Greater physical activity and higher androgen concentrations are independently associated with lower cardiometabolic risk in men.

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Abstract

Context

Male ageing is associated with lower circulating testosterone (T) and increased incidence of cardiovascular disease (CVD). Whether physical activity (PA) interacts with hormones to modify CVD risk is unclear.

Objective

We assessed whether PA and sex hormone concentrations were independently associated with measures of CVD risk.

Participants

1,649 men.

Methods

Leisure, home, work and total PA were ascertained. At baseline, serum T, dihydrotestosterone (DHT) and estradiol (E2) were assayed. Men were stratified into high PA+high hormone (H/H); low PA+high hormone (L/H); high PA+low hormone (H/L) and low PA+low hormone (L/L).

Results

Mean age was 49.8 years at outset with 415 CVD events and 127 CVD deaths occurring during 20-year follow-up. Men with higher PA and higher T or DHT had lower odds of metabolic syndrome (eg. leisure H/H vs L/L odds ratio [OR] 0.17 $p<0.001$ for T, 0.26 $p<0.001$ for DHT). Men with higher PA and E2 had lower risk of metabolic syndrome (eg. leisure PA H/H vs L/L OR 0.51, $p=0.001$). Men with higher leisure, work or total PA and higher DHT had the lowest risk of CVD death (eg. leisure H/H HR 0.55 vs L/L, $p=0.033$). Men with lower leisure, home or work PA and higher E2 were at greater risk of CVD death (eg. leisure L/H HR 1.60 vs L/L, $p=0.039$).

Conclusions

Considering T, DHT and E2 in the context of PA better informs consideration of cardiovascular risk. A 2x2 factorial RCT assessing PA and androgens would illuminate the scope for preventing CVD in men.

Introduction

As men grow older, circulating testosterone (T) concentrations decrease [1] while incidence of cardiovascular disease (CVD) increases.[2] Overweight older men have lower T levels compared to normal weight men of the same age [3] and reduced T levels are associated with poorer health outcomes including higher rates of metabolic syndrome and all-cause mortality.[4,5] This has raised the question of whether reduced circulating T might be a modifiable risk factor for cardiometabolic ill-health in ageing men, with increasing interest and controversy regarding the possible role of pharmacological T treatment as a means of preserving vascular health.[6]

Testosterone is the predominant androgen in men's circulation and drives the regulation of sexual development, virilisation, bone mineral density and body composition.[7] It is converted by 5α-reductase into dihydrotestosterone (DHT), a more potent androgen and by aromatase into estradiol (E2), a ligand for estrogen receptors.[8] Lower T, DHT, and higher E2 have been associated with features of the metabolic syndrome.[9] Furthermore, higher T and DHT are independent predictors for reduced incidence of stroke [10] with higher DHT also associated with reduced ischaemic heart disease mortality.[11] However, one randomised controlled trial (RCT) was terminated early due to excess cardiovascular events in a group of older men with limited mobility being treated with T,[12] although similar RCTs have not reproduced these findings [13,14] nor do meta-analyses associate testosterone supplementation with increased cardiovascular risk.[15]

Healthy lifestyle behaviours, including exercise, have been associated with higher circulating T in men aged 65+ years.[16] Of interest, the combination of T treatment and exercise training

improves upper and lower limb skeletal muscle strength and performance to a greater extent than exercise alone in young and middle-aged men.[17] One study–reported that body composition improved in older men (66±5 years) following 12 months of T treatment, regardless of whether they were randomised to exercise or usual care.[18] Thus it remains unclear whether men who exercise more and have higher circulating androgens would have lower risk of metabolic syndrome or CVD events compared to those who exercise less and have lower circulating androgens.

Our aim was to assess whether physical activity (PA) levels interact with sex hormone concentrations to influence cardiometabolic risk factors and disease. We tested the hypothesis that higher PA and higher circulating androgens are independently associated with more favourable cardiometabolic risk profiles and reduced incidence of CVD events in community-dwelling men.

