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Higher circulating androgens and higher physical activity levels are associated with less central adiposity and lower risk of cardiovascular death in older men

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Abstract

Objective

Low endogenous sex hormones and low physical activity (PA) levels have been associated with CVD risk. Whether these interact to influence CVD outcomes remains unclear. We assessed whether sex hormone concentrations and PA were additively associated with lower central adiposity and CVD risk.

Patients

3,351 community-dwelling men, mean age 77 years.

Measurements

Baseline testosterone (T), dihydrotestosterone (DHT) and estradiol (E2) were assayed. Levels of PA were ascertained by questionnaire. Men were stratified using median splits into high hormone+high PA (H/H), high hormone+low PA (H/L); low hormone+high PA (L/H) and low hormone+low PA (L/L) groups.

Results

865 CVD events and 499 CVD deaths occurred during 10-year mean follow-up. Men with higher T, DHT or SHBG and higher PA had the lowest BMI, waist circumference and risk of metabolic syndrome. Men with higher T had the lowest risk of incident CVD events, irrespective of PA level. Men with higher T or DHT and higher PA had the lowest risk of

dying from CVD (e.g. hazard ratios for T/PA H/H 0.76 p=0.031; H/L 0.85 p=0.222; L/H 0.80 p=0.075; L/L 1.00).

Conclusion

Higher circulating androgens and higher PA were associated with less central adiposity at baseline and fewer CVD deaths during follow-up. These findings are consistent with a potential additive effect of androgens and PA on cardiometabolic outcomes in older men.

Key terms: testosterone, dihydrotestosterone, physical activity, metabolic syndrome, cardiovascular disease.

Introduction

Older men, as a group, have lower T concentrations compared with younger men,[1] and have a higher prevalence of cardiovascular disease (CVD).[2] An inverse relationship exists between T concentrations and the risk of metabolic syndrome (MetS), mediated via associations of lower T with hypertriglyceridemia and abdominal obesity.[3] Epidemiological studies have associated lower endogenous T concentrations with a higher risk of CVD.[4] Higher concentrations of T and dihydrotestosterone (DHT), a ligand for the androgen receptor, have been shown to be independent predictors for reduced incidence of stroke[5] with DHT also linked to decreases in ischaemic heart disease mortality.[6]

Of note, the effects of T therapy on the cardiovascular system remain unclear. One randomised controlled trial (RCT) of T supplementation was terminated prematurely due to adverse cardiovascular events[7] and the Cardiovascular sub-study of T-Trials showed

increased non-calcified coronary plaque after 12 months of T treatment.[8] However, it is unclear how this change in non-calcified plaque volume relates to the risk of major adverse cardiovascular events.[9] Interestingly, in the T trials itself, there was a low and equal number of cardiovascular events in each group (7 in each arm).[10] Furthermore, retrospective case control studies have associated T prescriptions with a lower risk of CV events.[11-13] This includes a large (n=83,010) retrospective study in men with low T levels by Sharma, et al.[12] in which the authors concluded there was a significant reduction in all-cause mortality, myocardial infarction and stroke following normalization of T levels.

Low circulating T in older men is also associated with increased risk of frailty[14] and increased fat mass, decreased muscle strength and decreased bone mineral density,[15] all of which can respond positively to exercise-based interventions.[16] Physical activity (PA) levels are a predictor of reduced CVD risk and all-cause mortality.[2] T has important anabolic actions on skeletal muscle, which are comparable between younger and older men.[17] The T Trials were conducted in 790 men aged ≥65 years and older, with baseline T <9.5 nmol/L[10]. Although the primary outcome for the Physical Function Trial was negative, T supplementation was associated with modest improvement in walking distance.[10] Thus, a close relationship appears to exist between T and PA, and both have potential beneficial effects on physical performance.

These observations highlight the importance of exploring the association of T on CVD risk and on possible intermediate mediators such as central adiposity, as well as examining whether PA interacts with T to influence these outcomes. In this study, we performed a cross-sectional analysis to determine whether higher circulating androgens and higher PA levels are additively associated with lower risk of central adiposity and MetS. We then undertook a

longitudinal analysis to assess whether higher circulating androgens and higher PA were additively associated with lower rates of CVD-related events and mortality.

