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Systematic review: the Androgens In Men Study.

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ABSTRACT

Objectives

The overall study aim is to clarify the relation of endogenous sex hormones (primarily testosterone) with major health outcomes in men.

Setting

Community-dwelling men.

Participants

20,180 adult males participated in the final set of studies identified and selected from a systematic review. Eligible studies included prospective cohort studies with plasma or serum testosterone concentrations measured for adult males using mass spectrometry with at least 5 years of follow-up data, with incident cardiovascular, cancer, mortality, dementia or cognitive events recorded. Only published or grey literature items written in English were considered.

Primary and secondary outcome measures

Planned prospective outcome measures: cardiovascular disease (CVD) events, CVD deaths, all-cause mortality, cancer deaths, cancer diagnoses, cognitive decline, dementia. Outcome measures analysed in this paper were of the published estimates most frequently reported in selected studies: CVD deaths, all-cause mortality. All planned outcomes will be investigated for the selected studies as a separate series of individual participant data (IPD) meta-analyses.

Results

Screening of 1,994 de-duplicated items identified 9 suitable studies, with an additional two identified by colleagues (11 in total). Summary estimates of mean testosterone concentration

and age at recruitment for 20,180 adult males were 15.4±0.7nmol/L and 64.9±3.3yr. Despite considerable variation in mean testosterone, a meta-regression estimated no significant dependence on mean age at recruitment among studies (Slope = -0.03, 95% CI -0.11 – 0.06). Meta-analyses demonstrated no significant effect of a 5 nmol/L increase in testosterone on the risk of all-cause mortality (hazard ratio, HR = 0.96, 95% CI 0.89 – 1.03) or death from CVD (HR = 0.95, 95% CI 0.83 – 1.08).

Conclusions

Analyses of published estimates did not demonstrate associations of endogenous testosterone with CVD deaths or with all-cause mortality. Suggested further research includes the planned IPD meta-analyses for selected studies, including scope for investigating non-linear effects.

Registration

PROSPERO: CRD42019139668.

STRENGTHS AND LIMITATIONS OF THIS STUDY

- This is the first systematic review on this topic to restrict selections to prospective cohort studies of community-dwelling men with testosterone measured using mass spectrometry: the “gold standard” method.
- Systematic searches were made of both the published and grey literature using online search tools.
- Meta-analyses used estimates obtained from studies with at least five years of follow-up data and from fitted models which controlled for (at least) the age, smoking status, and body mass index or waist circumference of participants.
- Meta-analyses of published estimates were limited to assuming linear relationships, however subsequent IPD meta-analyses planned to arise from this work will look to explore non-linear associations.

- Analyses are of observational data, and so summary estimates will not fully eliminate the possibility of confounding arising from unadjusted effects.

1. INTRODUCTION

What does a low testosterone level mean for a man's health? In men, levels of testosterone, the key male sex hormone (androgen), decline with increasing age, yet the basis for and health consequences of this phenomenon remain unclear.[1-5] Many middle- and older-aged men are told their levels are "too low", explaining the 12-fold increase in global testosterone prescriptions over 2000-2011, costing $1.8 billion.[6] The Androgens In Men Study (AIMS) will seek to clarify the associations of androgens (primarily testosterone) with key health outcomes in men (mortality, cardiovascular disease, cancer, cognitive decline and dementia). The AIMS will conduct a systematic review and a series of individual participant data meta-analyses to address these questions. In this paper we present the systematic review and meta-analyses using published estimates from prospective cohort studies with at least 5 years of follow-up data and testosterone measured using only mass spectrometry, the most reliable method.[7]

2. METHODS

This systematic review, conducted 14 June—31 December 2019, was of "etiology and/or risk type" studies.[8, 9] The pre-specified purpose of the systematic review was to identify studies with suitable individual participant-level data (IPD) for collaborating with on a series of IPD meta-analyses. The PEO (Population, Exposure, Outcomes) characteristics included: adult men in the general community; endogenous circulating sex hormone concentration (primarily testosterone); incident cardiovascular disease (CVD), mortality, cancers, cognitive decline, dementia. Subgroup IPD meta-analyses are also planned for heart failure, myocardial

infarction, stroke; colorectal cancer, lung cancer, prostate cancer. A protocol was submitted to PROSPERO on 23 July 2019 and registered on 20 November 2019 (registration number CRD42019139668) and a protocol article has been published.[10]

2.1. Literature search and screening

Four online search tools were used to identify available published (MEDLINE, EMBASE) and grey literature (OpenGrey, Mednar) *items* (journal articles, reports, theses, webpage articles) reporting on suitable prospective cohort *studies* (the underlying unique sources of data). Two reviewers (RJM, JH) independently screened the de-duplicated items against pre-specified criteria using Rayyan.[11] To optimise efficiency, title and abstract screenings were initially conducted (Step 1), followed by full text screenings of the selected abstracts (Step 2). Disagreements were resolved through subsequent discussions between reviewers and agreement quantified using Cohen's Kappa and percent agreement. Only items reporting on prospective population-based cohort studies of adults (combined sexes or of men alone) with mass spectrometry measurements of testosterone and at least five years of subsequent follow-up data on incident CVD events, cancer or dementia diagnoses, cognition assessments, or on all-cause, CVD, or cancer deaths were selected. The Newcastle-Ottawa Quality Assessment Scale for Cohort Studies (NOS) was used to assess quality of the selected items.[12] The terms and full criteria used for the MEDLINE search, PRISMA checklist, NOS star ratings and additional methods details are included in Supplementary Material.

2.2. Meta-analyses of published estimates

Published estimates (author names, publication year, cohort study name, number of participants analysed, model covariates, testosterone statistics (overall and for individual exposure levels), participant age statistics, numbers of outcome events, follow-up time,

hazard ratios (HRs) and 95% CIs of the most fully-adjusted model) were extracted from selected articles by the first author (RJM). Testosterone statistics were converted into standard units (nmol/L) and values representing categorical ranges were determined following Wang et al.[13] If not reported, the numbers of participants and events within categories of testosterone, and the means of participant ages and testosterone concentrations at baseline, were calculated. The numbers of participants within quartile or quintile categories were calculated by dividing the total sample size by four or five. The numbers of events within categories were solved using Newton's method by applying the algorithm of Greenland and Longnecker.[14] Means and standard deviations for testosterone and age were calculated from presented quartile estimates using the Box-Cox method of McGrath et al., which does not make distributional assumptions.[15]

A random effects meta-regression of mean baseline testosterone concentration on the mean participant age at baseline was conducted using published estimates from: (i) only those items identified in systematic searches; and (ii) all suitable articles, including those found outside of systematic searches. A t-test of the meta-regression slope coefficient's departure from zero was done after applying the Knapp and Hartung adjustment.

Dose-response random effects meta-analyses (DR-MAs) were conducted to summarise published HR estimates for the associations of baseline testosterone concentrations with incident all-cause deaths and with CVD cause-specific deaths. Estimates from an additional article that had not been selected from systematic searches (Yeap et al[16]) were also used because it reported suitable estimates from one of the selected studies, and had been published within the literature search period. Contour-enhanced funnel plots were inspected for publication bias and patterns in heterogeneity and Cochran Q tests for heterogeneity (I^2),

as well as regression tests for funnel plot asymmetry,[17] were done.

The "metafor" package was used for meta-regressions, forest plots and funnel plots, the "doseresmeta" package for DR-MAs, and the "estmeansd" package for calculating study means and standard deviations from published quartile statistics in R version 4.0.2.[18-21]

2.3. Patient and public involvement

This work uses existing published data. Patients and public were not involved in the design, conduct, reporting, or dissemination plans of the systematic review or meta-analyses.

3. RESULTS

3.1. Literature search and study selection

The literature search returned 2,177 items (1,738 published and 439 from grey literature), with 1,994 items remaining after duplicates had been removed, and after excluding two Mednar items that had insufficient information available to review (Fig. 1). These included 1,764 journal articles, 111 webpage articles, 81 theses, and 38 unpublished reports/other documents. Systematic screening of the returned, deduplicated items excluded 1,968, classified five as "Maybe", and selected 20 as suitable. Most (92.1%) items were excluded from screening titles and abstracts at Step 1, with a much smaller percentage (6.6%) excluded from screening the 157 full text items in Step 2. One item could not be screened in Step 2 because the full text was not available. Inter-reviewer agreement was a Cohen's Kappa $\kappa = 0.69$ (or 96.0 percent agreement) for Step 1 and $\kappa = 0.82$ (or 98.1 percent agreement) for Step 2.

The 20 selected items collectively reported on eight prospective cohort studies: three from Australia (Busselton Health Study *BHS*, The Concord Health and Ageing in Men Project *CHAMP*, The Health In Men Study *HIMS*); three from Europe (European Male Ageing Study *EMAS*, The *MrOS* Osteoporotic Fractures in Men study in Sweden, Study of Health in Pomerania *SHIP*); and two from the USA (Atherosclerosis Risk in Communities *ARIC*, Cardiovascular Health Study *CHS*). Two of the five items classified as "Maybe" reported on the *MrOS USA* study, which were found, after further investigation, to be suitable for selection. Two additional studies were identified as suitable based on information external to the systematic searches and screenings: one from Australia (The Men Androgen Inflammation Lifestyle Environment and Stress study *MAILES*); and one from the USA (the Framingham Heart Study *FHS*. This is 11 cohort studies identified, in total. Additional details on returned and screened items, and selected article attributes are provided in Supplementary Material.

3.2. Meta-analysis and summary of selected articles.

The quality of selected articles ranged from six to nine (out of nine) stars on the Newcastle-Ottawa Scale. Relatively high scores reflected that all articles: were of population-based studies; accurately measured the exposure (baseline testosterone concentration); included multivariable models adjusting for participant age and other risk factors; had outcomes measured or collected from record linkage, with or without expert adjudication; and had sufficient follow-up (Tables S5-S7, Supplementary Material). Relevant outcomes included CVD deaths (n=7 articles); all-cause deaths (n=6); strokes or cerebrovascular disease (n=6); cognitive function or cognitive decline (n=5); coronary heart disease (n=4); CVD events (n=4); cancer diagnoses (n=3); myocardial infarction (n=2); heart failure (n=1); and dementia

(n=1). All were published between 2010 and 2018, reflecting the relatively recent adoption of mass spectrometry as the "gold standard" for measuring endogenous testosterone levels.[7]

The mean age of men at baseline ranged from middle-aged (49-54yr: BHS, FHS, MAILES, SHIP)[22-27] to elderly (72-77yr: CHAMP, CHS, HIMS, MrOS Sweden, MrOS USA).[28-36] Across the 11 studies, summary estimates for 20,180 adult males at baseline were 64.9±3.3yr for mean age and 15.4±0.7nmol/L for mean testosterone. Although there appeared to be a slight declining trend in mean testosterone with mean age among studies (Meta-regression Slope= -0.07, 95% CI -0.21 – 0.07), this estimate was not significantly different from zero (P=0.27; Fig. 2a). However, the distribution of model residuals demonstrated significant heterogeneity (P<0.001) and funnel plot asymmetry (P=0.02). Additional diagnostics highlighted a relatively high mean testosterone estimate from Pencina et al.[37] (FHS) and a low mean testosterone estimate (relative to mean age) from Chan et al.[24] (BHS), as compared to the other studies (Supplementary Material). When restricted to systematically selected items (reporting on ARIC, BHS, CHAMP, CHS, EMAS, HIMS, MAILES, MrOS Sweden, SHIP studies), tests of residual heterogeneity were significant (P<0.001), funnel plot asymmetry (P=0.91) was non-significant, and the slope estimate (Meta-regression Slope= -0.03, 95% CI -0.11 – 0.06) was not significantly different from zero (P=0.50; Fig. 2b). These results demonstrate that varying distributions of participant age (likely reflecting differences in study-specific objectives and recruitment methods) did not explain the observed heterogeneity in published estimates of testosterone among the studies.

Hazard ratios (HRs) for all-cause mortality were calculated from values in four of the selected articles (ARIC[38], BHS[24], CHS[39], EMAS[40]) and from one that was not selected, but had reported on the HIMS study during the literature search period.[16] All HRs

were adjusted for the age, smoking status, and body mass index (BMI) or waist circumference of participants. A DR-MA estimated a summary HR of 0.96 (95% CI 0.89-1.03) per 5nmol/L increase in testosterone (Fig. 3). The summary estimate was similar when calculated using an alternative estimate from Yeap et al[16] (HR=0.97, 95% CI 0.92-1.03). For both analyses, tests for residual heterogeneity (I^2=23.6%, P=0.26; I^2=0.0%, P=0.76) and funnel plot asymmetry (P=0.09; P=0.39) were non-significant. A comparable HR was calculated from a CHAMP study article[30] for inclusion in the forest plot but not in the DR-MA, because a corresponding estimate of variance per 5nmol/L increase in testosterone could not be calculated. Additional funnel plots, which included HR estimates from this CHAMP article[30] (per 1 standard deviation decrease in testosterone, as reported in that article), also demonstrated no significant asymmetry (Fig. S2c,d, Supplementary Material). These results demonstrate no overall effect of baseline testosterone concentration on the relative hazard of death from any cause after adjusting for factors including age, smoking status, and BMI or waist circumference.

HRs for death caused by CVD demonstrated similar findings. A DR-MA using estimates from the same five articles estimated a summary HR of 0.95 (95% CI 0.83-1.08) per 5nmol/L increase in testosterone, with no significant residual heterogeneity (I^2=28.3%, P=0.23) or funnel plot asymmetry (P=0.20; Fig. 4). Again, all HRs were adjusted for the age, smoking status, and BMI or waist circumference. The DR-MA repeated using an alternative estimate from Chasland et al.[25] for the BHS gave similar results (summary HR=0.93, 95% CI 0.83-1.03; heterogeneity I^2=17.5%, P=0.30; funnel plot asymmetry P=0.17). These results demonstrate no overall effect of baseline testosterone concentration on the relative hazard of death from CVD after adjusting for factors including age, smoking status, and BMI or waist circumference.

4. DISCUSSION

The systematic review identified nine studies, and when combined with an additional two identified by colleagues, comprises 11 in total, with data for over 20,000 men from Australia, Europe, USA and the United Kingdom. Meta-regressions revealed significant heterogeneity in testosterone measurements at baseline, which was not explained by the mean age of participants among studies. However, DR-MA summary estimates demonstrated no significant effects of baseline testosterone on the relative hazard of death from any cause or from CVD, with negligible heterogeneity present. The DR-MAs, which suitably accounted for correlations between estimates for different exposure categories within studies, were of published estimates that had been adjusted for age, smoking status, and BMI or waist circumference. Furthermore, only published estimates from prospective cohort studies of community-dwelling men that had measured testosterone accurately using mass spectrometry and had observed at least five years of follow-up data were used. Despite some of these studies having reported an association between testosterone and mortality,[16, 30] the collective body of evidence demonstrated no overall associations of endogenous testosterone concentration with mortality or CVD mortality.

Previous meta-analyses investigating associations of endogenous testosterone with the health outcomes of interest looked at CVD outcomes[41-43], all-cause mortality[41], and prostate cancer[44]. Boyle et al.[44] and Holmegard et al.[42] both reported negligible heterogeneity in their estimates. Boyle et al. found no significant association of a 5nmol/L increase in testosterone with prostate cancer and Holmegard et al. estimated a 43% increase in risk of ischemic stroke for men with testosterone levels below the 10th percentile, as compared to men in the 11th-90th percentile range, from a meta-analysis of four articles.[42, 44] Ruige et

al. estimated an 11% decrease in risk of a CVD event from a standard deviation increase in testosterone, and reported that significant heterogeneity was explained by larger effect sizes estimated for studies that recruited older men and for more recent articles.[43] Araujo et al estimated a 35% increase in risk of all-cause mortality and a non-significant effect on CVD mortality from a 2.18 standard deviation decrease in testosterone, although reported significant heterogeneity, and suggested that effects were driven by differences between the cohorts, such as underlying health status.[41] Two of these meta-analyses did not restrict selections to prospective cohort studies[41, 44] and none restricted selections based on testosterone assay method, although Ruige et al.[43] did find that assay method did not explain heterogeneity in that study.

The presented meta-analyses are the first to restrict selections to items of prospective cohort studies of community-dwelling men with testosterone measured using mass spectrometry, which is widely regarded as the reference method,[7] and with at least five years of follow-up data. Accordingly, the presented summary estimates could arguably be viewed as the most reliable to date. However, summary estimates represented associations that were assumed to be linear at the scale of log hazards, which was a key limitation of the analyses and likely to result in an oversimplification of true effects. For instance, although the 95% CI for the Pye et al[40] study (calculated from HR estimates for quintile categories of testosterone) overlapped one, an alternative set of estimates in that article (which could not be included in the DR-MAs) reported a two-fold increase in the risk of all-cause mortality for men with very low testosterone (<8nmol/L), as compared to “eugonadal” men (>11nmol/L). Pye et al[40] postulated that their reported differences in estimates might be reflective of a nonlinear association that emerges only when endogenous testosterone declines into the lower part of the range (<8nmol/L). Furthermore, Yeap et al.[16] estimated an “U”-shaped association

between endogenous testosterone and all-cause mortality, as consistent with a lower relative risk of health impacts for adult males with mid-range levels of testosterone. However, Shores et al.[39] also used non-linear modelling but did not find any significant associations of testosterone with all-cause or CVD mortality. Clearly, the investigation of non-linear associations is required to more comprehensively investigate the associations of testosterone concentrations with health outcomes in men.

Individual participant data (IPD) meta-analyses that incorporate flexible non-linear modelling techniques will provide improved scope to clarify the nature of such associations. The ability to apply a consistent statistical model to all studies, incorporate a more extended set of covariates than may have been included at the individual study level, and to estimate effects with increased statistical power, should result in more reliable summary estimates with reduced bias. Furthermore, other hitherto unpublished variables may be available for sharing by the collaborating studies to use in IPD meta-analyses, which could be useful for constructing analysis covariates or outcome variables. For instance, articles from the ARIC study that were identified from the systematic review reported on incident CVD event and death outcomes, but documentation on the ARIC study website shows that data on other prospective health outcomes, including cause-specific deaths and dementia diagnoses, are also available upon request.[45] Although there have been recent advances with non-linear modelling methods for the meta-analyses of published estimates,[18, 46] sufficient information in the published articles, as is required for implementing these methods, was not available. In future work, estimates from analyses of the IPD-level data will be used to estimate and plot non-linear summary effects, and so will provide further improvements to estimates of associations between androgen levels and health outcomes in men.

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AUTHORS' CONTRIBUTIONS

BBY, KM, RJM, JH contributed to the design of the systematic review. RJM conducted the literature search and RJM, JH independently screened the returned items. RJM, KM conducted the statistical analyses. All authors were involved in manuscript preparation and subsequent revisions, and approved this submission.

COMPETING INTERESTS

None declared.

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DATA SHARING STATEMENT

No additional data available.

PATIENT CONSENT

This manuscript does not contain patient personal data.

ETHICS APPROVAL

The AIMS study has been assessed as exempt from ethics review by the Human Ethics office at the University of Western Australia (file reference number RA/4/20/5014).

PROVENANCE AND PEER REVIEW

Not commissioned; externally peer reviewed.

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FIGURE LEGENDS

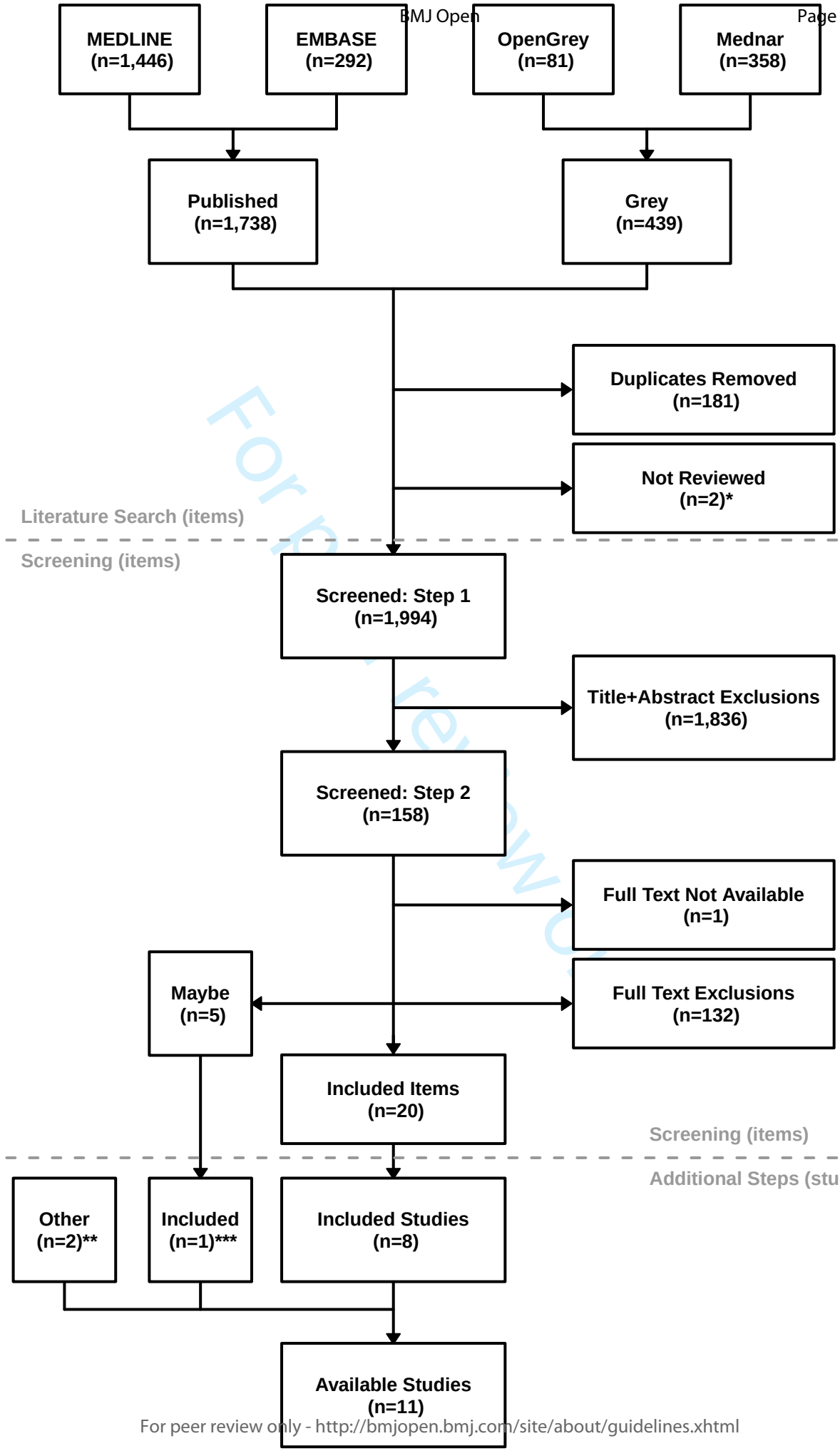
Figure 1. Studies returned from systematic review of the published and grey literature. Step 1 involved screening of titles and abstracts only and Step 2 the screening of full text items not excluded at Step 1 (see Tables 1, 2). "Items" are individual articles or reports, with multiple items returned for some studies (the purpose was to identify studies with suitable IPD-level data). * = Mednar items with insufficient information available to review; ** = Additional studies identified through known contacts; *** = Screening criteria for five items selected as "Maybe" in Step 2 were further investigated using information external to systematic searches and screenings, resulting in the identification of one additional study with suitable IPD-level data.

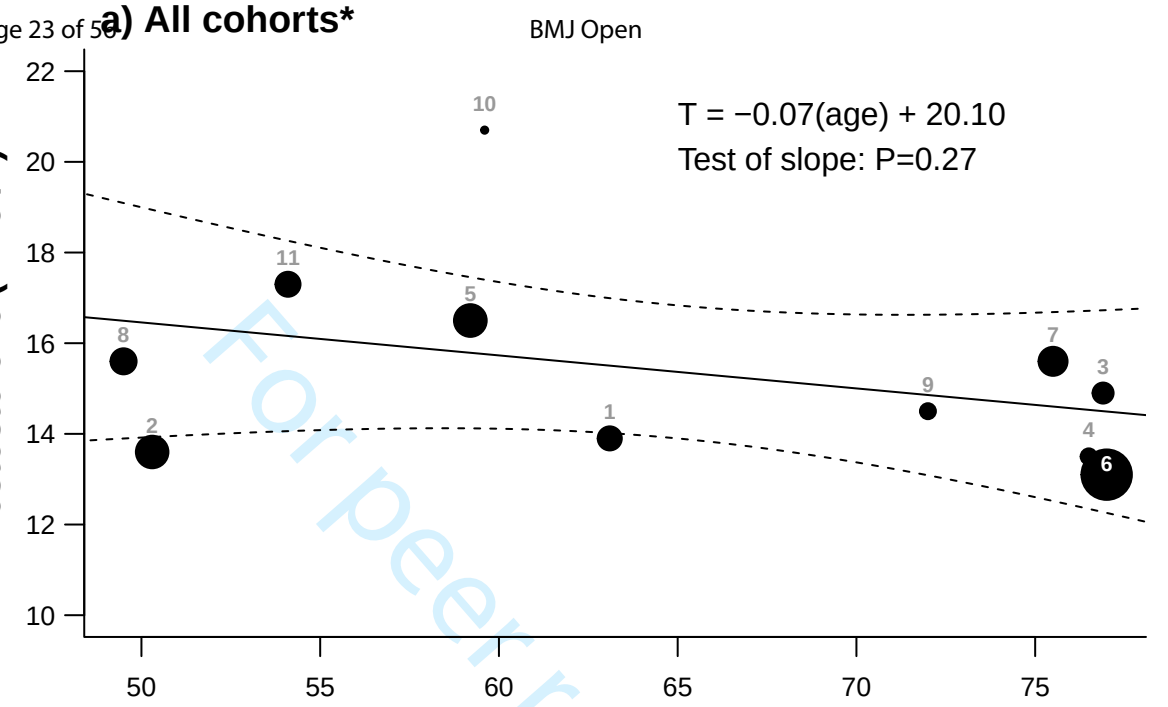
Figure 2. Meta-regression of mean testosterone on mean age for (a) all 11 cohort studies and (b) 9 studies with articles that were selected by systematic literature searches and screening. The size of plotted points refers are proportional to the inverse of the corresponding standard errors (indicative of relative weightings), with lines demonstrating the fitted model and 95% CIs. Plotted estimates are numbered as from the following articles (cohort studies): 1= Srinath et al.[38] (ARIC); 2= Chan et al.[24] (BHS); 3= Hsu et al.[30] (CHAMP); 4= Shores et al.[34] (CHS); 5= Lee et al.[47] (EMAS); 6= Chan et al.[28] (HIMS); 7= Ohlsson et al.[32] (MrOS Sweden); 8= Kische et al.[26] (SHIP); 9= Sueoka et al.[35] (MrOS USA); 10= Pencina et al.[37] (FHS); 11= Li et al.[27] (MAILES). * = includes articles from two additional studies (FHS, MAILES) that were not identified from systematic searches but by colleagues.