Methods

Study population and sample

Busselton is in the coastal region of Western Australia with a predominantly Anglo-Celtic population. The Busselton Health Survey (BHS) includes a series of cross-sectional surveys conducted from 1966 to 1987.[19] Surviving participants of these surveys were invited to a follow-up survey in 1994/95. A total of 2143 men participated and provided blood samples for analysis. The relevant Human Research Ethics Committees approved the study and all participating men provided written consent.

Survey measurement methods

Methods used in the 1994/95 Busselton Health Survey have previously been described.[19] All men completed a comprehensive health and lifestyle questionnaire and underwent a physical assessment that included anthropometry (height, weight, waist circumference via standardised protocols) and blood pressure (systolic and diastolic via mercury sphygmomanometer after five minutes rest seated) and a fasting blood sample. Smoking, diabetes, medication use and hours of PA for exercise/leisure, at home and at work per usual week were obtained by questionnaire.[20] Participants were asked how many hours were spent engaging in moderate or vigorous activities in each of the three environments with examples given for each. For each physical activity setting we calculated (hours/week of moderate intensity activities) + 2 × (hours/week of vigorous intensity activity). Body mass index (BMI) was calculated as weight (kg) divided by square of height (m).

Laboratory assays

Serum cholesterol, high-density lipoprotein (HDL) and triglycerides (TG) were assayed using a Hitachi 747 analyser (Roche Diagnostics, Castle Hill, NSW, Australia) and glucose using a hexokinase assay at time of survey. Serum was stored at -70°C and serum T, DHT and E2 were measured from 200µl samples in 2013 using a single liquid chromatography-tandem mass spectrometry (LC-MS) run without derivatization using atmospheric pressure photo-ionization for positive mode for androgens and negative mode for oestrogens, as previously described.[9,21] Between run imprecision for T was 8.6% at ~~a~~ 5.3 nmol/L and 7.9% at 26.9 nmol/L. For DHT between run imprecision was 11.3% at 1.3 nmol/L and 9.1% at 5.3 nmol/L. For E2 between run imprecision was 14.5% at 73 pmol/L and 9.9% at 279 pmol/L.[9] LH was assayed using immunoassay (Abbott Architect, Abbott Diagnostics, Australia) with between run imprecision of 5.6% at 4.8 IU/L. SHBG was assayed using a solid-phase, two site

enzyme immunometric assay with chemiluminescent substrate (Immulite 2000XPi; Siemens Healthcare, Bayswater, Vic., Australia) with between-run imprecision of 3.4% at 39.4 nmol/l. Free T was calculated using empirical formulae, which provides closer concordance with measured free T compared with calculations based on equilibrium binding equations.[22]

Definition of metabolic syndrome and prevalent cardiovascular disease

The metabolic syndrome score was defined using five risk components (hypertension, hyperglycemia, hypertriglyceridemia, high density lipoprotein (HDL) cholesterol, waist circumference) according to the National Cholesterol Education Program *Adult Treatment Panel* III 2005 criteria.[23] Hypertension was defined as systolic blood pressure ≥130 mmHg, or diastolic blood pressure ≥85 mmHg or drug treatment for hypertension. Hyperglycemia was defined as fasting glucose ≥5.6 mmol/L. Hypertriglyceridemia was defined as triglycerides ≥1.7 mmol/L or receipt of fibrates or nicotinic acid. Low high density lipoprotein (HDL) cholesterol was defined as HDL ≤1.0 mmol/L. Central obesity was defined as waist circumference ≥102 cm. A participant was regarded as having metabolic syndrome if three or more criteria were met. History of CVD at the time of survey attendance in 1994/95 was defined as-any hospital admission for CVD (ICD-9 390-459) in the 15-year period before the survey.

