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Chasland, LC, Knuiman, MW, Divitini, ML, Chan, YX, Handelsman, DJ, Naylor, LH, Green, DJ and Yeap, BB (2017) Greater physical activity and higher androgen concentrations are independently associated with lower cardiometabolic risk in men. Clinical Endocrinology. ISSN 0300-0664

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

3
4 **Lauren C. Chasland, BSc¹, Matthew W. Knuiman, BSc, PhD², Mark L. Divitini, BSc²,**
5 **Yi X. Chan, MBBS^{3,4}, David J. Handelsman, MBBS, PhD⁵, Louise H. Naylor, BSc,**
6 **PhD¹, Daniel J. Green, BSc, PhD¹, Bu B. Yeap MBBS, PhD^{3,4}**

7
8 ¹School of Sport Science, Exercise and Health, University of Western Australia, Perth,
9 Western Australia,

10 ²School of Population Health, University of Western Australia, Perth, Western Australia,

11 ³School of Medicine, University of Western Australia, Perth, Western Australia,

12 ⁴Department of Endocrinology and Diabetes, Fiona Stanley Hospital, Perth, Western
13 Australia,

14 ⁵ANZAC Research Institute, Sydney, New South Wales, Australia
15

16 **Abbreviated title:** Physical activity, androgens and health

17 **Key terms:** testosterone, physical activity, metabolic syndrome, cardiovascular disease

18 **Word Count:** 3781

19 **Number of figures and tables:** 6
20

21 **Corresponding author:**

22 Bu B Yeap

23 School of Medicine

24 University of Western Australia

25 Perth, 6009

26 Tel: +61 8 6151 1149

27 Fax: +61 6151 1199

28 Email: bu.yeap@uwa.edu.au
29

30 **Nothing to disclose:** LCC, MWK, MLD, YXC, DJH, LHN DJG, BBY.
31
32

33 **Sources of research support:** This study was supported by Project Grant 1021326 from the
34 National Health and Medical Research Council of Australia. The 1994/1995 Busselton
35 Health Survey was supported and funded by the Health Promotion Foundation of Western
36 Australia.

37 **Abstract**

38 **Context**

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

42 **Objective**

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

45 **Participants**

46 1,649 men.

47 **Methods**

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

52 **Results**

53 Mean age was 49.8 years at outset with 415 CVD events and 127 CVD deaths occurring
54 during 20-year follow-up. Men with higher PA and higher T or DHT had lower odds of
55 metabolic syndrome (eg. leisure H/H vs L/L odds ratio [OR] 0.17 $p < 0.001$ for T, 0.26
56 $p < 0.001$ for DHT). Men with higher PA and E2 had lower risk of metabolic syndrome (eg.
57 leisure PA H/H vs L/L OR 0.51, $p = 0.001$). Men with higher leisure, work or total PA and
58 higher DHT had the lowest risk of CVD death (eg. leisure H/H HR 0.55 vs L/L, $p = 0.033$).
59 Men with lower leisure, home or work PA and higher E2 were at greater risk of CVD death
60 (eg. leisure L/H HR 1.60 vs L/L, $p = 0.039$).

61 **Conclusions**

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

65 **Introduction**

66

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

75

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

87

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

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

97

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

103

104 **Methods**

105 *Study population and sample*

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

112

113 Survey measurement methods

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

125

126 Laboratory assays

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

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

142

143 Definition of metabolic syndrome and prevalent cardiovascular disease

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

156

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

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

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

167

168 Statistical analysis

169 Characteristics of the survey sample are expressed as mean (SD) and median (interquartile
170 range) for continuous data, and N (%) for categorical data. Men were divided into four
171 groups (1) high PA and high hormone (H/H), (2) low PA and high hormone (L/H), (3) high
172 PA and low hormone (H/L) and (4) low PA and low hormone (L/L) based on using median
173 splits to determine high/low. For frequencies of the four groups, see Supplementary Table 1.
174 In the cross-sectional analyses linear regression was used to compare mean BMI and waist
175 circumference (after adjustment for age) across the four groups. Logistic regression was used
176 to compare the prevalence of metabolic syndrome (after adjustment for age) across the four
177 groups. In the longitudinal follow-up analysis, Cox proportional hazards regression analysis
178 was used to compare risk of fatal and non-fatal CVD events (after adjustment for age,
179 prevalent CVD, smoking, waist circumference, cholesterol, HDL, lipids medication, diabetes,
180 SBP and hypertension medication) across the four groups. PA*hormone interaction analyses
181 were conducted using PA and hormones as continuous variables.

