Title: Salivary testosterone levels and health status in men and women in the British general population: findings from the third National Survey of Sexual Attitudes and Lifestyles (Natsal-3)

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Conflicts of interest

AMJ has been a Governor of the Wellcome Trust since 2011. FCWW has acted as a consultant for Bayer-Schering, Eli Lilly and Besins Healthcare and also participated in advisory board meetings and lectured on their behalf. FCWW has received lecture fees from Bayer-Schering and Besins Healthcare. FCWW has received grant support (2010-2014) from Bayer Schering AG and Besins Healthcare.

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Abstract

Context

Availability of highly specific and sensitive salivary measurement for testosterone by mass spectrometry presents the opportunity to examine health correlates of salivary testosterone (Sal-T) in a large-scale population survey.

Objective

To examine associations between Sal-T and health-related factors in men and women aged 18-74 years.

Design & Setting

Morning saliva samples were obtained from participants in a cross-sectional probability sample survey of the general British population ('Natsal-3').

Participants

1,599 men; 2,123 women.

Methods

Sal-T was measured using liquid chromatography tandem mass spectrometry; linear regression was used to examine associations between self-reported health factors and mean Sal-T.

Results

In men, mean Sal-T was associated with a range of health factors after age-adjustment, with multivariable analysis showing a strong independent negative association with BMI. Men reporting cardiovascular disease or currently taking medication for depression had lower age-adjusted Sal-T. The decline in Sal-T with increasing age remained after adjustment for health-related factors. In women, Sal-T declined with increasing age, however there were no age-independent associations with health-related factors or specific heath conditions, with the exception of higher Sal-T in smokers.

Conclusions

Sal-T levels were associated, independently of age, with a range of self-reported health markers, particularly BMI, in men but not women. The findings support the view that there is an age-related decline in Sal-T in men and women, which cannot be explained by an increase in ill-health. Our results demonstrate the potential of Sal-T as a convenient measure of tissue androgen exposure for population and clinical research.

Introduction

In men, testosterone deficiency with pituitary or testicular disease (male hypogonadism) is known to cause a wide range of symptoms and conditions including sexual dysfunction, loss of vigour, poor physical performance, fractures, and low mood, which can be effectively treated with Testosterone Replacement Therapy (TRT).(1) However, the more general relationship between male testosterone levels and ill-health is less well understood. Community-based studies in men have shown associations between lower average serum testosterone (serum-T) and poorer health outcomes, including frailty, obesity, insulin resistance, cardiovascular diseases and mortality, although findings have not always been consistent.(2–5) It is suggested that lower testosterone levels in older men may contribute to these ageing-related conditions although questions remain about direction and causality.(3) Some studies have also cast doubt as to whether there truly is an independent age-related decrease in testosterone, or whether the observed decline is a biomarker of accumulated co-morbidities.(6) Currently, whether testosterone treatment would benefit symptomatic older men with low testosterone remains a subject of intense debate and research.(7,8) Many of the existing studies have been carried out in middle-aged or older men, and the health implications of lower testosterone in younger men are unclear. In women, circulating concentrations of testosterone are typically around 5-10% of those in men, with distinct age-related declines, independent of the menopause.(9,10) The evidence for associations between testosterone and health in women is even more equivocal than for men.(10–13) Furthermore, research efforts in women have been seriously hampered by inadequate sensitivity of serum-T measurements, due to the low concentration of testosterone and poor specificity of commonly-used immunoassay methods.(14)

Salivary testosterone (Sal-T) is believed to represent tissue hormone levels which are unaffected by variations in circulating binding proteins(15) thereby providing an alternative to serum free testosterone (free-T), in the assessment of androgen status. In contrast to the collection of serum, which is invasive and expensive, collection of saliva is relatively straightforward and requires minimal training. We have recently demonstrated that Sal-T can be reliably and accurately measured by a highly sensitive and specific liquid chromatography tandem mass spectrometry (LC-MS/MS) method (Macdonald 2011). In a validation study comparing samples from the same individuals, we

found that Sal-T in adult men and women not only correlated more strongly with calculated serum free-T than serum total-T but was also unaffected by variations in SHBG.(16) Fiers at al (Add ref) confirmed the good correlation in both men and women between Sal-T and serum free-T measured by equilibrium dialysis but there was a significant systematic positive bias in women, which may reflect the influence of salivary protein binding to the lower female concentrations of Sal-T. We have established age-specific reference ranges for Sal-T in British men aged 18-69 years and women aged 18–74 years.(17) However, the physiological and behavioural correlates of Sal-T have not yet been explored.

Using data from the third National Survey of Sexual Attitudes and Lifestyles (Natsal-3), a probability sample survey of British men and women, we investigated whether Sal-T is associated, independently of age, with demographic characteristics, lifestyle, general health, and reported health conditions. We hypothesised that relationships between Sal-T and health-related factors in men would be similar to those previously observed with serum free-T. To our knowledge, this is the first study to have examined the associations between Sal-T and health in a large community sample of men and women using a highly sensitive and specific assay, exploiting the theoretical and practical advantages of salivary measurements to the full.

Methods

Participants and procedures

Full details of the Natsal-3 methods, including details of the saliva sample collection and testing, have are described elsewhere.(17–19) Briefly, Natsal-3 was a probability sample survey of 15,162 men and women aged 16-74 years resident in Britain. Interviews took place between September 2010 and August 2012 using a combination of computer-assisted personal interviewing (CAPI) and computer-assisted self-interview (CASI) for the more sensitive questions. The response rate was 57.7%.

Single morning saliva samples were self-collected from a subsample of men and women aged 18-74 years, who did not regularly work night shifts. Altogether, 9,170 eligible participants were invited to provide a saliva sample: 6,515 (71.0%) agreed to do so and 4,591 samples were received by the

laboratory and matched to the survey data (50.1% of those invited). A total of 463 samples were excluded due to problems with the sample quality(17) leaving 4,128 participants (45.0% of those invited) with a testosterone result (1,675 men; 2,453 women). This paper examines associations between Sal-T and health in the general population, therefore those with clinical conditions or taking medication likely to affect testosterone levels were excluded from analysis: currently taking medication for epilepsy (15 men; 15 women) or prostate disease (43 men); treatment for ovarian, testicular, or pituitary condition (16 men; 23 women) or for polycystic ovaries (35 women) in the past year; pregnant at interview (42 women); current receipt of HRT (62 women); ever receipt of HRT together with having had a hysterectomy (proxy measure for having had ovaries removed; 181 women); missing data for these questions (3 men; 15 women); resulting in 1,599 men and 2,123 women being included in the analysis. These exclusions aimed to minimise confounding of the relationship between testosterone and health caused by these factors, and differ from those used to define the 'healthy population' in our previous reference range analysis, which were based on criteria typically used in clinical reference range studies. (17)

Measures

Health and medication questions were self-reported, and body mass index (BMI) was calculated from self-reported height and weight. Due to small numbers of underweight individuals (BMI<18.5), these participants were excluded from analysis of BMI (14 men and 44 women). Most of the health questions were asked in the CAPI, with the exception of depressive symptoms (past 2 weeks) which were assessed in the CASI using a validated two-item patient health questionnaire (PHQ-2).(20) Cardiac, vascular disease, or hypertension were defined as ever being given such diagnoses by a doctor.

