

New Horizons:

Testosterone or Exercise for Cardiometabolic Health in Older Men

Daniel J Green¹, Lauren C Chasland^{1,2}, Louise H Naylor^{1,2}, Bu B Yeap^{3,4}

¹School of Human Sciences (Exercise and Sport Science),
The University of Western Australia, Perth, WA, Australia

²Allied Health Department, Fiona Stanley Hospital, Perth, Western Australia

³Medical School, University of Western Australia, Perth, Western Australia

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

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Author for correspondence:

Winthrop Professor Daniel J. Green, PhD
School of Human Sciences (Exercise and Sport Science),
The University of Western Australia, Perth, WA, Australia, 6009
E: danny.green@uwa.edu.au; Ph: +61 8 6488 2361; F: +61 8 6488 1039
ORCID 0000-0003-3226-2921

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3 **Abstract**

4 Middle-aged and older men have typically accumulated comorbidities, are increasingly
5 sedentary, and have lower testosterone concentrations (T) compared to younger men. Both
6 reduced physical activity (PA) and lower T are associated with, and may predispose to,
7 metabolically adverse changes in body composition, which contribute to higher risks of
8 cardiometabolic disease. Exercise improves cardiometabolic health, but sustained participation is
9 problematic. By contrast, rates of T prescription have increased, particularly in middle-aged and
10 older men without organic diseases of the hypothalamus, pituitary or testes, reflecting the
11 unproven concept of a restorative hormone that preserves health. Two recent large randomised
12 trials of T, and meta-analyses of randomised trials, did not show a signal for adverse
13 cardiovascular (CV) events and T treatment on a background of lifestyle intervention reduced
14 type 2 diabetes by 40% in men at high risk. Men with both higher endogenous T and higher PA
15 levels have lower CV risk, but causality remains unproven. Exercise training interventions
16 improve blood pressure and endothelial function in middle-aged and older men, without
17 comparable benefits or additive effects of T treatment. Therefore, exercise training improves
18 cardiometabolic health in middle-aged and older men when effectively applied as a supervised
19 regimen incorporating aerobic and resistance modalities. Treatment with T may have indirect
20 cardiometabolic benefits, mediated via favourable changes in body composition. Further
21 evaluation of testosterone as a pharmacological intervention to improve cardiometabolic health
22 in aging men could consider longer treatment durations and combination with targeted exercise
23 programs.

1 Introduction

2 Physical inactivity is now considered a key risk factor for non-communicable diseases and the
3 fourth leading cause of death worldwide (1). There is clear evidence of an inverse linear dose-
4 response relationship between physical activity (PA) volume and all-cause mortality (2), with the
5 greatest improvement in cardiovascular (CV) risk occurring when moving from being sedentary
6 to engaging in low levels of activity (3). One prospective cohort study (n=416,175) reported a
7 14% decrease in all-cause mortality and 3-year longer life expectancy in those who engaged in
8 15 mins of daily physical activity, compared to being inactive (4). Moreover, the lower all-cause
9 mortality associated with moderate levels of PA may be more apparent in older (60-74 years, -
10 34%) than younger adults (35-59 years, -26%) (5), and recent studies utilizing wearable
11 technologies suggest a greater decrease in all-cause mortality than historical studies which relied
12 upon self-report (6). The beneficial influence of physical activity may be mediated by
13 improvements in cardiorespiratory fitness and indices of vascular health such as blood pressure
14 (BP) and endothelial function, and via exercise-induced increases in muscle mass, strength or
15 function. Nonetheless, increasing PA remains a population health challenge (7), and
16 pharmacological strategies that emulate the impacts of exercise have recently been mooted.

17
18 Testosterone (T) plays an important role in sexual development, behaviour and body
19 composition, and symptoms of androgen deficiency include decreased energy levels, low sexual
20 desire, low mood, irritability, poor concentration and reduced muscle mass and strength (8,9).
21 Testosterone is the classical anabolic hormone which, like resistance exercise, acts to increase
22 muscle mass (10). Greater muscle mass protects against development of sarcopenia and frailty,
23 and also confers metabolic benefits. Transgenic mice expressing a constitutively active protein

1 kinase, Akt1, which stimulates muscle hypertrophy via growth of type IIb muscle fibres, exhibit
2 reduced fat accumulation and increased metabolic rate, hepatic fatty acid oxidation and ketone
3 body production (11). Furthermore, mice with a null mutation of myostatin, an endogenous
4 inhibitor of muscle growth, are resistant to diet-induced obesity and have markedly improved
5 insulin sensitivity (12). Highlighting the metabolic importance of muscle mass, a monoclonal
6 antibody targeting the activin type II receptor to facilitate skeletal muscle growth, increased lean
7 mass, reduced fat mass and improved glycemia in a phase 2 trial of people with diabetes (13).

8
9 T treatment has become increasingly prevalent in recent decades (14), but the safety of its use
10 beyond men with pathological androgen deficiency remains unclear. Nonetheless, T prescription
11 has increased 11-fold in recent decades, partly due to the unproven premise of a restorative
12 health-preserving hormone (14). Non-medically prescribed and off-label use may also occur,
13 with some middle-aged and older men considering T supplementation an anti-aging strategy
14 (15). Interestingly, although T concentrations can decrease with age (16,17), they are higher in
15 older men who engage in a healthy lifestyle, inclusive of regular exercise, compared to men who
16 have less healthy lifestyles (18). The influence of higher endogenous circulating T
17 concentrations, and the effects of pharmacological intervention with exogenous T treatment, on
18 cardiometabolic health in middle-aged and older men are pivotal to better understanding of the
19 endocrinology of male aging (19). An equally important question is whether a combination of T
20 treatment with exercise training might achieve optimal cardiometabolic outcomes in aging men.

