1.a. Full Title: Factors predicting optimum testosterone levels in men: The Androgens In Men Study.

b. Abbreviated Title (Length 26 characters): AIMS papers 2 to 4

2. Writing Group:
Writing group members: Bu Yeap (UWA), Kevin Murray (UWA), Ross Marriott (UWA), Christie Ballantyne (ARIC), David Couper (ARIC), Adrian Dobs (ARIC), authors from each of the other AIMS cohort studies. Order to be later determined.

I, the first author, confirm that all the coauthors have given their approval for this manuscript proposal.

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3. **Timeline:**

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**Notes:**
- Timeframe for data applications and harmonization (to be completed by 30 September 2019) provided in Manuscript Proposal for Paper 1.
- Concurrent analysis and writing tasks are considered feasible, since the IPD meta-analyses will use the same statistical models to analyse different health outcomes from the same datasets.
- Funding for the position of project manager and statistician presently exists until 20 May 2020. We are presently seeking additional funds to extend this position.

4. **Rationale:**

Low testosterone concentrations are associated with a range of poorer health outcomes in older men,\(^1\)\(^-\)\(^5\) but whether it is a biomarker for underlying ill-health or a causal factor for disease remains unclear.

Currently, testosterone treatment is recommended for men who have symptoms and signs of androgen deficiency and low testosterone concentrations, due to disease of the hypothalamus, pituitary or testes (organic or pathological hypogonadism).\(^6\)-\(^9\) Randomised controlled trials of testosterone treatment in older men with low-normal testosterone concentrations without organic hypogonadism have shown modest benefits on sexual function, anaemia and bone density, but not on cognition over 12 months.\(^10\)-\(^14\) The effect of testosterone on the cardiovascular system remains unclear.\(^15\),\(^16\) However, the selection criteria of these trials was such that the screening to enrolment ratio was 65:1, a highly selected population of older men.\(^10\),\(^11\),\(^16\) Importantly, the trials were underpowered for outcomes of cardiovascular events, incidence of dementia or fracture, and for mortality risk.\(^16\)
Therefore, an international collaboration of prospective cohort studies examining the associations of sex hormones with these outcomes will clarify the influence of sex hormone exposures on key health outcomes in men, provide information on lifestyle measures that maintain endogenous testosterone production and identify the scope and optimal recruitment criteria for future trials of testosterone therapy. These data will also allow reference ranges for testosterone in men across ages to be refined to inform recommendations for clinical practice.

References cited:

5. **Main Hypothesis/Study Questions:**

Specific hypotheses are that in men, lower plasma testosterone concentrations are independently associated with:

i) Incidence of cardiovascular events (Paper 2);

ii) Cardiovascular deaths (Paper 2);

iii) All-cause mortality (Paper 2);

iv) Cancer diagnoses (Paper 3);

v) Deaths from cancer (Paper 3);

vi) Cognitive decline (Paper 4);

vii) Dementia diagnoses (Paper 4).

6. **Design and analysis (study design, inclusion/exclusion, outcome and other variables of interest with specific reference to the time of their collection, summary of data analysis, and any anticipated methodologic limitations or challenges if present).**

6.1. **Paper 2:** Associations between testosterone concentrations and subsequent incidence of cardiovascular events, cardiovascular deaths, and all-cause mortality in men.

**Outcome Variable(s):** Incident cardiovascular events (e.g., diagnoses), incident deaths caused by cardiovascular disease, incident deaths (any cause). Cardiovascular events to be defined as any one of myocardial infarction, stroke or heart failure.

**Primary Outcomes:** Incident cardiovascular events (e.g., diagnoses), incident deaths (all causes).

**Secondary Outcomes:** Incident deaths caused by cardiovascular disease.
Main Independent Variable: Total testosterone.

- Analysis to be repeated for related hormonal parameters, if available: Sex Hormone Binding Globulin, Luteinising Hormone, estradiol, dihydrotestosterone.

Confounding Variables: Age, education level, ethnicity, marital status, site, alcohol consumption, BMI, waist, physical activity, blood pressure, hypertension, smoking status, general health, atrial fibrillation, COPD, diabetes, history of cardiovascular disease, cholesterol, LDL, HDL, creatinine level, lipid lowering medications.

Exclusion Criteria: Exclude males who at baseline were either: (i) taking androgens; (ii) taking anti-androgen medications; (iii) had prior orchidectomy.

Statistical Analysis:

Cox proportional hazards models will be used to assess the effect of androgen level on the incident risk of each outcome, with separate IPD meta-analyses conducted for each outcome (cardiovascular events including cardiovascular deaths, cardiovascular deaths, all-cause deaths) and each hormonal variable (testosterone, dihydrotestosterone, estradiol, LH, SHBG). Component study will be modelled either as a stratified variable or as a random term using frailty models. Participants with prevalent cardiovascular disease at baseline will be excluded. The length of follow-up will also be standardised among studies in order to maximise data from all datasets, whilst minimising the prospect for variable lengths-to-follow-up among studies introducing additional heterogeneity into results.

