Predictive value of serum testosterone for type 2 diabetes risk assessment in men

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Abstract

Background: Effective prevention of type 2 diabetes (T2D) requires early identification of high-risk individuals who might benefit from intervention. We sought to determine whether low serum testosterone, a novel risk factor for T2D in men, adds clinically meaningful information beyond current T2D risk models.

Methods: The Men Androgen Inflammation Lifestyle Environment and Stress (MAILES) study population consists of 2563 community-dwelling men aged 35–80 years in Adelaide, Australia. Of the MAILES participants, 2038 (80.0 %) provided information at baseline (2002–2006) and follow-up (2007–2010). After excluding participants with diabetes (n = 317), underweight (n = 5), and unknown BMI status (n = 11) at baseline; and unknown diabetes status (n = 50) at follow-up; 1655 participants were followed for 5 years. T2D at baseline and follow-up was defined by self-reported diabetes, or fasting plasma glucose (FPG) ≥7.0 mmol/L (126.1 mg/dL), or glycated haemoglobin (HbA1c) ≥6.5 %, or diabetes medications. Risk models were tested using logistic regression models. Sensitivity, specificity, positive predictive values (PPV) were used to identify the optimal cut-off point for low serum testosterone for incident T2D and the area under the receiver operating characteristic (AROC) curve was used to summarise the predictive power of the model. 15.5 % of men had at least one missing predictor variable; addressed through multiple imputation.

Results: The incidence rate of T2D was 8.9 % (147/1655) over a median follow-up of 4.95 years (interquartile range: 4.35–5.00). Serum testosterone level predicted incident T2D (relative risk 0.96 [95 % CI: 0.92,1.00], P = 0.032) independent of current risk models including the AUSDRISK, but did not improve corresponding AROC statistics. A cut-off point of <16 nmol/L for low serum testosterone, which classified about 43 % of men, returned equal sensitivity (61.3 % [95 % CI: 52.6,69.4]) and specificity (58.3 % [95 % CI: 55.6,60.9]) for predicting T2D risk, with a PPV of 12.9 % (95 % CI: 10.4,15.8).

Conclusions: Low serum testosterone predicts an increased risk of developing T2D in men over 5 years independent of current T2D risk models applicable for use in routine clinical practice. Screening for low serum testosterone in addition to risk factors from current T2D risk assessment models or tools, including the AUSDRISK, would identify a large subgroup of distinct men who might benefit from targeted preventive interventions.

Keywords: Testosterone, Diabetes, Incidence, Screening, Prognosis, ROC, Cohort

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**Key messages**

- Serum testosterone predicts risk of T2D in men independent of current risk assessment models or tools
- A cut-off point of <16 nmol/L for low serum testosterone was optimal for predicting T2D risk in men
- Over 40% of men had low serum testosterone (<16 nmol/L)
- Additional screening for low serum testosterone would identify a large group of distinct men who might benefit from targeted preventive interventions

**Background**

Type 2 diabetes (T2D) accounts for at least 90% of all cases of diabetes and is an increasingly prevalent and debilitating disease [1]. Diabetes is currently ranked the 14th leading cause of global disease burden, and has moved up several places since 1990 [2]. The International Diabetes Federation estimates that 387 million people worldwide had diabetes in 2014, and by 2035 this figure will rise to 592 million [1]. Preventing the rising prevalence of T2D in high-income countries like Australia, where healthcare expenditure for diabetes is among the highest in the world [1], could yield significant health and economic benefits [3].

It is generally accepted that people with diagnosed T2D have progressed from ‘pre-diabetes’; an intermediate stage of impaired glucose regulation defined by impaired fasting glucose (IFG) and/or impaired glucose tolerance (IGT) [4]. The prevalence of pre-diabetes could be as high as 20 to 30% in high-income countries [5, 6]. Progression from pre-diabetes to T2D is likely explained by non-modifiable risk factors including older age, male gender, ethnicity, and urbanisation [1]; as well as modifiable risk factors including smoking, obesity, unhealthy diet and physical activity behaviours [6–9]. Effective lifestyle programs targeting modifiable risk factors in people with pre-diabetes may delay or prevent the onset of T2D. Indeed, large-scale trials from Finland [10], China [11], and the United States [12] showed that lifestyle intervention can effectively halve the risk of developing T2D in people with pre-diabetes over three to six years.