21
22 This review compares the independent, and possible combined, effects of T and exercise on
23 cardiometabolic health and longevity in men. It considers the impacts of these factors from an

1 epidemiological perspective, and in terms of their effects on physiological outcomes such as
2 blood pressure and vascular function, as demonstrated in randomized trials. The consequences of
3 anabolic steroid abuse are beyond the scope of this review.

4 5 **Is Endogenous Testosterone Associated with Cardiometabolic Health?**

6 Older men on average have lower T concentrations compared to younger and middle-aged men,
7 in conjunction with greater accumulation of medical comorbidities (20-22). Lower T
8 concentrations have been inversely associated with CV risk factors such as BMI, waist
9 circumference, diabetes and hypertension (18,20,23,24). Men with lower T concentrations are
10 also more likely to have central adiposity and insulin resistance, and to develop metabolic
11 syndrome or type 2 diabetes (25-27). In a study of 195 men aged ≥ 70 years, calculated free T
12 values (but not total T concentrations) were inversely associated with CIMT progression (28).
13 However, in another study of 1,101 men aged 59 years, total T concentrations were inversely
14 associated with carotid plaque area in a cross-sectional analysis, but there were no prospective
15 associations with changes in plaque area or carotid intima media thickness (CIMT) (29). While
16 different studies have produced contrasting results (for review, see (19)), in the Osteoporotic
17 Fractures in Men (MrOS) Sweden study of 2,416 men aged 69-81 years, lower baseline T
18 concentrations (from blood samples mostly collected in the morning) were associated with
19 increased risk of cardiovascular events (30). In the Health In Men Study (HIMS) of 3,690 men
20 aged 70-89 years (with early morning blood sampling), lower baseline T concentration was
21 associated with higher risk of incident stroke but not myocardial infarction (MI) (31,32).
22 However, in 210,700 men from the United Kingdom (UK) Biobank aged 40-69 years followed
23 for 9 years, baseline T concentrations (from blood samples collected throughout the day) were

not associated with incident cardiovascular events, nor with incidence of MI, stroke or heart failure when analysed as distinct outcomes (33). Therefore, associations of lower endogenous T concentrations with cardiovascular events may be present in older but not middle-aged men, and driven more by stroke rather than MI.

Some studies suggest that lower T concentrations are associated with increased risk of cardiovascular and all-cause mortality (34-38), whilst others do not (39,40) (for detailed review, see (19)). Of note, an analysis of 149,436 men aged 40-69 years from the UK Biobank followed for 11 years showed no association of baseline T concentrations with cardiovascular mortality (41). In that analysis, men with lower baseline T concentrations had higher all-cause mortality, but the association was non-linear. A similar non-linear (U-shaped) association of baseline T concentrations with all-cause mortality was seen in the HIMS study of men aged ≥ 70 years (34). Thus, lower endogenous T concentrations may be related to all-cause rather than cardiovascular mortality risk, but the non-linear nature of the association merits consideration and causality remains unproven.

Does Exogenous Testosterone Treatment Impact on Cardiometabolic Health?

The Testosterone in Older Men with Mobility Limitations (TOM) trial of 209 men aged ≥ 65 years with mobility limitations and baseline T concentration 100-350 ng/dL (3.5-12.1 nmol/L) or calculated free T > 173 pmol/L was discontinued in response to a higher rate of loosely defined adverse cardiovascular events in the T (n=23) vs the placebo group (n=5) (42). However, another randomized controlled trial (RCT) in 274 men aged ≥ 65 years, who were frail or intermediate-frail with baseline T < 12 nmol/L (346 ng/dL) or calculated free T < 250 pmol/L, found no

1 increase in cardiovascular adverse events in T-treated men and reported an improvement in
2 physical function (assessed using gait, balance, and submaximal walking tests) (43). The
3 Testosterone Trials (T Trials) randomised 790 men aged ≥ 65 years with baseline T concentration
4 < 275 ng/dL (9.54 nmol/L) and symptoms of decreased libido, difficulty walking or climbing
5 stairs, or low vitality, to 12 months T treatment vs placebo, finding modest improvement in
6 sexual function but no benefit for walking distance or vitality (44). T treatment compared to
7 placebo was associated with improved sexual desire, erectile function, and overall sexual
8 activity, volumetric bone mineral density and estimated bone strength, and correction of
9 unexplained anaemia (39,45). In T Trials seven men in each arm had major cardiovascular
10 adverse events (MACE: comprising myocardial infarction, stroke, or death from cardiovascular
11 causes) during the treatment period.

12
13 In the T Trials cardiovascular sub-study, which analysed 73 men receiving T and 65 receiving
14 placebo, T treatment was associated with a greater increase in non-calcified coronary artery
15 plaque volume, and no change in coronary artery calcium score (46). No MACE events occurred
16 in either group. The groups were unbalanced: placebo-treated men had greater plaque volumes at
17 baseline and at the end of the one-year study, thus larger and longer duration studies of this
18 endpoint would be important (47). By comparison, in the Testosterone Effects on Atherosclerosis
19 Progression in Aging Men (TEAAM) trial involving 308 men aged ≥ 60 years, T treatment for
20 three years did not alter the rate of change in coronary artery calcium score, nor the rate of
21 progression of CIMT, compared with placebo (48).