Subjects and methods

Study population

The Health In Men Study (HIMS) is a population-based cohort study of community-dwelling older men from Perth, Western Australia, which has been described previously.[18] In brief, 12,203 men aged ≥65 years completed a questionnaire and attended for a physical examination in wave 1 (W1, 1996–1999); 4,248 of these men who were by then aged 70-89 years attended for reassessment and venesection in wave 2 (W2, 2001– 2004). Men were predominantly of Caucasian ethnic origin. The University of Western Australia Human Research Ethics Committee approved the study, and all men gave written informed consent.

Physical activity

Physical activity (PA) was assessed using questionnaire in W1. Men were asked if they currently undertook recreational exercise in a usual week and, if so, the time (hours and minutes) spent performing 'non-vigorous' and 'vigorous' exercise, separately. The questionnaire included examples of each type of activity: slow walking, slow cycling, Tai Chi and Yoga for non-vigorous activity; and fast walking, jogging, aerobics, vigorous swimming, vigorous cycling, tennis, football and squash for vigorous activity. For the purpose of this analysis we defined PA in a usual week as the sum of the number of hours of non-vigorous and 2x the number of hours of vigorous physical activities to reflect the higher intensities associated with vigorous activities.

Laboratory assays

Blood samples were collected between 8:00 and 10:30 AM at W2. Plasma was prepared immediately after phlebotomy and stored at -80°C until assayed. Total T, DHT, and E2 were quantified within a single LC-MS/MS run without derivatization using atmospheric pressure photoionization in positive mode for androgens and negative mode for estrogens[19] from 200-μL samples as reported previously.[20] Precision profiles displayed coefficients of variation <6%for T levels (>0.4 nmol/L), <13% for DHT levels (>0.7 nmol/L), and <8% for E2 levels (>25pmol/L). LH and SHBG had been determined previously by chemiluminescent immunoassays on an Immulite 2000 analyzer (DPC-Biomediq) with coefficients of variation of <7% for both.

Metabolic syndrome and prevalent cardiovascular disease definitions

The metabolic syndrome score was defined using five risk components (hypertension, hyperglycaemia, hypertriglyceridaemia, high-density lipoprotein (HDL) cholesterol and central obesity) according to the National Cholesterol Education Program *Adult Treatment Panel* III 2005 criteria.[21] Accordingly, hypertension was defined as systolic blood pressure ≥130mmHg, or diastolic blood pressure ≥85mmHg or drug treatment for hypertension. Hyperglycaemia was defined as fasting glucose ≥5.6mmol/L or non-fasting glucose >7.8mmol/L. Hypertriglyceridaemia was defined as triglycerides ≥1.7mmol/L. Low high-density lipoprotein (HDL) cholesterol was defined as HDL≤1.0mmol/L. Central obesity was defined as waist circumference ≥102cm. A participant was regarded as having metabolic syndrome if three or more criteria were met. Prevalent CVD was defined as any history of heart attack or stroke, heart bypass surgery or balloon angioplasty, aortic aneurysm, or surgery to the aorta, carotid or lower limb arteries at baseline.

Ascertainment of fatal and non-fatal cardiovascular events during follow-up

Follow-up of hospital admissions and deaths was undertaken using the Western Australian Data Linkage System (WADLS) which provides electronic linkage to records from hospital admissions and death registries.[22]. WADLS captures all admissions to public and private hospitals in Western Australia and all-cause and cause-specific mortality. Follow-up using data linkage for the longitudinal analysis was from the time of blood sampling (2001-2004) until 31 Dec 2013, representing an average of 10.4 years. Hospital admissions and deaths certificates were coded using the ICD-9/ICD-9-CM system up to mid-1999 and ICD10-AM thereafter. CVD events were classified as including fatal/non-fatal myocardial infarction (ICD9 410 or ICD10 I21-I22), heart failure (ICD9 428 or ICD10 I50), or stroke (ICD9 430-438 or ICD10 I60-I64, I69.0-I69.4). CVD deaths were classified as deaths where the underlying cause of death was CVD (ICD9 390-459 or ICD10 I00-I99).