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

Follow-up for hospital admissions and deaths were available until mid-2014, amounting to 20 years of follow-up. Outcome events were ascertained from hospital admissions and death records. Hospital admission codes used the ICD-9/ICD-9-CM system up to mid-1999 and ICD10-AM thereafter. Deaths from CVD were ascertained based on deaths with underlying cause of death coded as diseases of the circulatory system (ICD-9 390-459; ICD-10 I00-99,

G45). Non-fatal CVD events were defined as a hospital admission with a principal diagnosis of coronary heart disease (ICD-9 410-414; ICD-10 I20-25), stroke (ICD-9 430-437; ICD-10 I60-68, G45), congestive heart failure (ICD-9 428; ICD-10 I50) or peripheral arterial disease (ICD-9 440-448; ICD-10 I70-79).

Statistical analysis

Characteristics of the survey sample are expressed as mean (SD) and median (interquartile range) for continuous data, and N (%) for categorical data. Men were divided into four groups (1) high PA and high hormone (H/H), (2) low PA and high hormone (L/H), (3) high PA and low hormone (H/L) and (4) low PA and low hormone (L/L) based on using median splits to determine high/low. For frequencies of the four groups, see Supplementary Table 1. 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, cholesterol, HDL, lipids medication, diabetes, SBP and hypertension medication) across the four groups. PA*hormone interaction analyses were conducted using PA and hormones as continuous variables.

Results

Baseline characteristics of study population

After restricting the cohort to men aged 20-79 years and excluding men who were taking androgens, anti-androgens, or had a history of orchidectomy or prostate cancer, or with

missing PA or hormone variables, 1649 men were included in the analysis. Baseline demographic, physical and biochemical data are shown in Table 1.

TABLE 1

Associations of physical activity and sex hormones with BMI

There was an inverse association of higher PA and higher T with lower BMI (in the age-adjusted model (Table 2). Men in the H/H group had significantly lower BMI than those in the L/L group, with intermediate results for L/H and H/L groups (e.g. leisure H/H 25.4 $p<0.001$; L/H 25.8 $p<0.001$; H/L 27.1 $p<0.001$ vs L/L 27.9 kg/m^2). There were no PA*T interactions (all $p\geq0.18$) suggesting that higher PA and higher T are independently associated with lower BMI. There were similar results with DHT (e.g. leisure H/H 25.7 $p<0.001$; L/H 26.1 $p<0.001$; H/L 26.8 $p<0.003$ vs L/L 27.5). There were no PA*DHT interactions (all $p\geq0.05$). Higher PA and higher E2 were generally not associated with BMI (all group comparisons $p>0.05$ except leisure H/H vs L/L with $p=0.04$), and there were no PA*E2 interactions (all $p\geq0.16$).

TABLE 2

Associations of physical activity and sex hormones with waist circumference

There was an inverse association of higher PA and higher T with waist circumference in the age-adjusted model (Table 3). Men in the H/H group had significantly smaller waist circumference than those in the L/L group, with intermediate results for L/H and H/L groups

(e.g. leisure H/H 89.2 p<0.001; L/H 90.9 p<0.001; H/L 94.8 p<0.001 vs L/L 97.2 cm). There were similar results with DHT (e.g. leisure H/H 90.3, p<0.001; L/H 92.0 p<0.001; H/L 93.9 p=0.002 vs L/L 95.9 cm). Strong interactions were present for work PA*DHT (p=0.006) and total PA*DHT (p=0.001). There was a greater difference in waist circumference between men with low DHT vs men with high DHT irrespective of PA. For higher work and total PA, waist circumference was significantly lower in men with low DHT but not in men with high DHT (see Supplementary Table 7). Men with higher leisure, home or total PA and higher E2 had significantly smaller waist circumferences (e.g. leisure H/H 91.6, p<0.001 vs L/L 93.9 cm). There were no PA interactions for T (all p≥0.26) or E2 (all p≥0.09).