We previously identified seasonal variation in Sal-T, thus season of data collection was included as a potential confounder, defined as: winter (December, January, February), spring (March, April, May), summer (June, July, August), autumn (September, October, November).

The LC-MS/MS Sal-T assay was developed using strict validation criteria,(21) with a lower limit of quantification of 6.5 pmol/L. Full details of the laboratory methods, including the validation of the assay, have been published elsewhere.(16,17)

Statistical analyses

Statistical analyses were carried out using STATA (version 13.1) accounting for the complex survey design (stratification, clustering, and weighting of the sample).(22) We applied two weights: the survey weight corrected for unequal probability of selection and differential response (by age, sex, and region) to the survey itself; the additional saliva weight corrected for unequal probability of selection and differential response to the saliva sample. Factors found to be associated with providing a saliva sample included age at interview, ethnicity, self-reported general health, and sexual function; the saliva weighting significantly reduced these biases.(18)

Descriptive statistics are presented as means (standard errors), with multivariable linear regression used to assess differences between groups. Throughout, we censored very high Sal-T values so that, for each 10-year age group stratified by sex, values above the 99th percentile were assigned a value equal to that of the 99th percentile. The Sal-T data for men were normally distributed, however the distribution for women was positively skewed and so values were transformed on the natural log scale for analysis. Accordingly, for men we present linear regression coefficients representing differences in mean testosterone in pmol/l, whereas for women we present ratios of geometric mean Sal-T obtained from exponentiated coefficients. Interval regression was used to assign values to the range 0 to 6.5pmol/l for 3 men, and 0.5 (to allow log transformation) to 6.5pmol/l for 62 women with testosterone levels below the limit of detection (<6.5pmol/l). Age was adjusted for using both linear and quadratic terms to account for a non-linear relationship of testosterone with age. In our previous presentation of reference ranges,(17) we truncated analyses for men at age 69 as we were unable to calculate robust upper and lower limits based on standard deviation for older men. Here we have included data for the full age range of men (18-74 years) as the focus is on differences in mean Sal-T, rather than the upper and lower reference limits.

As several associations were found for men, multivariable analyses were used to determine which health factors were independently associated with Sal-T. Variables were grouped into a series of domains, in order to identify their individual contributions to the overall relationships with Sal-T after adjustment for earlier domains. The domains were: 1) age and season; 2) relationship status and children; 3) BMI and 4) general health. A fifth lifestyle domain (smoking, alcohol consumption; drug use) contained no significant associations (either age-adjusted or multivariable) and was therefore not presented in the final multivariable table. Within each domain the variables were entered into a forward stepwise model selection process (significance level for inclusion $p<0.1$) with variables selected from earlier domains included with certainty. The ordering of domains began with factors for which the evidence of association was best established (from earlier publications) or of a demographic nature, and then proceeded to health-related factors. In this way, any identified associations between Sal-T and health could be seen as robust and not explained by confounding factors in other domains. An equivalent analysis was not performed for women given the lack of age-adjusted associations. In further analysis we examined associations between specific health conditions or medical treatments and Sal-T, adjusting firstly for age and then for those factors that had been cumulatively selected from domains 1 to 3.

Ethics

The Natsal-3 study was approved by the Oxfordshire Research Ethics Committee A (reference: 09/H0604/27). Written informed consent was obtained for anonymised testing of saliva samples, without return of results.

Results

Age-adjusted associations of demographic and general health factors with mean salivary testosterone (Table 1 & 2, figures 1 & 2)

For both men and women, mean Sal-T decreased with increasing age, and seasonal variation was observed, as previously reported.(17) Among men, variation by relationship status was seen after adjustment for age (e.g. coefficient for those not in a steady relationship, compared with those

married/cohabiting: 20.12 (95% confidence interval: 6.41 to 33.82)); no association was found with having children. There were no associations with demographic factors for women.

Mean Sal-T was associated, independently of age, with a range of measures of general health in men but not in women. In age-stratified analyses, there was a clear association between mean Sal-T and increasing categories of BMI (normal, overweight, obese) in men but not women (figure 1), and a more moderate difference by self-reported general health among men, with no association for women (figure 2). Expressed as age-adjusted coefficients, we found levels were lower among men who were overweight or obese, (coefficients -23.63 (-34.81 to -12.46) and -51.26 (-64.64 to -37.88) respectively, compared with normal BMI) reported poorer general health (-16.14 (-27.50 to -4.78)), longstanding illness or disability (-13.36 (-27.50 to -4.78)); difficulty walking upstairs (-23.74 (-44.08 to -3.41) for those with much difficulty/inability, compared with no difficulty), or two or more comorbid health conditions (-21.57 (-35.10 to -8.04) compared with none). There was no association among men with smoking, alcohol consumption or illicit drug use.

The only health association found for women was with smoking, with higher Sal-T among women who smoked (ratio of geometric means 1.11 (1.02-1.22) for current smokers compared with non-smokers). Women who had taken hormonal contraception (HC) in the past year had lower mean Sal-T (Geometric mean ratio compared with those who hadn't taken HC: 0.85 (0.77-0.94), p=0.001). Adjusting the women's analysis for season as well as age did not change any of the results (data not shown). A sensitivity analysis excluding women on HC in the past year (n=590) produced generally similar results, except for smoking, which became non-significant (p=0.110; data not shown).