1 Testosterone for the Prevention of type 2 Diabetes Mellitus (T4DM) enrolled 1007 men aged 50-
2 74 years, waist ≥ 95 cm and baseline T concentration ≤ 14 nmol/L (404 ng/dL), with impaired
3 glucose tolerance or newly diagnosed type 2 diabetes (49,50). All men received a lifestyle
4 program (Weight Watchers) and were randomized to T treatment or placebo, for two years. Men
5 in the T arm had a 40% lower risk of diabetes at two years, defined using oral glucose tolerance
6 testing (OGTT) (50). T treatment was associated with favourable changes in body composition:
7 men in the placebo group lost 1.9kg of fat but also lost 1.3kg of muscle mass, whereas men in the
8 T arm lost 4.6kg of fat and gained 0.4kg of muscle. There was no signal for excess CV adverse
9 events in T4DM, with 17 men in the placebo and 12 in the T arms experiencing a MACE during
10 the trial (50). But as in the T Trials, and all other T RCTs reported to date, the number of men
11 experiencing such events was too low to permit definitive conclusions regarding safety of T
12 treatment.

13
14 In T4DM, T treatment resulted in lower fasting glucose and a reduced glucose increment at 2-
15 hours following OGTT, with no difference in HbA1c (50). In T Trials T treatment reduced
16 insulin resistance (assessed using homeostatic model assessment, HOMA-IR) with no changes in
17 fasting glucose nor HbA1c (51). The TIMES2 Study using transdermal T treatment vs placebo in
18 220 men with type 2 diabetes and/or metabolic syndrome also showed improvement in HOMA-
19 IR and stable HbA1c values at 6 and 12 months (52). The lack of change in HbA1c (despite
20 improved fasting glucose concentrations and OGTT responses in T4DM) may relate to T effects
21 on red blood cell stability, or possibly on deglycation mechanisms (53-55). There are key
22 differences between participants in T Trials and T4DM: men in T4DM were drawn from a wider
23 age range, had central adiposity (reflected in measured waist circumference) and impaired

1 glucose tolerance or newly diagnosed type 2 diabetes, with a higher cut-off for baseline T
2 concentrations. Importantly, T4DM involved a longer (2-year) intervention duration, and all
3 T4DM participants received a background lifestyle intervention, not a feature of T Trials or
4 TIMES2.

5
6 One meta-analysis by Xu *et al.* (56) of 27 RCTs with 2,994 participants associated T with
7 increased risk of cardiovascular-related events. However, contemporary meta-analyses (57-63)
8 do not associate T treatment with increased cardiovascular risk. For example, Corona *et al.* (57)
9 in a meta-analysis of 93 RCTs with 4,653 participants receiving active treatment and 3,826
10 placebo found no clear effect of T on the incidence of CV events, with an odds ratio for MACE
11 of 0.97 (95% confidence interval 0.64-1.46). Hudson *et al* (58) performed a two-stage individual
12 participant data (IPD) meta-analysis, including 35 primary studies with 5,601 participants, of
13 which 17 provided IPD on 3,431 participants, finding no evidence that testosterone increased
14 short- to medium-term cardiovascular risks, while noting the need for studies to evaluate long-
15 term safety (58).

16
17 Retrospective case-control studies, typically utilising large health insurance databases comparing
18 outcomes in men receiving T prescriptions versus those not prescribed T, have major limitations
19 including missing data on indications for prescribing, absence of randomisation and other
20 possible biases (64). Such studies have yielded contrasting results (65-75). Some have associated
21 T prescription with CV events (66,67,73), others reported neutral associations or reduced risk of
22 CV events and/or mortality (65,68-70,72,74,75). Some studies found differential associations

with CV risk based on duration of therapy (70), and whether or not “normal” T concentrations were reported on treatment (68,69,74).

Whilst not the primary focus of this review, erythrocytosis and elevation of haematocrit are recognised adverse events related to T treatment (9,59,61,76,77). The presence of prostate cancer is a contraindication to T therapy (8,9). Evidence to date suggests T treatment does not increase the risk of developing prostate cancer beyond that expected in eugonadal men, but it may be associated with detection of subclinical prostate cancer via PSA testing and prostate biopsy (8,9,59,61,63,77). The overall frequency of serious adverse events including prostate cancer events and MACE in large RCTs has been reassuringly low, albeit such trials, including T Trials and T4DM, generally excluded men at very high risk (44,49,50,63). The only T RCT powered to examine CV events as a primary endpoint, the “TRAVERSE” study, recruited hypogonadal men aged 45-80 years with T <300 ng/dL (10.4 nmol/L) with evidence of CVD or at increased risk for CVD (78). It commenced in 2018 and was completed in November 2022 with 5,246 participants (79). Its results will provide vital information for both cardiovascular and prostate safety of T therapy.

Are Higher Levels of Physical Activity and/or Exercise Associated with Better Cardiovascular Outcomes?