Statistical analysis

Characteristics of the survey sample are expressed as mean (SD) and median for continuous data, and N (%) for categorical data. Men were divided into four groups: high hormone and high PA (H/H), high hormone and low PA (H/L), low hormone and high PA (L/H), and low hormone and low PA (L/L) based on using median splits to determine high/low. In the cross-sectional analyses linear regression was used to compare mean BMI and waist circumference (after adjustment for age) across the four groups. Logistic regression was used to compare the prevalence of metabolic syndrome (after adjustment for age) across the four groups. In the longitudinal follow-up analysis, Cox proportional hazards regression analysis was used to compare risk of fatal and non-fatal CVD events (after adjustment for age, prevalent CVD, smoking, waist circumference, total cholesterol, HDL, lipids medication, diabetes, systolic blood pressure and hypertension medication) across the four groups. Hormone*PA

interaction analyses were conducted with hormones and PA analysed as continuous variables. In instances where there was clear evidence of an interaction ($p<0.01$) we have additionally provided the estimated effect (and 95% CI) of an additional 5 hours of PA for men with a low level of the hormone and for men with a high level of the hormone. In linear regression, a 95% CI for an effect that excludes 0 is significant at 5% level (i.e. $p<0.05$); in logistic regression, a 95% CI for an odds ratio that excludes 1 is significant at 5% level; and in Cox regression, a 95% CI for the hazard ratio that excludes 1 is significant at 5% level.

Results

Baseline characteristics of study population

After excluding men taking androgen-related medications (n=104), with a history of orchidectomy (n=51) or prostate cancer (n=559), missing physical activity data (n=7), and missing hormone data (n=157), 3351 men were included in the analysis. Baseline demographic, physical and biochemical data are displayed in Table 1. Mean age was 77years, mean BMI 26.4kg/m^2 and median level of PA was five hours/week. At baseline, 33% of the cohort had a history of CVD and 14% had diabetes.

Associations of sex hormones and physical activity with BMI

There was an inverse association of higher androgens (and SHBG) and higher PA with lower BMI in the age-adjusted model (Table 2). Men in the H/H group had a significantly lower BMI than those in the L/L group, with intermediate results for the H/L and L/H groups (e.g. for T/PA H/H 25.4 $p<0.001$; H/L 25.5 $p<0.001$; L/H 27.0 $p<0.001$; L/L 27.8 kg/m^2). There was strong evidence of hormone*PA interaction ($p<0.01$) for T, DHT and SHBG. In all cases the effect of PA on BMI was greater in men with lower circulating hormone (Supplementary Table 1). For example, an additional five hours of PA was associated with 0.32 kg/m^2 lower

BMI for men with low circulating T ($p<0.05$) and associated with 0.05kg/m^2 lower BMI for men with high circulating T ($p>0.05$). There was an inverse association of higher PA with lower BMI irrespective of E2 or LH concentrations (Table 2). There were no E2*PA or LH*PA interactions.

Associations of sex hormones and physical activity with waist circumference

There was an inverse association of higher androgens (and SHBG) and higher PA with lower waist circumference in the age-adjusted model (Table 3). Men in the H/H group had a significantly lower waist circumference than those in the L/L group, with intermediate results for the H/L and L/H groups (e.g. for T/PA H/H 95.1 $p<0.001$; H/L 96.5 $p<0.001$; L/H 100.3 $p<0.001$; L/L 103.1 cm). There was strong evidence of hormone*PA interaction ($p<0.01$) for DHT and SHBG. In both cases the effect of PA on waist circumference was greater in men with lower circulating hormone (Supplementary Table 2). For example, an additional five hours of PA was associated with 1.16cm lower waist circumference for men with low circulating DHT ($p<0.05$) and associated with 0.39cm lower waist circumference for men with high circulating DHT ($p<0.05$). There was an inverse association of higher PA with lower waist circumference irrespective of E2 or LH concentrations (Table 3). There were no E2*PA or LH*PA interactions.

