granulocyte count, although the confidence intervals included no association. AAG was also positively associated with hemoglobin in model 1 (0.70 g/L per standard deviation AAG, 95% CI 0.07 to 1.32).

DISCUSSION

Consistent with other observational studies serum testosterone was negatively associated with some markers of inflammation (Bonithon-Kopp et al., 1988;Brand et al., 2012;Gannage-Yared et al., 2011;Haring et al., 2012;Kupelian et al., 2010;Laaksonen et al., 2003;Yang et al., 1993;Zhang et al., 2013). This study adds by considering an additional

biomarker of androgen activity, AAG, which was not associated with lower values of any of the inflammatory markers considered, but was associated with higher fibrinogen as well as with hemoglobin as would be expected of an androgen.

Despite using data from a meticulously executed population representative study, some limitations do exist. First, serum testosterone is a well-accepted biomarker, whilst AAG is rarely used and might represent something other than androgen activity, such as liver function or obesity. However, that does not seem biologically plausible (Labrie et al., 2009). The positive association of AAG with hemoglobin was unchanged by adjustment for alanine aminotransferase (data not shown), and the general pattern of associations for AAG was similar with and without adjustment for adiposity (model 2 compared with model 1 in Table 2). AAG could also be specifically a marker of aging and ill-health rather than of androgen activity, although AAG had the same relation with hemoglobin as testosterone therapy (Fernandez-Balsells et al., 2010), suggesting AAG is a measure of androgen activity. Second, it may appear counter-intuitive that correlated factors (serum testosterone and AAG) have different associations with the same outcomes; however the correlation was relatively weak. Third, serum testosterone was measured using competitive electrochemiluminescence immunoassays rather than the gold-standard liquid chromatography tandem mass spectrometry. However, any measurement error is, most likely, non-differential, impairing the precision of the estimates rather than creating a bias. Fourth, this study was limited to men, because sex hormones were not measured for women in NHANES III. However, androgens are a more important hormone among men than women. Fifth, white blood cell count has been used by others as a marker of systemic low-grade (Lowe, 2005) and is associated prospectively with ischemic heart disease (Madjid and Willerson, 2011), particularly granulocyte count (Rana et al., 2007), white blood cell count could reflect general immune activity in response to chronic and infectious diseases. However, the pattern of associations was fairly consistent across all markers of inflammation.

The discrepancy between the associations of serum testosterone and AAG with markers of inflammation could be because they affect inflammation differently. For example, serum testosterone might suppress inflammation whilst dihydrotesterone which is metabolized from testosterone and also captured by AAG might promote inflammation. Interplays of this nature are very difficult to distinguish and no studies examining them could be identified. However, both testosterone and dihydrotestosterone operate via the same androgen receptor. Conversely, it is also possible that systemic inflammation, or its drivers, affect testosterone and AAG differently, reducing serum testosterone but not AAG. Pro-inflammatory cytokines inhibit testosterone secretion through their influence on the hypothalamic-pituitary-gonadotropic axis (Turnbull and Rivier, 1997;van der Poll et al., 1993). Notably, the associations of serum testosterone, but not AAG, with inflammatory markers were changed by adjustment for adiposity, perhaps because adiposity generates inflammation (Madsen et al., 2008;Welsh et al., 2010), which in turn reduces testosterone production (Turnbull and Rivier, 1997;van der Poll et al., 1993). However, it also possible that testosterone, but not AAG, operates by reducing fat which in turn reduces inflammation. These possibilities are difficult to distinguish in observational studies and RCTs targeting markers of systemic inflammation rarely assay androgens. Nevertheless, AAG had the

association with hemoglobin expected from RCTs whilst testosterone did not, suggesting perhaps that serum testosterone rather than AAG may be more sensitive to systemic inflammation or to common causes of systemic inflammation and ill-health. Given these uncertainties, it would be valuable to assess the role of endogenous testosterone and AAG in a study design less open to biases, such as Mendelian randomization. To date, no Mendelian randomization study examining the association of serum testosterone and/or AAG with markers of systemic inflammation could be identified A recent, small Mendelian randomization study did not corroborate the usually observed associations of serum testosterone with healthier values of conventional cardiovascular risk factors (Haring et al., 2013).