Inverse associations between physical activity levels and cardiovascular risk factors (80), risk of major vascular events (81-83) and mortality (84-88) are well established. Landmark studies in the 1960/70s highlighted the inverse association between job-related PA and CVD risk by demonstrating that those in more active roles (e.g. bus conductors and railroad switchmen) had a

lower risk of CVD than their more sedentary counterparts (bus drivers and railroad clerks) (89,90). The subsequent Harvard Alumni Study (91) was one of the first to suggest a dose-response between PA and risk of heart attack, whilst a recent prospective cohort study in 437,378 participants demonstrated that moderate to high occupational PA contributed to longevity in men after a 28-year follow-up (92). By assessing 44 studies, Lee *et al.* (2) concluded that there was clear evidence of an inverse linear dose-response relationship between the volume of PA and all-cause mortality. Collectively, these results highlight the impact of PA on CV risk and all-cause mortality into middle and older age. The feasibility of implementing lifestyle interventions in sedentary older adults was demonstrated in the LIFE Study, in which 818 participants randomised to a moderate intensity structured physical activity program had lower risk of major mobility disability compared to 817 who received a health education program, over a mean follow-up of 2.6 years (93).

Do Testosterone and Exercise Have Additive Effects on Cardiometabolic Health in Aging Men?

Few epidemiological or interventional studies have simultaneously assessed the effect of T and PA levels on measures of cardiovascular risk and/or mortality, a significant gap in the literature. A large study using data from the UK Biobank (n=208,677) recently evaluated the impact of sociodemographic, lifestyle and medical factors on T levels in men aged 40-69 years (94). This study reported that T was lower in those with a less favourable combination of sociodemographic, lifestyle and behavioural factors, including insufficient physical activity (to the order of 2 nmol/L (58 ng/dL)). Men with a diagnosis of CVD or diabetes also had lower T concentrations (approximately 0.3-1.0 nmol/L lower). This study suggests that lower PA, as well

1 as chronic conditions such as CVD and diabetes, may be associated with lower T levels,
2 highlighting their inter-related nature.

3
4 Two epidemiological studies have indicated that men with the combination of higher endogenous
5 T concentrations and higher self-reported physical activity (PA) levels tend to have more
6 favourable cardiometabolic outcomes. In a study of 1,649 men with a mean age of 49.8 years
7 (95), higher T concentrations and higher PA levels were consistently associated with lower BMI,
8 waist circumference and odds of metabolic syndrome. This is congruent with previous work
9 assessing T concentrations and PA levels independently (18,20,23,24,80). However,
10 improvements in CV risk factors did not translate into risk reduction of CVD events or death.
11 Another epidemiological study (96) using a larger dataset of older men (the Health in Men Study
12 HIMS n=3,351 age=77 years) reported that men with higher circulating androgens and higher
13 PA levels had the lowest BMI, waist circumference and risk of metabolic syndrome, reinforcing
14 earlier findings. In this study, which had twice the number of CVD events and almost four times
15 the number of CVD deaths compared to the previous study, men with higher circulating
16 androgens (T or dihydrotestosterone, DHT) and higher PA levels had the lowest risk of dying
17 from CVD (96). These results provided the rationale for the design and implementation of a 2x2
18 factorial randomised, double-blind, placebo-controlled trial investigating the effects of
19 combining T treatment with supervised, center-based EXercise training (the TEX trial) in middle
20 to older-aged men with low-normal serum T levels (97-99) (see Figure 1).

21
22 Collectively, epidemiological studies suggest that men with lower endogenous T concentrations
23 have poorer health outcomes, whereas men engaging in healthy lifestyle behaviours are more

likely to have higher T concentrations, and better outcomes. This raises the question as to whether T may be a modifiable risk factor for poor health in aging men. Epidemiological studies which assess the interaction between androgens and PA levels, and their association with CVD risk factors, are limited, but suggest that higher circulating androgens (T or DHT) combined with higher PA levels may be associated with favourable cardiometabolic outcomes. While outside the scope of this review, it is also possible that higher T concentrations or treatment with T, combined with higher PA levels, may have additional potential benefits on sexual and physical function, and wellbeing (61,63). Important gaps exist in our understanding of the inter-relationships between T concentrations, physical in/activity and cardiovascular risk. We now consider what is known about the impact of testosterone, exercise and their combination on key measures of cardiovascular risk, namely blood pressure and vascular health and function.

What Are the Effects of Testosterone, Exercise, and Their Combination on Blood Pressure?

High blood pressure (BP) is recognised as a leading risk factor for mortality, responsible for 13% of deaths globally (1). The prevalence of hypertension increases with age, and it is forecast to affect more than 1.5 billion people worldwide by 2025 (100,101). A 2 mmHg lower office systolic BP (SBP) has been associated with a 10% lower risk of stroke and 7% lower risk of ischemic heart disease, in primarily middle-aged adults (102).

What Impact Does T Have on Blood Pressure?

Traditional single measurement “office” blood pressure

The majority of cross-sectional studies in men have reported inverse associations between T concentrations and office BP and/or associated lower T concentrations with prevalent or incident hypertension (103). In contrast, two studies that assessed the relationship between T and components of the metabolic syndrome did not find a relationship between T and hypertension (104,105). However, a meta-analysis of 20 observational studies examining T concentrations and risk of metabolic syndrome reported an association of lower baseline T concentrations with prevalent but not incident hypertension (25).

Although many T intervention trials assess office blood pressure at baseline, only four have reported blood pressure as an outcome (106-109). These studies all report no significant difference in blood pressure following T treatment compared to placebo over durations from 16-52 weeks. In contrast, a study conducted by Zitzmann *et al.* (110), designed to assess the safety of a new intramuscular injection of long-acting ester T undecanoate (1000 mg at 10-14 week intervals) in 66 men aged 17-66 years, reported that SBP and diastolic BP (DBP) decreased by 6 and 7 mmHg respectively. No placebo group was included, so results must be interpreted with caution.