Multivariable analyses of men (table 3)

In men, all associations with variables in domains 1-3 in the age-adjusted analyses remained significant in the multivariable analyses: age, season, relationship status, and BMI. Only one variable from domain 4 - self-reported general health - was selected in the model, although providing inconclusive evidence of an association (-11.39 (-22.69 to -0.10) p=0.048). In further exploratory analysis we fitted a model excluding BMI but including age, season, and relationship status, in which

self-reported general health was found to be more strongly associated with Sal-T (-19.01 (-30.48 to -7.54) p=0.001; data not shown).

Specific health conditions (table 4)

Lower mean Sal-T was associated with reported cardiovascular disease (including hypertension) in men, independently of age (-18.06 (-29.43 to -6.68)). There was also an association with reporting currently taking medication for depression (-24.56 (-48.33 to -0.79)), but not with current depressive symptoms. There was no association with self-reported diabetes. Further adjustment for season, relationship status, and BMI attenuated the association with cardiovascular disease (-8.98 (-20.27 to 2.31); p=0.119) but strengthened the association with depression medication (-28.02 (-51.64 to -4.40); p=0.020). There was no evidence of associations between Sal-T and any of these conditions among women.

Discussion

Summary of findings and comparison with other studies

This study is the first to show associations between salivary testosterone (Sal-T) and health in a large national probability sample survey of men and women, across a wide age range. In men, we found significant age-independent associations between lower Sal-T and higher BMI, poorer self-reported general health, mobility problems, longstanding illness, and comorbid conditions (cardiovascular disease, treatment for depression). The association between Sal-T and self-reported general health in men was attenuated after adjustment for BMI, suggesting that the relationship is at least partly explained by obesity. We found no associations between Sal-T and health factors in women, except smoking, with higher Sal-T among women who smoked.

We showed that the age-related decline in Sal-T remains after adjusting for health and demographic factors. This is consistent with some previous serum studies,(3,5) although others have attributed the age-related decline in serum total T entirely to declining health.(6) This inconsistency may partly be explained by the age-related increase in sex hormone binding globulin (SHBG) which attenuated the age-trend in total serum-T, but not Sal-T.

Our findings of associations with health-related factors in men are largely consistent with evidence from serum studies. There is a large body of evidence that, in men, obesity is strongly associated with lower serum-T, independently of age.(3) Associations have also been reported between lower serum-T and insulin resistance and diabetes, pre-clinical indicators for cardiovascular disease (CVD), cardiovascular events, physical frailty and increased mortality.(3,5,23,24) Cross-sectional data cannot shed light on the direction of these relationships but longitudinal studies have found that obesity leads to decreases in testosterone, and that weight loss increases testosterone levels in obese men,(25) although there is also evidence of bidirectional associations.(3) Longitudinal serum-T studies have shown that low testosterone precedes cardiovascular events.(23) The mechanisms of this association are unclear and may involve low testosterone affecting several cardiovascular risk factors, central adiposity and inflammation.(26) Unlike most serum-T studies we did not find an association with self-reported diabetes for men, but the number of men with diabetes in our sample was small. We found no association with current depressive symptoms but did observe an association with reporting treatment for depression. Little research exists on the effects of antidepressants on the hypothalamic-pituitary-gonadal axis, although one study has found higher salivary testosterone levels among men and women using Selective Serotonin Reuptake Inhibitors (SSRIs), which contrasts with our results.(27)

We examined the associations between Sal-T and several demographic factors in order to address potential confounding of the associations with health. A previous study found ethnic variation in testosterone levels, however we were unable to examine this due to small numbers of participants in ethnic minority groups. The findings of this study only partly concur with those from (generally smaller) studies which have reported lower testosterone among men and women in established relationships (28) and parents, especially those actively involved in childcare(29,30). We found associations with relationship status for men only, and no associations with parenthood for either men or women, however our measures of parenthood capture a broad range of circumstances in terms of children's age and parents' involvement in child-rearing, which may explain this apparent discrepancy.

We found no association between Sal-T and smoking, alcohol consumption, or drug use in men. Previous serum-T in men studies have generally, although not always consistently, shown smoking to be associated with increased total-T, yet few studies have shown an association with free-T.(31) Smoking, via direct effects on liver function, increases SHBG levels, which may explain why total-, but not free-, testosterone is increased in smokers.(31) The evidence regarding whether testosterone is associated with alcohol consumption is also mixed,(24,32) and few studies have examined associations with other drugs.

Previous research about the health correlates of testosterone in women has not only yielded inconsistent findings, but has largely been carried out within narrow age ranges, and using sub-optimal measures of testosterone.(14) We found no evidence of associations between Sal-T and general health indicators, or specific conditions or medications in women. We did find a positive association with smoking, which is consistent with other studies.(11,33) We also found lower mean Sal-T among women who had taken hormonal contraception in the past year, which supports evidence from smaller experimental studies showing increases in SHBG and corresponding decreases in serum free-T among women taking oral contraceptives.(34) Our findings regarding the different relationship with health for men and women may relate to the different sites of production, with testosterone coming from the adrenal glands as well as the ovaries in women, but exclusively from the testes in men, the menstrual cyclical fluctuations and the much lower concentrations of Sal-T in women compared to men.

Strengths and limitations

A key strength of this study is the highly sensitive and specific Sal-T assay, enabling accurate measurement in men as well as women, (albeit there is a systematic positive bias due to salivary protein binding among women, and permitting measurement of testosterone on a large-scale probability sample survey across a wide age range. Although less invasive than serum, large-scale home-based collection of saliva has presented a number of challenges including: coping with the

diurnal variation in testosterone levels, preventing contamination, ensuring prompt receipt at the laboratory before deterioration, and minimising non-response bias. Considerable attention was paid to the development of protocols for sample collection, with extensive validation and piloting,(18) yet although 71.0% of participants agreed to provide a sample, useable samples were received from only 45.0% of those invited, highlighting the challenge of obtaining self-collected and self-posted samples. This response rate is similar to community-based serum testosterone studies,(35,36) and the response rate to the survey overall was similar to other major British social surveys. To minimise potential non-response bias, both to the survey and to the saliva sample, we applied statistical weights during analysis.(18)

The health data collected were self-reported and are therefore reliant on knowledge of conditions and medications, and accurate reporting. This may particularly affect BMI based on self-reported height and weight, as people tend to underestimate weight and overestimate height. However, previous studies have shown self-reported height and weight to be sufficient for examining associations in epidemiological studies.(37) As a sexual health survey, only a limited number of questions about general health could be asked, therefore we were unable to look at associations with some factors that may have been of interest such as frailty, osteoporosis, or sleep disturbance. We were also unable to measure preclinical disease indicators.