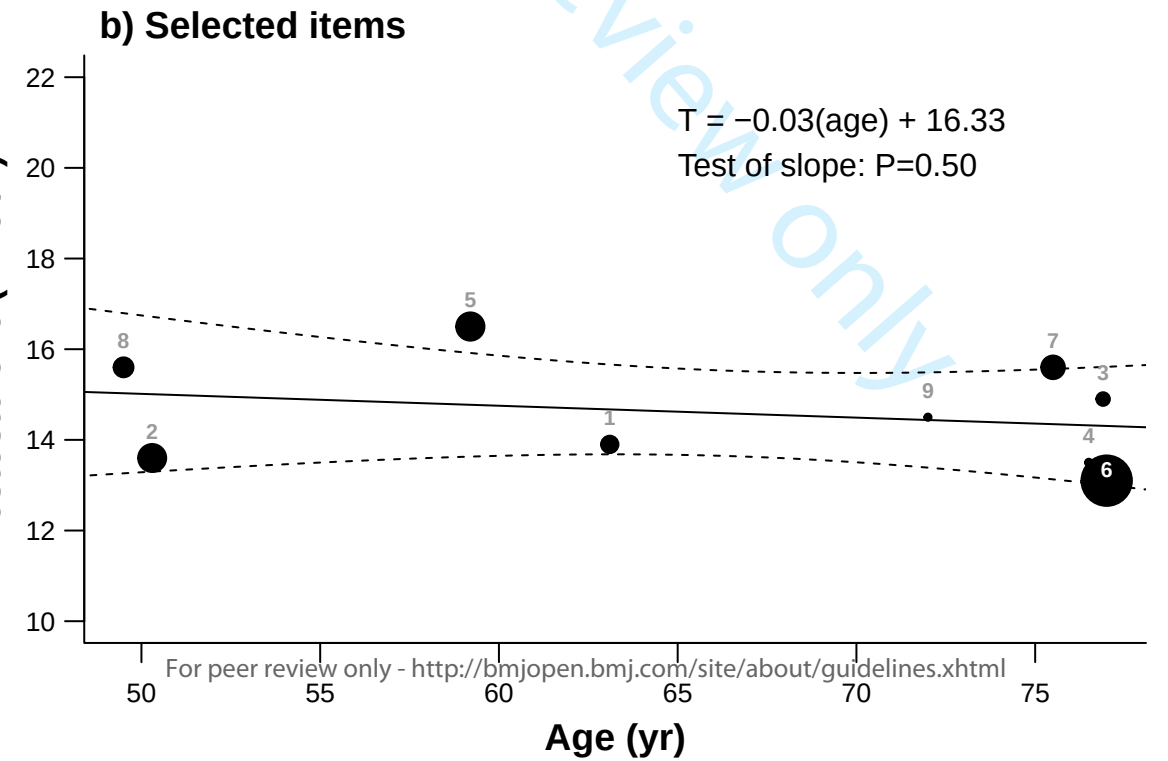
Figure 3. Forest plot of a meta-analysis of published estimates: association of testosterone with all-cause mortality. Plotted values are the estimated hazard ratios (HR) for death from

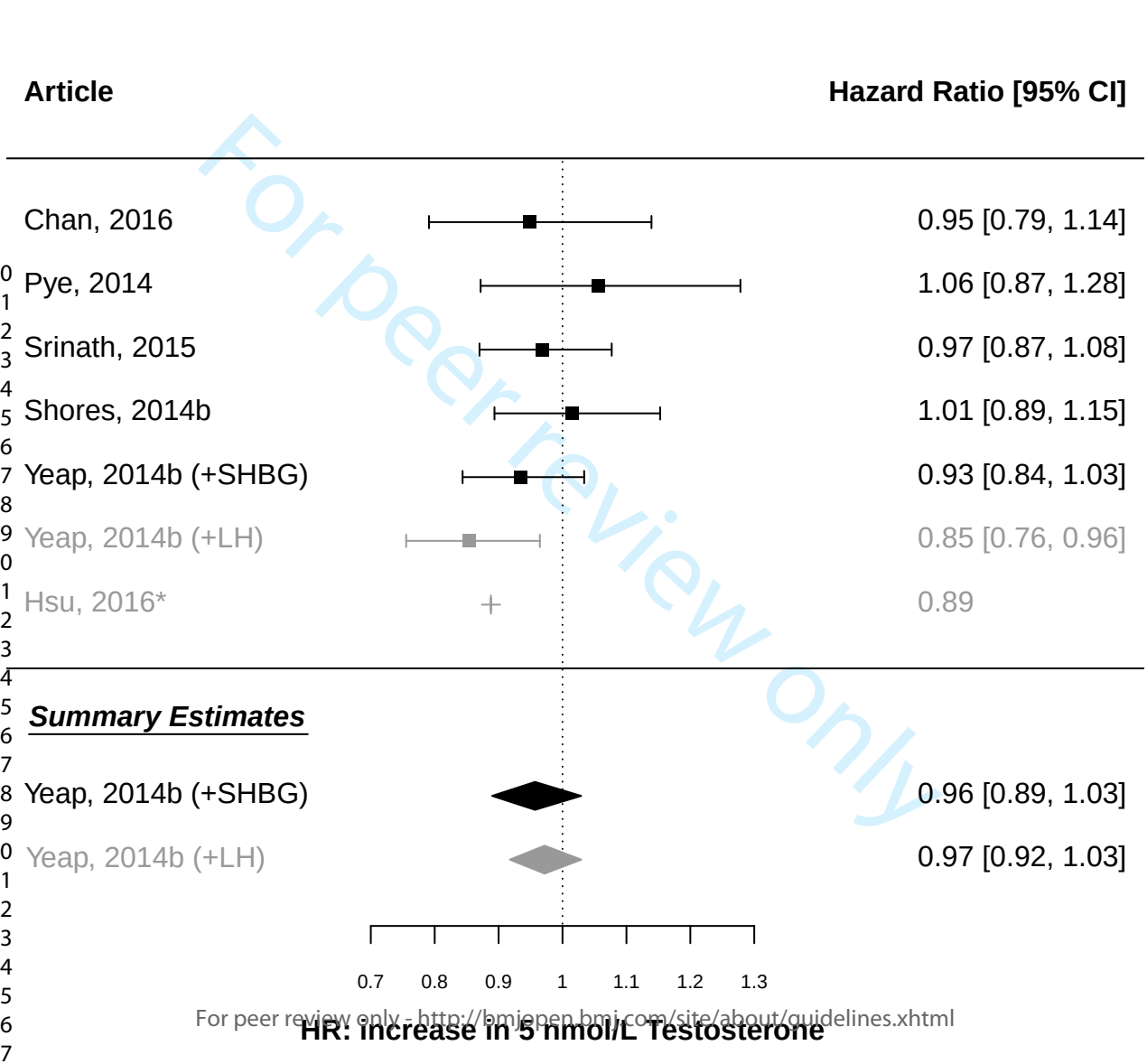
any cause, as attributed to an increase in endogenous testosterone concentration by 5 nmol/L. The vertical reference line is HR=1. Study-specific estimates are presented for six of the selected studies: BHS (Chan, 2016)[24]; EMAS (Pye, 2014)[40]; ARIC (Srinath, 2015)[38]; CHS (Shores, 2014b)[39]; HIMS (Yeap, 2014b)[16]; CHAMP (Hsu, 2016).[30] Summary estimates are colour-coded as calculated using either the estimates from Yeap et al.[16] calculated from the model including SHBG (black) or from the model including LH (grey). * This estimate from Hsu et al.[30] could not be used to calculate the summary estimate because a variance estimate was not calculable for a 5nmol/L change in testosterone using the published information.

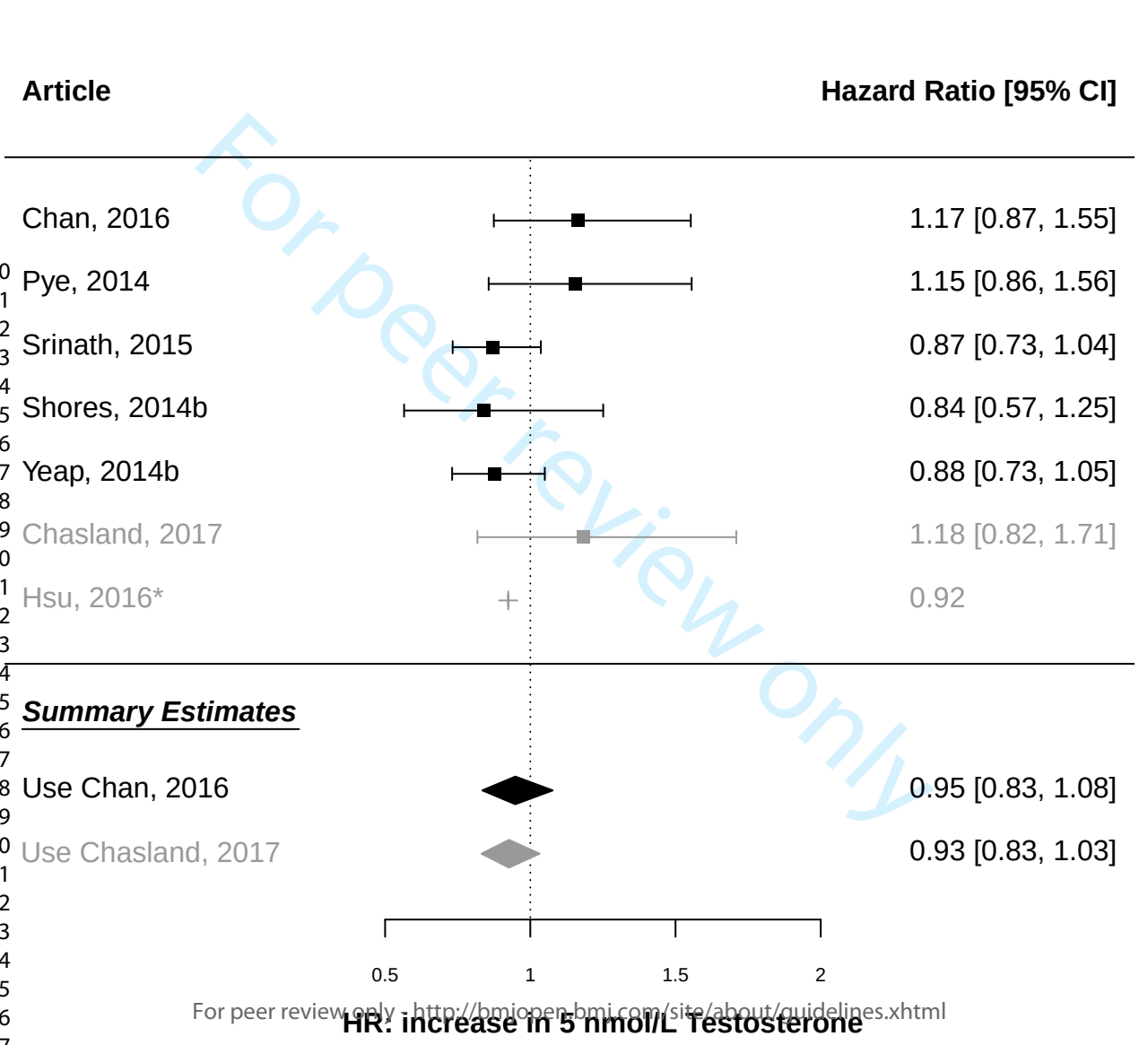
Figure 4. Forest plot of a meta-analysis of published estimates: association of testosterone with mortality caused by cardiovascular disease. Plotted values are the estimated hazard ratios (HR) for death from any cause, as attributed to an increase in endogenous testosterone concentration by 5 nmol/L. The vertical reference line is HR=1. Study-specific estimates are presented for six of the selected studies: BHS (Chan, 2016; Chasland, 2017)[24, 25]; EMAS (Pye, 2014)[40]; ARIC (Srinath, 2015)[38]; CHS (Shores, 2014b)[39]; HIMS (Yeap, 2014b)[16]; CHAMP (Hsu, 2016).[30] Summary estimates are colour-coded as calculated using either the estimates from Chan et al.[24] (black) or Chasland et al.[25] (grey) for the BHS. * This estimate from Hsu et al.[30] could not be used to calculate the summary estimate because a variance estimate was not calculable for a 5nmol/L change in testosterone using the published information.











Supplementary Material: Systematic Review for the Androgens In Men Study.

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Supplementary Material: Additional details on systematic searches and screening

We used online search tools to identify available published (MEDLINE, EMBASE) and grey literature (OpenGrey, Mednar) *items* (journal articles, reports, theses, webpage articles) reporting on suitable prospective cohort *studies* (the underlying unique sources of data). We used OpenGrey and Mednar because both were free search tools that we considered likely to identify additional grey literature items and studies in an expanded search beyond the mainstream publications. Mednar is a medically-focussed search engine of public and deep web resources, excluding subscription services.[1] OpenGrey is a searchable database containing citations for items including technical or research reports, theses, conference papers, and other types of grey literature.[2] Literature searches were conducted on 18-22 July 2019, with no date restrictions set.

Where possible (as functionality varied among the different tools), we placed the following restrictions on the search: items reporting on the results of a research study, longitudinal or prospective cohort studies, not of hormone therapy or deprivation treatments. Due to study timeframe and language translation limitations, we opted to search for only those items that were reported in the English language. The terms and full criteria used for the MEDLINE search are provided in Table S1, and the PRISMA checklist as Table S8.

Selection criteria were set as applicable to the planned sets of IPD meta-analyses (Table S2).[3] Only items reporting on prospective population-based cohort studies, adults of combined sexes or of men alone, with individuals free of the disease at baseline, were sought. Items reporting a different design for the analysis of longitudinal data, such as nested case-control or case-cohort design, were also considered acceptable. A minimum of five years follow-up was selected, to ensure a sufficient number of incident events for statistical

modelling. We excluded items that did not measure testosterone using mass spectrometry, which is regarded to be the 'gold standard' method,[4] although testosterone was not required to be mentioned in the title or abstract, nor modelled as the primary exposure variable. Selected items were to be studies of humans, reported in English, and reporting on analyses of at least one of the AIMS outcomes.

Two reviewers (RJM, JH) independently screened the de-duplicated items against these pre-specified criteria. To optimise efficiency, the selection of items proceeded in two steps. Title and abstract screenings (Step 1) were followed by full text screening of items selected in Step 1 (Step 2). If an item was selected for exclusion, then the main reason for that decision was recorded. If there was uncertainty in the decision to exclude, in Step 1 the reviewer selected "include" (in Step 1) or "maybe" (in Step 2). At the end of each step, the two reviewers sought to achieve consensus, through discussion, for each item that did not achieve agreement. Exclusion reasons were used to inform discussions for achieving consensus. Items with a consensus decision of "maybe" were further investigated by Reviewer 1 (RJM) using information external to the systematic searches and screenings (reading further details of methods used in cited articles, and from correspondence with authors or other researchers currently working on the research study).

This screening procedure was adjusted to accommodate the different types of items reviewed (published articles, theses, webpage articles, unpublished reports; Table S3). A pilot set of title-only screenings for 30 randomly chosen articles suggested that sufficient information was contained within the titles alone for the purpose of Step 1 screenings.[a] Therefore, in cases

[a] 30 titles were initially screened at random. 18 were flagged as not suitable, leaving 12 as potentially suitable. Subsequent Step 1 screening of titles with abstracts selected 25 of these articles for exclusion, with 5 retained for Step 2 (full text screening). All 5 were flagged as being potentially suitable in the pilot set of title-only screenings.

when an abstract was not available, only the titles were screened. Website items identified by the Mednar search tool were the type of item that most often did not have abstract or summary text, and in these cases the webpage text was reviewed in place of an abstract (Table S3).

Endnote X8[5] was used for collating and storing the citations returned from literature searches, and for de-duplicating and storing the selected references. The full citations, including abstracts, were exported from Endnote for uploading into Rayyan[6], which is a free web tool that was used for screening, recording exclusion decisions, and downloading selection results.

The literature search identified 2,177 items (1,738 published and 439 from grey literature), with 1,994 items remaining after duplicates had been removed, and after excluding two Mednar items that had insufficient information available to review (Fig. 1). Table S4 shows the frequencies of returned items by search terms present in the titles and abstracts. Most (72.7%) had the word “cancer”, and 1,107 (55.5%) of these had the word “prostate cancer”, in the title or abstract. This, combined with frequent mentions of “androgen deprivation” (29.2%), “radiotherapy” (18.6%), and “brachytherapy” (8.3%), show that items reporting aspects of testosterone deprivation or suppression for treating prostate cancer were a predominant feature of the returned items. Different types of returned items included 1,764 published articles, 111 webpage articles, 81 theses, and 38 unpublished reports/other documents, and the percentages without abstract or webpage text screened in Step 1 were 2.6%, 1.8%, 24.7%, 65.8%, respectively (i.e., 4.7% overall).

One thousand nine hundred sixty-eight items were excluded, five items were classified as "Maybe", and one item could not be screened because the full text version was not available, leaving n = 20 suitable items selected (Fig. 1). Most (92.1%) of the exclusions were made from reviewing titles and abstracts at Step 1, with a further 6.6% excluded from screening of the 157 full text items in Step 2. Inter-reader agreement was a Cohen's Kappa $\kappa = 0.69$ (or 96.0 percent agreement) for Step 1 and $\kappa = 0.82$ (or 98.1 percent agreement) for Step 2. Percentages of items with search terms (AIMS outcomes) in the title or abstract increased after Step 1 in most cases except for "cancer" and "prostate cancer" (Table S4). This reflects many exclusions in Step 1 that were of items reporting research on testosterone deprivation or suppression treatments for prostate cancer.

The systematic approach to literature searching and screening is widely held to be beneficial to identifying studies that otherwise may not have been considered for inclusion, and thus to minimise the prospect for reviewer biases affecting study selections and summary results.[7] This process is not perfect though, and in our case it did not identify two prospective cohort studies that were known to be suitable, prior to commencing this review (FHS, MAILES).[3] In the case of MAILES, this was one of the more recently commenced of the selected studies, with its cohort profile article published in 2014,[8] and accordingly has had a comparatively short timeframe within which to analyse and publish suitable findings. In the case of FHS, associations of endogenous testosterone with male health outcomes had previously been investigated and published, but not using mass spectrometry for measuring testosterone.[9, 10] Those articles were identified in the literature search but had been excluded on account of assay method. Only relatively recently have testosterone measures been re-assayed for FHS participants using mass spectrometry methods.[11] One article by Pencina et al[12] was possibly within scope but not identified because it had not been entered into the MEDLINE

database prior to the literature search (article entry date = 14 May 2020). Furthermore, an article that presented suitable estimates from one of the selected studies by Yeap et al[13] was not identified from the literature search because it did not have "prospective", "follow-up", "cohort study" or "longitudinal study" terms in its title or abstract, nor any of the corresponding MeSH terms listed (refer to Table S1 for search terms used).

In expanding our literature search to unpublished grey literature, it successfully located one suitable item, which was a link to a Web MD webpage article, with further details published in a conference abstract by Sueoka et al[14] that would otherwise have not been returned from searching only the MEDLINE and EMBASE databases.

Supplementary Material: Tables

Table S1. Full electronic search strategy used for MEDLINE database.

The following is the search that was conducted on 18 July 2019 using MEDLINE.

1. Testosterone/ or Androgens/
2. (testosterone or androgen* or sex hormone* or sex steroid*).ti.
3. (testosterone or androgen*).ab.
4. cardiovascular diseases/ or heart diseases/ or heart failure/ or vascular diseases/ or stroke/ or myocardial infarction/ or coronary disease/ or cerebrovascular disorders/
5. (cardiovascular or stroke or myocardial infarction or heart failure).ti.
6. neoplasms/ or colorectal neoplasms/ or lung neoplasms/ or prostatic neoplasms/
7. cancer.ti.
8. mortality/ or mortality.ti.
9. dementia/ or cognition/ or dementia.ti. or cognit*.ti.
10. Aging/psychology or Neuropsychological Tests/
11. 1 or 2 or 3
12. 4 or 5 or 6 or 7 or 8 or 9 or 10
13. 11 and 12
14. longitudinal studies/ or prospective studies/ or follow-up studies/ or cohort studies/
15. (prospective or follow-up or cohort study or longitudinal study).ti,ab.
16. 14 or 15
17. 13 and 16
18. (exogenous or replacement or therapy or hormone treatment).ti.
19. Hormone Replacement Therapy/
20. 18 or 19
21. 17 not 20
22. limit 21 to humans
23. limit 22 to english language
24. limit 23 to (adaptive clinical trial or address or autobiography or bibliography or biography or case reports or clinical trial, all or clinical trial, phase i or clinical trial, phase ii or clinical trial, phase iii or clinical trial, phase iv or clinical trial, veterinary or clinical trials, veterinary as topic or clinical trial protocol or clinical trial or comment or controlled clinical trial or dictionary or editorial or lecture or legislation or meta analysis or practice guideline or pragmatic clinical trial or published erratum or randomized controlled trial or retracted publication or "retraction of publication" or "review" or "scientific integrity review" or "systematic review")
25. Retrospective Studies/ or Case-Control Studies/ or (retrospective analysis or case-control).ti.
26. 24 or 25
27. 23 not 26

Notes:

Terms with a trailing "/" are MeSH terms and those with a trailing "*" are truncated search strings. Beforehand, a search of PROSPERO was conducted for another suitable strategy but none were found. However, the above strategy is based upon one that has been used for a similar study.[15] This search strategy is also published in the protocol article for the Androgens In Men Study.[3]

Table S2: Selection criteria for screening items returned from the literature search. If neither Include nor Exclude could be selected for Step 1, then reviewer selected "Include".

	Exclude	Include	Rationale	Used in Step 1		Used in Step 2
				Title & Abstract		Full-text
				Title only (no abstract)	Title & Abstract	
Article type:	Reviews, comments/opinion pieces, systematic reviews, dictionary, fact sheet, website information about diseases, fact sheets, etc.	Research study article / report, or an article that specifically refers to the results of one (e.g., a webpage referring to unpublished data).	These searches were of both published and unpublished scientific literature for the purpose of identifying prospective cohort studies that are likely to have the relevant data for planned IPD meta-analyses	Yes	Yes	Yes
Study type:	Retrospective or cross-sectional designs, case studies, case-control, surveys, RCTs or other trials, experiments, evaluation of androgen / testosterone therapy / deprivation / HRT or the effectiveness of any other type of intervention / surgery / treatment, genetics, etc.	Prospective cohort study.	A prospective cohort study design is of incident health outcomes for investigating etiology or disease risk for a cohort free of disease at baseline, and ideally should be representative of the local population, but may or may not be some demographic subset: e.g., age range, sex, ethnicity type. Further details in Table 2.	Yes	Yes	Yes
Population (at baseline/date of recruitment to study)	Studies of juveniles only Studies of females only Individuals with some specific health condition/characteristic or following surgery / other medical treatment for specific illness	Adults (18 yr or older) Not females only Community-dwelling men	The study is of community-dwelling men.	Yes	Yes	Yes
Exposure (at baseline)	Do not exclude studies that do not model testosterone as the exposure: although it should be shown that it was measured for participants. If not mentioned in Step 2 then Exclude.	Endogenous testosterone	This will be the focal exposure for all IPD meta-analyses. However, as we are focussing on the identification of only those studies who have suitable androgen measurements available in IPD data, then testosterone does not necessarily need to be modelled as the focal exposure in included items. It is likely that details on the methods will be available only from full-text review.	Only if available	Only if available	**Yes**
	Testosterone not measured using mass spectrometry	Testosterone assay of serum or plasma sample using mass spectrometry (lc-ms or gc-ms)		Only if available	Only if available	**Yes**
Outcome (at follow-up)	Incident outcome not one of those type of events specified for inclusion.	Diagnosis/event of: cardiovascular disease (any); cancer (any); dementia. Deaths (any cause); deaths due to any type of cardiovascular disease; deaths due to any type of cancer. Cognition change / outcome	These are the outcomes for the planned IPD meta-analyses so it is important to seek IPD datasets from those studies who have already modelled these outcomes. We refer to these as the "AIMS outcomes".	Yes	Yes	Yes
	Less than 5 years of follow-up data	Five or more years of follow-up data, with outcomes identified using systematic follow-up or data linkage.	As consistent across all included studies for IPD meta-analyses and set *a priori.* Likely that this will be available only from full-text review so not included Step 1.	No	No	**Yes**
Language	Title and/or abstract of article not in English	Title and/or abstract of article in English	As limited by the timeframe of this study and the native language of reviewers (a practicality).	Yes	Yes	Yes
Species	Studies not of humans	Studies of humans	We are studying humans.	Yes	Yes	Yes

Table S3: Adaptation of screening rules for different types of published and unpublished items.

Item Type	Step 1	Step 2
Published article	Screen title (and abstract[a])	Screen full text article
Thesis	Screen title (and abstract[a])	Screen full thesis
Unpublished report / other document	Screen title (and abstract[a,b])	Screen full document
Webpage	Screen title and webpage[c]	Screen full text article/document as identified from the webpage, or from a google search of information provided about the article, from the webpage.

[a] = when an abstract was available, otherwise title-only decisions were made (see Table 1).

[b] = or, if not an abstract, other suitable document summary, as returned by the search tool.

[c] = for webpage articles, the webpage text served as the proxy for an abstract, with the proviso that the reviewer did not navigate to additional webpages during Step 1.

Table S4. Words mentioned in the titles or abstracts of reviewed items.[a]

Word(s)	Step 1 items (n=1,994)	Step 2 items (n=158)	Selected items (n=20)
Search terms (AIMS outcomes)			
cancer	1,449 (72.7)	72 (45.6)	6 (30.0)
colorectal cancer	9 (0.5)	4 (2.5)	2 (10.0)
lung cancer	10 (0.5)	6 (3.8)	2 (10.0)
prostate cancer	1,107 (55.5)	40 (25.3)	2 (10.0)
cardiovascular	219 (11.0)	49 (31)	15 (75.0)
heart failure	29 (1.5)	2 (1.3)	1 (5.0)
stroke	31 (1.6)	12 (7.6)	4 (20.0)
myocardial infarction	33 (1.7)	7 (4.4)	1 (5.0)
mortality	232 (11.6)	45 (28.5)	9 (45.0)
dementia	22 (1.1)	8 (5.1)	2 (10.0)
cognit*	87 (4.4)	20 (12.7)	4 (20.0)
Other frequently observed (not search terms)			
androgen deprivation	583 (29.2)	2 (1.3)	0 (0.0)
androgen receptor	235 (11.8)	10 (6.3)	0 (0.0)
brachytherapy	165 (8.3)	0 (0.0)	0 (0.0)
breast cancer	153 (7.7)	9 (5.7)	0 (0.0)
radiotherapy	371 (18.6)	0 (0.0)	0 (0.0)

[a] = Items summarised as numbers (percentages); *= wildcard character designating truncation

Table S5. Attributes of selected items.