A meta-analysis of 30 trials comprising 1,642 men concluded that T treatment in men with low T concentrations resulted in modest changes in BP (SBP: +0.8 and DBP: +2mmHg) (111).

However, it is important to note that this meta-analysis also included populations with chronic

health conditions (such as COPD and cancer) and only 6/30 trials reported allocation concealment.

Ambulatory (24-hr) blood pressure

Measurement of 24-hour ambulatory blood pressure (ABP) provides a rich BP profile, inclusive of night-time readings. Ambulatory BP has greater prognostic accuracy (112), with higher levels of ABP significantly associated with increased risk of CV events (113,114) and mortality (115,116).

Studies assessing the association of circulating T with 24-hour ABP are scant and cross-sectional. Malan *et al.* (117) assessed 194 African and Caucasian men (25-65 years) stratified into low (mean 11.7 nmol/L (337 ng/dL)) and high (mean 22.2 nmol/L (640 ng/dL)) T groups, finding that lower T concentrations were associated with higher (+15 mmHg SBP) ABP in African, but not Caucasian men. In 106 normotensive men, Jimenez *et al.* (118) reported that T concentrations displayed a significant inverse association with both office and 24-hr ABP. These studies suggest that endogenous T concentrations are inversely associated with ABP, although additional larger studies are required.

Few T intervention studies have assessed the effect of T on 24-hr ABP (119,120). Gittelman *et al.* (119) assessed the safety of a new sub-cutaneous T enanthate auto-injector and reported ABP outcomes in 133 men aged 18-75 years. At week 12, while 24hr SBP and DBP were higher (3.7 and 1.3 mmHg respectively), as were office BPs at week 26 (3.4 and 1.8 mmHg respectively), there was no correlation between serum T concentrations and changes in BP. Swerdloff *et al.*

(120) compared a new oral formulation to topical T in a randomized open-label study (n=222; 18–65 years). The oral formulation resulted in a mean SBP increase in ABP of 4.9 mmHg after 16 weeks which was also reflected in the office SBP (+2.8 mmHg). There were no significant BP changes in the topical group. These results suggest that short-term (12-16 week) T use may increase SBP, when measured over a 24-hr period. These results are consistent with studies involving other oral testosterone formulations, one study (n=138; 26-75 years) associating T treatment with increases in 24-hr SBP (+3.8 mmHg) and DBP (+1.2 mmHg) after 4 months therapy (121); the other (n=155; mean age 51.2 years) with increases in 24-hr SBP (+1.7 and +1.8 mmHg) at 120 and 180 days (122).

What Impact Does Exercise Training Have on Blood Pressure?

Traditional office blood pressure

The evidence supporting the BP-lowering effect of exercise is well-established (123-126) and peak bodies such as the American College of Sports Medicine (ACSM) and American Heart Association strongly promote exercise for the prevention, and treatment, of hypertension (127,128). A meta-analysis comprising 2,419 participants concluded that regular aerobic exercise decreased SBP by 3.8 mmHg and DBP by 2.6 mmHg over 12 weeks (126). Consequently, physically active men have a relative risk of developing hypertension that is 35-70% lower than their sedentary peers (80,129,130). For those with hypertension, exercise is recommended as initial lifestyle therapy capable of decreasing SBP by 5-7 mmHg (131). Furthermore, a recent meta-analysis including 391 RCTs (n=39,742) reported the SBP-lowering effects of aerobic or resistance exercise were similar to that of antihypertensive medication (132).

Ambulatory (24-hr) blood pressure

Palatini *et al.* (133) assessed 602 men aged 19-45 years and reported that 24-hr SBP was significantly higher in non-exercisers than their exercising (>one aerobic session per week) counterparts. Kokkinos *et al.* (134) assessed 407 men (30-79 years) and reported pre-hypertensive men (resting SBP 120–139 mmHg or DBP 80-89 mmHg) with moderate fitness levels had 8/4, 7/3 and 6/4 mmHg lower daytime, night-time and 24-hr ABP (SBP/DBP) values compared to men with low fitness levels (134).

In terms of the impact of distinct forms of exercise, Lima *et al.* (135) (n=44, aged 60-75 years) reported greater decreases in ABP with combined (aerobic and resistance) training than aerobic alone. Saco-Ledo *et al.* (136) conducted a meta-analysis that assessed the effect of exercise on ABP in individuals with hypertension and concluded that aerobic exercise provided the most significant benefits for all measures of ABP compared to resistance or ‘multi-component’ training. However, only 4/15 studies in that analysis included a combined (aerobic and resistance training) program with one involving water exercise (137), another designed to compare continuous vs interval training (138). This highlights the paucity of data surrounding the effects of distinct forms of exercise training on ABP, even more so in healthy middle and older aged men.

What Impact Does the Combination of T Treatment and Exercise Training Have on Blood Pressure?