Associations of sex hormones and physical activity with metabolic syndrome

There was an inverse association of higher androgens (and SHBG) and higher PA with lower (age-adjusted) risk of metabolic syndrome (Table 4). Men in the H/H group had a significantly lower risk of metabolic syndrome than those in the L/L group, with intermediate results for the H/L and L/H groups (e.g. odds ratios for T/PA H/H 0.24 $p<0.001$; H/L 0.32 $p<0.001$; L/H 0.64 $p<0.001$; L/L 1.00). There was no evidence of any hormone*PA

interaction in their association with metabolic syndrome. There was an inverse association of higher PA with lower risk of metabolic syndrome irrespective of E2 or LH concentrations. There were no PA*E2 or PA*LH interactions.

Associations of sex hormones and physical activity with incident CVD events

In the fully-adjusted model, men with higher T and higher PA (H/H) had a lower risk of CVD events compared with the L/L reference group, as did men with higher T and lower PA (H/L) (Table 5) (e.g. hazard ratio for T/PA H/H 0.81 p=0.032; H/L 0.73 p=0.002; L/H 0.90 p=0.240; L/L 1.00). Thus higher T was associated with lower incidence of CVD events irrespective of PA levels. There was no evidence of any hormone*PA interaction. There were no significant associations of DHT, E2, SHBG or LH and PA with incidence of CVD events. In a combined model with T and SHBG, these results were not altered (data not shown).

Associations of sex hormones and physical activity with CVD deaths

In the fully-adjusted model, men with higher T or DHT and higher PA had a significantly reduced risk of CVD death than those in the L/L group (Table 6) (e.g. hazard ratio for T/PA H/H 0.76 p=0.031; H/L 0.85 p=0.222; L/H 0.80 p=0.075; L/L 1.00 and for DHT/PA H/H 0.71 p=0.008; H/L 0.81 p=0.101; L/H 0.81 p=0.086; L/L 1.00). There was no evidence of any hormone*PA interaction. There were no significant associations of E2, SHBG or LH and PA with CVD deaths. In a combined model with T and SHBG, these results were not altered (data not shown).

Results for calculated free T

Results for calculated free T (cFT, see Supplementary Methods) mirrored those seen with total T (see Supplementary Tables S3 and S4) and did not contribute further information.

Discussion

Higher androgens (T, DHT) or SHBG and higher PA were strongly and additively associated with lower BMI and smaller waist circumference. Similarly there was an inverse relationship between higher androgens or SHBG and higher PA with risk of metabolic syndrome. Men with higher T had a significantly lower risk of experiencing CVD events. Of note, men with higher androgens and higher PA levels had a significantly lower risk of dying from CVD. The findings for cFT were essentially the same as for measured T without providing additional information.[23]

The cross-sectional associations of higher circulating androgens and higher PA levels with lower BMI and waist circumference allow differing interpretations. Men who do not have excessive central adiposity are likely to have higher circulating T, and either or both factors could facilitate PA. Furthermore, engagement in healthy lifestyle behaviours may lead to less central adiposity and also higher circulating T.[24] This is congruent with optimizing comorbidities and facilitating loss of excess weight the recommended course of action in older men who have low T concentrations in the absence of pituitary or testicular disease.[25] Alternatively, men with higher T levels are less likely to accumulate visceral fat[26] and may therefore have lower BMI and smaller waist circumference. However, the interaction between androgens and PA is noteworthy, as it suggests that additional PA may be associated with greater benefit (less central adiposity) in men with low compared to high circulating androgens. This provides some rationale for interventional studies targeting men with low circulating androgens for exercise-based interventions.