Item	Article	Country	Study name[§]	Baseline** No. adult males	Baseline period	Age (yr) Mean (sd)	T (nmol/L) Mean (sd)	Follow-up (relevant outcomes) Length of follow-up (yr) (person-years)[¶]	AIMS Longitudinal Outcomes (no. of events analysed)
Selected from systematic review									
1	Srinath, 2015[16]	USA	ARIC	1,558	1996-98	63.1 (5.6)	13.9 (5.7)	Md=12.8 (CHD); Md=13.1 (HF) (25,374; HF)	Coronary Heart Disease (CHD; 287) Heart Failure (HF; 104) CHD deaths (29) All-cause deaths (347)
2	Srinath, 2016[17]	USA	ARIC	1,558	1996-98	63.1 (5.6)	13.9 (5.7)	Md=14.1 (27,311)	Ischemic Stroke (79)
3	Chan, 2016[18]	Australia	BHS	1,804	1994-95	50.3 (16.8)	13.6 (4.9)	Mn=14.9 (31,930)	CVD events (234; 399)*** CVD deaths (71; 141)*** All-cause deaths (191; 319)***
4	Chasland, 2017[19]	Australia	BHS	1,649	1994-95	49.8 (15.3)	13.7 (4.9)	Tot=20	CVD events (415) CVD deaths (127)
5	Chan, 2018[20]	Australia	BHS	1,574	1994-95	51.1 (14.7)	13.5 (4.8)	Tot=20	Prostate cancer (116) Lung cancer (22) Colorectal cancer (48) Cancer (any; 289)
6	Hsu, 2015[21]	Australia	CHAMP	853	2005-07	76.9 (5.5)	14.6 (6.2)	Tot=5	Cognitive decline (95)
7	Hsu, 2016[22]	Australia	CHAMP	1,705	2005-07	76.9 (5.5)	14.9 (6.6)	Md=6.9; Tot=10 (11,764)	Cancer deaths (151) CVD deaths (185) Other deaths (174) All-cause deaths (510)
8	Hsu, 2018[23]	Australia	CHAMP	1,651	2005-07	76.9 (5.5)	14.7 (6.4)	Tot=5	All-cause deaths (382) CVD deaths (cases not reported) Cancer deaths (cases not reported) Other deaths (cases not reported) Change in: MMSE, SF-12 (Mental).

Supplementary Material: Systematic Review for the Androgens In Men Study.

				Baseline**				Follow-up (relevant outcomes)	
Item	**Article**	**Country**	**Study name§**	**No. adult males**	**Baseline period**	**Age (yr) Mean (sd)**	**T (nmol/L) Mean (sd)**	**Length of follow-up (yr) (person-years)¶**	**AIMS Longitudinal Outcomes (no. of events analysed)**
9	Rosenberg, 2018[24]	USA	CHS	1,019	1994	76.3 (4.9)	13.2 (6.2)	Md=9.5 (10,716)	Atrial Fibrillation (304)
10	Shores, 2014a[25]	USA	CHS	1,032	1994	76.5 (5.2)	13.5 (6.1)	Md=10; Tot=16 (19,220)	Ischemic stroke (114)
11	Shores, 2014b[26]	USA	CHS	NR	1994	NR	NR	Md=8.9 (CVD events) Md=10.8 yr (All-cause deaths). (9,184; CVD events)	CVD events (436) CVD deaths (157) All-cause deaths (777)
12	Lee, 2013[27]	Europe§§	EMAS	2,736	2003-05	59.2 (10.7)	16.5 (6)	Md=4.3; Tot=5 (14,486)	Cancer (any) Myocardial Infarction (MI) Heart Failure, Other heart conditions Stroke Cognitive function All-cause deaths (193)
13	Pye, 2014[28]	Europe§§	EMAS	2,599	NR	60 (11)	NR	Md=4.3; Tot=5 (11,140)	Cancer deaths (60) CVD deaths (56) All-cause deaths (147)
14	Chan, 2017[29]	Australia	HIMS	3,690	2001-04	77 (3.6)	13.1 (4.9)	Md=9.1, 9.2; Tot=11 (38,665)	Prostate cancer (348) Lung cancer (107) Colorectal cancer (137)
15	Ford, 2018[30]	Australia	HIMS	4,069	2001-04	NR	NR	Md=10.5; Tot=12 (44,404)	Dementia (499)
16	Yeap, 2014[31]	Australia	HIMS	3,690	2001-04	NR	NR	Mn=6.6 (2.3 sd) (28,036)	MI (344) Stroke (300)

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				Baseline**				Follow-up (relevant outcomes)	
Item	**Article**	**Country**	**Study name§**	**No. adult males**	**Baseline period**	**Age (yr) Mean (sd)**	**T (nmol/L) Mean (sd)**	**Length of follow-up (yr) (person-years)¶**	**AIMS Longitudinal Outcomes (no. of events analysed)**
17	Ohlsson, 2010[32]	Sweden	MrOS	2,644	2001-04	75.5 (3.2)	15.6 (6.5)	Mn=4.5† (11,880)	CVD deaths (123) Cancer deaths (127) All-cause deaths (328)
18	Ohlsson, 2011[33]	Sweden	MrOS	2,416	2001-04	75.4 (3.2)	15.7 (6)	Md=5.1 (11,605)	CVD events (485) Chronic Heart Disease events (302) Cerebrovascular events (225)
19	Tivesten, 2014[34]	Sweden	MrOS	2,416	2001-04	75.4 (3.2)	15.7 (6)	Md=5.2 (12,070; CHD) (12,137; CBD)	Chronic Heart Disease (302; CHD) Cerebrovascular Disease (225; CBD)
20	Kische, 2017[35]	Germany	SHIP	1,962	1997-01	49.5 (16.3)	15.6 (6.1)	Tot=10	Change in cognitive status
Decision = "Maybe". Item selected based on additional information									
21	LeBlanc, 2010[36]	USA	MrOS	1,602	NR	NR	NR	Mn=4.5†† (26,977)	Cognitive function (and change in) Cognitive decline
22*	Sueoka, 2010[14]	USA	MrOS	697	2000-05	72 (5.5)	14.5 (5.1)	Av=3.9†† (6,247)	Coronary Heart Disease events (100)
Other. Additional studies selected based on information external to the systematic review									
	No articles were selected.	USA	FHS	3,352[12]	1998-05	59.6 (9.1)[12] 49.4 (13.8)[11]	20.7 (8.0)[12]	Tot=10 (for Atrial Fibrillation)[37]	Cardiovascular outcomes[37, 38] Deaths[37] Cause-specific deaths[38] Cancer[39]
	No articles were selected.	Australia	MAILES	1,632[40]	2002-06[8]	54.1 (11.4)[40]	17.3 (5.7)[40]	Md=4.95; IQR=4.35-5.00[40] (12,686)	CVD events Deaths (99)[8] Cause-specific deaths[8]

§ Study name abbreviations: 'ARIC'= Atherosclerosis Risk in Communities; 'BHS'=Busselton Health Study; 'CHAMP'=The Concord Health and Ageing in Men Project; 'CHS'= Cardiovascular Health Study; 'FHS'= the Framingham Heart Study; 'HIMS'=The Health In Men Study; 'EMAS'=European Male Ageing Study; 'MAILES'= The Men Androgen Inflammation Lifestyle Environment and Stress study; 'MrOS Sweden'=The MrOS Osteoporotic Fractures in Men study in Sweden; 'MrOS USA' = The MrOS Osteoporotic Fractures in Men study USA; 'SHIP'=Study of Health in Pomerania SHIP.
§§ = UK, Italy, Belgium, Poland, Sweden, Spain, Hungary, Estonia

¶ 'Md'=median; 'Mn'=mean; 'Av'=average; 'Tot'=total follow-up for the cohort (i.e., maximum, rounded down to nearest whole year); 'IQR'=interquartile range. Unless provided in text, person-years was calculated by multiplying the median, mean, or average length of follow-up by the total number of adult male participants.
* = Note that this is a published conference abstract so is not technically a "Full Text" item.
** = Baseline statistics reported for whole cohort; 'NR' = statistics not reported for whole cohort; Means and standard deviations calculated by firstly transforming into standard units (for T: nmol/L) and then, where required, transforming from quartile statistics using the Box-Cox method of McGrath et al.[41]
*** = First number is for individuals without CVD at baseline.
† = Total follow-up exceeded 5 years, from baseline visit (2001-04) to end of mortality data collection (March 1, 2008).
†† = Note that since there was no published follow-up estimate exceeding 5 years (a requirement for selection) and it was not clear, based on the article information alone, whether the total follow-up was at least 5 years, these items were initially classified as "Maybe". The length of follow-up for collection of AIMS outcome data was determined to be satisfactory from subsequent correspondence with MrOS USA researchers.

Table S6. Exposure levels, outcome assessment, covariates.

Study	Article	Longitudinal measure of association	Exposure* (testosterone)	Outcome ascertainment	Covariates
ARIC	Srinath, 2015[16]	HR	T quartiles	CVD events and deaths identified by annual questionnaires and continuous surveillance, independent from hospital admissions data (ICD codes). Cause of death from death certificates.	Age, race/centre, BMI, waist circumference, smoking status, diabetes mellitus, hypertension, LDL, HDL.
	Srinath, 2016[17]	HR	T tertiles	Definite or probable stroke events identified from hospital admissions, annual phone calls, study examinations adjudicated by a physician, with secondary physician adjudication if it disagreed with a computer algorithm.	Age, race, centre, BMI, waist circumference, smoking status, diabetes mellitus, hypertension, LDL, HDL.
BHS	Chan, 2016[18]	HR	T quartiles (results not shown), Continuous T.	Linked hospital admissions and deaths records (ICD codes)	Age, smoking, vigorous exercise, alcohol, BMI, diabetes, CVD, COPD, non-skin cancer, systolic blood pressure, hypertension, lipid lowering therapy, cholesterol, HDL, triglycerides, C-reactive protein, creatinine
	Chasland, 2017[19]	HR	Categories: Low (L) v High (H) T, physical activity(PA) LT+LPA, LT+HPA, HT+LPA, HT+HPA	Linked hospital admissions and deaths records (ICD codes)	Age, prevalent CVD, smoking, waist circumference, cholesterol, HDL, lipids medication, diabetes, systolic blood pressure, hypertension medication
	Chan, 2018[20]	HR	T quartiles, Continuous T.	Linked cancer and death registry records (ICD codes)	Age, marital status, occupation, smoking, alcohol consumption, leisure time physical activity, BMI, diabetes
CHAMP	Hsu, 2015[21]	Slope estimate (change in MMSE on baseline hormone level or longitudinal change in hormone level)	Continuous T, cFT	Clinic assessment: MMSE, Informant Questionairre on Cognitive Decline as initial screen, followed by clinical assessment to diagnosis categories: normal cognition, MCI, dementia. During follow-up: A decline in MMSE by ≥3 points	Age, BMI, smoking status, years of education, depression score (GDS)
	Hsu, 2016[22]	RR	Continuous T, cFT	Deaths identified from 4-monthly phone calls or deaths registry. Cause of death identified on death certificates independently by 2 medical practitioners.	Age, BMI, smoking status, comorbidity score
	Hsu, 2018[23]	HR, RR (Death outcomes); Slope estimates (MMSE, SF-12 Mental)	Categories: Low (<20th centile) v Normal T combinations with Low (<20th centile) v Normal cFT	Cause of death identified on death certificates independently by 2 medical practitioners.	Age, BMI, smoking status, comorbidity score
CHS	Rosenberg, 2018[24]	HR	Continuous T and cFT, T and cFT quintiles	Independently verified from ECGs taken annually for participants and from hospital discharge diagnoses	Age (stratified), race, education, income, clinic, smoking status, diabetes mellitus, BMI, loop diuretics, height, hypertension, depressed left ventricular ejection fraction, kidney function, systolic blood pressure, SHBG

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Study	Article	Longitudinal measure of association	Exposure* (testosterone)	Outcome ascertainment	Covariates
	Shores, 2014a[25]	HR	Continuous T, cFT (linear & non-linear), T categories	Medicare data, hospital records, imaging studies, autopsy results, death certificates, physician interviews data used for adjudications by committee, which included a neurologist.	Age, systolic blood pressure, anti-hypertensive medications, atrial fibrillation, diabetes, smoking, lipid-lowering drugs, HDL, cholesterol, creatinine, fasting glucose, diabetes medications.
	Shores, 2014b[26]	HR	Continuous T, cFT (linear or non-linear) categories: Q1, Q2-4	Medicare data, hospital records, imaging studies, autopsy results, death certificates, physician interviews data used for adjudications by committee, which included a neurologist.	Age, race, site, smoking status, alcohol consumption, hypertensive use, HDL, BMI, waist circumference, diabetes, SHBG.
EMAS	Lee, 2013[27]	N/A	No modelling of longitudinal outcomes reported	MI, heart failure, other heart conditions, cancers, stroke identified from postal questionnaire, MMSE for participants ≥65 yr old from clinic assessments Variable methods for data capture + validation among centres.	No modelling of longitudinal outcomes reported
	Pye, 2014[28]	HR	T, free T categories: quintiles, low v eugonadal T, LOH status.	Deaths identified from follow-up postal questionnaire or enquiry if no reply received, with 89% of deaths verified from death certificates, death registers, or medical/hospital records.	Age, site, BMI, smoking status, general health.
HIMS	Chan, 2017[29]	SHR	Continuous T, cFT.	Linked hospital admissions, death and cancer registry records (ICD, ICD-O-3 codes).	Age, BMI, smoking status, physical activity, alcohol consumption, diabetes mellitus, HDL, triglycerides, prior cancer diagnosis.
	Ford, 2018[30]	HR	Continuous T, free T Quartile categories of T, free T	Linked data (ICD codes) from inpatient and outpatient mental health services, hospital admissions, community aged care services, cancer and death registries.	Age, baseline cognitive function, depression, BMI, hypertension, CVD, plasma homocysteine.
	Yeap, 2014[31]	HR	T, free T as quartile categories	Linked hospital admissions, death and cancer registry records (ICD codes).	Age, education, smoking status, BMI, waist to hip ratio, hypertension, dyslipidemia, diabetes, creatinine, prior cancer or existing CVD. Also SHBG for models with T.
MrOS Europe	Ohlsson, 2010[32]	HR (in relation to DHEA, DHEA-S)	No: T modelled as a covariate only	Linked data (death and hospital discharge registries), death certificates.	Age, site, BMI, C-reactive protein, ApoB/A1, smoking status, diabetes, hypertension, prior CVD, prior cancer, low testosterone (in lowest quartile), low estradiol
	Ohlsson, 2011[33]	HR	T, free T as quartile categories, T as binary categories.	Linked data (death and hospital discharge registries), death certificates.	Age, morning sample, site, BMI, ApoB/A1, physical activity, smoking status, diabetes, hypertension
	Tivesten, 2014[34]	HR (in relation to DHEA, DHEA-S)	No: T modelled as a covariate only	Linked data (death and hospital discharge registries), death certificates.	Age, morning sample, site, BMI, ApoB/A1, C-reactive protein, estradiol, testosterone (i.e., continuous T), SHBG, eGFR, smoking status, diabetes, hypertension.
SHIP	Kische, 2017[35]	Slope estimate (change in MMSE on baseline hormone)	T, free T as continuous and as 10-year age group quartile categories.	MMSE score.	Age, BMI, smoking status, alcohol consumption, physical activity, hypertension, occupational status, education level, civil status, baseline MMSE.

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Study	Article	Longitudinal measure of association	Exposure* (testosterone)	Outcome ascertainment	Covariates
MrOS USA	LeBlanc, 2010[36]	Change in mean score RR of clinically important decline	Free T quartiles and continuous free T	Cognitive tests at the baseline and follow-up visit from Part B of the Trail Making Test (Trails B) and the Modified Mental State Examination (3MS). Calculated from pre-defined drop in scores.	Age group, education level, race, general health, alcohol consumption, clinic, physical and mental health, physical activity, medications used at baseline, other sex steroids, SHBG.
	Sueoka, 2010[14]	HR	T quartiles	CHD events identified from 3-monthly contacts with participants. Incident events were reviewed and adjudicated by cardiologist using clinical records.	Age, clinic, BMI, blood pressure, lipid levels, smoking, hypertension, diabetes, use of lipid-lowering agents
FHS	N/A – no items were selected.	N/A	N/A	AF measured and adjudicated by cardiologists. Mortality data from death certificates, hospital or institutional records, obituaries, or direct notification[37] Medical records of CVD events reviewed by panel of experienced investigators. A heart study neurologist examined most participants with suspected stroke[38] Medical records of cancer diagnoses reviewed by two independent reviewers, with majority confirmed by pathology reports.[39]	N/A
MAILES	N/A – no items selected.	N/A	N/A	Self-reported and clinical follow-up data, death registry (linked data)[8]	N/A

* T = total testosterone; cFT = calculated free testosterone; Q1=quartile 1; Q2-4=quartiles 2 to 4 combined.

Table S7. Newcastle-Ottawa Quality Assessment Scale for Cohort Studies: selected articles.

Article	Study	Selection (4 stars)	Comparability (2 stars)	Outcome (3 stars)	Notes on Selection	Notes on Outcome
Srinath 2015[16]	ARIC	****	**	**		Losses to f/u not mentioned; linked data[b]
Srinath 2016[17]	ARIC	****	**	**		Losses to f/u not mentioned; linked data[b]
Chan 2016[18]	BHS	****	**	**		Losses to f/u not mentioned; linked data[b]
Chasland 2017[19]	BHS	***	**	**	Prevalent cases not excluded[a]	Losses to f/u not mentioned; linked data[b]
Chan 2018[20]	BHS	****	**	**		Losses to f/u not mentioned; linked data[b]
Hsu 2015[21]	CHAMP	****	**	***		
Hsu 2016[22]	CHAMP	***	**	***	Prevalent cases not excluded[c]	
Hsu 2018[23]	CHAMP	***	**	***	Prevalent cases not excluded[c]	
Rosenberg 2018[24]	CHS	****	**	**		Losses to f/u not mentioned; linked data[d]
Shores 2014a[25]	CHS	****	**	**		Losses to f/u not mentioned; linked data[d]
Shores 2014b[26]	CHS	****	**	**		Losses to f/u not mentioned; linked data[d]
Lee 2013[27]	EMAS	NA	NA	NA	No modelling of longitudinal outcomes reported	
Pye 2014[28]	EMAS	***	**	***	Prevalent cases not excluded[a]	
Chan 2017[29]	HIMS	****	**	**		Losses to f/u not mentioned; linked data[b]
Ford 2018[30]	HIMS	****	**	**		Losses to f/u not mentioned; linked data[b]
Yeap 2014[31]	HIMS	***	**	**	Prevalent cases not excluded[a]	Losses to f/u not mentioned; linked data[b]
Ohlsson 2010[32]	MrOS Sw.	NA	NA	NA	Testosterone was not the exposure variable in this article	
Ohlsson 2011[33]	MrOS Sw.	****	**	***		
Tivesten 2014[34]	MrOS Sw.	NA	NA	NA	Testosterone was not the exposure variable in this article	
Kische 2017[35]	SHIP	***	**	***	Prevalent cases not excluded[e]	
LeBlanc 2010[36]	MrOS USA	****	**	*		Bias from loss to f/u; F/u OK: additional steps[f]
Sueoka 2010[14]	MrOS USA	***	**	*	Prevalent cases not excluded[a]	F/u OK: additional steps[f]
Additional item not selected but included in DR-MA:						
Yeap 2014b[13]	HIMS	***	**	**	Prevalent cases not excluded[a]	Losses to f/u not mentioned; linked data[b]

'NA' = Not applicable (see Notes); 'f/u' = follow-up (of incident events); 'DR-MA' = dose-response meta-analyses of published estimates.
[a] = The influence of prevalent cases was statistically adjusted by including prevalent status as a model predictor.
[b] = Follow-up of cases was assumed to be almost complete because analyses were of linked administrative data.
[c] = The influence of prevalent cases was statistically adjusted by incorporating into a comorbidity status model predictor.
[d] = Follow-up of cases was assumed to be almost complete because analyses were of linked administrative data (with expert adjudications).
[e] = Outcome was change in cognition score, with baseline score (prevalent status) included as a model predictor.
[f] = Total length of follow-up period was not reported but determined to be satisfactory from correspondence with MrOS USA researchers.

Supplementary Material: Systematic Review for the Androgens In Men Study.

Table S8. PRISMA Checklist for Systematic Review: the Androgens In Men Study.

Section/topic	#	Checklist item	Reported on page #
TITLE			
Title	1	Identify the report as a systematic review, meta-analysis, or both.	1
ABSTRACT			
Structured summary	2	Provide a structured summary including, as applicable: background; objectives; data sources; study eligibility criteria, participants, and interventions; study appraisal and synthesis methods; results; limitations; conclusions and implications of key findings; systematic review registration number.	1
INTRODUCTION			
Rationale	3	Describe the rationale for the review in the context of what is already known.	5
Objectives	4	Provide an explicit statement of questions being addressed with reference to participants, interventions, comparisons, outcomes, and study design (PICOS).	5. For this type of review it is PEO instead.
METHODS			
Protocol and registration	5	Indicate if a review protocol exists, if and where it can be accessed (e.g., Web address), and, if available, provide registration information including registration number.	5
Eligibility criteria	6	Specify study characteristics (e.g., PICOS, length of follow-up) and report characteristics (e.g., years considered, language, publication status) used as criteria for eligibility, giving rationale.	5, Table S2
Information sources	7	Describe all information sources (e.g., databases with dates of coverage, contact with study authors to identify additional studies) in the search and date last searched.	5-6
Search	8	Present full electronic search strategy for at least one database, including any limits used, such that it could be repeated.	Table S1

Study selection	9	State the process for selecting studies (i.e., screening, eligibility, included in systematic review, and, if applicable, included in the meta-analysis).	6, Tables S2-S3.
Data collection process	10	Describe method of data extraction from reports (e.g., piloted forms, independently, in duplicate) and any processes for obtaining and confirming data from investigators.	6, Suppl. Data.
Data items	11	List and define all variables for which data were sought (e.g., PICOS, funding sources) and any assumptions and simplifications made.	6, Suppl. Data.
Risk of bias in individual studies	12	Describe methods used for assessing risk of bias of individual studies (including specification of whether this was done at the study or outcome level), and how this information is to be used in any data synthesis.	6, Table S7.
Summary measures	13	State the principal summary measures (e.g., risk ratio, difference in means).	7
Synthesis of results	14	Describe the methods of handling data and combining results of studies, if done, including measures of consistency (e.g., I^2) for each meta-analysis.	7

Section/topic	#	Checklist item	Reported on page #
Risk of bias across studies	15	Specify any assessment of risk of bias that may affect the cumulative evidence (e.g., publication bias, selective reporting within studies).	7
Additional analyses	16	Describe methods of additional analyses (e.g., sensitivity or subgroup analyses, meta-regression), if done, indicating which were pre-specified.	7
RESULTS			
Study selection	17	Give numbers of studies screened, assessed for eligibility, and included in the review, with reasons for exclusions at each stage, ideally with a flow diagram.	8, Fig. 1
Study characteristics	18	For each study, present characteristics for which data were extracted (e.g., study size, PICOS, follow-up period) and provide the citations.	Tables S5-6, S9
Risk of bias within studies	19	Present data on risk of bias of each study and, if available, any outcome level assessment (see item 12).	9, Table S7
Results of individual studies	20	For all outcomes considered (benefits or harms), present, for each study: (a) simple summary data for each intervention group (b) effect estimates and confidence intervals, ideally with a forest plot.	9-11, Figs. 2-4

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Synthesis of results	21	Present results of each meta-analysis done, including confidence intervals and measures of consistency.	10-11, Figs. 3-4
Risk of bias across studies	22	Present results of any assessment of risk of bias across studies (see Item 15).	10-11, Fig. S2
Additional analysis	23	Give results of additional analyses, if done (e.g., sensitivity or subgroup analyses, meta-regression [see Item 16]).	9-10, Fig. S1
DISCUSSION			
Summary of evidence	24	Summarize the main findings including the strength of evidence for each main outcome; consider their relevance to key groups (e.g., healthcare providers, users, and policy makers).	11-13
Limitations	25	Discuss limitations at study and outcome level (e.g., risk of bias), and at review-level (e.g., incomplete retrieval of identified research, reporting bias).	11-14
Conclusions	26	Provide a general interpretation of the results in the context of other evidence, and implications for future research.	11-14
FUNDING			
Funding	27	Describe sources of funding for the systematic review and other support (e.g., supply of data); role of funders for the systematic review.	15

From: Moher D, Liberati A, Tetzlaff J, Altman DG, The PRISMA Group (2009). Preferred Reporting Items for Systematic Reviews and Meta-Analyses: The PRISMA Statement. PLoS Med 6(7): e1000097. doi:10.1371/journal.pmed1000097

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Table S9. Extracted hazard ratio data for dose-response meta-analyses (DR-MAs).

Article	Study	Outcome	Testosterone	Units	HR	95% CI	Notes
Chan 2016[18]	BHS	All-cause mortality	<10.20	nmol/L	ref.		
Chan 2016	BHS	All-cause mortality	10.20 - <13.04	nmol/L	0.84	(0.62-1.14)	
Chan 2016	BHS	All-cause mortality	13.04 - <16.58	nmol/L	0.86	(0.62-1.19)	
Chan 2016	BHS	All-cause mortality	≥16.58	nmol/L	0.9	(0.62-1.3)	
Pye 2014[28]	EMAS	All-cause mortality	<11.65	nmol/L	1.1	(0.6-1.8)	
Pye 2014	EMAS	All-cause mortality	11.65-14.61	nmol/L	0.7	(0.4-1.3)	
Pye 2014	EMAS	All-cause mortality	14.61-17.28	nmol/L	0.7	(0.4-1.3)	
Pye 2014	EMAS	All-cause mortality	17.28-21.20	nmol/L	1.2	(0.7-2)	
Pye 2014	EMAS	All-cause mortality	>21.20	nmol/L	ref.		
Srinath 2015[16]	ARIC	All-cause mortality	≤288.4	ng/dL	0.96	(0.7-1.34)	
Srinath 2015	ARIC	All-cause mortality	288.5-377.6	ng/dL	0.99	(0.72-1.35)	
Srinath 2015	ARIC	All-cause mortality	377.7-480.1	ng/dL	1	(0.74-1.35)	
Srinath 2015	ARIC	All-cause mortality	≥480.2	ng/dL	ref.		
Shores 2014b[26]	CHS	All-cause mortality	<278	ng/dL	1.06	(0.88-1.29)	
Shores 2014b	CHS	All-cause mortality	≥278	ng/dL	ref.		
Yeap 2014b[13]	HIMS	All-cause mortality	0.25-9.82	nmol/L	ref.		Fully-adjusted model + SHBG
Yeap 2014b	HIMS	All-cause mortality	9.82-12.53	nmol/L	0.81	(0.68-0.98)	
Yeap 2014b	HIMS	All-cause mortality	12.56-15.75	nmol/L	0.75	(0.61-0.92)	
Yeap 2014b	HIMS	All-cause mortality	15.79-46.50	nmol/L	0.77	(0.61-0.97)	
Yeap 2014b	HIMS	All-cause mortality	0.25-9.82	nmol/L	ref.		Fully-adjusted model + LH
Yeap 2014b	HIMS	All-cause mortality	9.82-12.53	nmol/L	0.84	(0.7-1.01)	
Yeap 2014b	HIMS	All-cause mortality	12.56-15.75	nmol/L	0.81	(0.67-0.97)	
Yeap 2014b	HIMS	All-cause mortality	15.79-46.50	nmol/L	0.89	(0.73-1.07)	
Chan 2016	BHS	CVD mortality	<10.20	nmol/L	ref.		
Chan 2016	BHS	CVD mortality	10.20 - <13.04	nmol/L	1.12	(0.7-1.78)	
Chan 2016	BHS	CVD mortality	13.04 - <16.58	nmol/L	1.39	(0.86-2.25)	
Chan 2016	BHS	CVD mortality	≥16.58	nmol/L	1.25	(0.69-2.25)	
Chasland 2017[19]	BHS	CVD mortality	<13.1	nmol/L	ref.		Total PA, "Low" PA, NS PA x T: these estimates were used
	BHS	CVD mortality	≥13.1	nmol/L	1.25	(0.77-2.03)	
Chasland 2017	BHS	CVD mortality	<13.1	nmol/L	0.69	(0.4-1.2)	Total PA, "High" PA, NS PA x T
Chasland 2017	BHS	CVD mortality	≥13.1	nmol/L	0.8	(0.48-1.35)	
Pye 2014	EMAS	CVD mortality	<11.65	nmol/L	1	(0.4-2.2)	
Pye 2014	EMAS	CVD mortality	11.65-14.61	nmol/L	0.5	(0.2-1.4)	
Pye 2014	EMAS	CVD mortality	14.61-17.28	nmol/L	0.4	(0.2-1.2)	
Pye 2014	EMAS	CVD mortality	17.28-21.20	nmol/L	1.1	(0.5-2.4)	
Pye 2014	EMAS	CVD mortality	>21.20	nmol/L	ref.		
Srinath 2015	ARIC	CVD mortality	≤288.4	ng/dL	1.36	(0.45-4.08)	
Srinath 2015	ARIC	CVD mortality	288.5-377.6	ng/dL	1.26	(0.73-3.7)	
Srinath 2015	ARIC	CVD mortality	377.7-480.1	ng/dL	0.57	(0.16-1.99)	
Srinath 2015	ARIC	CVD mortality	≥480.2	ng/dL	ref.		