Few studies combining a T and an exercise intervention have reported BP outcomes, with varied results (139-141). Twelve weeks of supraphysiological T treatment (weekly injection of

3.5mg/kg bodyweight) combined with resistance training in men aged 19-45 years (n=21) resulted in an average 10 mmHg increase in office SBP (140). In contrast, Pasiakos *et al.* (142) reported no significant differences in office BP between males (n=50 aged 18-39 years) receiving T (200 mg weekly injection) or placebo in addition to a 28-d live-in, 55% exercise- and diet-induced energy deficit phase. Broadly in line with these results, Heufelder *et al.* (139) reported decreases in office BP following a 12-month diet and exercise intervention, independent of whether the men (n=32, age 57 years) newly diagnosed with type 2 diabetes mellitus also received T (50 mg gel daily) or placebo treatment. Despite supervised exercise interventions being employed in all three of the studies mentioned above, none incorporated a non-exercising control group to ascertain the relative effects of T and exercise in isolation. In contrast, Hildreth *et al.* (141) included a non-exercising control group over 12 months to assess the effect of T and/or progressive resistance exercise on supine (office) BP measures. No significant changes within, or between groups were reported in the cohort of 167 men ≥ 60 years (141). None of these T+Ex studies assessed ABP.

We recently completed a direct 2x2 factorial randomized placebo-controlled trial in which 80 men aged 50-70 years with waist circumference ≥ 95 cm and T concentration 6-14 nmol/L (173-403 ng/dL) were randomized to daily transdermal T or matching placebo (P) (double-blind), and to supervised exercise (Ex) or no additional exercise (NEx) for 12 weeks (the TEX trial – see Figure 1) (97). T treatment was with transdermal T cream, 2 mL (100 mg) administered to the upper body daily, a standard therapeutic dose (77). The exercise intervention was personalised and supervised, and comprised a mix of aerobic and resistance stations with gym attendance for one hour three times a week. There was a main effect of T treatment to increase 24-h SBP,

1 primarily seen when T was combined with Ex (97). Given that treatment-induced changes in
2 ABP tend to be smaller than for office BP (143), the 3 mmHg increase in 24-hr SBP observed in
3 the T and exercise group may be clinically meaningful. We also observed that exercise decreased
4 night-time DBP, and that this effect was attenuated by the addition of T treatment (97). This
5 finding is of interest given that smaller nocturnal declines in BP have been associated with
6 cardiovascular mortality risk (144).

7
8 Collectively, cross-sectional studies indicate that BP measurements (office and ABP) are
9 inversely related to T concentrations, however T intervention studies contradict these results. In
10 contrast, there is general consensus that exercise training reduces BP (office and ABP) although
11 which type of exercise (aerobic, resistance, combined) produces more favourable BP outcomes
12 remains a matter of debate. Few studies assessed the effect of combining T and exercise
13 interventions on office BP measurements with heterogenous results. Our data (97) from a head-
14 to-head comparison of the effects of exercise training and T treatment on BP outcomes suggests
15 that T treatment in isolation does not have beneficial effects on BP in middle to older aged men
16 with low-normal baseline T concentrations. BP should be carefully assessed and monitored when
17 prescribing T treatment to middle-aged and older men. Conversely, middle aged and older men
18 with low-normal T concentrations with a higher-than-optimal BP could benefit from a suitable
19 exercise program.

20
ACCEPTED MANUSCRIPT

What are the Effects of Testosterone, Exercise, and Their Combination on Vascular Function?

What is Vascular Function and How is it Assessed?

The vascular endothelium is integral to the maintenance of vascular health in humans, and arterial dysfunction precedes clinical manifestation of CVD (145). Flow-mediated dilation (FMD) measures the dilator response of a conduit artery to reactive hyperemia after temporary distal occlusion and ischemia (146). FMD is a validated surrogate measure for vascular health and a powerful predictor of future CVD events (147,148), with a 1% increase in FMD translating to a 9-13% improvement in CVD outcomes (148,149). In males, FMD decreases with age in a curvilinear fashion as a function of baseline arterial diameter. FMD is largely, though not solely, mediated by endothelial release of nitric oxide (NO), which has vasodilator and other anti-atherogenic effects (146,148). To specifically test vascular smooth muscle sensitivity to NO (i.e. nitric oxide mediated but endothelium-independent dilation), NO-donors such as glyceryl trinitrate (GTN) can be administered. Given that advancing age (150) and low testosterone (T) concentrations (151-153) are both associated with endothelial dysfunction and reduced FMD in men, whether T treatment has beneficial impacts on artery function in aging men is a relevant question.

What Impact does Testosterone Have on Vascular Function?

Endothelium-dependent dilation

Epidemiological studies associate higher circulating T concentrations with enhanced endothelial function, typically assessed as brachial artery FMD, in men across the age span (151-153). In contrast, interventional studies assessing the effect of T treatment on FMD in middle-aged and

1 older men have provided inconsistent results (154-160). For example, in eugonadal men with
2 coronary artery disease, intravenous T resulted in a dose-dependent improvement in FMD, one
3 hour after the infusion (155) and in 55 obese men with type 2 diabetes and $T < 11$ nmol/L (317
4 ng/dL) and/or calculated free T < 220 pmol/L, intramuscular T undecanoate resulted in increased
5 FMD over a two-year period (156). However, another study in men with coronary artery disease
6 and $T \leq 12$ nmol/L (346 ng/dL) reported no change in FMD following oral T treatment for 8
7 weeks (161) and other studies have reported decreased FMD following administration of T
8 (158,160). A meta-analysis by Sansone *et al.* (162) concluded that T does not significantly alter
9 vascular function in hypogonadal men but this meta-analysis only included a total of five
10 studies. However, highlighting the paucity of data concerning the effects of T intervention on
11 endothelial function. Given the limited and inconsistent findings from studies to date, further
12 studies using validated FMD techniques (163,164) are needed in men at risk of cardiometabolic
13 disease.