Men with higher circulating androgens or SHBG and higher PA levels had the lowest risk of metabolic syndrome with no hormone*PA interactions found. The same limitations of cross-sectional analysis apply. Metabolic syndrome is closely associated with central adiposity, and men with less central adiposity may have higher circulating T and be more active. The alternative explanation is that both higher androgens and higher levels of PA may reduce the risk of having metabolic syndrome in an additive fashion.

The parallel associations of SHBG with androgens for BMI, waist circumference and metabolic syndrome may reflect the close correlations between T and DHT with SHBG since most circulating androgens are bound to SHBG.[23] Consistent with our observations, it is important to acknowledge the relationship between SHBG and insulin sensitivity, with lower levels of SHBG found in the settings of obesity and insulin resistance.[27] Compared to PA levels alone, E2 and LH were not major determinants of BMI, waist circumference or risk of metabolic syndrome, suggesting that higher circulating androgens rather than estrogens are more strongly related to lesser degrees of central adiposity.

Previous epidemiological studies have often assessed hormone or PA levels without analyzing whether one may interact with the other in relation to CVD risk and mortality. Hsu et al. demonstrated in older men that a decline in androgens (serum T, DHT and cFT) and estrogens (E2) were significantly associated with all-cause and cancer mortality but not CVD mortality.[28] In a large cohort study of elderly men, Tivesten et al.[29] reported that lower T and E2 predicted mortality independent of each other when adjusted for by PA. In contrast, Yeap et al.[5], in analyses adjusting for PA, found E2 was not associated with either all-cause or ischaemic heart disease mortality, but higher T was associated with lower all-cause mortality and higher DHT with lower ischaemic heart disease mortality. Lower circulating T or DHT

predicted increased incidence of stroke and transient ischemic attacks after adjustment for conventional CVD risk factors.[30] However, as PA was included as a covariate in these analyses it was unclear whether higher circulating androgens and higher PA levels were independently or additively associated with lower CVD risk.

In a previous analysis conducted in a smaller cohort of predominantly middle-aged men who had experienced fewer outcome events, we were unable to clarify the influence of sex hormones and PA on the CVD events and mortality.[31] In our current larger study, men with higher circulating T had the lowest risk of CVD events, and men with higher circulating T or DHT and higher PA levels had a significantly reduced risk of dying from CVD. These associations were independent of waist circumference suggesting that that central adiposity was not a major mediating factor. One possible explanation is higher circulating androgens and higher PA levels may be biomarkers for better underlying health, which would then be reflected in lower incidence of CVD events and mortality. However, we adjusted for age and other conventional CVD risk factors in the longitudinal analyses making it less likely that the results reflected confounding from differences in baseline cardiovascular health. The lower risk of a CVD event (when compared to the L/L reference group) remained significant for men with higher T irrespective of PA level. However, the lowest risk of dying from CVD was seen in men with both higher circulating androgens and higher levels of PA. The absence of any significant hormone*PA interactions on this outcome supports the interpretation that these effects may be independent and additive.

Interventional studies are indispensable to determine the direction of causality. A RCT of 102 men indicated that a 12-month moderate-intensity exercise program did not alter T levels but increased DHT and SHBG.[32] When 143 older men with low-normal T levels were

randomised to T or placebo +/- exercise, the greatest improvement in body composition was seen in the T+exercise group consistent with an additive effect.[33] Furthermore, the same group recently reported that the aforementioned study also demonstrated that 12 months of T supplementation (+/- resistance exercise) did not alter vascular function.[34] In older men,[35] the addition of T to 10-12 week structured exercise interventions resulted in greater muscle hypertrophy than found with either T or exercise in isolation. In a study of 50 older men with erectile dysfunction, the combination of 12 weeks of T therapy + exercise resulted in higher T concentrations and greater improvement in symptom scores compared to T therapy alone.[36] Interestingly, these improvements were maintained in the exercise group for eight weeks after the T therapy was discontinued.[36] It is therefore possible T treatment may synergise with exercise training to improve body composition, but the impact of this combination on CVD risk remains unknown.