Only one sample was collected from each participant and so we were unable to take into account intra-individual variation in testosterone levels, which may be particularly relevant for pre-menopausal women as testosterone varies throughout the menstrual cycle.(38) However, some have argued that it is unnecessary to control for menstrual variation, given the relatively small effects compared with, for example, diurnal variation or individual differences.(38) This is consistent with our earlier validation work in which did not find significant within-individual differences when samples were taken at weekly and monthly intervals.(16) A small number of men and women (n=20 and n=37 respectively) included in our analysis reported receiving cancer treatment in the past year, and as the nature of the cancer or treatment was unknown, this could have affected testosterone levels for some. However, given the small numbers this is unlikely to have affected our findings overall.

Implications for clinical research, policy, and further research need

Our findings are broadly consistent with previous research using serum, and where differences exist it is not always clear whether these are due to differences in the measure used (serum total-T, free-T, or Sal-T), or due to other differences in the study population and design. Further observational research using reliable saliva T measurements linked to a broader range of clinical correlates would strengthen the evidence base in this respect.

There are concerns about inappropriate marketing and use of testosterone replacement therapy (TRT) for men, particularly in the USA.(7) Our cross-sectional finding of lower Sal-T among men with poorer health does not imply causality, nor indicate treatment. Although longitudinal research has shown that low testosterone precedes poor health outcomes(23) the benefits of TRT in the general population remain unclear. Further research is needed before conclusions can be reached regarding the nature of the relationship between testosterone and ill-health, and the risks and benefits of intervention.

Our finding of an independent age-related decline in Sal-T suggests that reproductive senescence in men, as in women, is not solely the consequence of poor health. The clinical significance of this merits further investigation using Sal-T as well as serum-T measurements. The application of Sal-T measurement in future research should make a significant contribution towards clarifying the role of low testosterone in health and ageing in men and women

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Table 1: Sample characteristics, mean salivary Testosterone (Sal-T), and associations between Sal-T and demographic and health factors: MEN

	Denominators (n) unwt, wt		mean sal-T (pmol/l)	(SE)	age-adjusted# coefficients	(95% CI)
All men	*1599*	*1886*	223.49	3.33		
Age, season, region						
Age group						p<0.0001
18-24	*186*	*242*	315.81	9.14	-	-
25-34	*245*	*328*	264.62	7.77	-51.19	(-74.44, -27.94)
35-44	*236*	*363*	234.16	8.42	-81.65	(-105.90, -57.41)
45-54	*297*	*388*	205.74	5.11	-110.08	(-130.78, -89.37)
55-64	*330*	*334*	174.63	4.34	-141.18	(-160.84, -121.52)
65-74	*305*	*231*	151.72	3.74	-164.09	(-183.51, -144.68)
Season						p=0.0360
winter	*400*	*500*	235.15	6.93	-	-
spring	*464*	*521*	223.77	6.09	-5.75	(-20.90, 9.40)
summer	*421*	*457*	207.52	6.02	-20.73	(-36.74, -4.72)
autumn	*314*	*408*	226.74	6.40	-2.95	(-19.42, 13.52)
Region						p=0.2432
Scotland & North of England	*558*	*639*	230.52	5.74	-	-
Midlands & Wales	*355*	*391*	224.33	6.48	-7.20	(-21.22, 6.81)
East of England & South of England	*686*	*856*	217.86	5.17	-11.08	(-24.14, 1.99)
Relationship and children						
Relationship status						p=0.0103
Married / civil partnership / cohabiting	*928*	*1277*	206.71	3.95	-	-
Steady relationship, not living together	*187*	*179*	263.63	8.34	14.62	(-1.89, 31.13)
Not in a steady relationship	*459*	*405*	261.46	6.97	20.12	(6.41, 33.82)
Any natural children						p=0.374
No	*642*	*736*	256.27	5.99	-	-
Yes	*928*	*1104*	204.97	3.62	-5.80	(-18.61, 7.00)
Lives with a child (aged <18y)						p=0.7611
No	*1281*	*1342*	220.84	3.76	-	-
Yes	*318*	*544*	230.05	6.18	-2.17	(-16.21, 11.86)
BMI						
BMI$						p<0.0001
Normal (BMI 18.5-25 kg/m2)	*599*	*723*	253.68	5.07	-	-
Overweight (BMI 25-30 kg/m2)	*612*	*715*	209.82	4.09	-23.63	(-34.81, -12.46)
Obese (BMI >30 kg/m2)	*334*	*365*	174.34	5.72	-51.26	(-64.64, -37.88)
General health and function						
Self-reported general health						p=0.0054
Very good/good	*1253*	*1510*	232.72	3.83	-	-
Fair/bad/very bad	*346*	*375*	186.37	5.08	-16.14	(-27.50, -4.78)
Longstanding illness or disability						p=0.0131
No	*977*	*1251*	238.63	4.15	-	-
Yes	*621*	*634*	193.69	4.54	-13.36	(-23.91, -2.81)
Difficulty walking upstairs because of a health problem						p=0.0499
No difficulty	*1369*	*1643*	230.41	3.59	-	-
Some difficulty	*166*	*177*	182.11	7.60	-8.31	(-23.34, 6.71)
Much difficulty or unable	*64*	*66*	162.09	12.08	-23.74	(-44.08, -3.41)
Number of comorbid conditions*						p=0.0073
0	*913*	*1169*	245.12	4.38	-	-
1	*397*	*435*	201.40	5.87	-11.10	(-24.18, 1.98)
2+	*289*	*282*	167.97	5.49	-21.57	(-35.10, -8.04)
Lifestyle						
Current smoker					p=0.6599	
No	*1247*	*1458*	219.63	3.60	-	-
Yes	*352*	*428*	236.67	7.20	3.01	(-10.42, 16.45)
Average alcohol consumption per week					p=0.3611	
None	*295*	*343*	213.51	7.44	-	-
Not more than recommended	*1130*	*1352*	226.02	3.98	9.51	(-5.57, 24.59)
More than recommended	*167*	*183*	223.35	9.83	14.50	(-7.32, 36.32)
Taken non-prescribed drugs, past year					p=0.7584	
No	*1363*	*1585*	218.03	3.52	-	-
Yes	*197*	*244*	264.34	9.03	2.89	(-15.56, 21.34)