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Article	Study	Outcome	Testosterone	Units	HR	95% CI	Notes
Shores 2014b	CHS	CVD mortality	<278	ng/dL	1.28	(0.94-1.75)	
Shores 2014b	CHS	CVD mortality	≥278	ng/dL	ref.		
Yeap 2014b	HIMS	CVD mortality	0.25-9.82	nmol/L	ref.		
Yeap 2014b	HIMS	CVD mortality	9.82-12.53	nmol/L	0.82	(0.61-1.11)	
Yeap 2014b	HIMS	CVD mortality	12.56-15.75	nmol/L	0.79	(0.58-1.09)	
Yeap 2014b	HIMS	CVD mortality	15.79-46.50	nmol/L	0.79	(0.56-1.11)	

Supplementary Material: Figures.

Figure S1. Meta-regression diagnostics.

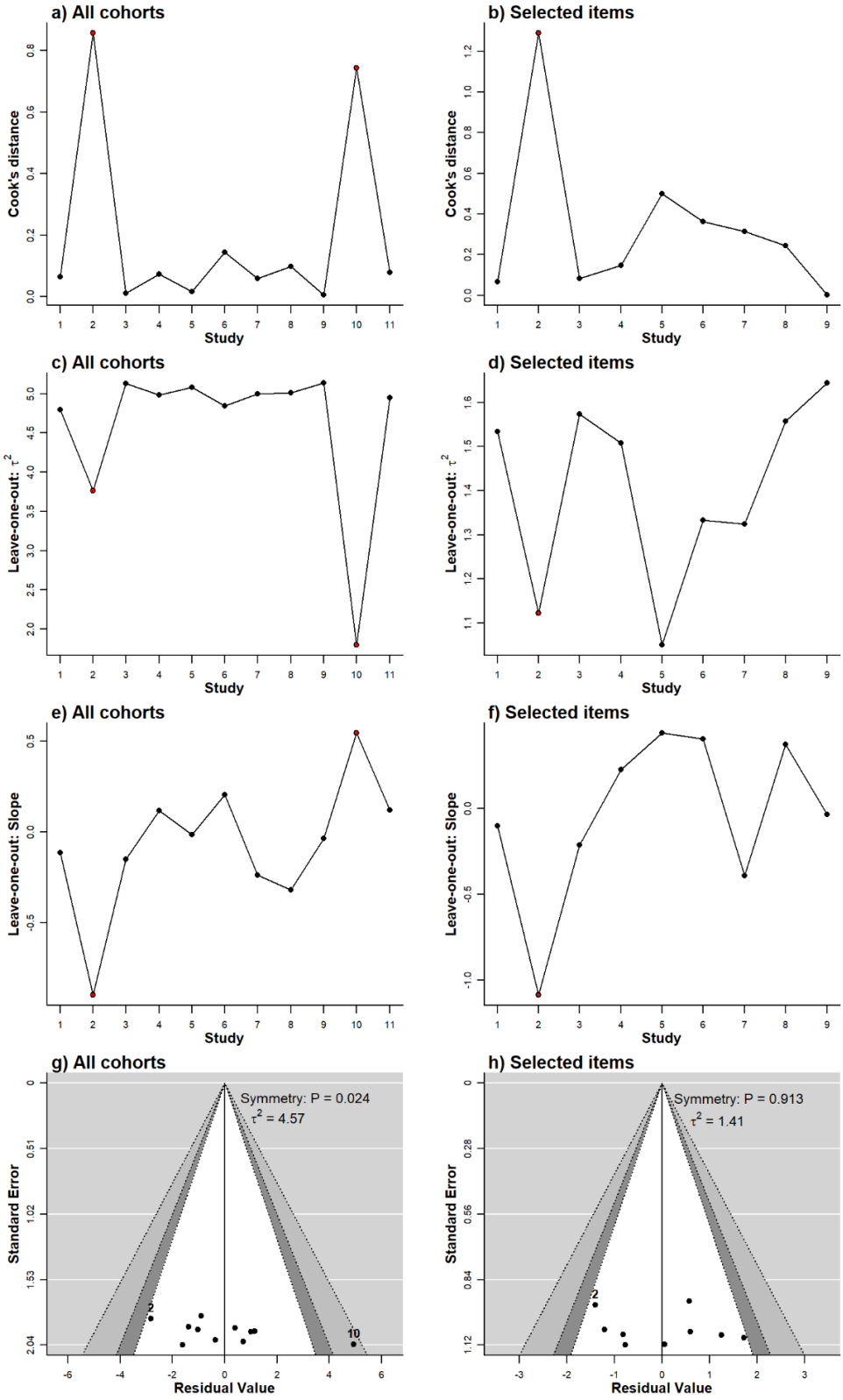

Figure S1: Meta-regression diagnostics showing the influence of studies on model fit (a,b), τ^2 (estimated amount of total heterogeneity: c,d), estimated slope (e,f), and distribution of residuals with funnel plots (g,h). Analysis repeated for all 11 cohort studies (a,c,e,g) and for 9

studies with selected articles (b,d,f,h). In cases where more than one article was available per cohort study, the article with the largest sample size was used. Highlighted estimates for cohort study 2 (BHS) were those from Chan et al.[18] (N=1,804) and for study 10 (FHS) were from Pencina et al.[12] (N=720). In funnel plots: light grey + dark grey + white shading = 99% pseudo confidence interval (CI); dark grey + white shading = 95% CI; white shading = 90% CI.

Figure S2. Funnel plots for dose-response meta-analyses.

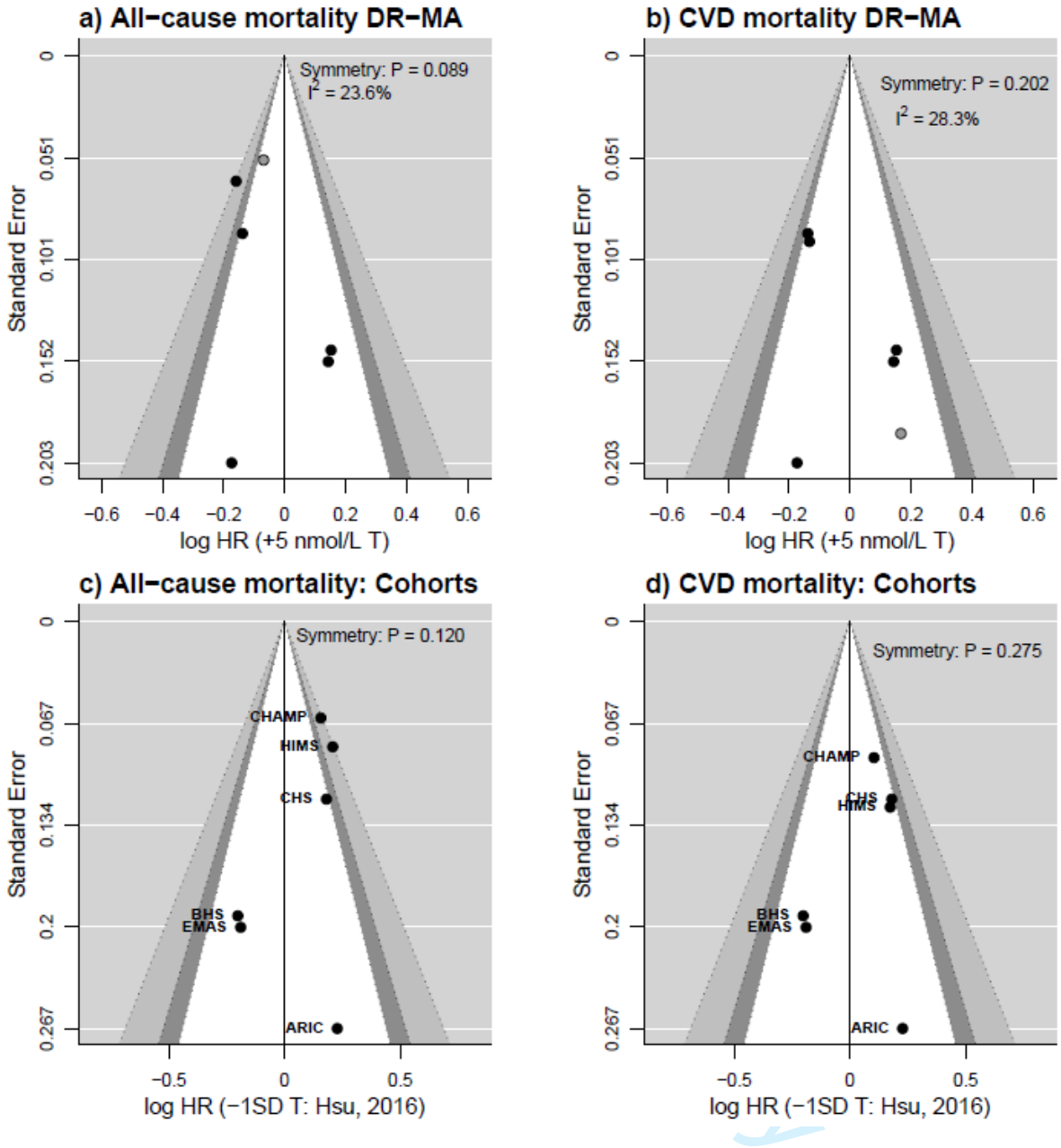

Figure S2: Contour-enhanced funnel plots showing the distribution of log hazard ratio (HR) estimates for all-cause mortality (a, c) and mortality caused by cardiovascular disease (CVD) (b, d) attributed to a 5 nmol/L increase (a, b), or a 1.9 nmol/L (1SD in Hsu et al. 2016[22]) decrease, in endogenous testosterone concentration. Log HR values and standard errors were calculated using generalised least squares regression of published estimates.[42, 43] In cases where more than one article was available per cohort study, the article with the largest sample size was used. Estimates represented by black dots in (a) and (b) were analysed in respective dose-response meta-analyses (DR-MA; results presented in Figs. 3, 4). The grey dot in (a) is the estimate for Yeap et al. 2014b[13] and in (b) is the estimate for Chasland et al. (2017)[19]; these estimates were substituted for others for the HIMS and BHS studies respectively for alternative summary estimates (i.e., the grey summary estimates presented in Figs. 3, 4). Estimates presented in (c) and (d) are shown for a more complete assessment of funnel plot symmetry: estimates are plotted for all studies with estimates, including those that

did not have sufficient information for including in the DR-MA. In funnel plots: light grey + dark grey + white shading = 99% pseudo confidence interval (CI); dark grey + white shading = 95% CI; white shading = 90% CI.

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PRISMA Checklist for Systematic Review: the Androgens In Men Study.

Section/topic	#	Checklist item	Reported on page #
TITLE			
Title	1	Identify the report as a systematic review, meta-analysis, or both.	1
ABSTRACT			
Structured summary	2	Provide a structured summary including, as applicable: background; objectives; data sources; study eligibility criteria, participants, and interventions; study appraisal and synthesis methods; results; limitations; conclusions and implications of key findings; systematic review registration number.	1
INTRODUCTION			
Rationale	3	Describe the rationale for the review in the context of what is already known.	5
Objectives	4	Provide an explicit statement of questions being addressed with reference to participants, interventions, comparisons, outcomes, and study design (PICOS).	5. For this type of review it is PEO instead.
METHODS			
Protocol and registration	5	Indicate if a review protocol exists, if and where it can be accessed (e.g., Web address), and, if available, provide registration information including registration number.	5
Eligibility criteria	6	Specify study characteristics (e.g., PICOS, length of follow-up) and report characteristics (e.g., years considered, language, publication status) used as criteria for eligibility, giving rationale.	5, Table S2
Information sources	7	Describe all information sources (e.g., databases with dates of coverage, contact with study authors to identify additional studies) in the search and date last searched.	5-6
Search	8	Present full electronic search strategy for at least one database, including any limits used, such that it could be repeated.	Table S1
Study selection	9	State the process for selecting studies (i.e., screening, eligibility, included in systematic review, and, if applicable, included in the meta-analysis).	6, Tables S2-S3.

Data collection process	10	Describe method of data extraction from reports (e.g., piloted forms, independently, in duplicate) and any processes for obtaining and confirming data from investigators.	6, Suppl. Data.
Data items	11	List and define all variables for which data were sought (e.g., PICOS, funding sources) and any assumptions and simplifications made.	6, Suppl. Data.
Risk of bias in individual studies	12	Describe methods used for assessing risk of bias of individual studies (including specification of whether this was done at the study or outcome level), and how this information is to be used in any data synthesis.	6, Table S7.
Summary measures	13	State the principal summary measures (e.g., risk ratio, difference in means).	7
Synthesis of results	14	Describe the methods of handling data and combining results of studies, if done, including measures of consistency (e.g., I^2) for each meta-analysis.	7

Section/topic	#	Checklist item	Reported on page #
Risk of bias across studies	15	Specify any assessment of risk of bias that may affect the cumulative evidence (e.g., publication bias, selective reporting within studies).	7
Additional analyses	16	Describe methods of additional analyses (e.g., sensitivity or subgroup analyses, meta-regression), if done, indicating which were pre-specified.	7
RESULTS			
Study selection	17	Give numbers of studies screened, assessed for eligibility, and included in the review, with reasons for exclusions at each stage, ideally with a flow diagram.	8, Fig. 1
Study characteristics	18	For each study, present characteristics for which data were extracted (e.g., study size, PICOS, follow-up period) and provide the citations.	Tables S5-6, S9
Risk of bias within studies	19	Present data on risk of bias of each study and, if available, any outcome level assessment (see item 12).	9, Table S7
Results of individual studies	20	For all outcomes considered (benefits or harms), present, for each study: (a) simple summary data for each intervention group (b) effect estimates and confidence intervals, ideally with a forest plot.	9-11, Figs. 2-4
Synthesis of results	21	Present results of each meta-analysis done, including confidence intervals and measures of consistency.	10-11, Figs. 3-4

Risk of bias across studies	22	Present results of any assessment of risk of bias across studies (see Item 15).	10-11, Fig. S2
Additional analysis	23	Give results of additional analyses, if done (e.g., sensitivity or subgroup analyses, meta-regression [see Item 16]).	9-10, Fig. S1
DISCUSSION			
Summary of evidence	24	Summarize the main findings including the strength of evidence for each main outcome; consider their relevance to key groups (e.g., healthcare providers, users, and policy makers).	11-13
Limitations	25	Discuss limitations at study and outcome level (e.g., risk of bias), and at review-level (e.g., incomplete retrieval of identified research, reporting bias).	11-14
Conclusions	26	Provide a general interpretation of the results in the context of other evidence, and implications for future research.	11-14
FUNDING			
Funding	27	Describe sources of funding for the systematic review and other support (e.g., supply of data); role of funders for the systematic review.	15

From: Moher D, Liberati A, Tetzlaff J, Altman DG, The PRISMA Group (2009). Preferred Reporting Items for Systematic Reviews and Meta-Analyses: The PRISMA Statement. PLoS Med 6(7): e1000097. doi:10.1371/journal.pmed1000097

BMJ Open

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Primary Subject Heading:	Epidemiology
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Keywords:	EPIDEMIOLOGY, Sex steroids & HRT < DIABETES & ENDOCRINOLOGY, STATISTICS & RESEARCH METHODS

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Systematic review and meta-analyses on associations of endogenous testosterone concentration with health outcomes in community-dwelling men.

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Word count, excluding title page, abstract, strengths and limitations, references, acknowledgements, contributions, figures and tables: 3,639 words.

ABSTRACT

Objectives

The overall study aim is to clarify the relation of endogenous sex hormones with major health outcomes in men. This paper reports a systematic review focussing on published estimates for testosterone associations.

Setting

Community-dwelling men.

Participants

20,180 adult males participated in the final set of studies identified and selected from a systematic review. Eligible studies included prospective cohort studies with plasma or serum testosterone concentrations measured for adult males using mass spectrometry with at least 5 years of follow-up data and one of the specified outcome measures recorded. Only published or grey literature items written in English were considered.

Primary and secondary outcome measures

Planned prospective outcome measures: cardiovascular disease (CVD) events, CVD deaths, all-cause mortality, cancer deaths, cancer diagnoses, cognitive decline, dementia. Meta-analyses were of the most frequently reported outcomes in selected studies: CVD deaths and all-cause mortality. Succinct characterisations of testosterone associations with other outcomes are also presented.

Results

Screening of 1,994 de-duplicated items identified 9 suitable studies, with an additional two identified by colleagues (11 in total). Summary estimates of mean testosterone concentration

and age at recruitment for 20,180 adult males were 15.4±0.7nmol/L and 64.9±3.3yr. Despite considerable variation in mean testosterone, a meta-regression estimated no significant dependence on mean age at recruitment among studies (Slope = -0.03, 95% CI -0.11 – 0.06). Meta-analyses demonstrated negligible heterogeneity and no significant effect of a 5 nmol/L increase in testosterone on the risk of all-cause mortality (hazard ratio, HR = 0.96, 95% CI 0.89 – 1.03) or death from CVD (HR = 0.95, 95% CI 0.83 – 1.08).

Conclusions

Analyses of published estimates did not demonstrate associations of endogenous testosterone with CVD deaths or with all-cause mortality. Suggested further research includes the planned individual participant data meta-analyses for selected studies, enabling the investigation of non-linear summary effects.

Registration

PROSPERO: CRD42019139668.

STRENGTHS AND LIMITATIONS OF THIS STUDY

- This is the first systematic review on this topic to restrict selections to prospective cohort studies of community-dwelling men with testosterone measured using mass spectrometry: the "gold standard" method.
- Systematic searches were made of both the published and grey literature using online search tools.
- Meta-analyses used estimates obtained from studies with at least five years of follow-up data and from fitted models which controlled for (at least) the age, smoking status, and body mass index or waist circumference of participants.
- Meta-analyses of published estimates were limited to assuming linear relationships, however subsequent IPD meta-analyses planned to arise from this work will look to explore non-linear associations.

- Analyses are of observational data, and so summary estimates will not fully eliminate the possibility of confounding arising from unadjusted effects.

1. INTRODUCTION

What does a low testosterone level mean for a man's health? In men, levels of testosterone, the key male sex hormone (androgen), decline with increasing age, yet the basis for and health consequences of this phenomenon remain unclear.[1-5] Endogenous testosterone concentrations reflect the function of the hypothalamic-pituitary-testicular (HPT) axis, and are relatively lower in men who are obese, or with metabolic syndrome or diabetes.[6-8] Others have reported associations of lower endogenous testosterone concentrations with higher risk of incident diseases, such as cardiovascular disease (CVD), and death.[9-16] Whether low testosterone concentrations might contribute directly to the risk of CVD or death or whether it may be associated indirectly through its relationship with aging and obesity is unknown.[17] And whether or not it is directly related, it is possible that endogenous testosterone could be useful as a biomarker for diagnostic and/or prognostic health care applications in men.[18-20] An improved understanding of the associations of testosterone to health outcomes could inform further exploration and development of this concept.

The Androgens In Men Study (AIMS) seeks to clarify the associations of androgens (primarily testosterone) with key health outcomes in men (mortality, cardiovascular disease, cancer, cognitive decline and dementia) by conducting a systematic review and a series of individual participant data meta-analyses.[21] In this paper we present the systematic review and meta-analyses using published estimates from prospective cohort studies with at least 5

years of follow-up data and testosterone measured using only mass spectrometry, the most reliable method.[22]

2. METHODS

This systematic review, conducted 14 June—31 December 2019, was of "etiology and/or risk type" studies.[23-24] The pre-specified purpose of the systematic review was to identify studies with suitable individual participant-level data (IPD) for collaborating with on a series of IPD meta-analyses. The PEO (Population, Exposure, Outcomes) characteristics included: adult men in the general community; endogenous circulating sex hormone concentration (primarily testosterone); incident cardiovascular disease (CVD), mortality, cancers, cognitive decline, dementia. Subgroup IPD meta-analyses are also planned for heart failure, myocardial infarction, stroke; colorectal cancer, lung cancer, prostate cancer. A protocol was submitted to PROSPERO on 23 July 2019 and registered on 20 November 2019 (registration number CRD42019139668) and a protocol article has been published.[21]

2.1. Literature search and screening

Four online search tools were used to identify available published (MEDLINE, EMBASE) and grey literature (OpenGrey, Mednar) *items* (journal articles, reports, theses, webpage articles) reporting on suitable prospective cohort *studies* (the underlying unique sources of data). Two reviewers (RJM, JH) independently screened the de-duplicated items against pre-specified criteria using Rayyan.[25] To optimise efficiency, title and abstract screenings were initially conducted (Step 1), followed by full text screenings of the selected abstracts (Step 2). Disagreements were resolved through subsequent discussions between reviewers and agreement quantified using Cohen's Kappa and percent agreement. Only items reporting on prospective population-based cohort studies of adults (combined sexes or of men alone) with

mass spectrometry measurements of testosterone and at least five years of subsequent follow-up data on incident CVD events, cancer or dementia diagnoses, cognition assessments, or on all-cause, CVD, or cancer deaths were selected. The Newcastle-Ottawa Quality Assessment Scale for Cohort Studies (NOS) was used to assess quality of the selected items.[26]

Additional details on the methods and results are provided in Supplementary Material (Supplementary Section 1). Specifically, additional details on systematic searches and screening (Supplementary Section 2; Supplementary tables 1-4), supplemental tables (Supplementary Section 3), including the PRISMA checklist (Supplementary table 5), supplemental figures (Supplementary Section 4), and references cited (Supplementary Section 5) have been included.

2.2. Meta-analyses of published estimates

Published estimates (author names, publication year, cohort study name, number of participants analysed, model covariates, testosterone statistics (overall and for individual exposure levels), participant age statistics, numbers of outcome events, follow-up time, hazard ratios (HRs) and 95% CIs of the most fully-adjusted model) were extracted from selected articles by the first author (RJM). For the purpose of these analyses, we present associations for endogenous total testosterone concentrations, comprising the sum of testosterone in the circulation, whether bound to sex hormone-binding globulin or albumin, or unbound. Testosterone statistics were converted into standard units (nmol/L) and values representing categorical ranges were determined following Wang et al.[27] If not reported, the numbers of participants and events within categories of testosterone, and the means of participant ages and testosterone concentrations at baseline, were calculated. The numbers of participants within quartile or quintile categories were calculated by dividing the total sample

size by four or five. The numbers of events within categories were solved using Newton's method by applying the algorithm of Greenland and Longnecker.[28] Means and standard deviations for testosterone and age were calculated from presented quartile estimates using the Box-Cox method of McGrath et al., which does not make distributional assumptions.[29]

A random effects meta-regression of mean baseline testosterone concentration on the mean participant age at baseline was conducted using published estimates from: (i) only those items identified in systematic searches; and (ii) all suitable articles, including those found outside of systematic searches. A t-test of the meta-regression slope coefficient's departure from zero was done after applying the Knapp and Hartung adjustment.

Dose-response random effects meta-analyses (DR-MAs) were conducted to summarise published HR estimates for the associations of baseline testosterone concentrations with incident all-cause deaths and with CVD cause-specific deaths, as these were the most frequently reported outcomes in selected articles. Estimates from an additional article that had not been selected from systematic searches (Yeap et al[30]) were also used because it reported suitable estimates from one of the selected studies, and had been published within the literature search period. Contour-enhanced funnel plots were inspected for publication bias and patterns in heterogeneity and Cochran Q tests for heterogeneity (I^2), as well as regression tests for funnel plot asymmetry,[31] were done. Forest plots were constructed to represent single HR estimates for each study, per 5nmol/L increase in testosterone. For completeness, HR estimates for the other outcomes are represented in a grouped forest plot, and other effect sizes in tables.

The "metafor" package was used for meta-regressions, forest plots and funnel plots, the

"doseresmeta" package for DR-MAs, and the "estmeansd" package for calculating study means and standard deviations from published quartile statistics in R version 4.0.2.[32-35]

2.3. Patient and public involvement

This work uses existing published data. Patients and public were not involved in the design, conduct, reporting, or dissemination plans of the systematic review or meta-analyses.

3. RESULTS

3.1. Literature search and study selection

The literature search returned 2,177 items (1,738 published and 439 from grey literature), with 1,994 items remaining after duplicates had been removed, and after excluding two Mednar items that had insufficient information available to review (Fig. 1). These included 1,764 journal articles, 111 webpage articles, 81 theses, and 38 unpublished reports/other documents. Systematic screening of the returned, deduplicated items excluded 1,968, classified five as "Maybe", and selected 20 as suitable. Most (92.1%) items were excluded from screening titles and abstracts at Step 1, with a much smaller percentage (6.6%) excluded from screening the 157 full text items in Step 2. One item could not be screened in Step 2 because the full text was not available. Inter-reviewer agreement was a Cohen's Kappa $\kappa = 0.69$ (or 96.0 percent agreement) for Step 1 and $\kappa = 0.82$ (or 98.1 percent agreement) for Step 2.

The 20 selected items collectively reported on eight prospective cohort studies: three from Australia (Busselton Health Study *BHS*,[36-38] The Concord Health and Ageing in Men Project *CHAMP*,[9, 39-40] The Health In Men Study *HIMS*);[14, 41-42] three from Europe

(European Male Ageing Study *EMAS*,[11, 43] The *MrOS* Osteoporotic Fractures in Men study in Sweden,[10, 44-45] Study of Health in Pomerania *SHIP*);[46] and two from the USA (Atherosclerosis Risk in Communities *ARIC*,[47-48] Cardiovascular Health Study *CHS*).[49-51] Two of the five items classified as "Maybe" reported on the *MrOS USA* study, which were found, after further investigation, to be suitable for selection.[52-53] Two additional studies were identified as suitable based on information external to the systematic searches and screenings: one from Australia (The Men Androgen Inflammation Lifestyle Environment and Stress study *MAILES*);[54] and one from the USA (the Framingham Heart Study *FHS*).[55] This is 11 cohort studies identified, in total. Additional details on returned and screened items, and selected article attributes are provided in Supplementary Material (Supplementary Section 2, Supplementary tables 4, 6-7).