The Cardiovascular sub-study of T-Trials reported a greater increase in non-calcified coronary plaque volume in men following one year of T treatment compared to placebo.[8] At baseline, 50% of these men had severe atherosclerosis, emphasizing that the highly specific selection of men with low circulating T produced a cohort with high rates of established atherosclerosis. Men with established CVD should be treated with lipid-lowering, antihypertensive and antiplatelet therapies, and revascularization, as appropriate. The role of T and exercise training to improve metabolic parameters and cardiovascular risk profiles should be investigated on a background of optimal management of any existing cardiovascular risk factors and disease.

Study strengths include the large cohort of community-dwelling older men, availability of sex steroid results measured using mass spectrometry and the collection of PA data. Our analyses, which were adjusted for potential confounders, were designed to illuminate additive influences of hormones and PA and also tested for interactions between these on each of the outcomes. The cohort size and longitudinal follow-up over an extended period provided ample numbers of CVD events and CVD-related deaths for longitudinal analysis. WADLS, an established data-linkage system, provided comprehensive ascertainment of hospital admissions and causes of death in the study participants.[22]

We acknowledge several limitations of the study. This was an observational study, which precludes inferring causality. Physical activity data were collected in 1996-1999 and hormones were assayed in 2001-2004 (ie. these were collected at separate timepoints). Nevertheless, a previous study (n=5022, mean age 61years) reported that PA levels were relatively stable over a 10-year period.[37]

Although, vigorous activity may have been replaced to some degree with lesser intensity activities, the rank order of individuals remained stable.[37] Therefore, variations in PA levels between the first and second wave of the study would be expected to mask any underlying associations thus favouring the null hypothesis. Men with low T are more likely to report a lower quality of life[38] and it is possible this may have reduced motivation to exercise. However, our a-priori hypothesis was to assess hormones and PA and, thus we did not explore underlying motivations for engaging in PA. Data collected on PA was via self-report questionnaires thus susceptible to recall bias. Although a more objective measure (e.g. accelerometry) would be preferable, validated questionnaires used to quantify PA are informative in large samples of community-dwelling older adults.[39] Hormones and PA data

were collected at a single point in time; serial measurements of these variables were not available. HIMS consisted primarily of Caucasian men so these results may not be applicable to men of other ethnicities or to women.

Conclusion

Higher circulating androgens and higher PA levels are associated with lower BMI, smaller waist circumference, and lower odds of metabolic syndrome in older men. Higher circulating androgens and higher PA levels are associated with a lower risk of dying from CVD. These results suggest a potential additive effect of androgens and PA and also provide a rationale for interventional studies combining T therapy with exercise training to test whether or not this combination might reduce cardiometabolic risk in men.

Declaration of interest

Nothing to disclose: LCC, MWK, MLD, KM, DJH, LF, GJH, OPA, JG, NR, LHN DJG, BBY.

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Table 1. Characteristics of study population comprising 3351 community-dwelling older men. Data are shown as mean ± SD, N (%) or medians as labeled.

Age, years	77.0 ± 3.6
BMI, kg/m^2	26.4 ± 3.6
Waist circumference, cm	98.8 ± 9.8
CVD history	1112 (33)
Metabolic syndrome	1010 (30)
Smoking Never	1114 (33)
Former	2052 (61)
Current	185 (5.5)
Cholesterol, mmol/L	4.89 ± 0.95
HDL, mmol/L	1.40 ± 0.36
Lipids medication	1278 (38)
Diabetes	469 (14)
Systolic BP, mmHg	148 ± 20
Hypertension medication	1837 (55)
Testosterone, nmol/L [ng/dL]	13.1 ± 4.9 [378 ± 140]
	Median 12.5 [361]
Dihydrotestosterone, nmol/L [ng/dL]	1.42 ± 0.73 [41.3 ± 21.2]
	Median 1.32 [38.0]
Estradiol, pmol/L [pg/mL]	73.5 ± 29.0 [20.0 ± 7.9]
	Median 70.2 [19.1]
SHBG, nmol/L	42.7 ± 16.9
	Median 39.9
LH, IU/L	5.85 ± 5.41
	Median 4.37
PA, hours/week	7.05 ± 7.23
	Median 5.00
CVD events during follow-up[1]	865 (25.8)
CVD deaths during follow-up	499 (14.9)

[1] Men with one or more CVD event. Outcome was time to first CVD event.