Multivariable versions of each of these models will also be fitted, with additional predictors for potential confounders included. Non-linear associations for continuous variables will be modelled using natural splines with pre-specified knots and linear boundary constraints. The (standardised) measure of effect size used will be the hazard ratio. Subgroup analyses will be conducted separately for each of three specific types of cardiovascular disease (outcomes): myocardial infarction, stroke and heart failure.

Multiply-imputed estimates for component studies and summary estimates will be suitably pooled using Rubin’s rules. Contour-enhanced funnel plots will be constructed to visually assess patterns in estimates of effect sizes and precision among studies, to investigate heterogeneity and possible meta-biases. The relative amount of heterogeneity will be estimated (e.g., using ) and forest plots presented. Subgroup or meta-regression analyses may be conducted if pronounced heterogeneity is estimated.

Planned subgroup analyses are for specific types of cardiovascular outcomes: heart failure; myocardial infarction; stroke.

**Outcome Variable(s):** Incident deaths caused by cancer, incident cancer diagnoses.

**Primary Outcome:** Incident deaths caused by cancer.

**Secondary Outcome:** Incident cancer diagnoses.

**Main Independent Variable:** Total testosterone.

- Analysis to be repeated for related hormonal parameters, if available: Sex Hormone Binding Globulin, Luteinising Hormone, estradiol, dihydrotestosterone.

**Confounding Variables:** Age, education level, ethnicity, marital status, site, alcohol consumption, BMI, waist, physical activity, blood pressure, hypertension, smoking status, general health, diabetes.

**Exclusion Criteria:** Exclude males who at baseline were either: (i) taking androgens; (ii) taking anti-androgen medications; (iii) had prior orchidectomy.

**Statistical Analysis:**

Cox proportional hazards models will be used to assess the effect of androgen level on the incident risk of each outcome, with separate IPD meta-analyses conducted for each outcome (cancer deaths, all-cause deaths) and each hormonal variable (testosterone, dihydrotestosterone, estradiol, LH, SHBG). Component study will be modelled either as a stratified variable\textsuperscript{17} or as a random term using frailty models.\textsuperscript{18-20} Participants with prevalent cancer at baseline will be excluded. The length of follow-up will also be standardised among studies in order to maximise data from all datasets, whilst minimising the prospect for variable lengths-to-follow-up among studies introducing additional heterogeneity into results.\textsuperscript{21}

Multivariable versions of each of these models will also be fitted, with additional predictors for potential confounders included. Non-linear associations for continuous variables will be modelled using natural splines with pre-specified knots and linear boundary constraints. The (standardised) measure of effect size used will be the hazard ratio. Subgroup analyses will be conducted separately for each of three common types of cancer in men (outcomes): colorectal cancer, lung cancer, prostate cancer.

Multiply-imputed estimates for component studies and summary estimates will be suitably pooled using Rubin’s rules.\textsuperscript{22} Contour-enhanced funnel plots will be constructed to visually assess patterns in estimates of effect sizes and precision among studies, to investigate heterogeneity and possible meta-biases. The relative amount of heterogeneity will be estimated (e.g., using $I^2$) and forest plots presented. Subgroup or meta-regression analyses may be conducted if pronounced heterogeneity is estimated.\textsuperscript{23}
Planned subgroup analyses are for specific types of common cancers in men: colorectal cancer; lung cancer; prostate cancer.

6.3. **Paper 4:** Associations of androgen levels with cognitive impairment and incident dementia in men.

**Outcome Variable(s):** Incident dementia diagnoses, baseline cognition score, and (if baseline and follow-up scores are available) the change in cognition score.

**Primary Outcome:** Incident dementia.

**Secondary Outcome:** Baseline cognition score, and (if baseline and follow-up scores are available) the change in cognition score.

**Main Independent Variable:** Total testosterone.

- Analysis to be repeated for related hormonal parameters, if available: Sex Hormone Binding Globulin, Luteinising Hormone, estradiol, dihydrotestosterone.

**Confounding Variables:** Age, education level, ethnicity, marital status, site, alcohol consumption, BMI, waist, physical activity, blood pressure, hypertension, smoking status, general health, diabetes, anxiety, depression, psychotropic drug use.

**Exclusion Criteria:** Exclude males who at baseline were either: (i) taking androgens; (ii) taking anti-androgen medications; (iii) had prior orchidectomy.