Table 1 footnotes:
#adjusted for age and age-squared to account for non-linear relationship between testosterone and age
*Measure of comorbidity includes arthritis, heart attack, coronary heart disease, angina, other forms of heart disease, hypertension, stroke, diabetes, broken hip or pelvis bone or hip replacement ever, backache lasting longer than 3 months, any other muscle or bone disease lasting longer than 3 months, treatment for depression, treatment for cancer, and treatment for any thyroid condition in the past year.
$Those with BMI<18.5 have been excluded from analysis due insufficient numbers to analyse this group separately

Table 2: Sample characteristics, mean salivary Testosterone (Sal-T), and associations between Sal-T and demographic and health factors: WOMEN

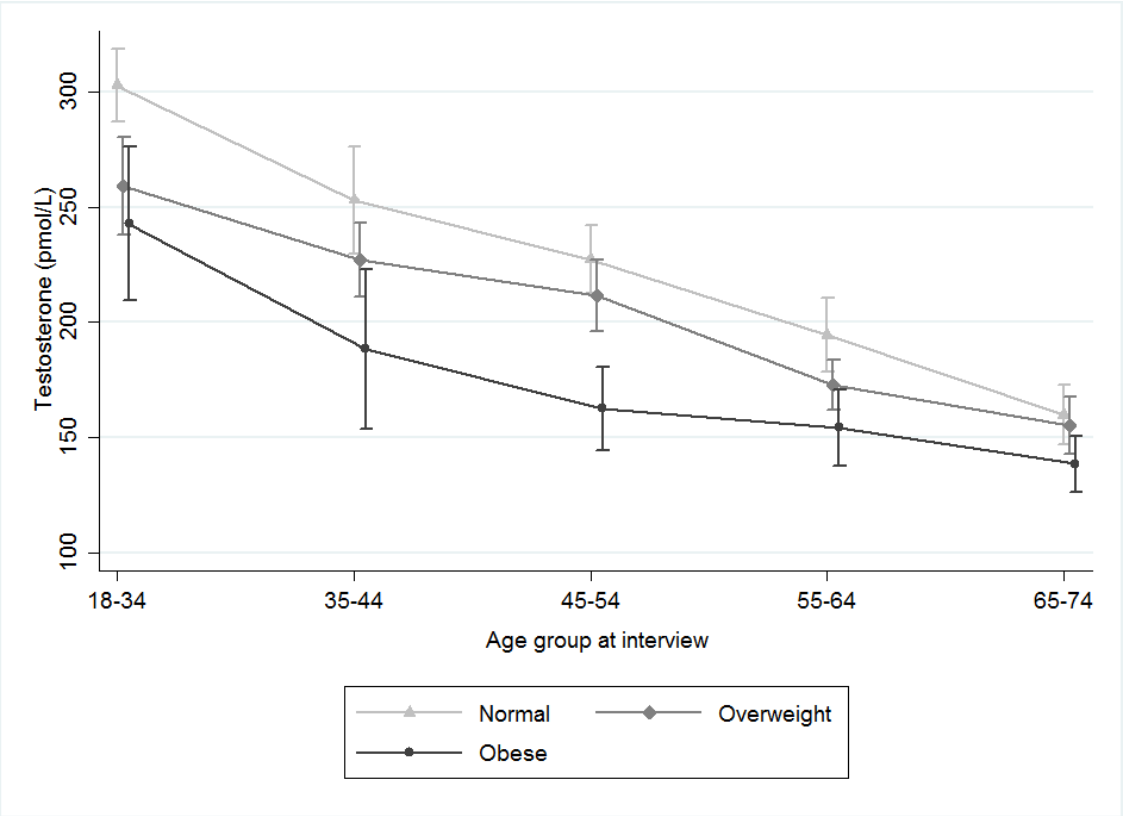
	Denominators (n) (unwt, wt)		mean sal-T (pmol/l)	(SE)	age-adjusted Ratios#	(95% CI)
All	*2123*	*1899*	37.09	0.86		
Age, season, region						
Age group					p<0.0001	
18-24	*231*	*252*	49.98	4.15	-	-
25-34	*390*	*359*	41.74	1.82	0.93	(0.78, 1.12)
35-44	*391*	*381*	40.49	1.92	0.91	(0.76, 1.09)
45-54	*408*	*390*	34.16	1.55	0.76	(0.63, 0.91)
55-64	*378*	*304*	27.35	0.98	0.63	(0.53, 0.75)
65-74	*325*	*213*	27.24	1.25	0.60	(0.50, 0.72)
Season					p<0.0001	
winter	*529*	*460*	31.57	1.09	-	-
spring	*652*	*556*	37.71	1.95	1.15	(1.05, 1.26)
summer	*491*	*394*	42.46	1.83	1.28	(1.16, 1.40)
autumn	*451*	*489*	37.27	1.65	1.09	(0.98, 1.20)
Region					p=0.2229	
Scotland & North of England	*741*	*632*	35.66	1.19	-	-
Midlands & Wales	*475*	*408*	35.09	2.19	0.96	(0.87, 1.05)
East of England & South of England	*907*	*859*	39.10	1.32	1.04	(0.96, 1.13)
Relationship and children						
Relationship status					p=0.9109	
Married / civil partnership / cohabiting	*1203*	*1234*	35.50	0.92	-	-
Steady relationship, not living together	*257*	*196*	42.54	2.39	1.01	(0.89, 1.14)
Not in a steady relationship	*646*	*449*	39.75	2.08	1.02	(0.94, 1.10)
Any natural children					p=0.6694	
No	*597*	*520*	43.25	2.25	-	-
Yes	*1497*	*1336*	34.67	0.78	1.02	(0.93, 1.12)
Lives with a child (aged <18y)					p=0.5441	
No	*1409*	*1179*	35.80	1.15	-	-
Yes	*711*	*717*	39.09	1.29	1.03	(0.94, 1.12)
BMI						
BMI$					p=0.7452	
Normal (BMI 18.5-25 kg/m2)	*965*	*870*	39.39	1.23	-	-
Overweight (BMI 25-30 kg/m2)	*586*	*504*	34.82	1.37	0.97	(0.89, 0.94)
Obese (BMI >30 kg/m2)	*428*	*355*	35.13	1.53	0.97	(0.90, 0.94)
General health and function						
Self-reported general health					p=0.3880	
Very good/good	*1753*	*1560*	37.91	0.98	-	-
Fair/bad/very bad	*370*	*339*	33.33	1.65	0.96	(0.88-1.05)
Longstanding illness or disability					p=0.8803	
No	*1360*	*1281*	38.21	1.11	-	-
Yes	*763*	*618*	34.77	1.37	1.01	(0.92-1.10)
Difficulty walking upstairs because of a health problem					p=0.4682	
No difficulty	*1787*	*1598*	38.11	0.95	-	-
Some difficulty	*241*	*221*	32.92	2.62	0.99	(0.87, 1.13)
Much difficulty or unable	*95*	*80*	28.4	2.79	0.87	(0.69, 1.09)
Number of comorbid conditions*					p=0.5587	
0	*1177*	*1101*	39.52	1.22	-	-
1	*546*	*474*	35.01	1.33	0.99	(0.91, 1.08)
2+	*400*	*324*	31.91	2.00	0.93	(0.82, 1.06)
Lifestyle						
Current smoker					p=0.0165	
No	*1714*	*1548*	35.88	0.82	-	-
Yes	*409*	*351*	42.44	2.45	1.11	(1.02, 1.22)
Average alcohol consumption per week					p=0.3272	
None	*640*	*606*	36.04	1.57	-	-
Not more than recommended	*1280*	*1113*	37.97	1.13	1.06	(0.98, 1.16)
More than recommended	*198*	*175*	35.23	2.08	1.02	(0.91, 1.15)
Taken non-prescribed drugs, past year					p=0.3064	
No	*1970*	*1746*	36.68	0.90	-	-
Yes	*115*	*106*	46.44	4.01	1.09	(0.93, 1.28)