Table 2. BMI in kg/m^2 in older men according to circulating hormones and physical activity levels stratified using median splits. Data are shown as age-adjusted mean (p-value) from linear regression for BMI according to hormone/physical activity groups.

Hormone variable	Low hormone & Low PA	Low hormone & High PA[1]	High hormone & Low PA[1]	High hormone & High PA[1]	Hormone × PA[2]
T	27.8	27.0 (< 0.001)	25.5 (< 0.001)	25.4 (< 0.001)	0.002
DHT	27.7	26.8 (< 0.001)	25.6 (< 0.001)	25.6 (< 0.001)	< 0.001
E2	26.7	26.0 (< 0.001)	26.7 (0.692)	26.3 (0.040)	0.225
SHBG	27.7	26.9 (< 0.001)	25.6 (< 0.001)	25.4 (< 0.001)	< 0.001
LH	26.8	26.2 (0.002)	26.7 (0.538)	26.1 (< 0.001)	0.682

Frequencies: Low T / Low PA, n=880; Low T / High PA, n=797; High T / Low PA, n=799; High T / High PA, n=875.

[1] p-value for comparison with Low hormone & Low PA group

[2] p-value for hormone*PA interaction, with hormones and PA modelled as continuous variables

Table 3. Waist circumference in cm in older men according to circulating hormones and physical activity levels stratified using median splits. Data are shown as age-adjusted mean (p-value) from linear regression for waist circumference according to hormone/physical activity groups.

Hormone variable	Low hormone & Low PA	Low hormone & High PA[1]	High hormone & Low PA[1]	High hormone & High PA[1]	Hormone × PA[2]
T	103.1	100.3 (< 0.001)	96.5 (< 0.001)	95.1 (< 0.001)	0.056
DHT	102.6	99.7 (< 0.001)	96.9 (< 0.001)	95.8 (< 0.001)	< 0.001
E2	99.9	97.3 (< 0.001)	100.0 (0.943)	98.0 (< 0.001)	0.294
SHBG	102.8	99.9 (< 0.001)	96.9 (< 0.001)	95.5 (< 0.001)	0.002
LH	100.0	97.7 (< 0.001)	99.9 (0.865)	97.6 (< 0.001)	0.809

Frequencies: Low T / Low PA, n=880; Low T / High PA, n=797; High T / Low PA, n=799; High T / High PA, n=875.

[1] p-value for comparison with Low hormone & Low PA group

[2] p-value for hormone*PA interaction with hormones and PA modelled as continuous variables

Table 4. Prevalence of metabolic syndrome according to circulating hormones and physical activity levels stratified using median splits. Data are shown as age adjusted odds ratio (p-value) and [95% CI] from logistic regression for presence of **metabolic syndrome** according to hormone/physical activity groups.

Hormone variable	Low hormone & Low PA	Low hormone[1] & High PA	High hormone & Low PA[1]	Low hormone & High PA[1]	Hormone × PA[2]
T	1.00	0.640 (< 0.001) [0.526, 0.779]	0.319 (< 0.001) [0.257, 0.395]	0.235 (< 0.001) [0.189, 0.294]	0.396
DHT	1.00	0.654 (< 0.001) [0.536, 0.798]	0.416 (< 0.001) [0.337, 0.513]	0.302 (< 0.001) [0.244, 0.374]	0.497
E2	1.00	0.577 (< 0.001) [0.469, 0.710]	0.712 (0.001) [0.581, 0.872]	0.540 (< 0.001) [0.437, 0.666]	0.195
SHBG	1.00	0.658 (< 0.001) [0.540, 0.801]	0.361 (< 0.001) [0.292, 0.447]	0.252 (< 0.001) [0.202, 0.315]	0.093
LH	1.00	0.619 (< 0.001) [0.502, 0.764]	0.942 (0.561) [0.769, 1.154]	0.664 (< 0.001) [0.535, 0.825]	0.919

Frequencies: Low T / Low PA, n=880; Low T / High PA, n=797; High T / Low PA, n=799; High T / High PA, n=875.