3.2. Meta-analysis and summary of selected articles.

The quality of selected articles ranged from six to nine (out of nine) stars on the Newcastle-Ottawa Scale. Relatively high scores reflected that all articles: were of population-based studies; accurately measured the exposure (baseline testosterone concentration); included multivariable models adjusting for participant age and other risk factors; had outcomes measured or collected from record linkage, with or without expert adjudication; and had sufficient follow-up, ranging from 5-20 years (Supplementary tables 6-8). Relevant outcomes included: all-cause deaths (n=8 articles); CVD deaths (n=7); strokes or cerebrovascular disease (n=6); cognitive function or cognitive decline (n=5); coronary heart disease (n=4); CVD events (n=4); cancer deaths (n=4); cancer diagnoses (n=3); myocardial infarction (MI; n=2); heart failure (HF; n=2); and dementia (n=1). However, one of these articles was a cohort profile description that did not report effect size estimates but the availability of all-cause deaths, cause-specific deaths, stroke, cognitive function, CVD, cancer, MI, and HF

outcome data.[43]. Two articles reported testosterone not as the exposure but as a covariate in analyses investigating associations with cerebrovascular events and with all-cause, cancer, and CVD deaths.[44-45] The supplementary material for one article[11] was sought to obtain effect size estimates for cancer deaths but these were not obtained as at the time of writing. All were published between 2010 and 2018, reflecting the relatively recent adoption of mass spectrometry as the "gold standard" for measuring endogenous testosterone levels.[22]

The mean age of men at baseline ranged from middle-aged (49-54yr: BHS, FHS, MAILES, SHIP)[18, 36-38, 46, 56] to elderly (72-77yr: CHAMP, CHS, HIMS, MrOS Sweden, MrOS USA).[9-10, 39, 41, 44-45, 49-50, 53] Across the 11 studies, summary estimates for 20,180 adult males at baseline were 64.9±3.3yr for mean age and 15.4±0.7nmol/L for mean testosterone. Although there appeared to be a slight declining trend in mean testosterone with mean age among studies (Meta-regression Slope= -0.07, 95% CI -0.21 – 0.07), this estimate was not significantly different from zero (P=0.27; Fig. 2a). However, the distribution of model residuals demonstrated significant heterogeneity (P<0.001) and funnel plot asymmetry (P=0.02). Additional diagnostics highlighted a relatively high mean testosterone estimate from Pencina et al.[57] (FHS) and a low mean testosterone estimate (relative to mean age) from Chan et al.[37] (BHS), as compared to the other studies (Supplementary figure 1). When restricted to systematically selected items (reporting on ARIC, BHS, CHAMP, CHS, EMAS, HIMS, MAILES, MrOS Sweden, SHIP studies), tests of residual heterogeneity were significant (P<0.001), funnel plot asymmetry (P=0.91) was non-significant, and the slope estimate (Meta-regression Slope= -0.03, 95% CI -0.11 – 0.06) was not significantly different from zero (P=0.50; Fig. 2b). These results demonstrate that varying distributions of participant age (likely reflecting differences in study-specific objectives and recruitment methods) did not explain the observed heterogeneity in published estimates of testosterone

among the studies.

Hazard ratios (HRs) for all-cause mortality were calculated from values in four of the selected articles (ARIC,[48] BHS,[37] CHS,[51] EMAS[11]) and from one that was not selected, but had reported on the HIMS study during the literature search period.[30] All HRs were adjusted for the age, smoking status, and body mass index (BMI) or waist circumference of participants. A DR-MA estimated a summary HR of 0.96 (95% CI 0.89-1.03) per 5nmol/L increase in testosterone (Fig. 3). The summary estimate was similar when calculated using an alternative estimate from Yeap et al[30] (HR=0.97, 95% CI 0.92-1.03). For both analyses, tests for residual heterogeneity (I^2=23.6%, P=0.26; I^2=0.0%, P=0.76) and funnel plot asymmetry (P=0.09; P=0.39) were non-significant (Supplementary figure 2a). A comparable HR was calculated from a CHAMP study article[9] for inclusion in the forest plot but not in the DR-MA, because a corresponding estimate of variance per 5nmol/L increase in testosterone could not be calculated. An additional funnel plot, which included the HR estimate from this CHAMP article[9] (per 1 standard deviation decrease in testosterone, as reported in that article), also demonstrated no significant asymmetry (Supplementary figure 2b). These results demonstrate no overall effect of baseline testosterone concentration on the relative hazard of death from any cause after adjusting for factors including age, smoking status, and BMI or waist circumference.

HRs for death caused by CVD demonstrated similar findings. A DR-MA using estimates from the same five articles estimated a summary HR of 0.95 (95% CI 0.83-1.08) per 5nmol/L increase in testosterone, with no significant residual heterogeneity (I^2=28.3%, P=0.23) or funnel plot asymmetry (P=0.20; Fig. 4; Supplementary figure 2c). Again, all HRs were adjusted for the age, smoking status, and BMI or waist circumference. The DR-MA repeated

using an alternative estimate from Chasland et al.[38] for the BHS gave similar results (summary HR=0.93, 95% CI 0.83-1.03; heterogeneity I^2=17.5%, P=0.30; funnel plot asymmetry P=0.17; Supplementary figure 2d). These results demonstrate no overall effect of baseline testosterone concentration on the relative hazard of death from CVD after adjusting for factors including age, smoking status, and BMI or waist circumference.

Summary estimates calculated for the combined outcome of incident stroke and cerebrovascular disease (summary HR=0.93, 95% CI 0.83-1.03; heterogeneity I^2=43.3%, P=0.15) and incident CVD diagnosis (summary HR=0.93, 95% CI 0.84-1.03; heterogeneity I^2=34.7%, P=0.22) demonstrated no overall effect of testosterone (Supplementary figure 3). Funnel plot asymmetry was not assessed due to the low number of studies ($n \leq 4$),[58] and 95% confidence intervals could not be calculated for several studies[36-37, 41] using the published information. Although a summary estimate could not be calculated, the study-specific estimates demonstrated some significant associations with cancer outcomes (Supplementary figure 3, Supplementary table 9). Estimates showed an increased risk of lung cancer for men with higher concentrations,[41] an increased risk of death from cancer for men with lower[9] or the lowest (<8nmol/L)[11] concentrations, and an increased risk of diagnosis for any cancer or for prostate cancer for men with the lowest (<10.17nmol/L) concentrations of testosterone.[36] However, results were varied and not all articles reported these associations as being significant.[39] Furthermore, aside from an average increase in MMSE of 0.067 per ng/mL decrease in testosterone concentration during follow-up,[40] there were no significant associations of baseline testosterone with cognitive function, or with change in cognitive function reported in the selected articles (Supplementary table 10).

4. DISCUSSION

The systematic review identified nine studies, and when combined with an additional two identified by colleagues, comprises 11 in total, with data for over 20,000 men from Australia, Europe, USA and the United Kingdom. Meta-regressions revealed significant heterogeneity in testosterone measurements at baseline, which was not explained by the mean age of participants among studies. However, DR-MA summary estimates demonstrated no significant effects of baseline testosterone on the relative hazard of death from any cause or from CVD, with negligible heterogeneity present. The DR-MAs, which suitably accounted for correlations between estimates for different exposure categories within studies, were of published estimates that had been adjusted for age, smoking status, and BMI or waist circumference. Furthermore, only published estimates from prospective cohort studies of community-dwelling men that had measured testosterone accurately using mass spectrometry and had observed at least five years of follow-up data were used. Despite some of these studies having reported an association between testosterone and mortality,[9, 30] the collective body of evidence demonstrated no overall associations of endogenous testosterone concentration with mortality or CVD mortality.

Previous meta-analyses investigating associations of endogenous testosterone with the health outcomes of interest looked at CVD outcomes,[59-61] all-cause mortality,[59] and prostate cancer.[62] Boyle et al.[62] and Holmegard et al.[60] both reported negligible heterogeneity in their estimates. Boyle et al. found no significant association of a 5nmol/L increase in testosterone with prostate cancer and Holmegard et al. estimated a 43% increase in risk of ischemic stroke for men with testosterone levels below the 10^{th} percentile, as compared to men in the 11^{th}-90^{th} percentile range, from a meta-analysis of four articles.[60, 62] Ruige et al. estimated an 11% decrease in risk of a CVD event from a standard deviation increase in testosterone, and reported that significant heterogeneity was explained by larger effect sizes

estimated for studies that recruited older men and for more recent articles.[61] Araujo et al estimated a 35% increase in risk of all-cause mortality and a non-significant effect on CVD mortality from a 2.18 standard deviation decrease in testosterone, although reported significant heterogeneity, and suggested that effects were driven by differences between the cohorts, such as underlying health status.[59] Two of these meta-analyses did not restrict selections to prospective cohort studies[59, 62] and none restricted selections based on testosterone assay method, although Ruige et al.[61] did find that assay method did not explain heterogeneity in that study.

The presented meta-analyses are the first to restrict selections to items of prospective cohort studies of community-dwelling men with testosterone measured using mass spectrometry, which is widely regarded as the reference method,[22] and with at least five years of follow-up data. Accordingly, the presented summary estimates could arguably be viewed as the most reliable to date. These restrictions also resulted in the selection of a relatively small number of publications with estimates suitable for use in DR-MAs. Follow-up times for all-cause and CVD mortality ranged from a median of 4.3 years (total = 5 years; EMAS)[11] to a mean of 14.9 years (total = 16 years; BHS).[37] The number of incident deaths ranged from 147 (EMAS)[40] to 777 (CHS),[51] or to 974 with the additional HIMS article[30] included. The number of CVD deaths ranged from 29 (ARIC)[48] to 264 (CHS)[51], or to 325 with the additional HIMS article.[30] However, despite these differences, there was negligible heterogeneity in estimates and no significant funnel plot asymmetry detected.

Linear models were fitted because the HR estimates were reported for insufficient numbers of testosterone categories to have fitted non-linear DR-MA models. This was a key limitation

of the analyses and likely to have resulted in an oversimplification of true effects. For instance, although the 95% CI for the Pye et al[11] study (calculated from HR estimates for quintile categories of testosterone) overlapped one, an alternative set of estimates in that article (which could not be included in the DR-MAs) reported a two-fold increase in the risk of all-cause mortality for men with very low testosterone (<8nmol/L), as compared to "eugonadal" men (>11nmol/L). Pye et al[11] postulated that their reported differences in estimates might be reflective of a nonlinear association that emerges only when endogenous testosterone declines into the lower part of the range (<8nmol/L). Furthermore, Yeap et al.[30] estimated an "U"-shaped association between endogenous testosterone and all-cause mortality, as consistent with a lower relative risk of health impacts for adult males with mid-range levels of testosterone. However, Shores et al.[51] also used non-linear modelling but did not find any significant associations of testosterone with all-cause or CVD mortality. Clearly, the investigation of non-linear associations is required to more comprehensively investigate the associations of testosterone concentrations with health outcomes in men.

In addition to the linearity assumption, there were other methodological limitations. Several articles reported estimated HRs per increase or decrease in standard deviation (SD) and it was not possible to use these estimates in DR-MAs. Although it was possible to convert the per SD estimates to a standardised scale (i.e., per 5nmol/L increase), there was no information to determine adjustments to respective estimates of precision. Estimates for those studies could therefore not be included in the calculation of summary estimates and 95% confidence intervals could not be calculated in forest plots. Summary estimates were calculated from a relatively low number (n = 3-5) of articles and for most outcomes a summary estimate could not be calculated, which impacts upon the generalisability of findings. Furthermore, these analyses were of observational data so summary estimates will not fully eliminate the

possibility of confounding arising from unadjusted effects.

The implications of these findings are that associations of endogenous testosterone concentrations with key health outcomes should not be overstated, as they are not readily portrayed by meta-analyses of summary estimates. A more nuanced approach may be required, to capture non-linear or U-shaped associations.[11, 30] Also, while testosterone concentrations across ages were relatively stable when considering estimates from different cohorts, associations of testosterone with health outcomes may differ with age, for example with all-cause mortality in middle-aged men[37] compared to older men.[9, 30] A deeper understanding of associations of endogenous testosterone concentrations with key health outcomes, would provide a foundation for analyses of the effects of exogenous testosterone, administered via therapeutic or pharmacologic interventions, on men's health.

Individual participant data (IPD) meta-analyses that incorporate flexible non-linear modelling techniques will provide improved scope to clarify the nature of such associations. The ability to apply a consistent statistical model to all studies, incorporate a more extended set of covariates than may have been included at the individual study level, and to estimate effects with increased statistical power, should result in more reliable summary estimates with reduced bias. Furthermore, other hitherto unpublished variables may be available for sharing by the collaborating studies to use in IPD meta-analyses, which could be useful for constructing analysis covariates or outcome variables. For instance, articles from the ARIC study that were identified from the systematic review reported on incident CVD event and death outcomes, but documentation on the ARIC study website shows that data on other prospective health outcomes, including cause-specific deaths and dementia diagnoses, are also available upon request.[63] Although there have been recent advances with non-linear

modelling methods for the meta-analyses of published estimates,[32, 64] sufficient information in the published articles, as is required for implementing these methods, was not available. In future work, estimates from analyses of the IPD-level data will be used to estimate and plot non-linear summary effects, and so will provide further improvements to estimates of associations between androgen levels and health outcomes in men.

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AUTHORS' CONTRIBUTIONS

BBY, KM, RJM, JH contributed to the design of the systematic review. RJM conducted the literature search and RJM, JH independently screened the returned items. RJM, KM conducted the statistical analyses. All authors were involved in manuscript preparation and subsequent revisions, and approved this submission.

COMPETING INTERESTS

None declared.

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PATIENT CONSENT

This manuscript does not contain patient personal data.

PROVENANCE AND PEER REVIEW

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FIGURE LEGENDS

Figure 1. Studies returned from systematic review of the published and grey literature. Step 1 involved screening of titles and abstracts only and Step 2 the screening of full text items not excluded at Step 1 (see Supplementary tables 2, 3). “Items” are individual articles or reports, with multiple items returned for some studies (the purpose was to identify studies with suitable IPD-level data). * = Mednar items with insufficient information available to review; ** = Additional studies identified through known contacts; *** = Screening criteria for five items selected as “Maybe” in Step 2 were further investigated using information external to systematic searches and screenings, resulting in the identification of one additional study with suitable IPD-level data.

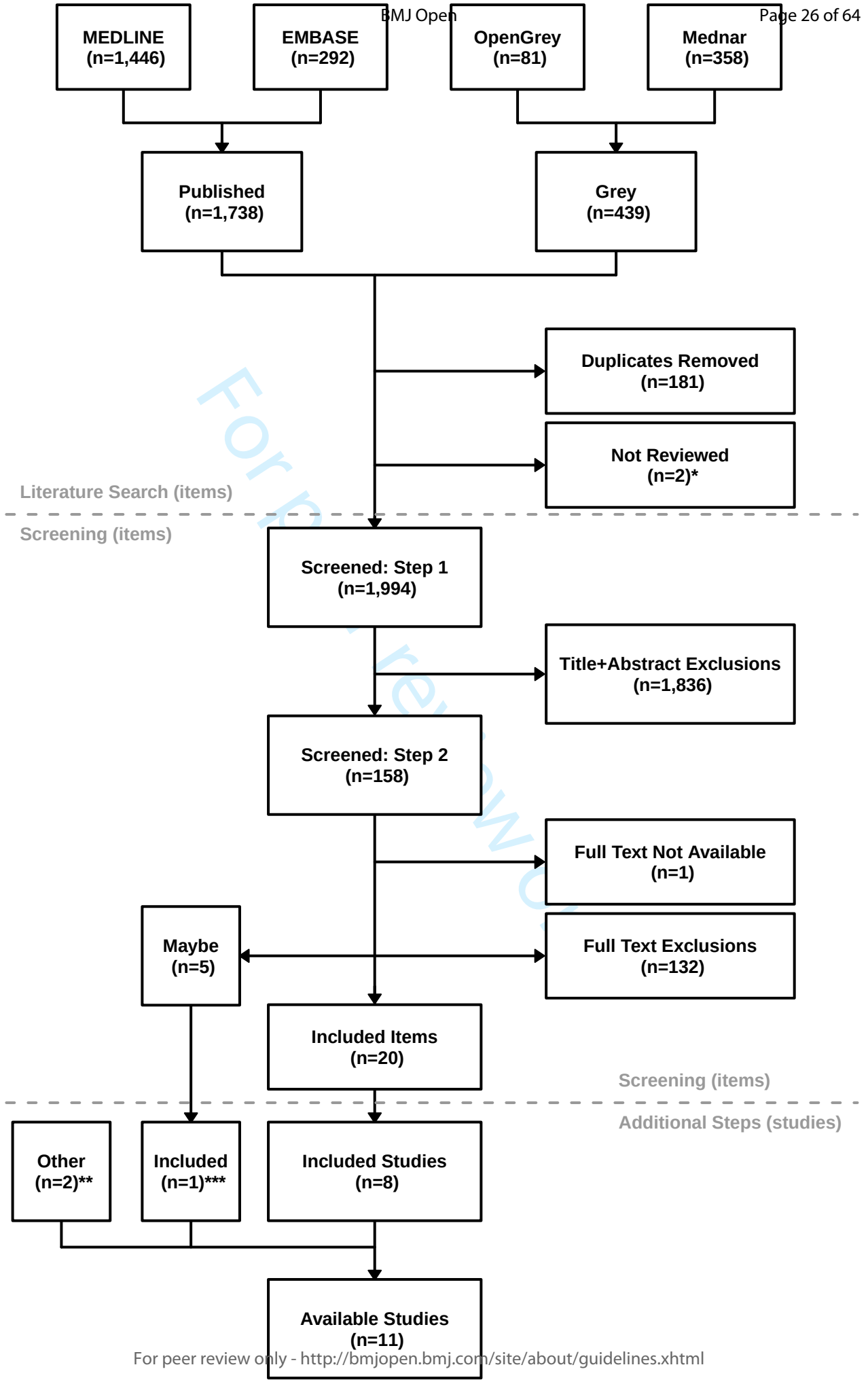
Figure 2. Meta-regression of mean testosterone on mean age for (a) all 11 cohort studies and (b) 9 studies with articles that were selected by systematic literature searches and screening. The size of plotted points refers are proportional to the inverse of the corresponding standard errors (indicative of relative weightings), with lines demonstrating the fitted model and 95% CIs. Plotted estimates are numbered as from the following articles (cohort studies):

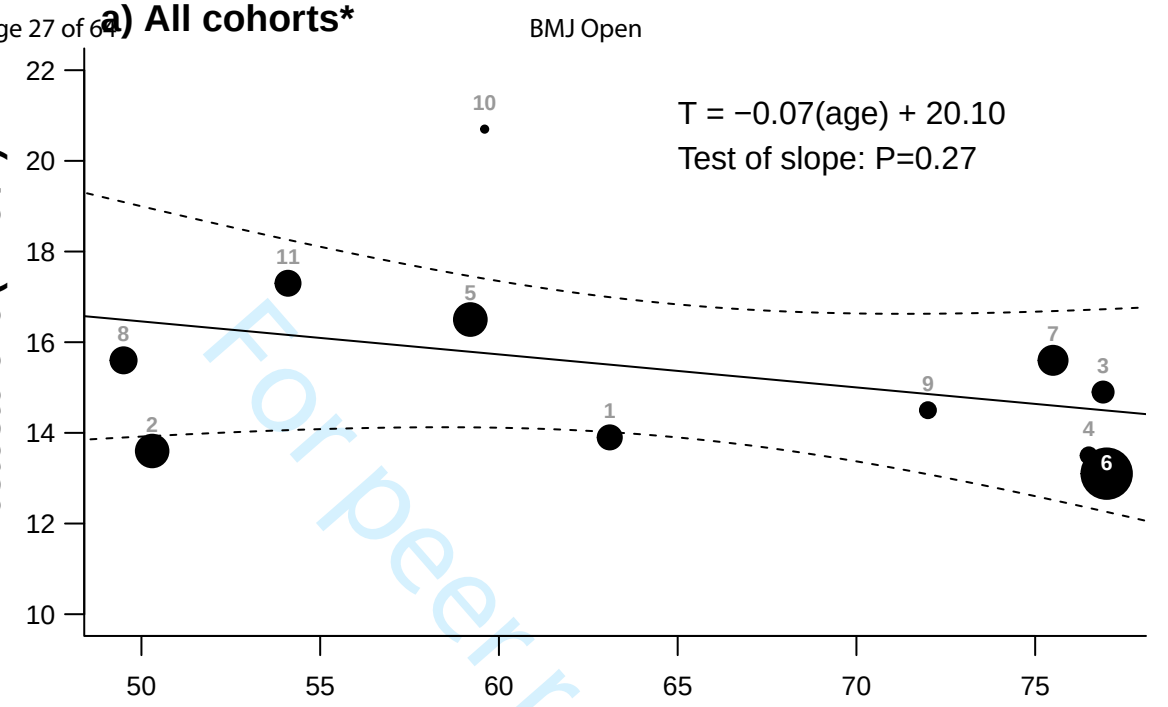
1= Srinath et al.[48] (ARIC); 2= Chan et al.[37] (BHS); 3= Hsu et al.[9] (CHAMP); 4= Shores et al.[50] (CHS); 5= Lee et al.[43] (EMAS); 6= Chan et al.[41] (HIMS); 7= Ohlsson et al.[44] (MrOS Sweden); 8= Kische et al.[46] (SHIP); 9= Sueoka et al.[53] (MrOS USA); 10= Pencina et al.[57] (FHS); 11= Li et al.[56] (MAILES). * = includes articles from two additional studies (FHS, MAILES) that were not identified from systematic searches but by colleagues.

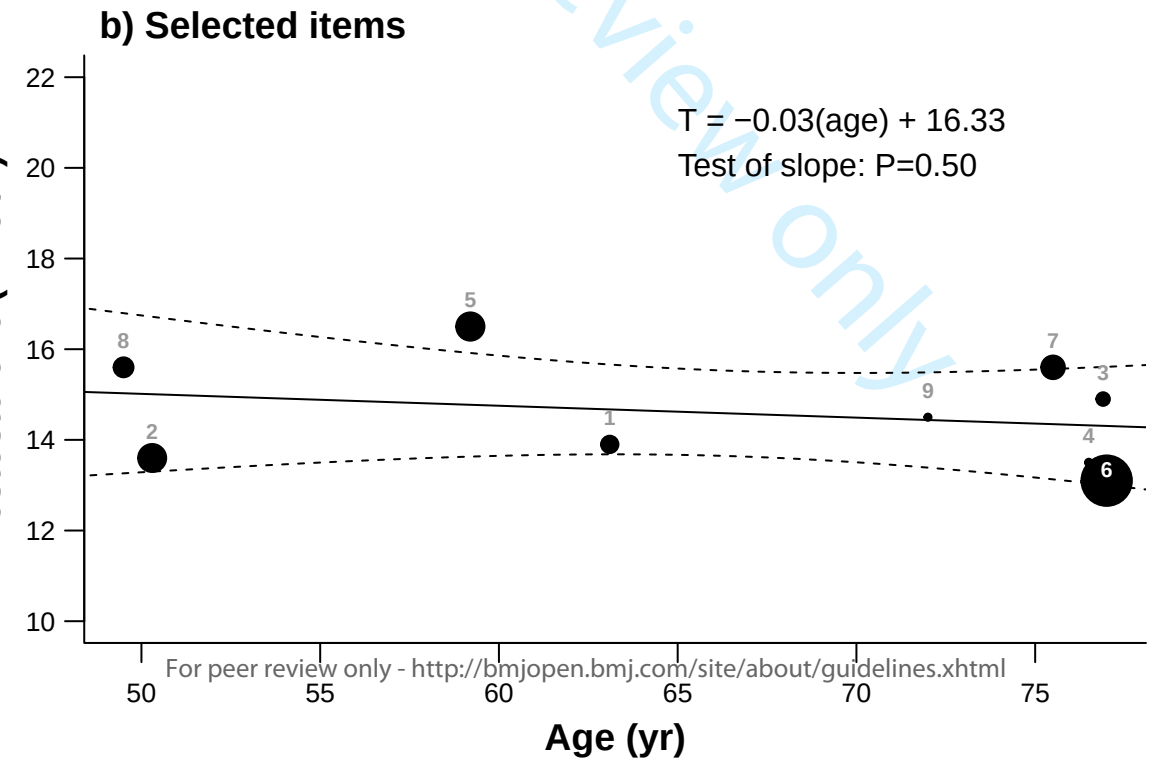
Figure 3. Forest plot of a meta-analysis of published estimates: association of testosterone with all-cause mortality. Plotted values are the estimated hazard ratios (HR) for death from any cause, as attributed to an increase in endogenous testosterone concentration by 5 nmol/L. The vertical reference line is HR=1. Study-specific estimates are presented for six of the selected studies: BHS (Chan, 2016)[37]; EMAS (Pye, 2014)[11]; ARIC (Srinath, 2015)[48]; CHS (Shores, 2014b)[51]; HIMS (Yeap, 2014b)[30]; CHAMP (Hsu, 2016).[9] Summary estimates are colour-coded as calculated using either the estimates from Yeap et al.[30] calculated from the model including SHBG (black) or from the model including LH (grey). * This estimate from Hsu et al.[9] could not be used to calculate the summary estimate because a variance estimate was not calculable for a 5nmol/L change in testosterone using the published information.