TABLE 3

Associations of physical activity and sex hormones with metabolic syndrome

For all settings of PA, men with higher PA and higher T had lowest (age-adjusted) odds of metabolic syndrome (Table 4). There were intermediate results for the L/H and H/L groups (e.g. leisure H/H odds ratio 0.166 p<0.001; L/H 0.323 p<0.001; H/L 0.744 p=0.078 vs L/L 1.00). There were no PA*T interactions (all p≥0.05). Similar results were seen for DHT (e.g. leisure H/H 0.255 p<0.001; L/H 0.475 p<0.001; H/L 0.823 p=0.270 vs L/L 1.00). There were no PA*DHT interactions (all p≥0.27). For leisure, home, work and total PA, men with higher PA and higher E2 had the lowest odds of metabolic syndrome (e.g. leisure H/H 0.507, p=0.001; L/H 1.013, p=0.945; H/L 0.910, p=0.624 vs L/L 1.00). There were significant interactions between E2 and leisure PA (p=0.027), total PA (p=0.037), and home PA (p=0.026), with little difference in odds of metabolic syndrome in men with higher vs lower PA in the presence of low E2.

TABLE 4

Cross sectional analyses of PA and cFT, and PA and SHBG

For outcomes of BMI, waist and metabolic syndrome, the results of PA and cFT, and PA and SHBG largely mirror those seen with (total) T (Supplementary Tables 2-4).

SUPPLEMENTARY TABLES 2-4

Associations of physical activity and sex hormones with incident CVD events

In the fully-adjusted model, there were no differences in the hazard ratio for CVD events for PA and hormones and no PA*hormone interactions (Table 5).

TABLE 5

Associations of physical activity and sex hormones with CVD deaths

In the fully-adjusted model, men with higher leisure, work or total PA and higher DHT had the lowest risk of CVD death (e.g. leisure hazard ratio H/H 0.55, p=0.033; L/H 0.81, p=0.346; H/L 0.73, p=0.243 vs L/L 1.00; Table 6). There were no PA*DHT interactions (all p≥0.461). Men with lower leisure, home, or work PA and higher E2 have an increased hazard ratio of CVD death (eg. Leisure L/H HR 1.60 vs L/L, p=0.039). There were no PA*E2 interactions (all p≥0.22).

TABLE 6

Longitudinal analyses of PA and cFT, and PA and SHBG

For the outcome of CVD events and CVD deaths, there were no consistent associations observed (Supplementary Tables 5-6). Men with high work PA and high cFT had a lower hazard ratio for CVD death but this was not seen for men with high leisure, home or total PA and high cFT (Supplementary Table 6). Men with high work or total PA and low SHBG had a lower hazard ratio for CVD death but this was not seen for men with high leisure or home PA and low SHBG (Supplementary Table 6).

SUPPLEMENTARY TABLES 5-6

Discussion

Higher PA and higher T or DHT were associated with lower BMI, waist circumference and odds of metabolic syndrome. There was an interaction between PA and DHT, with less difference in odds of metabolic syndrome attributable to PA in the presence of higher DHT. Men with higher leisure, work or total PA+higher DHT had the lowest risk of CVD death. Men with higher PA and higher E2 had the lowest odds of metabolic syndrome. There was an interaction such that the difference attributable to higher PA was less in men with lower E2. Men with lower levels of leisure, home or work PA and higher E2 had the highest hazard ratio for CVD death. There were no PA*hormone interactions for the longitudinal outcomes.

Previous epidemiological studies have assessed associations of either PA or hormone levels [3,9-11,16,20,24-30] with outcomes related to cardiometabolic health, but not analyzed for interactions between the two. Some studies have adjusted for PA when examining hormones vs cardiometabolic outcomes. Tivesten *et al.* reported low serum T and E2 increased risk of mortality in a population of 3014 men,[24] when adjusted for by age, BMI, smoking and PA but the role of PA alone was not assessed. Similarly, a 2014 meta-analysis demonstrated men with low total T were more likely to have prevalent metabolic syndrome compared to men with high total T [4] and adjustment for lifestyle factors (smoking status, alcohol consumption, and PA) was reported to not materially change the odds ratio but again the role of PA in its own right was not evaluated. These approaches have left unanswered the question whether, and to what extent, higher levels of PA and higher circulating androgens might be independently or additively associated with lower CVD risk.