15 **Endothelium-independent dilation**

16 Testosterone-induced relaxation primarily involves endothelium-independent dilation in
17 experimental studies in animals (e.g. canine, porcine), where T induces relaxation in coronary,
18 mesenteric, iliac, renal, and femoral arteries, both *in vivo* and *in vitro* conditions (165-168). In
19 comparison, few studies have sought to assess the effect of T on endothelium-independent
20 dilation in humans. Supraphysiological doses of T were inversely associated with function of the
21 NO-dilator system in a case study (169), whilst physiological T doses may confer beneficial
22 vasodilator impacts on vascular smooth muscle and myocardial perfusion in middle-aged and
23 older-aged men with coronary artery disease (170-173). Others studies have reported no benefit

(156,158,160,161). It is likely that differences in study populations (especially comorbidities), T doses, route of administration, and study duration have contributed to the heterogeneous endothelium-independent results reported.

What Impact Does Exercise Training Have on Vascular Health and Function?

Endothelium dependent dilation

In contrast to the vascular effects of T, the beneficial effect of exercise training on endothelial dependent dilation is well established (174-183). A review and meta-analysis of 51 RCTs (n=2,260) by Ashor *et al.* (184) reported that exercise training (4-52 weeks) improved FMD by 2-2.8% which may translate into a reduction in CVD risk of 26–36% (149). The authors also assessed different exercise modalities and reported that aerobic, resistance and combined (aerobic and resistance) exercise training improved FMD by a weighted mean difference (WMD) of 2.79, 2.52 and 2.07% respectively (184). Although a significant positive relationship was reported between aerobic intensity and endothelial function, for resistance exercise, greater frequency (as opposed to intensity) proved more influential (184).

Endothelium-independent dilation

In comparison to studies assessing endothelium-dependent dilation, fewer have reported results concerning endothelium-independent dilation. In the Ashor *et al.* (184) meta-analysis mentioned previously, only 26/51 trials (1159 participants) included data on endothelial-independent dilation, measured in response to nitroglycerin. When compared to control groups, there was a trend towards improvement in endothelial-independent dilation following exercise training (WMD 0.47%), but this was not statistically significant ($P=0.055$). This suggests the effect of

exercise on vascular function is predominantly endothelium mediated. However, it is important to note that the trials reporting endothelium-independent dilation primarily consisted of populations with significant CV risk factors (hypertension, diabetes, hyperlipidemia) or documented CVD (coronary artery disease, chronic heart failure).

Does the Combination of Exercise Training and Testosterone Have Additive Impacts on Vascular Health and Function?

As previous exercise studies have demonstrated improvements in endothelial function (184), and others indicated that T may confer beneficial vasodilatory impacts on smooth muscle (170), it is surprising that very few studies have sought to assess the effects of this combination on vascular health and function. In a 54-week open-label study where participants could choose their own study arm, Francomano *et al.* (157) reported that the addition of T to a diet and exercise intervention significantly improved endothelial function compared to placebo in obese, hypogonadal men (n=24, 40-65 years). However, the exercise intervention was not supervised, nor was adherence recorded. Furthermore, endothelial function was measured using peripheral artery tonometry which may be less reliable and reproducible compared to flow-mediated dilation technique (185-188).

In addition to their main outcomes paper (discussed earlier in this review) (189), Hildreth *et al.* separately published FMD results (141). Despite 12 months of supervised exercise training in 132 older men +/- T treatment, Hildreth *et al.* (141) reported no main effects of T or exercise, and no differences between exercise and no-exercise groups, alone or with T treatment. Differences in results between Hildreth *et al.* (141) and Francomano *et al.* (157) may be

1 attributed to not only the assessment type (peripheral artery tonometry vs FMD) but also the
2 modality of exercise training. The exercise intervention conducted by Hildreth *et al.* (141) was
3 primarily resistance-based, in contrast to the intervention employed by Francomano *et al.* (157)
4 which consisted of aerobic exercise. The dose-response relationship between aerobic exercise
5 intensity and FMD may also influence results (184,190).

6
7 In our recent 2x2 factorial randomized placebo-controlled trial, the TEX study (n=80, 50-70
8 years) of T treatment and exercise training over 12 weeks (99), exercise training improved
9 endothelium-dependent vasodilator function, whereas administration of testosterone at
10 therapeutic doses did not impact FMD, or add to the benefit of exercise (see Figure 1). Vascular
11 smooth muscle sensitivity to NO was not modified by exercise, testosterone, or their
12 combination. Thus our study, using state-of-the-art methodology for assessing endothelium
13 dependent and independent dilation and supervised exercise to which participants were highly
14 compliant, found no evidence that T had beneficial impact on endothelial function beyond those
15 associated with exercise.

16
17 In summary, the effect of T treatment alone on vascular function in middle-aged and older men
18 is unclear, with inconsistent results reported for both endothelium-dependent and -independent
19 dilation. In contrast, there is a mature body of evidence supporting the effect of exercise on
20 vascular health and function (174,175). Whether the combination of T treatment and an exercise
21 program provides benefit beyond that experienced with exercise alone has not been widely
22 studied. However, the evidence that is available indicates that exercise is beneficial for vascular,

1 and in particular endothelial, function whereas short-term T administration does not confer
2 benefit, or provide any additional effect in addition to the impact of exercise *per se*.