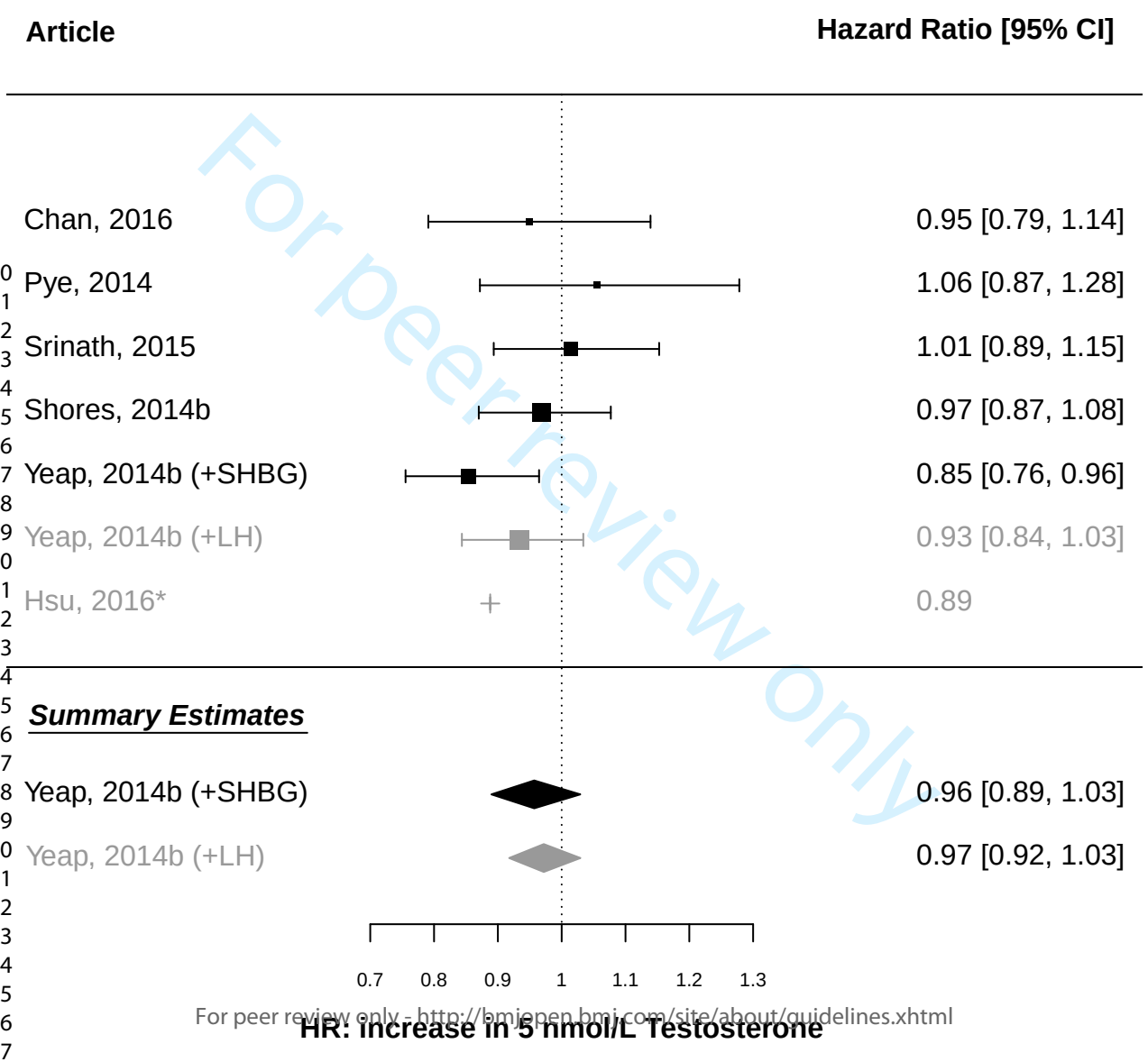
Figure 4. Forest plot of a meta-analysis of published estimates: association of testosterone with mortality caused by cardiovascular disease. Plotted values are the estimated hazard ratios (HR) for death from any cause, as attributed to an increase in endogenous testosterone concentration by 5 nmol/L. The vertical reference line is HR=1. Study-specific estimates are presented for six of the selected studies: BHS (Chan, 2016; Chasland, 2017)[37-38]; EMAS (Pye, 2014)[11]; ARIC (Srinath, 2015)[48]; CHS (Shores, 2014b)[51]; HIMS (Yeap,

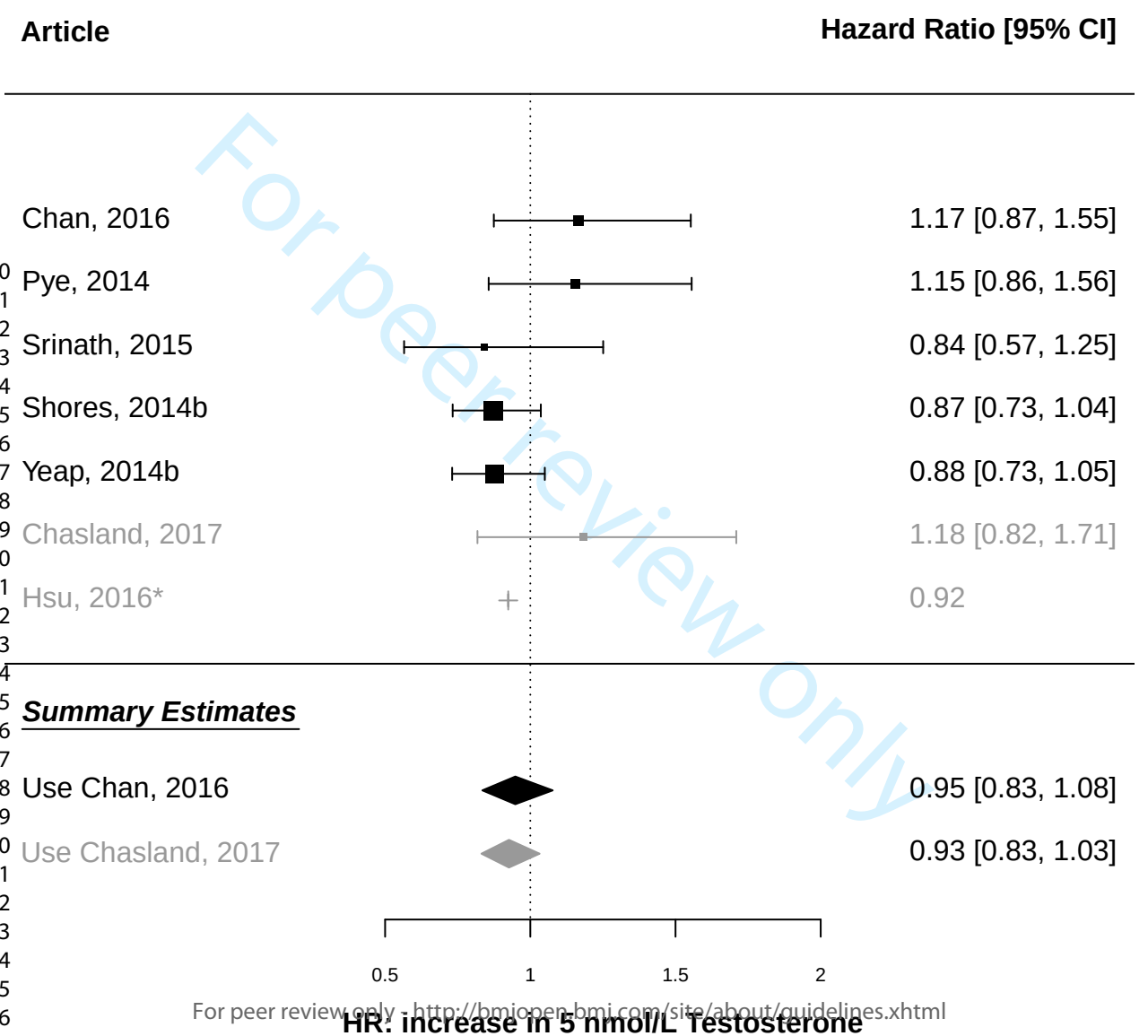
2014b)[30]; CHAMP (Hsu, 2016).[9] Summary estimates are colour-coded as calculated using either the estimates from Chan et al.[37] (black) or Chasland et al.[38] (grey) for the BHS. * This estimate from Hsu et al.[9] could not be used to calculate the summary estimate because a variance estimate was not calculable for a 5nmol/L change in testosterone using the published information.











Supplementary Material. Systematic review: associations of testosterone with men's health.

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2. Additional details on systematic searches and screening

We used online search tools to identify available published (MEDLINE, EMBASE) and grey literature (OpenGrey, Mednar) *items* (journal articles, reports, theses, webpage articles) reporting on suitable prospective cohort *studies* (the underlying unique sources of data). We used OpenGrey and Mednar because both were free search tools that we considered likely to identify additional grey literature items and studies in an expanded search beyond the mainstream publications. Mednar is a medically-focussed search engine of public and deep web resources, excluding subscription services.[1] OpenGrey is a searchable database containing citations for items including technical or research reports, theses, conference papers, and other types of grey literature.[2] Literature searches were conducted on 18-22 July 2019, with no date restrictions set.

Where possible (as functionality varied among the different tools), we placed the following restrictions on the search: items reporting on the results of a research study, longitudinal or prospective cohort studies, not of hormone therapy or deprivation treatments. Due to study timeframe and language translation limitations, we opted to search for only those items that were reported in the English language. The terms and full criteria used for the MEDLINE search are provided in Supplementary table 1, and the PRISMA checklist as Supplementary table 5.

Selection criteria were set as applicable to the planned sets of IPD meta-analyses (Supplementary table 2).[3] Only items reporting on prospective population-based cohort studies, adults of combined sexes or of men alone, with individuals free of the disease at baseline, were sought. Items reporting a different design for the analysis of longitudinal data, such as nested case-control or case-cohort design, were also considered acceptable. A

minimum of five years follow-up was selected, to ensure a sufficient number of incident events for statistical modelling. We excluded items that did not measure testosterone using mass spectrometry, which is regarded to be the 'gold standard' method,[4] although testosterone was not required to be mentioned in the title or abstract, nor modelled as the primary exposure variable. Selected items were to be studies of humans, reported in English, and reporting on analyses of at least one of the AIMS outcomes.

Two reviewers (RJM, JH) independently screened the de-duplicated items against these pre-specified criteria. To optimise efficiency, the selection of items proceeded in two steps. Title and abstract screenings (Step 1) were followed by full text screening of items selected in Step 1 (Step 2). If an item was selected for exclusion, then the main reason for that decision was recorded. If there was uncertainty in the decision to exclude, in Step 1 the reviewer selected "include" (in Step 1) or "maybe" (in Step 2). At the end of each step, the two reviewers sought to achieve consensus, through discussion, for each item that did not achieve agreement. Exclusion reasons were used to inform discussions for achieving consensus. Items with a consensus decision of "maybe" were further investigated by Reviewer 1 (RJM) using information external to the systematic searches and screenings (reading further details of methods used in cited articles, and from correspondence with authors or other researchers currently working on the research study).

This screening procedure was adjusted to accommodate the different types of items reviewed (published articles, theses, webpage articles, unpublished reports; Supplementary table 3). A pilot set of title-only screenings for 30 randomly chosen articles suggested that sufficient

information was contained within the titles alone for the purpose of Step 1 screenings.[a] Therefore, in cases when an abstract was not available, only the titles were screened. Website items identified by the Mednar search tool were the type of item that most often did not have abstract or summary text, and in these cases the webpage text was reviewed in place of an abstract (Supplementary table 3).

Endnote X8[5] was used for collating and storing the citations returned from literature searches, and for de-duplicating and storing the selected references. The full citations, including abstracts, were exported from Endnote for uploading into Rayyan[6], which is a free web tool that was used for screening, recording exclusion decisions, and downloading selection results.

The literature search identified 2,177 items (1,738 published and 439 from grey literature), with 1,994 items remaining after duplicates had been removed, and after excluding two Mednar items that had insufficient information available to review (Fig. 1). Supplementary table 4 shows the frequencies of returned items by search terms present in the titles and abstracts. Most (72.7%) had the word "cancer", and 1,107 (55.5%) of these had the word "prostate cancer", in the title or abstract. This, combined with frequent mentions of "androgen deprivation" (29.2%), "radiotherapy" (18.6%), and "brachytherapy" (8.3%), show that items reporting aspects of testosterone deprivation or suppression for treating prostate cancer were a predominant feature of the returned items. Different types of returned items included 1,764 published articles, 111 webpage articles, 81 theses, and 38 unpublished reports/other documents, and the percentages without abstract or webpage text screened in

[a] 30 titles were initially screened at random. 18 were flagged as not suitable, leaving 12 as potentially suitable. Subsequent Step 1 screening of titles with abstracts selected 25 of these articles for exclusion, with 5 retained for Step 2 (full text screening). All 5 were flagged as being potentially suitable in the pilot set of title-only screenings.

Step 1 were 2.6%, 1.8%, 24.7%, 65.8%, respectively (i.e., 4.7% overall).

One thousand nine hundred sixty-eight items were excluded, five items were classified as "Maybe", and one item could not be screened because the full text version was not available, leaving n = 20 suitable items selected (Fig. 1). Most (92.1%) of the exclusions were made from reviewing titles and abstracts at Step 1, with a further 6.6% excluded from screening of the 157 full text items in Step 2. Inter-reader agreement was a Cohen's Kappa $\kappa = 0.69$ (or 96.0 percent agreement) for Step 1 and $\kappa = 0.82$ (or 98.1 percent agreement) for Step 2. Percentages of items with search terms (AIMS outcomes) in the title or abstract increased after Step 1 in most cases except for "cancer" and "prostate cancer" (Supplementary table 4). This reflects many exclusions in Step 1 that were of items reporting research on testosterone deprivation or suppression treatments for prostate cancer.

The systematic approach to literature searching and screening is widely held to be beneficial to identifying studies that otherwise may not have been considered for inclusion, and thus to minimise the prospect for reviewer biases affecting study selections and summary results.[7] This process is not perfect though, and in our case it did not identify two prospective cohort studies that were known to be suitable, prior to commencing this review (FHS, MAILES).[3] In the case of MAILES, this was one of the more recently commenced of the selected studies, with its cohort profile article published in 2014,[8] and accordingly has had a comparatively short timeframe within which to analyse and publish suitable findings. In the case of FHS, associations of endogenous testosterone with male health outcomes had previously been investigated and published, but not using mass spectrometry for measuring testosterone.[9, 10] Those articles were identified in the literature search but had been excluded on account of assay method. Only relatively recently have testosterone measures been re-assayed for FHS

participants using mass spectrometry methods.[11] One article by Pencina et al[12] was possibly within scope but not identified because it had not been entered into the MEDLINE database prior to the literature search (article entry date = 14 May 2020). Furthermore, an article that presented suitable estimates from one of the selected studies by Yeap et al[13] was not identified from the literature search because it did not have "prospective", "follow-up", "cohort study" or "longitudinal study" terms in its title or abstract, nor any of the corresponding MeSH terms listed (refer to Supplementary table 1 for search terms used).

In expanding our literature search to unpublished grey literature, it successfully located one suitable item, which was a link to a Web MD webpage article, with further details published in a conference abstract by Sueoka et al[14] that would otherwise have not been returned from searching only the MEDLINE and EMBASE databases.

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3. Tables

Supplementary table 1. Full electronic search strategy used for MEDLINE database.

The following is the search that was conducted on 18 July 2019 using MEDLINE.

1. Testosterone/ or Androgens/
2. (testosterone or androgen* or sex hormone* or sex steroid*).ti.
3. (testosterone or androgen*).ab.
4. cardiovascular diseases/ or heart diseases/ or heart failure/ or vascular diseases/ or stroke/ or myocardial infarction/ or coronary disease/ or cerebrovascular disorders/
5. (cardiovascular or stroke or myocardial infarction or heart failure).ti.
6. neoplasms/ or colorectal neoplasms/ or lung neoplasms/ or prostatic neoplasms/
7. cancer.ti.
8. mortality/ or mortality.ti.
9. dementia/ or cognition/ or dementia.ti. or cognit*.ti.
10. Aging/psychology or Neuropsychological Tests/
11. 1 or 2 or 3
12. 4 or 5 or 6 or 7 or 8 or 9 or 10
13. 11 and 12
14. longitudinal studies/ or prospective studies/ or follow-up studies/ or cohort studies/
15. (prospective or follow-up or cohort study or longitudinal study).ti,ab.
16. 14 or 15
17. 13 and 16
18. (exogenous or replacement or therapy or hormone treatment).ti.
19. Hormone Replacement Therapy/
20. 18 or 19
21. 17 not 20
22. limit 21 to humans
23. limit 22 to english language
24. limit 23 to (adaptive clinical trial or address or autobiography or bibliography or biography or case reports or clinical trial, all or clinical trial, phase i or clinical trial, phase ii or clinical trial, phase iii or clinical trial, phase iv or clinical trial, veterinary or clinical trials, veterinary as topic or clinical trial protocol or clinical trial or comment or controlled clinical trial or dictionary or editorial or lecture or legislation or meta analysis or practice guideline or pragmatic clinical trial or published erratum or randomized controlled trial or retracted publication or "retraction of publication" or "review" or "scientific integrity review" or "systematic review")
25. Retrospective Studies/ or Case-Control Studies/ or (retrospective analysis or case-control).ti.
26. 24 or 25
27. 23 not 26

Notes:

Terms with a trailing "/" are MeSH terms and those with a trailing "*" are truncated search strings. Beforehand, a search of PROSPERO was conducted for another suitable strategy but none were found. However, the above strategy is based upon one that has been used for a similar study.[15] This search strategy is also published in the protocol article for the Androgens In Men Study.[3]

Supplementary table 2: Selection criteria for screening items returned from the literature search. If neither Include nor Exclude could be selected for Step 1, then reviewer selected "Include".

	Exclude	Include	Rationale	Used in Step 1		Used in Step 2
				Title & Abstract		Full-text
				Title only (no abstract)	Title & Abstract	
Article type:	Reviews, comments/opinion pieces, systematic reviews, dictionary, fact sheet, website information about diseases, fact sheets, etc.	Research study article / report, or an article that specifically refers to the results of one (e.g., a webpage referring to unpublished data).	These searches were of both published and unpublished scientific literature for the purpose of identifying prospective cohort studies that are likely to have the relevant data for planned IPD meta-analyses	Yes	Yes	Yes
Study type:	Retrospective or cross-sectional designs, case studies, case-control, surveys, RCTs or other trials, experiments, evaluation of androgen / testosterone therapy / deprivation / HRT or the effectiveness of any other type of intervention / surgery / treatment, genetics, etc.	Prospective cohort study.	A prospective cohort study design is of incident health outcomes for investigating etiology or disease risk for a cohort free of disease at baseline, and ideally should be representative of the local population, but may or may not be some demographic subset: e.g., age range, sex, ethnicity type.	Yes	Yes	Yes
Population (at baseline/date of recruitment to study)	Studies of juveniles only Studies of females only Individuals with some specific health condition/characteristic or following surgery / other medical treatment for specific illness	Adults (18 yr or older) Not females only Community-dwelling men	The study is of community-dwelling men.	Yes	Yes	Yes
Exposure (at baseline)	Do not exclude studies that do not model testosterone as the exposure: although it should be shown that it was measured for participants. If not mentioned in Step 2 then Exclude.	Endogenous testosterone	This will be the focal exposure for all IPD meta-analyses. However, as we are focussing on the identification of only those studies who have suitable androgen measurements available in IPD data, then testosterone does not necessarily need to be modelled as the focal exposure in included items. It is likely that details on the methods will be available only from full-text review.	Only if available	Only if available	Yes
	Testosterone not measured using mass spectrometry	Testosterone assay of serum or plasma sample using mass spectrometry (lc-ms or gc-ms)		Only if available	Only if available	Yes
Outcome (at follow-up)	Incident outcome not one of those type of events specified for inclusion.	Diagnosis/event of: cardiovascular disease (any); cancer (any); dementia. Deaths (any cause); deaths due to any type of cardiovascular disease; deaths due to any type of cancer. Cognition change / outcome	These are the outcomes for the planned IPD meta-analyses so it is important to seek IPD datasets from those studies who have already modelled these outcomes. We refer to these as the "AIMS outcomes".	Yes	Yes	Yes
	Less than 5 years of follow-up data	Five or more years of follow-up data, with outcomes identified using systematic follow-up or data linkage.	As consistent across all included studies for IPD meta-analyses and set *a priori.* Likely that this will be available only from full-text review so not included Step 1.	No	No	Yes
Language	Title and/or abstract of article not in English	Title and/or abstract of article in English	As limited by the timeframe of this study and the native language of reviewers (a practicality).	Yes	Yes	Yes
Species	Studies not of humans	Studies of humans	We are studying humans.	Yes	Yes	Yes

Supplementary table 3: Adaptation of screening rules for different types of published and unpublished items.

Item Type	Step 1	Step 2
Published article	Screen title (and abstract[a])	Screen full text article
Thesis	Screen title (and abstract[a])	Screen full thesis
Unpublished report / other document	Screen title (and abstract[a,b])	Screen full document
Webpage	Screen title and webpage[c]	Screen full text article/document as identified from the webpage, or from a google search of information provided about the article, from the webpage.

[a] = when an abstract was available, otherwise title-only decisions were made (see Supplementary table 2).

[b] = or, if not an abstract, other suitable document summary, as returned by the search tool.

[c] = for webpage articles, the webpage text served as the proxy for an abstract, with the proviso that the reviewer did not navigate to additional webpages during Step 1.

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Supplementary table 4. Words mentioned in the titles or abstracts of reviewed items.[a]

Word(s)	Step 1 items (n=1,994)	Step 2 items (n=158)	Selected items (n=20)
Search terms (AIMS outcomes)			
cancer	1,449 (72.7)	72 (45.6)	6 (30.0)
colorectal cancer	9 (0.5)	4 (2.5)	2 (10.0)
lung cancer	10 (0.5)	6 (3.8)	2 (10.0)
prostate cancer	1,107 (55.5)	40 (25.3)	2 (10.0)
cardiovascular	219 (11.0)	49 (31)	15 (75.0)
heart failure	29 (1.5)	2 (1.3)	1 (5.0)
stroke	31 (1.6)	12 (7.6)	4 (20.0)
myocardial infarction	33 (1.7)	7 (4.4)	1 (5.0)
mortality	232 (11.6)	45 (28.5)	9 (45.0)
dementia	22 (1.1)	8 (5.1)	2 (10.0)
cognit*	87 (4.4)	20 (12.7)	4 (20.0)
Other frequently observed (not search terms)			
androgen deprivation	583 (29.2)	2 (1.3)	0 (0.0)
androgen receptor	235 (11.8)	10 (6.3)	0 (0.0)
brachytherapy	165 (8.3)	0 (0.0)	0 (0.0)
breast cancer	153 (7.7)	9 (5.7)	0 (0.0)
radiotherapy	371 (18.6)	0 (0.0)	0 (0.0)

[a] = Items summarised as numbers (percentages); *= wildcard character designating truncation

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Supplementary table 5. PRISMA Checklist.

Section/topic	#	Checklist item	Reported on page #
TITLE			
Title	1	Identify the report as a systematic review, meta-analysis, or both.	1
ABSTRACT			
Structured summary	2	Provide a structured summary including, as applicable: background; objectives; data sources; study eligibility criteria, participants, and interventions; study appraisal and synthesis methods; results; limitations; conclusions and implications of key findings; systematic review registration number.	2-3
INTRODUCTION			
Rationale	3	Describe the rationale for the review in the context of what is already known.	4-5
Objectives	4	Provide an explicit statement of questions being addressed with reference to participants, interventions, comparisons, outcomes, and study design (PICOS).	5. For this type of review it is PEO instead.
METHODS			
Protocol and registration	5	Indicate if a review protocol exists, if and where it can be accessed (e.g., Web address), and, if available, provide registration information including registration number.	5
Eligibility criteria	6	Specify study characteristics (e.g., PICOS, length of follow-up) and report characteristics (e.g., years considered, language, publication status) used as criteria for eligibility, giving rationale.	5, Supplementary table 2
Information sources	7	Describe all information sources (e.g., databases with dates of coverage, contact with study authors to identify additional studies) in the search and date last searched.	5-6
Search	8	Present full electronic search strategy for at least one database, including any limits used, such that it could be repeated.	Supplementary table 1
Study selection	9	State the process for selecting studies (i.e., screening, eligibility, included in systematic review, and, if applicable, included in the meta-analysis).	5-6, Supplementary tables 2-3.

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Section/topic	#	Checklist item	Reported on page #
Data collection process	10	Describe method of data extraction from reports (e.g., piloted forms, independently, in duplicate) and any processes for obtaining and confirming data from investigators.	6, Suppl. Data.
Data items	11	List and define all variables for which data were sought (e.g., PICOS, funding sources) and any assumptions and simplifications made.	6, Suppl. Data.
Risk of bias in individual studies	12	Describe methods used for assessing risk of bias of individual studies (including specification of whether this was done at the study or outcome level), and how this information is to be used in any data synthesis.	6, Supplementary table 8.
Summary measures	13	State the principal summary measures (e.g., risk ratio, difference in means).	7
Synthesis of results	14	Describe the methods of handling data and combining results of studies, if done, including measures of consistency (e.g., I^2) for each meta-analysis.	7
Risk of bias across studies	15	Specify any assessment of risk of bias that may affect the cumulative evidence (e.g., publication bias, selective reporting within studies).	7
Additional analyses	16	Describe methods of additional analyses (e.g., sensitivity or subgroup analyses, meta-regression), if done, indicating which were pre-specified.	7
RESULTS			
Study selection	17	Give numbers of studies screened, assessed for eligibility, and included in the review, with reasons for exclusions at each stage, ideally with a flow diagram.	8, Fig. 1
Study characteristics	18	For each study, present characteristics for which data were extracted (e.g., study size, PICOS, follow-up period) and provide the citations.	Supplementary tables 6-7, 9-10
Risk of bias within studies	19	Present data on risk of bias of each study and, if available, any outcome level assessment (see item 12).	9, Supplementary table 8
Results of individual studies	20	For all outcomes considered (benefits or harms), present, for each study: (a) simple summary data for each intervention group (b) effect estimates and confidence intervals, ideally with a forest plot.	10-12, Figs 3-4; Supplementary figure 3

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Section/topic	#	Checklist item	Reported on page #
Synthesis of results	21	Present results of each meta-analysis done, including confidence intervals and measures of consistency.	10-12, Figs 3-4; Supplementary figure 3
Risk of bias across studies	22	Present results of any assessment of risk of bias across studies (see Item 15).	10-11, Supplementary figure 2
Additional analysis	23	Give results of additional analyses, if done (e.g., sensitivity or subgroup analyses, meta-regression [see Item 16]).	10-12, Fig 2; Supplementary figures 1-3
DISCUSSION			
Summary of evidence	24	Summarize the main findings including the strength of evidence for each main outcome; consider their relevance to key groups (e.g., healthcare providers, users, and policy makers).	12-16
Limitations	25	Discuss limitations at study and outcome level (e.g., risk of bias), and at review-level (e.g., incomplete retrieval of identified research, reporting bias).	14-15
Conclusions	26	Provide a general interpretation of the results in the context of other evidence, and implications for future research.	13-16
FUNDING			
Funding	27	Describe sources of funding for the systematic review and other support (e.g., supply of data); role of funders for the systematic review.	17

From: Moher D, Liberati A, Tetzlaff J, Altman DG, The PRISMA Group (2009). Preferred Reporting Items for Systematic Reviews and Meta-Analyses: The PRISMA Statement. PLoS Med 6(7): e1000097. doi:10.1371/journal.pmed1000097

Supplementary table 6. Attributes of selected items.

Item	Article	Country	Study name§	Baseline** No. adult males	Baseline period	Age (yr) Mean (sd)	T (nmol/L) Mean (sd)	Follow-up (relevant outcomes) Length of follow-up (yr) (person-years)¶	AIMS Longitudinal Outcomes (no. of events analysed)
Selected from systematic review									
1	Srinath, 2015[16]	USA	ARIC	1,558	1996-98	63.1 (5.6)	13.9 (5.7)	Md=12.8 (CHD); Md=13.1 (HF) (25,374; HF)	Coronary Heart Disease (CHD; 287) Heart Failure (HF; 104) CHD deaths (29) All-cause deaths (347)
2	Srinath, 2016[17]	USA	ARIC	1,558	1996-98	63.1 (5.6)	13.9 (5.7)	Md=14.1 (27,311)	Ischemic Stroke (79)
3	Chan, 2016[18]	Australia	BHS	1,804	1994-95	50.3 (16.8)	13.6 (4.9)	Mn=14.9 (31,930)	CVD events (234; 399)*** CVD deaths (71; 141)*** All-cause deaths (191; 319)***
4	Chasland, 2017[19]	Australia	BHS	1,649	1994-95	49.8 (15.3)	13.7 (4.9)	Tot=20	CVD events (415) CVD deaths (127)
5	Chan, 2018[20]	Australia	BHS	1,574	1994-95	51.1 (14.7)	13.5 (4.8)	Tot=20	Prostate cancer (116) Lung cancer (22) Colorectal cancer (48) Cancer (any; 289)
6	Hsu, 2015[21]	Australia	CHAMP	853	2005-07	76.9 (5.5)	14.6 (6.2)	Tot=5	Cognitive decline (95)
7	Hsu, 2016[22]	Australia	CHAMP	1,705	2005-07	76.9 (5.5)	14.9 (6.6)	Md=6.9; Tot=10 (11,764)	Cancer deaths (151) CVD deaths (185) Other deaths (174) All-cause deaths (510)
8	Hsu, 2018[23]	Australia	CHAMP	1,651	2005-07	76.9 (5.5)	14.7 (6.4)	Tot=5	All-cause deaths (382) CVD deaths (cases not reported) Cancer deaths (cases not reported) Other deaths (cases not reported) Change in: MMSE, SF-12 (Mental).