[1] p-value for comparison with Low hormone & Low PA group

[2] p-value for hormone*PA interaction, with hormones and PA modelled as continuous variables

Table 5. Incident CVD events in older men according to circulating hormones and physical activity levels stratified using median splits. Data are shown as multivariate adjusted* hazard ratio (p-value) and [95% CI] from Cox regression with the outcome of incident **CVD events** according to hormone/PA groups.

Hormone variable	Low hormone & Low PA	Low hormone & High PA[1]	High hormone & Low PA[1]	High hormone & High PA[1]	Hormone × PA[2]
T	1.00	0.896 (0.240) [0.746, 1.076]	0.730 (0.002) [0.598, 0.891]	0.811 (0.032) [0.670, 0.982]	0.583
DHT	1.00	0.939 (0.504) [0.779, 1.130]	0.890 (0.239) [0.733, 1.081]	0.925 (0.423) [0.764, 1.119]	0.426
E2	1.00	0.891 (0.220) [0.740, 1.072]	0.831 (0.053) [0.689, 1.002]	0.912 (0.336) [0.756, 1.100]	0.583
SHBG	1.00	0.993 (0.942) [0.818, 1.205]	1.014 (0.888) [0.836, 1.230]	0.989 (0.909) [0.814, 1.201]	0.729
LH	1.00	0.974 (0.791) [0.803, 1.182]	0.961 (0.682) [0.796, 1.161]	0.950 (0.615) [0.780, 1.158]	0.195

Frequencies: Low T / Low PA, n=880; Low T / High PA, n=797; High T / Low PA, n=799; High T / High PA, n=875.

* Models adjusted for age, prevalent CVD, smoking, waist circumference, total cholesterol, HDL, lipids medication, diabetes, systolic BP and hypertension medication.

[1] p-value for comparison with Low hormone & Low PA group

[2] p-value for hormone*PA interaction, with hormones and PA modelled as continuous variables

Table 6. CVD deaths in older men according to circulating hormones and physical activity levels stratified using median splits. Data are shown as multivariate adjusted* hazard ratio (p-value) and [95% CI] from Cox regression with the outcome of **CVD death** according to hormone/physical activity groups.

Hormone variable	Low hormone & Low PA	Low hormone & High PA[1]	High hormone & Low PA[1]	High hormone & High PA[1]	Hormone × PA[2]
T	1.00	0.800 (0.075) [0.625, 1.023]	0.854 (0.222) [0.664, 1.100]	0.757 (0.031) [0.589, 0.975]	0.969
DHT	1.00	0.810 (0.086) [0.637, 1.030]	0.812 (0.101) [0.633, 1.041]	0.711 (0.008) [0.552, 0.916]	0.889
E2	1.00	0.803 (0.079) [0.628, 1.026]	0.914 (0.466) [0.719, 1.163]	0.799 (0.081) [0.622, 1.028]	0.350
SHBG	1.00	0.830 (0.175) [0.634, 1.086]	1.113 (0.398) [0.868, 1.427]	0.934 (0.603) [0.721, 1.209]	0.563
LH	1.00	0.822 (0.136) [0.636, 1.063]	0.974 (0.830) [0.764, 1.241]	0.826 (0.150) [0.636, 1.072]	0.401

Frequencies: Low T / Low PA, n=880; Low T / High PA, n=797; High T / Low PA, n=799; High T / High PA, n=875.

* Models adjusted for age, prevalent CVD, smoking, waist circumference, total cholesterol, HDL, lipids medication, diabetes, systolic BP and hypertension medication.

[1] p-value for comparison with Low hormone & Low PA group

[2] p-value for hormone*PA interaction, with hormones and PA modelled as continuous variables