The findings from this study for AAG, but not serum testosterone, are consistent with the findings of RCTs testosterone or dihydrotestosterone therapy, where no significant effect on CRP, white blood cell count or fibrinogen was found, apart from for one trial where CRP was only reported for about half the participants (Kalinchenko et al., 2010). Just as observed here for AAG, such therapy produced non-significant increases in CRP (Aversa et al., 2010;Kapoor et al., 2007;Nakhai Pour et al., 2007;Ng et al., 2002), white blood cell count (Kalinchenko et al., 2010) and fibrinogen (English et al., 2000). Larger trials, or new information from completed trials, are needed to determine whether testosterone or dihyrotestosterone therapy has the positive relation with markers of systemic inflammation, as indicators of a less well-functioning immune system, which might be expected from a theoretical life history perspective. Notably such a perspective provides a general explanation for several diverse observations, by generating the hypothesis that androgens might generate both low-grade systemic inflammation and cardiovascular disease (Xu et al., 2013), and thereby an association of such inflammation with cardiovascular disease. Moreover, clarification of the effects of androgens on components of the immune system relating to adaptive immunity, such as lymphocytes, and to innate immunity, such as granulocytes (Munoz-Cruz et al., 2011) might clarify life history trade-offs concerning general and specific immune processes, given granulocytes contribute to inflammatory status and adaptive immune response (Kolaczkowska and Kubes, 2013).

This study is consistent with and extends the evidence to date concerning the associations of androgen activity with markers of systemic inflammation. It suggests that the negative association of serum testosterone with markers of systemic inflammation should not necessarily be interpreted as indicating that testosterone has a beneficial effect on systemic inflammation, whilst keeping open the possibility that androgens might have detrimental effects on the immune system among men. As such, this study raises the possibility that androgens in men rather than protecting against inflammation may be a factor generating inflammatory processes by suppressing the immune system. Whether these potential actions are part of, or a marker of, any causal process involved in any major chronic disease remains to be determined. This study also strongly suggest that observational studies assessing the role of androgens need to consider other biomarkers complimentary to serum testosterone, for which inexpensive assays need to be developed.

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Table 1

Testosterone and androstanediol glucuronide by socio-demographic characteristics among 1,490 men from NHANES III phase 1 (1988-1991) - unweighted

		Testosterone (ng/mL)				Androstanediol glucuronide (ng/mL)			
		n	mean	SD	ANOVA p-value	n	mean	SD	ANOVA p-value
Age	<45 years	735	5.96	1.96		741	14.5	10.7	
	45+ years	735	4.63	1.87	<0.001	739	12.0	12.1	<0.001
Years of formal education	≤8 years	350	4.93	2.12		355	12.2	9.03	
	9-11 years	259	5.46	1.94		258	13.5	10.4	
	12 years	399	5.44	2.09		401	13.0	11.9	
	13+ years	462	5.35	1.93	0.002	466	14.2	13.2	0.08
Race/ethnicity	Non-Hispanic black	362	5.67	2.15		364	12.4	8.9	
	Mexican-American	383	5.50	2.02		388	13.7	11.6	
	Other	56	4.78	2.01		56	10.2	5.9	
	Non-Hispanic white	669	5.01	1.92	<0.001	672	13.8	12.9	0.04
Smoking status	Current	462	6.10	2.09		465	12.7	8.1	
	Former	479	4.52	1.83		484	13.4	14.7	
	Never	529	5.29	1.88	<0.001	531	13.6	10.7	0.45
Alcohol use	<1/week	214	5.54	2.09		212	15.4	16.8	
	1-3/week	341	5.68	1.96		342	13.4	8.8	
	4+/week	302	5.32	2.06		306	12.7	9.1	
	Ex-drinkers	418	4.79	1.98		423	12.5	11.6	
	unknown	86	5.48	2.06		86	13.7	14.4	
	never	109	5.34	1.84	<0.001	111	13.0	8.8	0.08

SD: standard deviation

Table 2

Adjusted association of standard deviations of testosterone and androstanediol glucuronide with inflammatory markers among 1,490 men from NHANES III phase 1 (1988-1991).