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				Baseline**				Follow-up (relevant outcomes)	
Item	**Article**	**Country**	**Study name§**	**No. adult males**	**Baseline period**	**Age (yr) Mean (sd)**	**T (nmol/L) Mean (sd)**	**Length of follow-up (yr) (person-years)¶**	**AIMS Longitudinal Outcomes (no. of events analysed)**
9	Rosenberg, 2018[24]	USA	CHS	1,019	1994	76.3 (4.9)	13.2 (6.2)	Md=9.5 (10,716)	Atrial Fibrillation (304)
10	Shores, 2014a[25]	USA	CHS	1,032	1994	76.5 (5.2)	13.5 (6.1)	Md=10; Tot=16 (19,220)	Ischemic stroke (114)
11	Shores, 2014b[26]	USA	CHS	NR	1994	NR	NR	Md=8.9 (CVD events) Md=10.8 yr (All-cause deaths). (9,184; CVD events)	CVD events (436) CVD deaths (157) All-cause deaths (777)
12	Lee, 2013[27]	Europe§§	EMAS	2,736	2003-05	59.2 (10.7)	16.5 (6)	Md=4.3; Tot=5 (14,486)	Cancer (any) Myocardial Infarction (MI) Heart Failure, Other heart conditions Stroke Cognitive function All-cause deaths (193)
13	Pye, 2014[28]	Europe§§	EMAS	2,599	NR	60 (11)	NR	Md=4.3; Tot=5 (11,140)	Cancer deaths (60) CVD deaths (56) All-cause deaths (147)
14	Chan, 2017[29]	Australia	HIMS	3,690	2001-04	77 (3.6)	13.1 (4.9)	Md=9.1, 9.2; Tot=11 (38,665)	Prostate cancer (348) Lung cancer (107) Colorectal cancer (137)
15	Ford, 2018[30]	Australia	HIMS	4,069	2001-04	NR	NR	Md=10.5; Tot=12 (44,404)	Dementia (499)
16	Yeap, 2014[31]	Australia	HIMS	3,690	2001-04	NR	NR	Mn=6.6 (2.3 sd) (28,036)	MI (344) Stroke (300)

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				Baseline**				Follow-up (relevant outcomes)	
Item	**Article**	**Country**	**Study name§**	**No. adult males**	**Baseline period**	**Age (yr) Mean (sd)**	**T (nmol/L) Mean (sd)**	**Length of follow-up (yr) (person-years)¶**	**AIMS Longitudinal Outcomes (no. of events analysed)**
17	Ohlsson, 2010[32]	Sweden	MrOS	2,644	2001-04	75.5 (3.2)	15.6 (6.5)	Mn=4.5† (11,880)	CVD deaths (123) Cancer deaths (127) All-cause deaths (328)
18	Ohlsson, 2011[33]	Sweden	MrOS	2,416	2001-04	75.4 (3.2)	15.7 (6)	Md=5.1 (11,605)	CVD events (485) Chronic Heart Disease events (302) Cerebrovascular events (225)
19	Tivesten, 2014[34]	Sweden	MrOS	2,416	2001-04	75.4 (3.2)	15.7 (6)	Md=5.2 (12,070; CHD) (12,137; CBD)	Chronic Heart Disease (302; CHD) Cerebrovascular Disease (225; CBD)
20	Kische, 2017[35]	Germany	SHIP	1,962	1997-01	49.5 (16.3)	15.6 (6.1)	Tot=10	Change in cognitive status
Decision = "Maybe". Item selected based on additional information									
21	LeBlanc, 2010[36]	USA	MrOS	1,602	NR	NR	NR	Mn=4.5†† (26,977)	Cognitive function (and change in) Cognitive decline
22*	Sueoka, 2010[14]	USA	MrOS	697	2000-05	72 (5.5)	14.5 (5.1)	Av=3.9†† (6,247)	Coronary Heart Disease events (100)
Other. Additional studies selected based on information external to the systematic review									
	No articles were selected.	USA	FHS	3,352[12]	1998-05	59.6 (9.1)[12] 49.4 (13.8)[11]	20.7 (8.0)[12]	Tot=10 (for Atrial Fibrillation)[37]	Cardiovascular outcomes[37, 38] Deaths[37] Cause-specific deaths[38] Cancer[39]
	No articles were selected.	Australia	MAILES	1,632[40]	2002-06[8]	54.1 (11.4)[40]	17.3 (5.7)[40]	Md=4.95; IQR=4.35-5.00[40] (12,686)	CVD events Deaths (99)[8] Cause-specific deaths[8]

§ Study name abbreviations: 'ARIC'= Atherosclerosis Risk in Communities; 'BHS'=Busselton Health Study; 'CHAMP'=The Concord Health and Ageing in Men Project; 'CHS'= Cardiovascular Health Study; 'FHS'= the Framingham Heart Study; 'HIMS'=The Health In Men Study; 'EMAS'=European Male Ageing Study; 'MAILES'= The Men Androgen Inflammation Lifestyle Environment and Stress study; 'MrOS Sweden'=The MrOS Osteoporotic Fractures in Men study in Sweden; 'MrOS USA' = The MrOS Osteoporotic Fractures in Men study USA; 'SHIP'=Study of Health in Pomerania SHIP.
§§ = UK, Italy, Belgium, Poland, Sweden, Spain, Hungary, Estonia

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¶ 'Md'=median; 'Mn'=mean; 'Av'=average; 'Tot'=total follow-up for the cohort (i.e., maximum, rounded down to nearest whole year); 'IQR'=interquartile range. Unless provided in text, person-years was calculated by multiplying the median, mean, or average length of follow-up by the total number of adult male participants.

* = Note that this is a published conference abstract so is not technically a "Full Text" item.

** = Baseline statistics reported for whole cohort; 'NR' = statistics not reported for whole cohort; Means and standard deviations calculated by firstly transforming into standard units (for T: nmol/L) and then, where required, transforming from quartile statistics using the Box-Cox method of McGrath et al.[41]

*** = First number is for individuals without CVD at baseline.

† = Total follow-up exceeded 5 years, from baseline visit (2001-04) to end of mortality data collection (March 1, 2008).

†† = Note that since there was no published follow-up estimate exceeding 5 years (a requirement for selection) and it was not clear, based on the article information alone, whether the total follow-up was at least 5 years, these items were initially classified as "Maybe". The length of follow-up for collection of AIMS outcome data was determined to be satisfactory from subsequent correspondence with MrOS USA researchers.

Supplementary table 7. Exposure levels, outcome assessment, covariates.*

Study	Article	Longitudinal measure of association	Exposure (testosterone)	Outcome ascertainment	Covariates
ARIC	Srinath, 2015[16]	HR	T quartiles	CVD events and deaths identified by annual questionnaires and continuous surveillance, independent from hospital admissions data (ICD codes). Cause of death from death certificates.	Age, race/centre, BMI, waist circumference, smoking status, diabetes mellitus, hypertension, LDL, HDL.
	Srinath, 2016[17]	HR	T tertiles	Definite or probable stroke events identified from hospital admissions, annual phone calls, study examinations adjudicated by a physician, with secondary physician adjudication if it disagreed with a computer algorithm.	Age, race, centre, BMI, waist circumference, smoking status, diabetes mellitus, hypertension, LDL, HDL.
BHS	Chan, 2016[18]	HR	T quartiles (results not shown), Continuous T.	Linked hospital admissions and deaths records (ICD codes)	Age, smoking, vigorous exercise, alcohol, BMI, diabetes, CVD, COPD, non-skin cancer, systolic blood pressure, hypertension, lipid lowering therapy, cholesterol, HDL, triglycerides, C-reactive protein, creatinine
	Chasland, 2017[19]	HR	Categories: Low (L) v High (H) T, physical activity(PA) LT+LPA, LT+HPA, HT+LPA, HT+HPA	Linked hospital admissions and deaths records (ICD codes)	Age, prevalent CVD, smoking, waist circumference, cholesterol, HDL, lipids medication, diabetes, systolic blood pressure, hypertension medication
	Chan, 2018[20]	HR	T quartiles, Continuous T.	Linked cancer and death registry records (ICD codes)	Age, marital status, occupation, smoking, alcohol consumption, leisure time physical activity, BMI, diabetes
CHAMP	Hsu, 2015[21]	Slope estimate (change in MMSE on baseline hormone level or longitudinal change in hormone level)	Continuous T, cFT	Clinic assessment: MMSE, Informant Questionairre on Cognitive Decline as initial screen, followed by clinical assessment to diagnosis categories: normal cognition, MCI, dementia. During follow-up: A decline in MMSE by ≥3 points	Age, BMI, smoking status, years of education, depression score (GDS)
	Hsu, 2016[22]	RR	Continuous T, cFT	Deaths identified from 4-monthly phone calls or deaths registry. Cause of death identified on death certificates independently by 2 medical practitioners.	Age, BMI, smoking status, comorbidity score
	Hsu, 2018[23]	HR, RR (Death outcomes); Slope estimates (MMSE, SF-12 Mental)	Categories: Low (<20th centile) v Normal T combinations with Low (<20th centile) v Normal cFT	Cause of death identified on death certificates independently by 2 medical practitioners.	Age, BMI, smoking status, comorbidity score
CHS	Rosenberg, 2018[24]	HR	Continuous T and cFT, T and cFT quintiles	Independently verified from ECGs taken annually for participants and from hospital discharge diagnoses	Age (stratified), race, education, income, clinic, smoking status, diabetes mellitus, BMI, loop diuretics, height, hypertension, depressed left ventricular ejection fraction, kidney function, systolic blood pressure, SHBG

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Study	Article	Longitudinal measure of association	Exposure (testosterone)	Outcome ascertainment	Covariates
	Shores, 2014a[25]	HR	Continuous T, cFT (linear & non-linear), T categories	Medicare data, hospital records, imaging studies, autopsy results, death certificates, physician interviews data used for adjudications by committee, which included a neurologist.	Age, systolic blood pressure, anti-hypertensive medications, atrial fibrillation, diabetes, smoking, lipid-lowering drugs, HDL, cholesterol, creatinine, fasting glucose, diabetes medications.
	Shores, 2014b[26]	HR	Continuous T, cFT (linear or non-linear) categories: Q1, Q2-4	Medicare data, hospital records, imaging studies, autopsy results, death certificates, physician interviews data used for adjudications by committee, which included a neurologist.	Age, race, site, smoking status, alcohol consumption, hypertensive use, HDL, BMI, waist circumference, diabetes, SHBG.
EMAS	Lee, 2013[27]	N/A	No modelling of longitudinal outcomes reported	MI, heart failure, other heart conditions, cancers, stroke identified from postal questionnaire, MMSE for participants ≥65 yr old from clinic assessments Variable methods for data capture + validation among centres.	No modelling of longitudinal outcomes reported
	Pye, 2014[28]	HR	T, cFT categories: quintiles, low v eugonadal T, LOH status.	Deaths identified from follow-up postal questionnaire or enquiry if no reply received, with 89% of deaths verified from death certificates, death registers, or medical/hospital records.	Age, site, BMI, smoking status, general health.
HIMS	Chan, 2017[29]	SHR	Continuous T, cFT.	Linked hospital admissions, death and cancer registry records (ICD, ICD-O-3 codes).	Age, BMI, smoking status, physical activity, alcohol consumption, diabetes mellitus, HDL, triglycerides, prior cancer diagnosis.
	Ford, 2018[30]	HR	Continuous T, cFT Quartile categories of T, cFT	Linked data (ICD codes) from inpatient and outpatient mental health services, hospital admissions, community aged care services, cancer and death registries.	Age, baseline cognitive function, depression, BMI, hypertension, CVD, plasma homocysteine.
	Yeap, 2014[31]	HR	T, cFT as quartile categories	Linked hospital admissions, death and cancer registry records (ICD codes).	Age, education, smoking status, BMI, waist to hip ratio, hypertension, dyslipidemia, diabetes, creatinine, prior cancer or existing CVD. Also SHBG for models with T.
MrOS Europe	Ohlsson, 2010[32]	HR (in relation to DHEA, DHEA-S)	No: T modelled as a covariate only	Linked data (death and hospital discharge registries), death certificates.	Age, site, BMI, C-reactive protein, ApoB/A1, smoking status, diabetes, hypertension, prior CVD, prior cancer, low testosterone (in lowest quartile), low estradiol
	Ohlsson, 2011[33]	HR	T, cFT as quartile categories, T as binary categories.	Linked data (death and hospital discharge registries), death certificates.	Age, morning sample, site, BMI, ApoB/A1, physical activity, smoking status, diabetes, hypertension
	Tivesten, 2014[34]	HR (in relation to DHEA, DHEA-S)	No: T modelled as a covariate only	Linked data (death and hospital discharge registries), death certificates.	Age, morning sample, site, BMI, ApoB/A1, C-reactive protein, estradiol, testosterone (i.e., continuous T), SHBG, eGFR, smoking status, diabetes, hypertension.
SHIP	Kische, 2017[35]	Slope estimate (change in MMSE on baseline hormone)	T, cFT as continuous and as 10-year age group quartile categories.	MMSE score.	Age, BMI, smoking status, alcohol consumption, physical activity, hypertension, occupational status, education level, civil status, baseline MMSE.

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Study	Article	Longitudinal measure of association	Exposure (testosterone)	Outcome ascertainment	Covariates
MrOS USA	LeBlanc, 2010[36]	Change in mean score RR of clinically important decline	cFT quartiles and continuous cFT and T (data not shown)	Cognitive tests at the baseline and follow-up visit from Part B of the Trail Making Test (Trails B) and the Modified Mental State Examination (3MS). Calculated from pre-defined drop in scores.	Age group, education level, race, general health, alcohol consumption, clinic, physical and mental health, physical activity, medications used at baseline, other sex steroids, SHBG.
	Sueoka, 2010[14]	HR	T quartiles	CHD events identified from 3-monthly contacts with participants. Incident events were reviewed and adjudicated by cardiologist using clinical records.	Age, clinic, BMI, blood pressure, lipid levels, smoking, hypertension, diabetes, use of lipid-lowering agents
FHS	N/A – no items were selected.	N/A	N/A	AF measured and adjudicated by cardiologists. Mortality data from death certificates, hospital or institutional records, obituaries, or direct notification[37] Medical records of CVD events reviewed by panel of experienced investigators. A heart study neurologist examined most participants with suspected stroke[38] Medical records of cancer diagnoses reviewed by two independent reviewers, with majority confirmed by pathology reports.[39]	N/A
MAILES	N/A – no items selected.	N/A	N/A	Self-reported and clinical follow-up data, death registry (linked data)[8]	N/A

* ApoB/A1 = apolipoprotein-B to apolipoprotein-A1 ratio; BMI = body mass index; cFT = calculated free testosterone; CHD = coronary heart disease; COPD = chronic obstructive pulmonary disease; CVD = cardiovascular disease; DHEA = dehydroepiandrosterone; DHEA-S = DHEA sulfate; ECG = electrocardiogram; eGFR = estimated glomerular filtration rate; GDS = Geriatric Depression Scale; HDL = high-density lipoprotein; HR = hazard ratio; ICD = International Classification of Diseases; ICD-O-3 = International Classification of Diseases for Oncology; LDL = low-density lipoprotein; LOH = late-onset hypogonadism; MCI = mild cognitive impairment; MMSE = mini-mental state examination; N/A = not applicable; Q1=quartile 1; Q2-4=quartiles 2 to 4 combined; RR = relative risk; SF-12 = The Short Form (12) Health Survey; SHBG = sex hormone binding globulin; SHR = subhazard ratio, as estimated from competing-risks regression; T = total endogenous testosterone.

Supplementary table 8. Newcastle-Ottawa Quality Assessment Scale for Cohort Studies: selected articles.

Article	Study	Selection (4 stars)	Comparability (2 stars)	Outcome (3 stars)	Notes on Selection	Notes on Outcome
Srinath 2015[16]	ARIC	****	**	**		Losses to f/u not mentioned; linked data[b]
Srinath 2016[17]	ARIC	****	**	**		Losses to f/u not mentioned; linked data[b]
Chan 2016[18]	BHS	****	**	**		Losses to f/u not mentioned; linked data[b]
Chasland 2017[19]	BHS	***	**	**	Prevalent cases not excluded[a]	Losses to f/u not mentioned; linked data[b]
Chan 2018[20]	BHS	****	**	**		Losses to f/u not mentioned; linked data[b]
Hsu 2015[21]	CHAMP	****	**	***		
Hsu 2016[22]	CHAMP	***	**	***	Prevalent cases not excluded[c]	
Hsu 2018[23]	CHAMP	***	**	***	Prevalent cases not excluded[c]	
Rosenberg 2018[24]	CHS	****	**	**		Losses to f/u not mentioned; linked data[d]
Shores 2014a[25]	CHS	****	**	**		Losses to f/u not mentioned; linked data[d]
Shores 2014b[26]	CHS	****	**	**		Losses to f/u not mentioned; linked data[d]
Lee 2013[27]	EMAS	NA	NA	NA	No modelling of longitudinal outcomes reported	
Pye 2014[28]	EMAS	***	**	***	Prevalent cases not excluded[a]	
Chan 2017[29]	HIMS	****	**	**		Losses to f/u not mentioned; linked data[b]
Ford 2018[30]	HIMS	****	**	**		Losses to f/u not mentioned; linked data[b]
Yeap 2014[31]	HIMS	***	**	**	Prevalent cases not excluded[a]	Losses to f/u not mentioned; linked data[b]
Ohlsson 2010[32]	MrOS Sw.	NA	NA	NA	Testosterone was not the exposure variable in this article	
Ohlsson 2011[33]	MrOS Sw.	****	**	***		
Tivesten 2014[34]	MrOS Sw.	NA	NA	NA	Testosterone was not the exposure variable in this article	
Kische 2017[35]	SHIP	***	**	***	Prevalent cases not excluded[e]	
LeBlanc 2010[36]	MrOS USA	****	**	*		Bias from loss to f/u; F/u OK: additional steps[f]
Sueoka 2010[14]	MrOS USA	***	**	*	Prevalent cases not excluded[a]	F/u OK: additional steps[f]
Additional item not selected but included in DR-MA:						
Yeap 2014b[13]	HIMS	***	**	**	Prevalent cases not excluded[a]	Losses to f/u not mentioned; linked data[b]

'NA' = Not applicable (see Notes); 'f/u' = follow-up (of incident events); 'DR-MA' = dose-response meta-analyses of published estimates.
[a] = The influence of prevalent cases was statistically adjusted by including prevalent status as a model predictor.
[b] = Follow-up of cases was assumed to be almost complete because analyses were of linked administrative data.
[c] = The influence of prevalent cases was statistically adjusted by incorporating into a comorbidity status model predictor.
[d] = Follow-up of cases was assumed to be almost complete because analyses were of linked administrative data (with expert adjudications).
[e] = Outcome was change in cognition score, with baseline score (prevalent status) included as a model predictor.
[f] = Total length of follow-up period was not reported but determined to be satisfactory from correspondence with MrOS USA researchers.

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Supplementary table 9. Extracted hazard ratio data for dose-response meta-analyses (DR-MAs).*

Article	Study	Outcome	Testosterone	Units	HR	95% CI	Notes
Chan 2016[18]	BHS	All-cause mortality	<10.20	nmol/L	ref.		
Chan 2016	BHS	All-cause mortality	10.20 - <13.04	nmol/L	0.84	(0.62-1.14)	
Chan 2016	BHS	All-cause mortality	13.04 - <16.58	nmol/L	0.86	(0.62-1.19)	
Chan 2016	BHS	All-cause mortality	≥16.58	nmol/L	0.9	(0.62-1.3)	
Pye 2014[28]	EMAS	All-cause mortality	<11.65	nmol/L	1.1	(0.6-1.8)	
Pye 2014	EMAS	All-cause mortality	11.65-14.61	nmol/L	0.7	(0.4-1.3)	
Pye 2014	EMAS	All-cause mortality	14.61-17.28	nmol/L	0.7	(0.4-1.3)	
Pye 2014	EMAS	All-cause mortality	17.28-21.20	nmol/L	1.2	(0.7-2)	
Pye 2014	EMAS	All-cause mortality	>21.20	nmol/L	ref.		
Srinath 2015[16]	ARIC	All-cause mortality	≤288.4	ng/dL	0.96	(0.7-1.34)	
Srinath 2015	ARIC	All-cause mortality	288.5-377.6	ng/dL	0.99	(0.72-1.35)	
Srinath 2015	ARIC	All-cause mortality	377.7-480.1	ng/dL	1	(0.74-1.35)	
Srinath 2015	ARIC	All-cause mortality	≥480.2	ng/dL	ref.		
Shores 2014b[26]	CHS	All-cause mortality	<278	ng/dL	1.06	(0.88-1.29)	
Shores 2014b	CHS	All-cause mortality	≥278	ng/dL	ref.		
Yeap 2014b[13]	HIMS	All-cause mortality	0.25-9.82	nmol/L	ref.		Fully-adjusted model + SHBG
Yeap 2014b	HIMS	All-cause mortality	9.82-12.53	nmol/L	0.81	(0.68-0.98)	
Yeap 2014b	HIMS	All-cause mortality	12.56-15.75	nmol/L	0.75	(0.61-0.92)	
Yeap 2014b	HIMS	All-cause mortality	15.79-46.50	nmol/L	0.77	(0.61-0.97)	
Yeap 2014b	HIMS	All-cause mortality	0.25-9.82	nmol/L	ref.		Fully-adjusted model + LH
Yeap 2014b	HIMS	All-cause mortality	9.82-12.53	nmol/L	0.84	(0.7-1.01)	
Yeap 2014b	HIMS	All-cause mortality	12.56-15.75	nmol/L	0.81	(0.67-0.97)	
Yeap 2014b	HIMS	All-cause mortality	15.79-46.50	nmol/L	0.89	(0.73-1.07)	
Hsu 2016[22]	CHAMP	All-cause mortality		ng/mL	1.17	(1.03-1.32)	Per SD decrease in T. RR estimate used.
Chan 2016	BHS	CVD mortality	<10.20	nmol/L	ref.		

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Article	Study	Outcome	Testosterone	Units	HR	95% CI	Notes
Chan 2016	BHS	CVD mortality	10.20 - <13.04	nmol/L	1.12	(0.7-1.78)	
Chan 2016	BHS	CVD mortality	13.04 - <16.58	nmol/L	1.39	(0.86-2.25)	
Chan 2016	BHS	CVD mortality	≥16.58	nmol/L	1.25	(0.69-2.25)	
Chasland 2017[19]	BHS	CVD mortality	<13.1	nmol/L	ref.		Total PA, "Low" PA, NS PA x T: these estimates were used
	BHS	CVD mortality	≥13.1	nmol/L	1.25	(0.77-2.03)	
Chasland 2017	BHS	CVD mortality	<13.1	nmol/L	0.69	(0.4-1.2)	Total PA, "High" PA, NS PA x T
Chasland 2017	BHS	CVD mortality	≥13.1	nmol/L	0.8	(0.48-1.35)	
Pye 2014	EMAS	CVD mortality	<11.65	nmol/L	1	(0.4-2.2)	
Pye 2014	EMAS	CVD mortality	11.65-14.61	nmol/L	0.5	(0.2-1.4)	
Pye 2014	EMAS	CVD mortality	14.61-17.28	nmol/L	0.4	(0.2-1.2)	
Pye 2014	EMAS	CVD mortality	17.28-21.20	nmol/L	1.1	(0.5-2.4)	
Pye 2014	EMAS	CVD mortality	>21.20	nmol/L	ref.		
Srinath 2015	ARIC	CVD mortality	≤288.4	ng/dL	1.36	(0.45-4.08)	
Srinath 2015	ARIC	CVD mortality	288.5-377.6	ng/dL	1.26	(0.73-3.7)	
Srinath 2015	ARIC	CVD mortality	377.7-480.1	ng/dL	0.57	(0.16-1.99)	
Srinath 2015	ARIC	CVD mortality	≥480.2	ng/dL	ref.		
Shores 2014b	CHS	CVD mortality	<278	ng/dL	1.28	(0.94-1.75)	
Shores 2014b	CHS	CVD mortality	≥278	ng/dL	ref.		
Yeap 2014b	HIMS	CVD mortality	0.25-9.82	nmol/L	ref.		
Yeap 2014b	HIMS	CVD mortality	9.82-12.53	nmol/L	0.82	(0.61-1.11)	
Yeap 2014b	HIMS	CVD mortality	12.56-15.75	nmol/L	0.79	(0.58-1.09)	
Yeap 2014b	HIMS	CVD mortality	15.79-46.50	nmol/L	0.79	(0.56-1.11)	
Hsu 2016	CHAMP	CVD mortality		ng/mL	1.11	(0.93-1.32)	Per SD decrease in T. RR estimate used
Srinath 2016[17]	ARIC	Stroke / CBD	≤317.7	ng/dL	1.47	(0.83-2.61)	
	ARIC	Stroke / CBD	317.8-441.2	ng/dL	ref.		
Srinath 2016	ARIC	Stroke / CBD	≥441.3	ng/dL	1.15	(0.62-2.14)	
Shores 2014a[25]	CHS	Stroke / CBD	<200	ng/dL	1.46	(0.77-2.75)	
Shores 2014a	CHS	Stroke / CBD	200-400	ng/dL	0.9	(0.56-1.45)	

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Article	Study	Outcome	Testosterone	Units	HR	95% CI	Notes
Shores 2014a	CHS	Stroke / CBD	400-600	ng/dL	ref.		
Shores 2014a	CHS	Stroke / CBD	600-800	ng/dL	1.73	(0.88-3.39)	
Shores 2014a	CHS	Stroke / CBD	>800	ng/dL	1.69	(0.51-5.60)	
Yeap 2014[31]	HIMS	Stroke / CBD	0.25-9.82	nmol/L	ref.		
Yeap 2014	HIMS	Stroke / CBD	9.82-12.53	nmol/L	0.8	(0.59-1.09)	
Yeap 2014	HIMS	Stroke / CBD	12.56-15.75	nmol/L	0.72	(0.52-0.99)	
Yeap 2014	HIMS	Stroke / CBD	15.79-46.5	nmol/L	0.56	(0.39-0.81)	
Ohlsson 2011[33]	MrOS(Sw)	Stroke / CBD	≤340	ng/dL	ref.		
	MrOS(Sw)	Stroke / CBD	341-438	ng/dL	ref.		
Ohlsson 2011	MrOS(Sw)	Stroke / CBD	439-549	ng/dL	ref.		Quartile 4 vs. quartiles 1 to 3 of testosterone
Ohlsson 2011	MrOS(Sw)	Stroke / CBD	≥550	ng/dL	0.76	(0.55-1.05)	
Ohlsson 2011	MrOS(Sw)	CVD	≤340	ng/dL	ref.		
Ohlsson 2011	MrOS(Sw)	CVD	341-438	ng/dL	1.02	(0.80-1.30)	
Ohlsson 2011	MrOS(Sw)	CVD	439-549	ng/dL	0.96	(0.75-1.23)	
Ohlsson 2011	MrOS(Sw)	CVD	≥550	ng/dL	0.71	(0.54-0.93)	
Chan 2016	BHS	CVD		nmol/L	1.03	(0.92-1.15)	Per SD increase in T
Chasland 2017	BHS	CVD	<13.1	nmol/L	ref.		Total PA, "Low" PA, NS PA x T: these estimates were used
Chasland 2017	BHS	CVD	≥13.1	nmol/L	1.09	(0.83-1.44)	
Chasland 2017	BHS	CVD	<13.1	nmol/L	0.93	(0.70-1.23)	Total PA, "High" PA, NS PA x T
Chasland 2017	BHS	CVD	≥13.1	nmol/L	1.04	(0.78-1.38)	
Shores 2014b	CHS	CVD	<278	ng/dL	1.11	(0.87-1.43)	
Shores 2014b	CHS	CVD	≥278	ng/dL	ref.		
Yeap 2014	HIMS	CVD: MI	0.25-9.82	nmol/L	ref.		
Yeap 2014	HIMS	CVD: MI	9.82-12.53	nmol/L	1.07	(0.79-1.44)	
Yeap 2014	HIMS	CVD: MI	12.56-15.75	nmol/L	1.03	(0.76-1.41)	
Yeap 2014	HIMS	CVD: MI	15.79-46.5	nmol/L	0.92	(0.66-1.28)	
Srinath 2015	ARIC	CVD: HF	≤288.4	ng/dL	0.77	(0.46-1.29)	
Srinath 2015	ARIC	CVD: HF	288.5-377.6	ng/dL	0.72	(0.43-1.21)	