3 4 **Conclusions**

5 Often in parallel with declining T concentrations, aging men may experience decreases in fitness,
6 strength, and vascular function; all of which are associated with increased mortality (191-193).

7 From an epidemiological perspective, having higher endogenous T concentrations and engaging
8 in higher levels of PA are associated with more favourable cardiometabolic outcomes in men.

9 From a clinical perspective, exercise interventions are of undoubted benefit, but require effective
10 implementation and sustained adherence. Effects of T pharmacotherapy in men who do not have
11 organic hypogonadism on cardiometabolic outcomes require further study. Such T intervention
12 may be more effective if sustained over a period of time and if administered in conjunction with
13 lifestyle interventions (as in T4DM), or tailored exercise programs. It is possible that
14 cardiometabolic benefits of T may be indirect, reflecting changes in body composition, rather
15 than directly improving BP or endothelial function. Exercise remains a first line strategy for
16 aging men to improve their cardiometabolic health.

17 18 **Data Availability**

19 Data sharing is not applicable to this article as no datasets were generated or analyzed during the
20 current study.

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FIGURE LEGEND:

Figure 1: Summary of our 2x2 factorial, randomized, double-blind, placebo-controlled TEX (Testosterone and Exercise) trial. Left: Hypothesised synergistic impacts of T and Ex on vascular function: Ex has endothelium-dependent impacts on vascular function, whilst some evidence suggests that T impacts on smooth muscle function. Right Upper: Changes from baseline following the 12 week intervention in flow mediated dilation (FMD), glyceryl trinitrate (GTN) mediated dilation and 24 hour blood pressure responses. Right Lower: Infographic summary of results: exercise benefits BP and vascular function, T does not provide additive benefit. ** $P < 0.001$, * $P < 0.05$ for week 12 change from baseline compared with Placebo + No Exercise group change. '+' indicates improvement, '-' indicates no significant change, 'x' indicates no synergistic benefit.

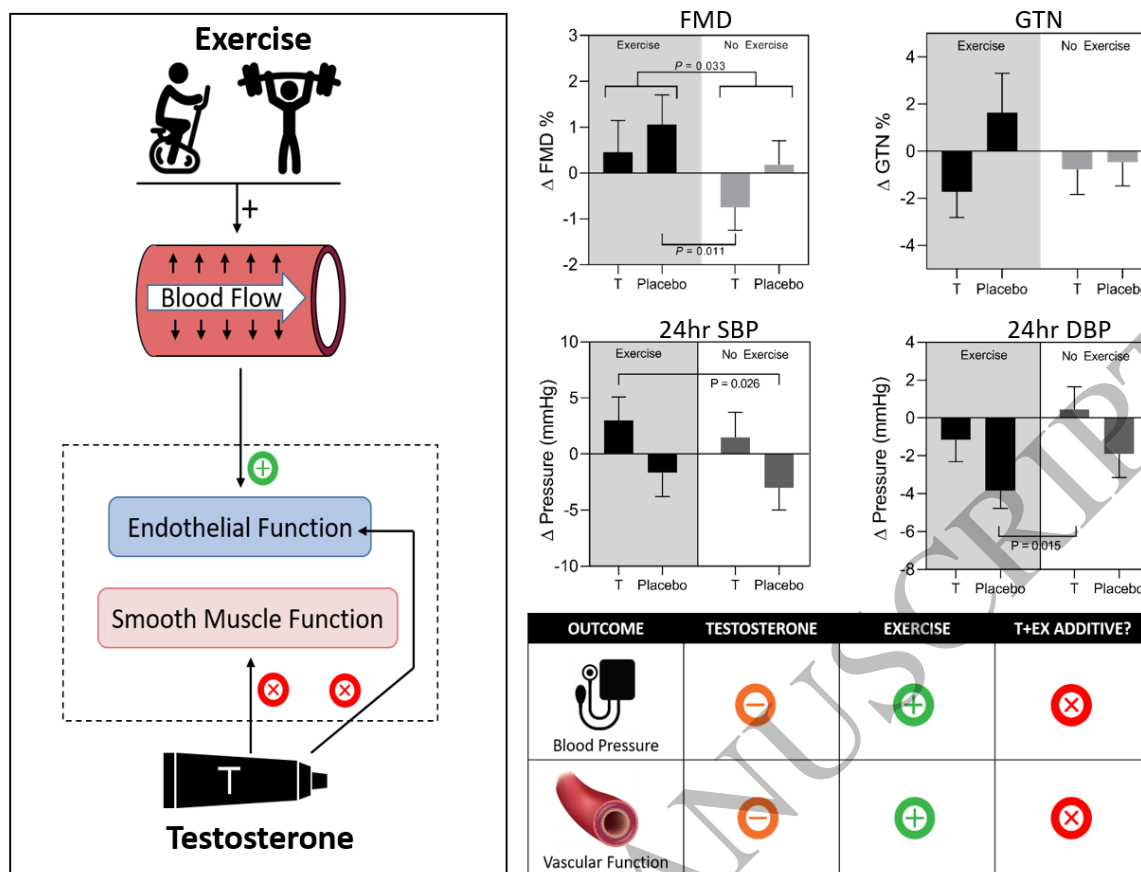


Figure 1
159x118 mm (1.7 x DPI)