			Testosterone					Androstanediol glucuronide				
			standard deviation	Tertile				standard deviation	Tertile			
	Model	n	b	Low	Medium	High	n	b	Low	Medium	High	
			95% CI		95% CI	95% CI		95% CI		95% CI	95% CI	
C-reactive protein (mg/dL)	1	1469	−0.08	ref	0.01	**−0.16**	1479	−0.003	ref	0.10	0.02	
			−0.16 to 0.004		−0.09 to 0.12	**−0.29 to −0.02**		−0.05 to 0.04		−0.04 to 0.25	−0.08 to 0.11	
	2	1421	−0.05	ref	0.03	−0.10	1432	−0.004	ref	0.09	0.001	
			−0.14 to 0.04		−0.07 to 0.13	−0.24 to 0.03		−0.05 to 0.04		−0.05 to 0.23	−0.10 to 0.10	
White blood cell count (10^{-9})	1	1443	**−0.26**	ref	−0.17	**−0.55**	1453	0.10	ref	0.15	0.22	
			−0.37 to −0.14		−0.45 to 0.11	**−0.79 to −0.31**		−0.05 to 0.25		−0.32 to 0.62	−0.25 to 0.70	
	2	1396	−0.07	ref	0.01	−0.09	1407	0.10	ref	0.04	0.15	
			−0.19 to 0.05		−0.21 to 0.24	−0.38 to 0.20		−0.06 to 0.25		−0.42 to 0.50	−0.32 to 0.63	
Lymphocyte count (10^{-9})	1	1443	−0.04	ref	−0.01	**−0.08**	1453	−0.01	ref	0.02	0.05	
			−0.09 to 0.02		−0.11 to 0.08	**−0.16 to −0.002**		−0.05 to 0.02		−0.10 to 0.13	−0.07 to 0.18	
	2	1396	0.01	ref	0.03	0.03	1407	−0.02	ref	−0.001	0.03	
			−0.05 to 0.06		−0.06 to 0.11	−0.05 to 0.11		−0.05 to 0.02		−0.11 to 0.11	−0.09 to 0.15	
Granulocyte count (10^{-9})	1	1430	**−0.21**	ref	−0.17	**−0.47**	1440	0.12	ref	0.11	0.15	
			−0.29 to −0.13		−0.40 to 0.07	**−0.65 to −0.29**		−0.02 to 0.25		−0.32 to 0.54	−0.27 to 0.60	
	2	1383	−0.08	ref	−0.04	−0.14	1394	0.12	ref	0.02	0.11	
			−0.18 to 0.02		−0.24 to 0.16	−0.39 to 0.10		−0.02 to 0.26		−0.39 to 0.44	−0.32 to 0.53	
Fibrinogen (g/L)	1	845	−0.07	ref	**−0.15**	−0.17	851	0.05	ref	0.04	0.05	
			−0.17 to 0.02		**−0.30 to −0.01**	−0.35 to 0.01		−0.004 to 0.11		−0.16 to 0.24	−0.15 to 0.24	
	2	809	−0.04	ref	−0.12	−0.09	816	**0.06**	ref	0.01	0.03	
			−0.13 to 0.05		−0.26 to 0.03	−0.24 to 0.07		**0.002 to 0.11**		−0.19 to 0.22	−0.17 to 0.23	
Hemoglobin (g/L)	1	1443	0.02	ref	0.88	−0.02	1453	**0.70**	ref	**1.82**	**1.91**	
			−0.89 to 0.92		−0.71 to 2.47	−2.01 to 1.97		**0.07 to 1.32**		**0.50 to 3.14**	**0.27 to 3.55**	
	2	1396	**0.85**	ref	**1.83**	**1.91**	1407	0.61	ref	**1.58**	1.51	

		Testosterone				Androstanediol glucuronide			
			Tertile				Tertile		
Model	n	standard deviation b 95% CI	Low	Medium b 95% CI	High b 95% CI	n	standard deviation b 95% CI	Low	Medium b 95% CI	High b 95% CI
		0.07 to 1.63		**0.27 to 3.40**	**0.11 to 3.72**		−0.07 to 1.29		**0.34 to 2.81**	−0.08 to 3.10

Model 1 adjusted for age, education, race/ethnicity, smoking and alcohol use

Model 2 additionally adjusted for body mass index and waist-hip ratio