182

183 **Results**

184 Baseline characteristics of study population

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

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

189

190

TABLE 1

191

Associations of physical activity and sex hormones with BMI

193 There was an inverse association of higher PA and higher T with lower BMI (in the age-
194 adjusted model (Table 2). Men in the H/H group had significantly lower BMI than those in
195 the L/L group, with intermediate results for L/H and H/L groups (e.g. leisure H/H 25.4
196 $p < 0.001$; 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
197 interactions (all $p \geq 0.18$) suggesting that higher PA and higher T are independently associated
198 with lower BMI. There were similar results with DHT (e.g. leisure H/H 25.7 $p < 0.001$; L/H
199 26.1 $p < 0.001$; H/L 26.8 $p < 0.003$ vs L/L 27.5). There were no PA*DHT interactions (all
200 $p \geq 0.05$). Higher PA and higher E2 were generally not associated with BMI (all group
201 comparisons $p > 0.05$ except leisure H/H vs L/L with $p = 0.04$), and there were no PA*E2
202 interactions (all $p \geq 0.16$).

203

204

TABLE 2

205

Associations of physical activity and sex hormones with waist circumference

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

210 (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
211 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
212 $p=0.002$ vs L/L 95.9 cm). Strong interactions were present for work PA*DHT ($p=0.006$) and
213 total PA*DHT ($p=0.001$). There was a greater difference in waist circumference between
214 men with low DHT vs men with high DHT irrespective of PA. For higher work and total PA,
215 waist circumference was significantly lower in men with low DHT but not in men with high
216 DHT (see Supplementary Table 7). Men with higher leisure, home or total PA and higher E2
217 had significantly smaller waist circumferences (e.g. leisure H/H 91.6, $p<0.001$ vs L/L 93.9
218 cm). There were no PA interactions for T (all $p\geq 0.26$) or E2 (all $p\geq 0.09$).

219 **TABLE 3**

220

221 Associations of physical activity and sex hormones with metabolic syndrome

222 For all settings of PA, men with higher PA and higher T had lowest (age-adjusted) odds of
223 metabolic syndrome (Table 4). There were intermediate results for the L/H and H/L groups
224 (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
225 1.00). There were no PA*T interactions (all $p\geq 0.05$). Similar results were seen for DHT (e.g.
226 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
227 no PA*DHT interactions (all $p\geq 0.27$). For leisure, home, work and total PA, men with higher
228 PA and higher E2 had the lowest odds of metabolic syndrome (e.g. leisure H/H 0.507,
229 $p=0.001$; L/H 1.013, $p=0.945$; H/L 0.910, $p=0.624$ vs L/L 1.00). There were significant
230 interactions between E2 and leisure PA ($p=0.027$), total PA ($p=0.037$), and home PA
231 ($p=0.026$), with little difference in odds of metabolic syndrome in men with higher vs lower
232 PA in the presence of low E2.

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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).

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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.

278 Previous epidemiological studies have assessed associations of either PA or hormone levels
279 (3,9-11,16,20,24-30) with outcomes related to cardiometabolic health, but not analyzed for
280 interactions between the two. Some studies have adjusted for PA when examining hormones
281 vs cardiometabolic outcomes. Tivesten *et al.* reported low serum T and E2 increased risk of
282 mortality in a population of 3014 men,⁽²⁴⁾ when adjusted for by age, BMI, smoking and PA
283 but the role of PA alone was not assessed. Similarly, a 2014 meta-analysis demonstrated men
284 with low total T were more likely to have prevalent metabolic syndrome compared to men
285 with high total T⁽⁴⁾ and adjustment for lifestyle factors (smoking status, alcohol consumption,
286 and PA) was reported to not materially change the odds ratio but again the role of PA in its
287 own right was not evaluated. These approaches have left unanswered the question whether,
288 and to what extent, higher levels of PA and higher circulating androgens might be
289 independently or additively associated with lower CVD risk.

290

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

300

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

316

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

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

328

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

340

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

349

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

359

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

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

381

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

394

395 A 2x2 factorial RCT in men with low-normal baseline T would be needed to clarify whether
396 an exercise intervention combined with T would reduce cardiovascular and mortality risk
397 more than either alone, or neither. Analyses of on-treatment concentrations of the T
398 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.

409

410 **Acknowledgements**

411 The authors would like to thank the staff of PathWest Laboratory Medicine, Sir Charles
412 Gairdner Hospital and the ANZAC Research Institute for performing biochemical and
413 hormonal assays. We thank the staff of the Western Australian Data Linkage Branch, the
414 Hospital Inpatient Data Collection System and Registrar General for their assistance in
415 providing linked data. The authors especially thank the Busselton men who took part in the
416 survey and the Busselton Population Medical Research Institute for access to the survey data.

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418 **Grants and Funding**

419 This study was funded by Project Grant 1021326 from the National Health and Medical
420 Research Council of Australia. BBY is the recipient of a clinical investigator award from the
421 Sylvia and Charles Viertel Charitable Foundation, NSW, Australia. The 1994/1995 Busselton
422 Health Survey was supported and funded by the Health Promotion Foundation of Western

423 Australia. DJG is supported by a National Health and Medical Research Council (NHMRC)
424 Principal Research Fellowship (APP1080914). LCC was supported by an Australian
425 Postgraduate Award and a REDiMED PhD Top-Up scholarship. The funding sources had no
426 involvement in the planning, analysis or writing of the manuscript.

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428 **Disclosures**

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

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