Table 2 footnotes:
unwt=unweighted; wt=weighted
#Ratio of geometric means, obtained from exponentiated age-adjusted linear regression coefficients of log-transformed data for women. Adjusted for age and age-squared to account for non-linear relationship between testosterone and age
*Measure of comorbidity includes arthritis, heart attack, coronary heart disease, angina, other forms of heart disease, hypertension, stroke, diabetes, broken hip or pelvis bone or hip replacement ever, backache lasting longer than 3 months, any other muscle or bone disease lasting longer than 3 months, treatment for depression, treatment for cancer, and treatment for any thyroid condition in the past year.
$Those with BMI<18.5 have been excluded from analysis due insufficient numbers to analyse this group separately

Figure 1: Mean (95% Confidence Interval) Sal-T (pmol/l) by age group and BMI

Note: figures for men and women have different scales

a) Men



b) Women

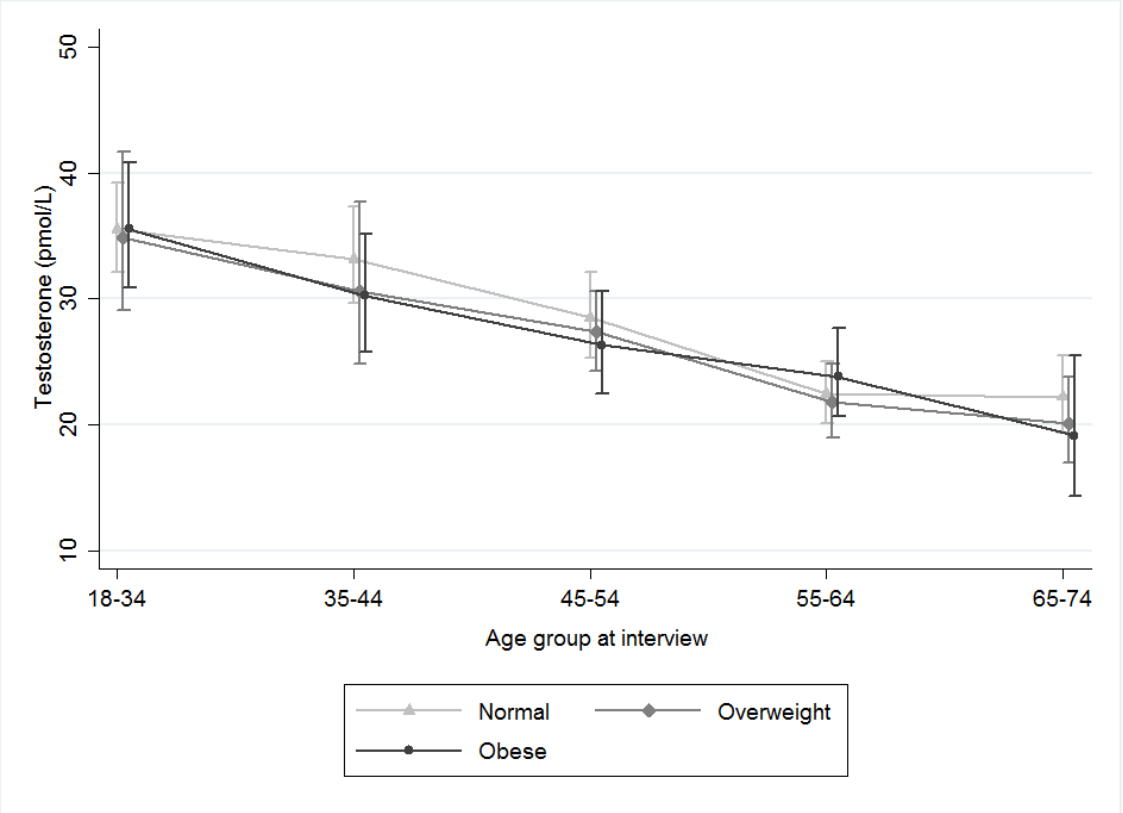
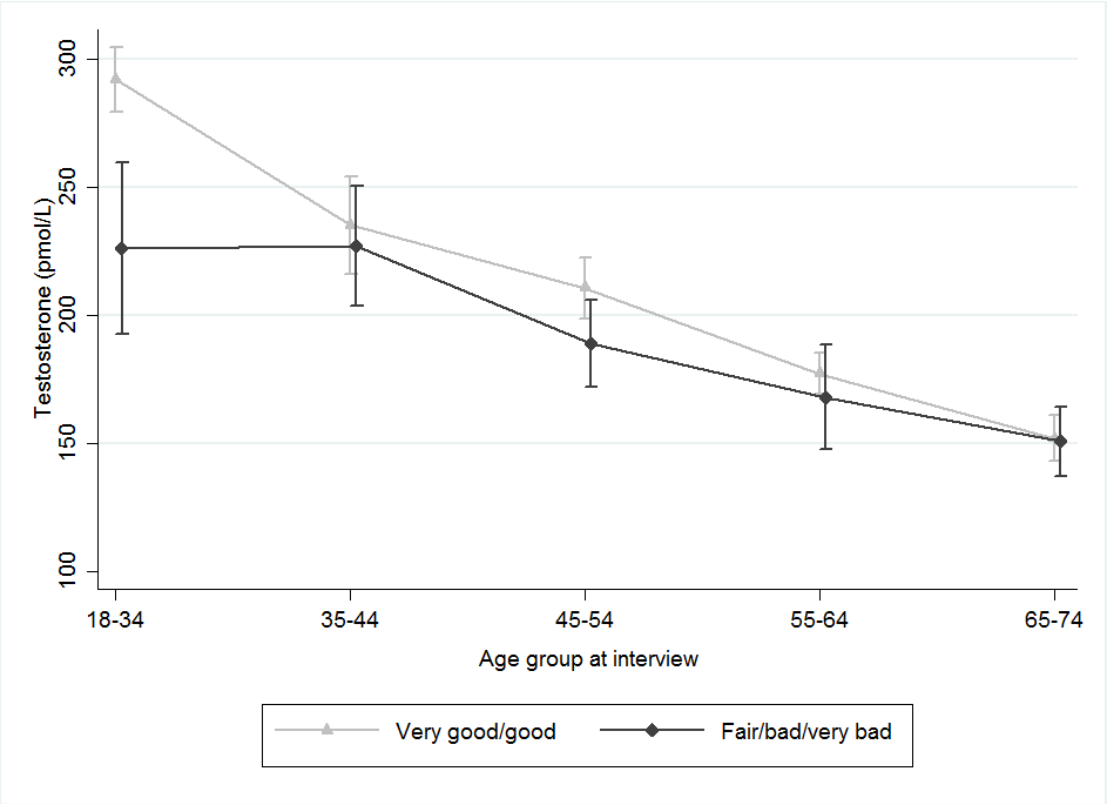