We found that men with higher PA levels and higher levels of T had the lowest BMI, waist circumference and risk of metabolic system. Interestingly, this did not translate to any reduction in risk of CVD events or CVD mortality. Several epidemiological studies have reported associations of low T with poorer CVD-related outcomes in men [10,24] while others have reported negative or neutral results.[30-32] Low T concentrations have been associated with increased risk of mortality in the European Male Ageing Study (EMAS).[27] However, in older men, an optimal or mid-range T is the best predictor of longevity.[11] In this cohort of men, T appears to have less predictive utility for longitudinal CVD-related outcomes compared with DHT.

Men with higher PA and higher DHT had a lower BMI, waist circumference and risk of metabolic syndrome than men who had lower PA levels and/or lower DHT levels. There was an interaction between PA and DHT with respect to waist circumference. As a group men with high DHT had lower waist circumference compared to men with low DHT, and the relationship of higher PA with lower waist circumference was strong (and significant) in men with low DHT and weak (and non-significant) in men with high DHT. Men with higher leisure, work or total PA and higher DHT also had the lowest risk of CVD mortality. There were no interactions between PA and DHT for this longitudinal outcome. This is concordant with our previous finding that older men with higher DHT have a lower mortality from ischaemic heart disease.[11] Furthermore, in the Cardiovascular Health Study there was a curvilinear association between DHT and CVD but an inverse association with all-cause mortality.[28] Those studies did not examine whether PA and T or DHT might interact to influence outcomes. Our results from men across a range of ages extend these observations demonstrating additive or independent associations of higher PA and higher DHT with lower CVD mortality, highlighting the value of DHT as an informative biomarker.

Men who had higher leisure PA levels had a significantly lower BMI and waist circumference than men who exercised less, independent of E2 levels. However men with higher (leisure, home, work or total) PA levels and higher E2 had significantly reduced risk of metabolic syndrome compared to men with high E2 and low PA. The difference associated with PA was not as apparent in men with low E2. Low leisure, home or work PA coupled with a high E2 level was predictive of increased CVD mortality. Previous epidemiological studies have not associated E2 with mortality from ischaemic heart disease or all-cause mortality.[11,26] However, other studies have reported an inverse association with E2 and CVD mortality [29] and all-cause mortality.[24,30] Results from the present study indicate that in men

with higher E2 levels, having higher rather than lower PA levels are associated with more favourable cross-sectional and longitudinal outcomes.

We acknowledge several limitations of the present study. As this was an observational study it precludes the ability to infer causality. Physical activity data were obtained via questionnaires susceptible to recall bias. Both PA and hormones were assessed at baseline and we did not include serial measures of these variables over time. We dichotomized variables for ease of presentation and interpretation of results. However we also analysed interactions with PA and hormones as continuous variables. While there is the possibility of false-positive findings occurring by chance due to multiple comparisons, it is reassuring that we have found consistently significant results in specific PA and hormone groups across the different categories of PA. As men in the analysis had attended previous surveys, a 'healthy survivor' effect may be present. Lastly, the BHS men are predominantly Anglo-Celtic so results may not apply to men from other ethnic backgrounds or to women.

The androgen receptor (AR) gene contains a CAG repeat sequence, and receptors with longer CAG repeat sequences exhibit impaired transcriptional activity. [33] In men with type 2 diabetes, the AR CAG repeat was positively associated with waist circumference and BMI independently of testosterone and estradiol concentrations. [34] Conversely in a different study men with shorter CAG repeats had higher systolic blood pressure, and lower HDL cholesterol. [35] As we did not measure AR CAG repeat length in our cohort we are unable to comment on the role of AR sensitivity, or AR-independent mechanisms by which testosterone might modulate cardiovascular risk.