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Article	Study	Outcome	Testosterone	Units	HR	95% CI	Notes
Srinath 2015	ARIC	CVD: HF	377.7-480.1	ng/dL	0.87	(0.53-1.42)	
Srinath 2015	ARIC	CVD: HF	≥480.2	ng/dL	ref.		
Chan 2018[20]	BHS	Cancer	<10.17	nmol/L	ref.		
Chan 2018	BHS	Cancer	10.17-<12.95	nmol/L	0.72	(0.53-0.99)	
Chan 2018	BHS	Cancer	12.95-<16.49	nmol/L	0.71	(0.51-0.98)	
Chan 2018	BHS	Cancer	≥16.49	nmol/L	0.81	(0.57-1.14)	
Chan 2018	BHS	Cancer: Prostate	<10.17	nmol/L	ref.		
Chan 2018	BHS	Cancer: Prostate	10.17-<12.95	nmol/L	0.62	(0.37-1.03)	
Chan 2018	BHS	Cancer: Prostate	12.95-<16.49	nmol/L	0.75	(0.46-1.23)	
Chan 2018	BHS	Cancer: Prostate	≥16.49	nmol/L	0.58	(0.33-1.01)	
Chan 2018	BHS	Cancer: Colorectal		nmol/L	1.04	(0.76-1.42)	Per SD increase in T
Chan 2018	BHS	Cancer: Lung		nmol/L	0.65	(0.39-1.09)	Per SD increase in T
Chan 2017[29]	HIMS	Cancer: Prostate		nmol/L	1.00	(0.90-1.12)	Per SD increase in T.
Chan 2017	HIMS	Cancer: Colorectal		nmol/L	0.96	(0.80-1.15)	Per SD increase in T
Chan 2017	HIMS	Cancer: Lung		nmol/L	1.30	(1.06-1.60)	Per SD increase in T
Hsu 2016	CHAMP	Cancer mortality		ng/mL	1.30	(1.02-1.65)	Per SD decrease in T. RR estimate used.
Ford 2018[30]	HIMS	Dementia	Not reported	nmol/L	1.39	(1.04-1.85)	
Ford 2018	HIMS	Dementia			1.31	(1.00-1.73)	
Ford 2018	HIMS	Dementia			1.23	(0.93-1.61)	
Ford 2018	HIMS	Dementia			ref.		
Ford 2018	HIMS	Dementia	SD for cohort not reported	nmol/L	1.11	(1.01-1.21)	Per SD decrease in T

* = Estimates were also reported for all-cause and CVD mortality mortality for the CHAMP study in another of the selected articles,[23] but were not used because they were reported for combinations of free testosterone and total testosterone, and so were not comparable to the above published estimates.

CBD = cerebrovascular disease; CVD = cardiovascular disease; HF = heart failure; LH = luteinising hormone; MI = myocardial infarction; NS = non-significant result at *a priori* selected threshold for test; PA = physical activity level; ref. = referent level; RR = relative risk; SD = standard deviation; SHBG = sex hormone-binding globulin; T = endogenous total testosterone concentration.

Supplementary table 10. Published estimates for selected studies investigating associations of total testosterone with cognitive status or decline.*

Article	Study	Outcome	Testosterone	Units	Effect size parameter	Estimate	95% CI	p value	Notes
Hsu 2015[21]	CHAMP	Cognitive change	Baseline	ng/mL	Slope: Per unit decline	0.012	NR	0.7	Longitudinal change in MMSE score
Hsu 2015	CHAMP	Cognitive change	Longitudinal	ng/mL		0.067	NR	0.03	Longitudinal change in MMSE score
Hsu 2015	CHAMP	Cognitive decline	Baseline	ng/mL	Odds Ratio	NR	NR	NR	Longitudinal decline in MMSE ≥ 3 points. Non-significant association (data not shown)
Hsu 2018[23]	CHAMP	Baseline cognition	Baseline NN	nmol/L	Slope	0	0	NR	MMSE** at baseline
Hsu 2018	CHAMP	Baseline cognition	Baseline NL	nmol/L	Slope	0.1	-0.8-1.1	NR	MMSE at baseline
Hsu 2018	CHAMP	Baseline cognition	Baseline LN	nmol/L	Slope	0.02	-1.0-1.02	NR	MMSE at baseline
Hsu 2018	CHAMP	Baseline cognition	Baseline LL	nmol/L	Slope	0.8	-0.5-0.4	NR	MMSE at baseline
Hsu 2018	CHAMP	Cognitive change	Baseline NN	nmol/L	Slope	0	0	NR	Longitudinal change in MMSE score
Hsu 2018	CHAMP	Cognitive change	Baseline NL	nmol/L	Slope	0.007	-0.5-0.5	NR	Longitudinal change in MMSE score
Hsu 2018	CHAMP	Cognitive change	Baseline LN	nmol/L	Slope	-0.2	-0.7-0.4	NR	Longitudinal change in MMSE score
Hsu 2018	CHAMP	Cognitive change	Baseline LL	nmol/L	Slope	-0.004	-0.3-0.3	NR	Longitudinal change in MMSE score
Kische 2017[35]	SHIP	Cognitive change	Baseline	nmol/L	Slope	0.02	-0.15-0.20	≥0.05	Longitudinal change in MMSE score after 5 years
Kische 2017	SHIP	Cognitive change	Baseline	nmol/L	Slope	0.01	-0.22-0.24	≥0.05	Longitudinal change in MMSE score after 10 years
LeBlanc 2010[36]	MrOS US	Baseline cognition	Baseline	nmol/L	-	NR	NR	≥0.29	3MS score (includes MMSE)
LeBlanc 2010	MrOS US	Follow-up cognition	Baseline	nmol/L	-	NR	NR	≥0.29	3MS score (includes MMSE)
LeBlanc 2010	MrOS US	Cognitive change	Baseline	nmol/L	-	NR	NR	≥0.29	3MS score (includes MMSE)
LeBlanc 2010	MrOS US	Baseline cognition	Baseline	nmol/L	-	NR	NR	≥0.63	Trails B: test of executive function and motor speed
LeBlanc 2010	MrOS US	Follow-up cognition	Baseline	nmol/L	-	NR	NR	≥0.63	Trails B: test of executive function and motor speed
LeBlanc 2010	MrOS US	Cognitive change	Baseline	nmol/L	-	NR	NR	≥0.63	Trails B: test of executive function and motor speed

* NR = Not reported; NN = Normal (>10.2 nmol/L) total testosterone (T), normal (>156 pmol/L) calculated free testosterone (cFT); NL = normal T, low (<156 pmol/L) cFT; LN = low (<10.2 nmol/L) T, normal cFT; LL = low T, low cFT. MMSE = mini-mental state examination; 3MS = modified mini-mental state examination.

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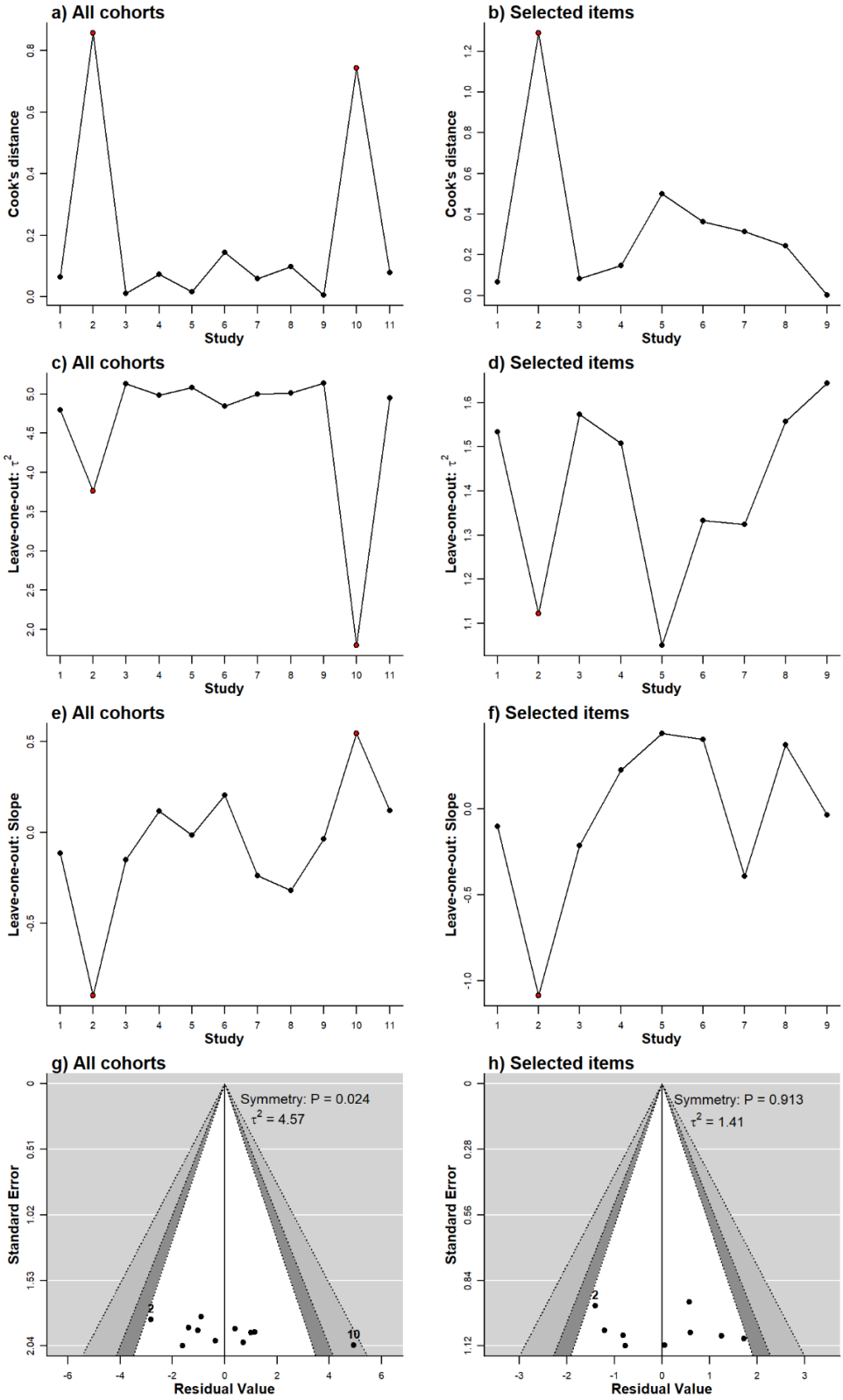
4. Figures.



Supplementary figure 1. Meta-regression diagnostics. Meta-regression diagnostics showing the influence of studies on model fit (a,b), τ^2 (estimated amount of total heterogeneity: c,d), estimated slope (e,f), and distribution of residuals with funnel plots (g,h). Analysis repeated for all 11 cohort studies (a,c,e,g) and for 9 studies with selected articles (b,d,f,h). In cases where more than one article was available per cohort study, the article with the largest sample

size was used. Highlighted estimates for cohort study 2 (BHS) were those from Chan et al.[18] (N=1,804) and for study 10 (FHS) were from Pencina et al.[12] (N=720). In funnel plots: light grey + dark grey + white shading = 99% pseudo confidence interval (CI); dark grey + white shading = 95% CI; white shading = 90% CI.

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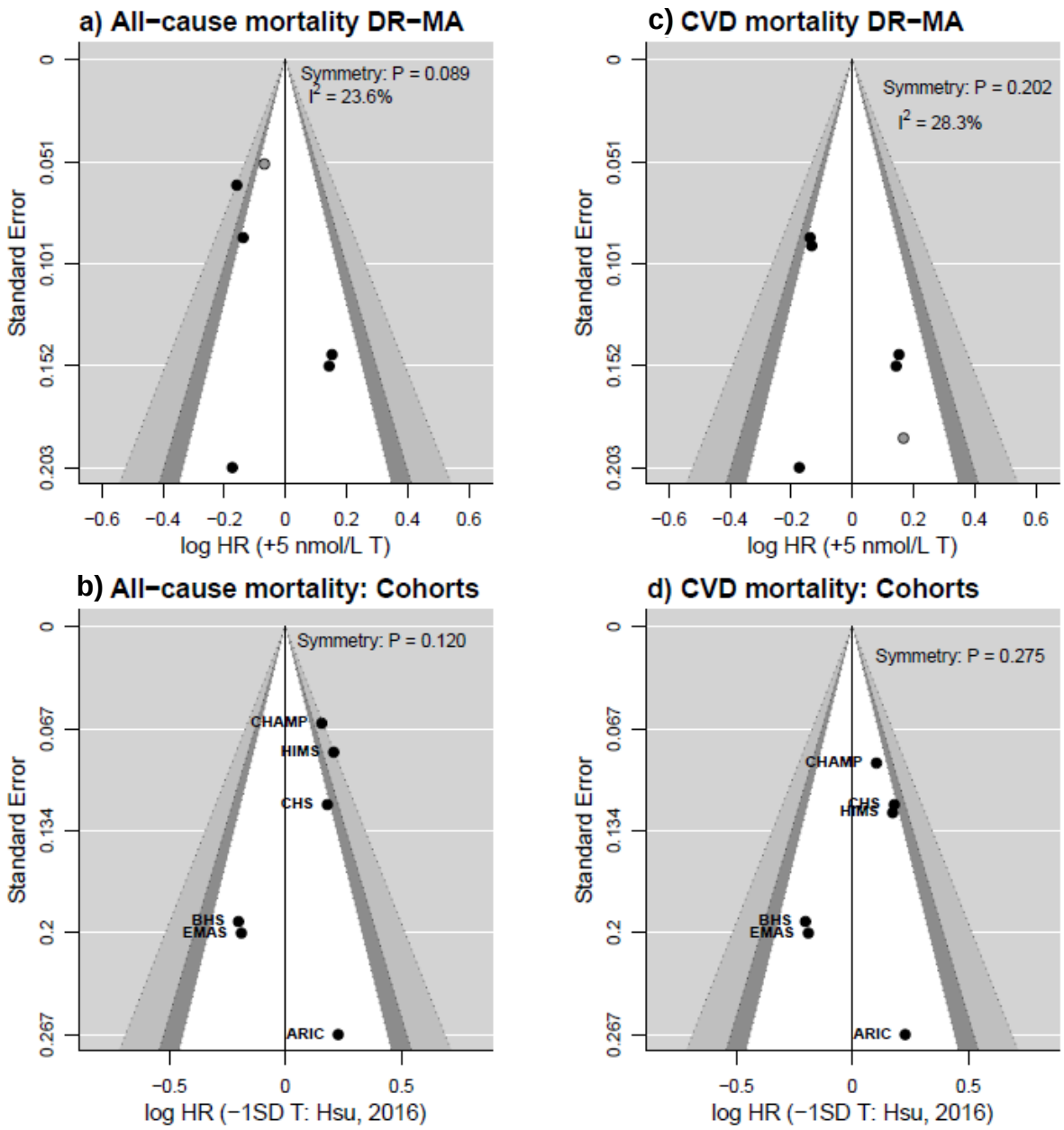

Supplementary figure 2. Funnel plots for dose-response meta-analyses. Contour-enhanced funnel plots showing the distribution of log hazard ratio (HR) estimates for all-cause mortality (a, b) and mortality caused by cardiovascular disease (CVD) (c, d) attributed to a 5 nmol/L increase (a, c), or a 1.9 nmol/L (1SD in Hsu et al. 2016[22]) decrease (b, d), in endogenous testosterone concentration. Log HR values and standard errors were calculated using generalised least squares regression of published estimates.[42, 43] In cases where more than one article was available per cohort study, the article with the largest sample size was used. Estimates represented by black dots in (a) and (c) were analysed in respective dose-response meta-analyses (DR-MA; results presented in Figs. 3, 4). The grey dot in (a) is the estimate for Yeap et al. 2014b[13] and in (c) is the estimate for Chasland et al. (2017)[19]; these estimates were substituted for others for the HIMS and BHS studies respectively for alternative summary estimates (i.e., the grey summary estimates presented in Figs. 3, 4). Estimates presented in (b) and (d) are shown for a more complete assessment of funnel plot symmetry: estimates are plotted for all studies with estimates, including those that did not have sufficient information for including in the DR-MA. In funnel plots: light grey + dark

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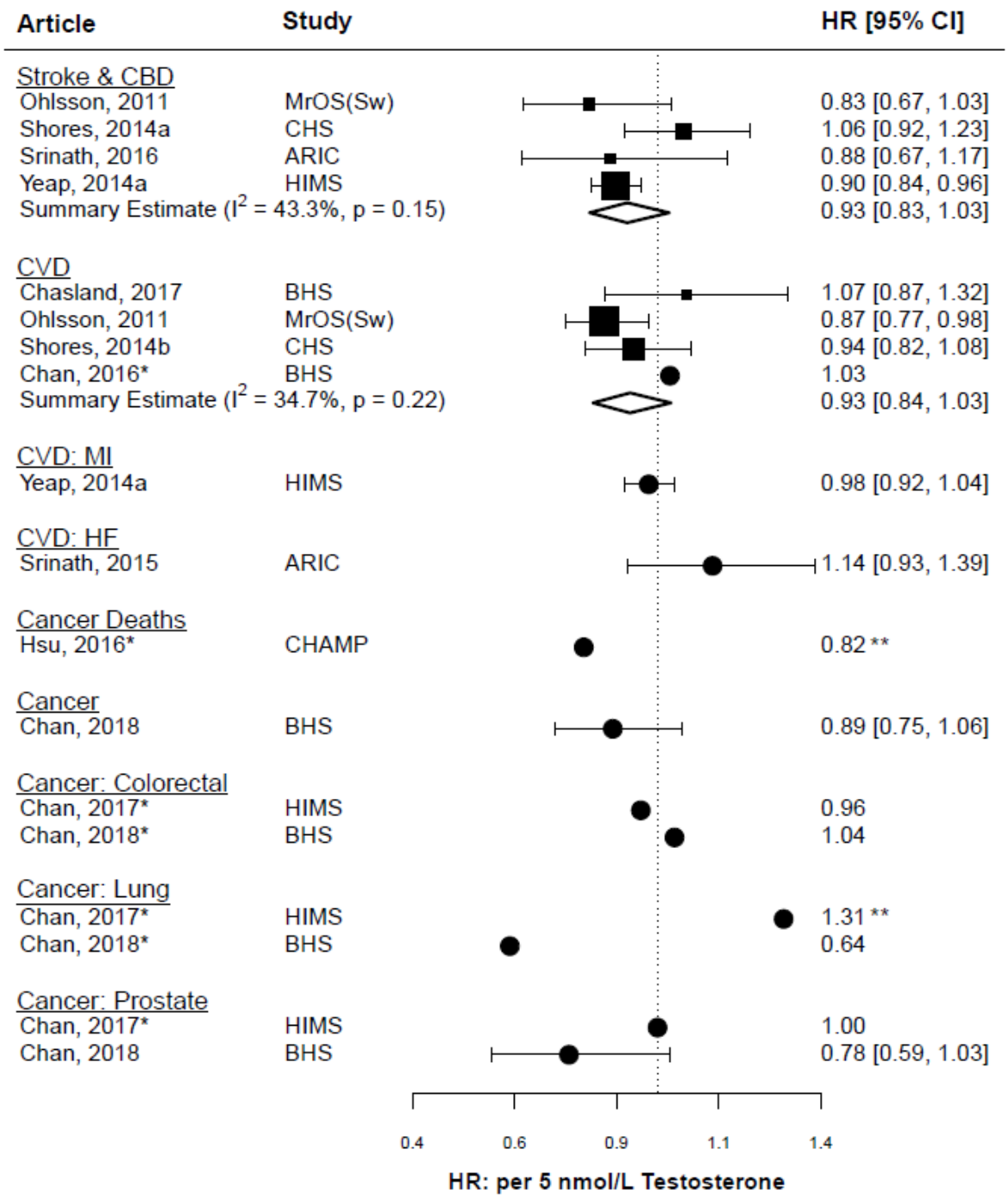
grey + white shading = 99% pseudo confidence interval (CI); dark grey + white shading = 95% CI; white shading = 90% CI.



Supplementary figure 3: Forest plot of published hazard ratio (HR) estimates: association of testosterone with other AIMS outcomes. Plotted estimates for other outcomes, as listed in the AIMS protocol article,[3] have been standardised to the HR for a 5nmol/L increase in testosterone. The size of squares are scaled to the precision of estimates, as used for obtaining the corresponding summary estimate for that outcome (diamonds). Estimates presented as circles were not used to obtain a summary estimate and so the size of circles is not scaled to estimated precision. * = 95 % confidence intervals (CIs) were not calculable for these

estimates, which were reported as the HR per standard deviation change (see Supplementary table 9).
** = for 'per SD' estimates: to show that the published HR 95% CIs did not overlap 1. 'CBD' = Cerebrovascular disease; 'CVD' = cardiovascular disease; 'MI' = myocardial infarction; 'HF' = heart failure. Study-specific estimates presented for MrOS Sweden (Ohlsson, 2011)[33]; CHS (Shores, 2014a, b)[25-26]; ARIC (Srinath, 2015; 2016)[16-17]; HIMS (Yeap, 2014a, b)[13,31]; BHS (Chan, 2016; Chasland, 2017; Chan, 2018)[18-20].

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PRISMA Checklist: Systematic review and meta-analyses on associations of endogenous testosterone concentration with health outcomes in community-dwelling men.

Section/topic	#	Checklist item	Reported on page #
TITLE			
Title	1	Identify the report as a systematic review, meta-analysis, or both.	1
ABSTRACT			
Structured summary	2	Provide a structured summary including, as applicable: background; objectives; data sources; study eligibility criteria, participants, and interventions; study appraisal and synthesis methods; results; limitations; conclusions and implications of key findings; systematic review registration number.	2-3
INTRODUCTION			
Rationale	3	Describe the rationale for the review in the context of what is already known.	4-5
Objectives	4	Provide an explicit statement of questions being addressed with reference to participants, interventions, comparisons, outcomes, and study design (PICOS).	5. For this type of review it is PEO instead.
METHODS			
Protocol and registration	5	Indicate if a review protocol exists, if and where it can be accessed (e.g., Web address), and, if available, provide registration information including registration number.	5
Eligibility criteria	6	Specify study characteristics (e.g., PICOS, length of follow-up) and report characteristics (e.g., years considered, language, publication status) used as criteria for eligibility, giving rationale.	5, Supplementary table 2
Information sources	7	Describe all information sources (e.g., databases with dates of coverage, contact with study authors to identify additional studies) in the search and date last searched.	5-6
Search	8	Present full electronic search strategy for at least one database, including any limits used, such that it could be repeated.	Supplementary table 1
Study selection	9	State the process for selecting studies (i.e., screening, eligibility, included in systematic review, and, if applicable, included in the meta-analysis).	5-6, Supplementary tables 2-3.

Section/topic	#	Checklist item	Reported on page #
Data collection process	10	Describe method of data extraction from reports (e.g., piloted forms, independently, in duplicate) and any processes for obtaining and confirming data from investigators.	6, Suppl. Data.
Data items	11	List and define all variables for which data were sought (e.g., PICOS, funding sources) and any assumptions and simplifications made.	6, Suppl. Data.
Risk of bias in individual studies	12	Describe methods used for assessing risk of bias of individual studies (including specification of whether this was done at the study or outcome level), and how this information is to be used in any data synthesis.	6, Supplementary table 8.
Summary measures	13	State the principal summary measures (e.g., risk ratio, difference in means).	7
Synthesis of results	14	Describe the methods of handling data and combining results of studies, if done, including measures of consistency (e.g., I^2) for each meta-analysis.	7
Risk of bias across studies	15	Specify any assessment of risk of bias that may affect the cumulative evidence (e.g., publication bias, selective reporting within studies).	7
Additional analyses	16	Describe methods of additional analyses (e.g., sensitivity or subgroup analyses, meta-regression), if done, indicating which were pre-specified.	7
RESULTS			
Study selection	17	Give numbers of studies screened, assessed for eligibility, and included in the review, with reasons for exclusions at each stage, ideally with a flow diagram.	8, Fig. 1
Study characteristics	18	For each study, present characteristics for which data were extracted (e.g., study size, PICOS, follow-up period) and provide the citations.	Supplementary tables 7-8, 9-10
Risk of bias within studies	19	Present data on risk of bias of each study and, if available, any outcome level assessment (see item 12).	9, Supplementary table 8
Results of individual studies	20	For all outcomes considered (benefits or harms), present, for each study: (a) simple summary data for each intervention group (b) effect estimates and confidence intervals, ideally with a forest plot.	10-12, Figs 3-4; Supplementary figure 3

Section/topic	#	Checklist item	Reported on page #
Synthesis of results	21	Present results of each meta-analysis done, including confidence intervals and measures of consistency.	10-12, Figs. 3-4, Supplementary figure 3
Risk of bias across studies	22	Present results of any assessment of risk of bias across studies (see Item 15).	10-11, Supplementary figure 2
Additional analysis	23	Give results of additional analyses, if done (e.g., sensitivity or subgroup analyses, meta-regression [see Item 16]).	10-12, Fig 2; Supplementary figures 1-3
DISCUSSION			
Summary of evidence	24	Summarize the main findings including the strength of evidence for each main outcome; consider their relevance to key groups (e.g., healthcare providers, users, and policy makers).	12-16
Limitations	25	Discuss limitations at study and outcome level (e.g., risk of bias), and at review-level (e.g., incomplete retrieval of identified research, reporting bias).	14-15
Conclusions	26	Provide a general interpretation of the results in the context of other evidence, and implications for future research.	13-16
FUNDING			
Funding	27	Describe sources of funding for the systematic review and other support (e.g., supply of data); role of funders for the systematic review.	17

From: Moher D, Liberati A, Tetzlaff J, Altman DG, The PRISMA Group (2009). Preferred Reporting Items for Systematic Reviews and Meta-Analyses: The PRISMA Statement. PLoS Med 6(7): e1000097. doi:10.1371/journal.pmed1000097