Figure 2: Mean (95% Confidence Interval) Sal-T (pmol/l) by age group and self-reported general health status
Note: figures for men and women have different scales

a) Men



b) Women

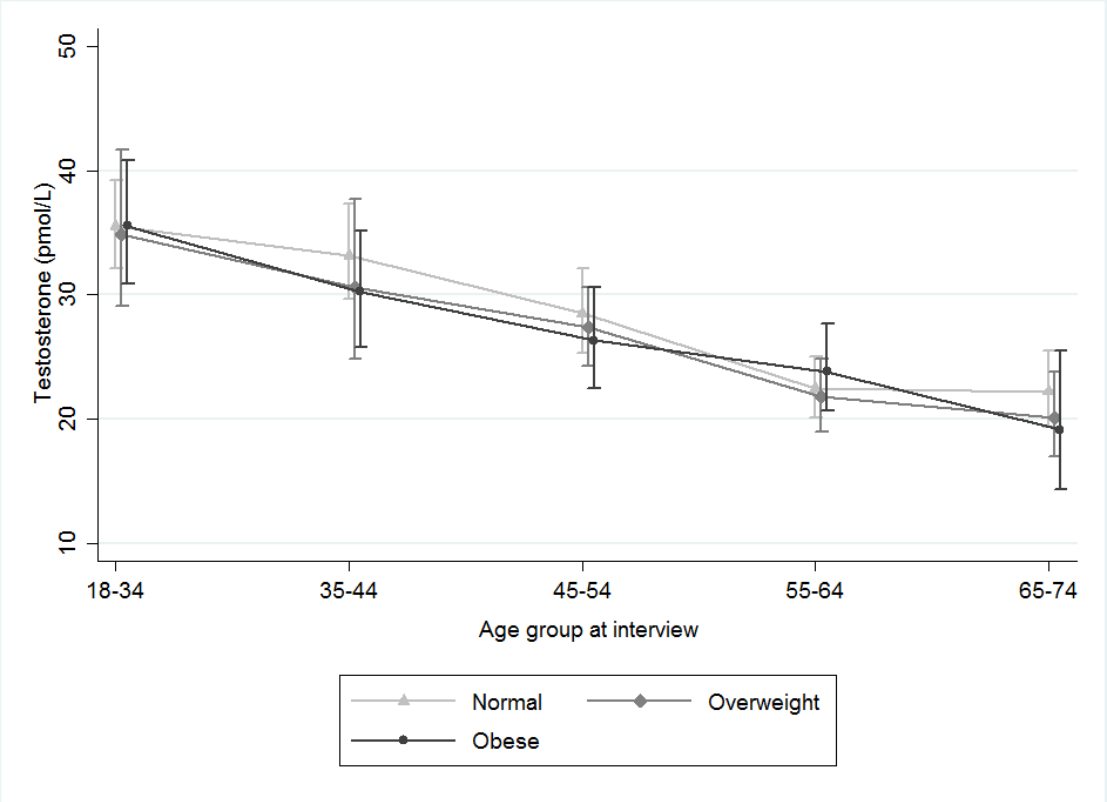


Table 3: Adjusted associations between demographic and health factors and salivary Testosterone: MEN