Strengths of our study include the large cohort of community-dwelling men with detailed baseline characterization. The different classifications of PA (leisure, home, work, total) allowed us to investigate to what extent each type influenced cardiometabolic health, and whether results were consistent across categories. In the majority of our examples, we have used leisure PA as is it has been consistently informative in our analyses and is also associated with longer life expectancy.[36] Serum T, DHT and E2 were assayed using LC-MS. We analyzed associations of PA and hormones with cardiometabolic outcomes, specifically evaluating for PA*hormone interactions. The long period of follow-up (20-years) facilitated assessment of outcome events.

Previous interventional studies have assessed the effect of T treatment on cardiometabolic outcomes in men with low-normal baseline T and metabolic syndrome and/or Type 2 diabetes mellitus (T2DM).[31,32] Jones *et al.* reported improvements in insulin resistance, cholesterol and sexual health in men following six months of T treatment.[31] Similarly, following 30 weeks of T administration, Kalinchenko *et al.* reported improvements in features of the metabolic syndrome and inflammatory markers.[32] However, these studies used background lifestyle interventions, generally encouraging uptake of healthy lifestyle behaviors rather than testing for additive effects of exercise and T. With a structured 10-week exercise intervention, Bhasin *et al.* reported supraphysiologic doses of T were additive to the increases in strength and muscle size in healthy men.[17] In a cohort of 71 frail elderly men (mean age 78.2 ± 6.4yrs), the addition of T to 12-weeks of high-intensity resistance exercise led to greater muscle hypertrophy.[37] One study assessed the effect of T treatment in apparently healthy older men following 12-months of strength training [18] where findings were generally consistent with previous studies indicating that T+PA improved body composition measures more so than either intervention alone. However, the aforementioned

studies were designed to detect differences in body composition and muscle strength and performance, rather than cardiometabolic outcomes. Of note, a recent meta-analysis limited to rigorous double-blind placebo-controlled RCTs of testosterone in men with metabolic syndrome or diabetes showed a marginal improvement in indices of insulin sensitivity, but no evidence of better glycemic control in men with relatively well controlled diabetes, or improvement in the Aging Male Symptom score.[38]

Our findings are consistent with independent and additive associations of PA and T on indices of body composition, with no evidence of interaction to suggest that one might modify the association of the other. Men with higher PA and higher DHT had lower BMI, waist circumference and metabolic syndrome, with an interaction between PA and DHT on waist circumference. Unlike the longitudinal analyses of PA and T, which showed no associations with CVD mortality, men with higher PA and DHT levels had lower risk of CVD mortality. Of note, men with high E2 and high PA were less likely to have metabolic syndrome, but those with high E2 and low PA had increased risk of CVD death. Overall, our results suggest that high PA and androgens, particularly DHT, predict more favourable outcomes. Conversely, high E2 and low PA predict less favourable outcomes. Key questions remain as to the direction of causation, and whether manipulation of both PA and hormone levels could modify cardiovascular risk.

A 2x2 factorial RCT in men with low-normal baseline T would be needed to clarify whether an exercise intervention combined with T would reduce cardiovascular and mortality risk more than either alone, or neither. Analyses of on-treatment concentrations of the T metabolites, DHT and E2, in relation to the study outcomes would also provide greater

399 understanding of the potential contribution of each hormone in the presence of PA. However,
400 benefits of such interventions need to be weighed carefully against the potential risks in light
401 of a recent systematic review demonstrating a possible increased risk of CV events during the
402 first 12 months for men ≥65 years receiving T supplementation.[15]
403
404 **Conclusions**
405 The most favourable combination of PA and hormone variables appears to be higher T and
406 DHT with greater PA. Conversely men with higher E2 levels have less favourable outcomes
407 in the presence of lower PA. Causality remains to be proven by appropriately designed
408 randomised controlled trials.
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Disclosures

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

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