**Statistical Analysis:**

Linear Mixed Models (LMMs) and Generalised Linear Mixed Models (GLMMs) will be used to model the association of androgen concentrations on cognitive impairment (cross-sectional analyses of baseline data). Cox proportional hazards models will be used to assess the effect of androgen concentrations on the incident risk of dementia. Men with prevalent dementia will be excluded from this analysis. We will also ask for follow-up cognition test scores, and if available, will run an analysis of changes in cognition test scores since baseline as an outcome. Separate IPD meta-analyses will be conducted for each hormonal variable as a focal predictor (testosterone, dihydrotestosterone, estradiol, LH, SHBG). Component study will be modelled as a random intercept term in LMMs and GLMMs and as a stratified factor or frailty model random term in Cox regressions. Multivariable versions of each of these models will also be fitted, with additional predictors for potential confounders included. Suspected non-linear relationships, at the scale of the linear predictor, will be investigated and modelled using splines with pre-specified knots and boundary constraints. Measures of effect size may include: $\eta^2$, Pearson’s $r$, standardised mean difference to the reference level (categorical predictors), standardised difference for an increase in one standard deviation (continuous predictors), odds ratio for LMMs and GLMMs and hazard ratio for Cox regressions.
Multiply-imputed estimates for component studies and summary estimates will be suitably pooled using Rubin’s rules. Contour-enhanced funnel plots will be constructed to visually assess patterns in estimates of effect sizes and precision among studies, to investigate heterogeneity and possible meta-biases. The relative amount of heterogeneity will be estimated (e.g., using $I^2$) and forest plots presented. Subgroup or meta-regression analyses may be conducted if pronounced heterogeneity is estimated.

Complexities (all papers):

- IPD level data are not available from all component studies, so aggregate level data will be sought from at least one additional study, and any others identified from systematic review. Aggregate-level data will be formally incorporated into the meta-analysis and compared against the IPD-level only meta-analysis to assess/evaluate potential biases in estimates.

- Harmonization for some variables where recorded differently by the different component studies (e.g., physical activity, alcohol consumption). The project manager is requesting data for some of these variables in multiple formats, which might help facilitate harmonization in these circumstances.

7.a. Will the data be used for non-CVD analysis in this manuscript? _✓_ Yes ____ No

b. If Yes, is the author aware that the file ICTDER03 must be used to exclude persons with a value RES_OTH = “CVD Research” for non-DNA analysis, and for DNA analysis RES_DNA = “CVD Research” would be used? _✓_ Yes ____ No
(This file ICTDER has been distributed to ARIC PIs, and contains the responses to consent updates related to stored sample use for research.)

8.a. Will the DNA data be used in this manuscript? ____ Yes _✓_ No

8.b. If yes, is the author aware that either DNA data distributed by the Coordinating Center must be used, or the file ICTDER03 must be used to exclude those with value RES_DNA = “No use/storage DNA”? ____ Yes ____ No

9. The lead author of this manuscript proposal has reviewed the list of existing ARIC Study manuscript proposals and has found no overlap between this proposal and previously approved manuscript proposals either published or still in active status. ARIC Investigators have access to the publications lists under the Study Members Area of the web site at: http://www.cscc.unc.edu/aric/mantrack/maintain/search/dtSearch.html

______ Yes ____ ✓ ____ No

Three published papers (PMID: 25584720, 27729576, 31133217) looked at the association of measured testosterone level at baseline with incident risk of cardiovascular outcomes, which is similar to Paper 2. A point of difference with the planned articles for the AIMS project is that they are IPD meta-analyses that incorporate data from multiple component studies, including the ARIC study. Accordingly, the planned AIMS papers will complement the
above-listed articles, but with a much broader scope of inference (i.e., none of the other overlapping ARIC studies are IPD meta-analyses).

10. What are the most related manuscript proposals in ARIC (authors are encouraged to contact lead authors of these proposals for comments on the new proposal or collaboration)?

11.a. Is this manuscript proposal associated with any ARIC ancillary studies or use any ancillary study data? ✓ Yes    ____ No

11.b. If yes, is the proposal
   ✓   A. primarily the result of an ancillary study (list number* _________)
   ✓   B. primarily based on ARIC data with ancillary data playing a minor role (usually control variables; list number(s)* AS #2013.21)

*ancillary studies are listed by number at https://www2.cscc.unc.edu/aric/approved-ancillary-studies

12a. Manuscript preparation is expected to be completed in one to three years. If a manuscript is not submitted for ARIC review at the end of the 3-years from the date of the approval, the manuscript proposal will expire.

12b. The NIH instituted a Public Access Policy in April, 2008 which ensures that the public has access to the published results of NIH funded research. It is your responsibility to upload manuscripts to PubMed Central whenever the journal does not and be in compliance with this policy. Four files about the public access policy from http://publicaccess.nih.gov/ are posted in http://www.cscc.unc.edu/aric/index.php, under Publications, Policies & Forms. http://publicaccess.nih.gov/submit_process_journals.htm shows you which journals automatically upload articles to PubMed central.