	Denominators (n) (unwt, wt)		age-adjusted coefficients	(95% CI)	Domain 1 adjusted coefficients	(95% CI)	Domain 2 adjusted coefficients	(95% CI)	Domain 3 adjusted coefficients	(95% CI)	Domain 4 adjusted coefficients	(95% CI)
Age, season, region												
Age				p<0.0001		p<0.0001		p<0.0001		p<0.0001		p<0.0001
18-24	*186*	*242*	-	-	-	-	-	-	-	-	-	-
25-34	*245*	*328*	-51.19	(-74.44, -27.94)	-52.08	(-75.40, -28.75)	-41.05	(-65.54, -16.56)	-33.70	(-58.31, -9.09)	-31.90	(-56.44, -7.35)
35-44	*236*	*363*	-81.65	(-105.90, -57.41)	-81.66	(-105.55, -57.78)	-69.74	(-94.08, -45.39)	-62.79	(-86.52, -39.07)	-60.66	(-84.61, -36.71)
45-54	*297*	*388*	-110.08	(-130.78, -89.37)	-109.69	(-130.35, -89.03)	-97.21	(-118.62, -75.81)	-82.80	(-104.26, -61.35)	-79.74	(-101.33, -58.16)
55-64	*330*	*334*	-141.18	(-160.84, -121.52)	-140.78	(-160.37, -121.18)	-126.87	(-147.78, -105.96)	-111.77	(-132.95, -90.59)	-107.91	(-128.99, -86.82)
65-74	*305*	*231*	-164.09	(-183.51, -144.68)	-163.73	(-183.04, -144.42)	-149.80	(-170.91, -128.70)	-134.69	(-155.81, -113.56)	-130.48	(-151.83, -109.13)
Season				p=0.0360		p=0.0360		p=0.0256		p=0.0368		p=0.0294
winter	*400*	*500*	-	-	-	-	-	-	-	-	-	-
spring	*464*	*521*	-5.75	(-20.90, 9.40)	-5.75	(-20.90, 9.40)	-6.57	(-21.79, 8.66)	-1.93	(-15.68, 11.81)	-2.00	(-15.76, 11.75)
summer	*421*	*457*	-20.73	(-36.74, -4.72)	-20.73	(-36.74, -4.72)	-21.48	(-37.47, -5.49)	-16.41	(-30.21, -2.61)	-16.76	(-30.56, -2.96)
autumn	*314*	*408*	-2.95	(-19.42, 13.52)	-2.95	(-19.42, 13.52)	-2.90	(-19.44, 13.64)	1.24	(-13.03, 15.52)	1.46	(-12.79, 15.71)
Relationship and children												
Relationship status				p=0.0103				p=0.0073		p=0.0185		p=0.0110
Married / civil partnership / cohabiting	*928*	*1277*	-	-			-	-	-	-	-	-
Steady relationship, not living together	*187*	*179*	14.62	(-1.89, 31.13)			16.02	(-0.55, 32.58)	11.18	(-4.54, 26.91)	11.68	(-4.07, 27.42)
Not in a steady relationship	*459*	*405*	20.12	(6.41, 33.82)			20.50	(6.85, 34.15)	18.52	(5.13, 31.91)	20.10	(6.50, 33.70)
Any natural children				p=0.374								
No	*642*	*736*	-	-								
Yes	*928*	*1104*	-5.80	(-18.61, 7.00)								
Lives with a child (aged <18y)				p=0.7611								
No	*1281*	*1342*	-	-								
Yes	*318*	*544*	-2.17	(-16.21, 11.86)								
BMI										p<0.0001		p<0.0001
BMI$				p<0.0001								
Normal (BMI 18.5-25 kg/m2)	*599*	*723*	-	-					-	-	-	-
Overweight (BMI 25-30 kg/m2)	*612*	*715*	-23.63	(-34.81, -12.46)					-22.40	(-33.63, -11.17)	-22.33	(-33.56, -11.11)
Obese (BMI >30 kg/m2)	*334*	*365*	-51.26	(-64.64, -37.88)					-49.69	(-62.84, -36.54)	-47.59	(-61.22, -33.96)
General health and function												
Self-reported general health				p=0.0054								p=0.048
Very good/good	*1253*	*1510*	-	-							-	-
Fair/bad/very bad	*346*	*375*	-16.14	(-27.50, -4.78)							-11.39	(-22.69, -0.10)
Longstanding illness or disability				p=0.0131								
No	*977*	*1251*	-	-								
Yes	*621*	*634*	-13.36	(-23.91, -2.81)								
Difficulty walking up stairs because of a health problem				p=0.0499								
No difficulty	*1369*	*1643*	-	-								
Some difficulty	*166*	*177*	-8.31	(-23.34, 6.71)								
Much difficulty or unable	*64*	*66*	-23.74	(-44.08, -3.41)								
Number of comorbid conditions*				p=0.0073								
0	*913*	*1169*	-	-								
1	*397*	*435*	-11.10	(-24.18, 1.98)								
2+	*289*	*282*	-21.57	(-35.10, -8.04)								

Table 3 footnotes:

unwt=unweighted; wt=weighted

Variables were grouped into a hierarchy of 'domains' as follows: 1. Age, season, region; 2. Relationship status and family; 3. BMI; 4. General health and function. 'Lifestyle' factors (smoking, drinking, drug use' were not included in the adjusted analysis given lack of associations seen in age-adjusted analysis.

The variables were entered in these groups in order, and including any variables found to be significant from previous domains, into forwards stepwise linear (interval) regression models to generate adjusted coefficients. The significance criteria for retention in the model was $p<0.1$.

Table 4: Associations between reporting specific health conditions and medical treatmentsand salivary Testosterone (Sal-T) by gender

	Men						Women					
	Denominators (n) unwt, wt		mean sal-T (pmol/l)	(SE)	age-adjusted coefficients	(95% CI)	*Denominators (n) unwt, wt*		mean sal-T (pmol/l)	(SE)	age-adjusted ratios#	(95% CI)
All	*1599*	*1886*	223.49	3.33			*2123*	*1899*	37.09	0.86		
Any cardiac or vascular disease or hypertension					p=0.0019						p=0.6863	
no	*1257*	*1548*	235.47	3.75	-	-	*1789*	*1625*	37.99	0.95	1.00	-
yes	*342*	*338*	168.6	4.92	-18.06	(-29.43, -6.68)	*334*	*275*	31.79	1.71	0.98	(0.88, 1.09)
Diabetes					p=0.0932						p=0.4689	
no	*1482*	*1769*	227.47	3.4	-	-	*2036*	*1828*	37.34	0.87	1.00	-
yes	*117*	*116*	162.98	8.82	-15.55	(-33.71, 2.61)	*87*	*71*	30.67	2.54	0.93	(0.77, 1.13)
Currently taking medication for depression					p=0.0428						p=0.5670	
no	*1513*	*1810*	224.79	3.42	-	-	*1930*	*1737*	37.29	0.92	1.00	-
yes	*86*	*75*	192.34	11.61	-24.56	(-48.33, -0.79)	*193*	*163*	34.99	2.11	0.97	(0.86, 1.09)
Depressive symptoms^					p=0.7057						p=0.7079	
no	*1386*	*1644*	223.84	3.41	-	-	*1864*	*1646*	37.13	0.81	1.00	-
yes	*176*	*186*	228.68	11.25	-4.00	(-24.77, 16.77)	*222*	*206*	38.04	3.74	0.97	(0.85, 1.12)

Table 4 footnotes:

unwt=unweighted; wt=weighted

^Participants were asked whether they had often been bothered by feeling down, depressed, or hopeless in the past 2 weeks, and whether they had often been bothered by little interest or pleasure in doing things in the past 2 weeks, using a validated two-question patient health questionnaire (PHQ-2).

#Ratio of geometric means, obtained from exponentiated age-adjusted linear regression coefficients of log-transformed data for women