Effective prevention of T2D requires early identification of high-risk individuals who might benefit from intervention. Screening for T2D risk factors can be cost-effective, especially when followed by lifestyle intervention [13]. There are at least seven diabetes risk models or scoring systems (often called ‘risk assessment tools’) with potential adaptation for use in routine clinical practice [14]. However, these tools may need to be updated for novel biomarkers that have emerged since these risk models were published.

Evidence from observational studies suggests that low endogenous testosterone level may be a reversible risk factor for T2D in men. For instance, a recent systematic review with meta-analysis showed that men with testosterone levels >15.5 nmol/L have a 42% reduced risk of developing T2D compared with men with testosterone levels ≤15.5 nmol/L [15]. Cross-sectional studies in Australia show that a high proportion of men with T2D have low testosterone level, and that low testosterone level is inversely associated with glycaemia and insulin resistance [16, 17]. On average, men with T2D and metabolic syndrome (MetS) have a testosterone level that is 2.6 nmol/L lower than controls [15, 18]. Sex hormones may explain why men are more likely to develop T2D than women, as shown in several T2D risk models [19–21]. Therefore, the aim of this study was to determine whether low serum testosterone level adds clinically meaningful information beyond current T2D risk models in men, to inform guidelines and clinical practice.

**Methods**

**Study design and setting**

The Men Androgen Inflammation Lifestyle Environment and Stress (MAILES) prospective cohort study was established in 2009 to investigate the associations of sex steroids, inflammation, environmental and psychosocial factors with cardio-metabolic disease risk in men [22]. The MAILES study population consists of 2563 community-dwelling men aged 35–80 years at enrolment in Adelaide, Australia, from the harmonisation of two cohort studies: all participants of the Florey Adelaide Male Ageing Study (FAMAS) and a sub-set of male participants of the North West Adelaide Health Study (NWAHS). Written informed consent for participation in the study was obtained. The methods used in both FAMAS [23, 24] and NWAHS [25, 26] including the validity of subject selection for achieving a representative population sample, have been described previously.

The FAMAS is a cohort study of 1195 randomly selected men aged 35 years and over from the metropolitan region of Adelaide. Participants in the study were required to be male, aged between 35 and 80 years at the time of recruitment, living in the defined catchment area of North and West Adelaide with a connected telephone and number listed in the Electronic White Pages, be willing and able to comply with the protocol and give written, informed consent. Exclusion criteria were limited to living outside the catchment area and telephone numbers that belonged to non-residential properties. Recruiters were also instructed to exclude responders if they were: (i) of insufficient mental or physical ability to
understand the requirements of participation or adequately participate; (ii) ill or otherwise incapacitated to attend clinics; (iii) currently residing in an institution (e.g. aged care facility) and (iv) non-English speaking. Prior to the study commencing, approval for the research was obtained from the Royal Adelaide Hospital Research Ethics Committee and, where appropriate, the Aboriginal Health Research Ethics Committee. The FAMAS achieved an overall response rate of 45.1 % at baseline (2002–05), and the sample was representative of the male population for most key demographics [24].

The NWAHS is a cohort study of 4060 randomly selected adults aged 18 years and over from the north-west region of Adelaide. The sample was a randomly selected population from the northern and western suburbs of Adelaide. All households in the northern and western areas of Adelaide with a telephone connected and a telephone number listed in the Electronic White Pages were eligible for selection in the study. Telephone numbers that belonged to businesses, institutions and residential care facilities were excluded from the sample. However, people who had their own telephone number and who were living in individual units attached to a nursing home were eligible to participate. Within each household, the person who had their birthday last and was aged 18 years and over, was selected for interview and invited to attend the clinic for a biomedical examination. The study excluded those people from a non-English speaking background who could not communicate sufficiently well with the telephone interviewer and who could not answer questions at the initial recruitment stage, although every effort was made to encourage family members to assist in translating. The sub-set of men aged 35 years and over was 1368. Prior to the study commencing, approval for the research was obtained from the North West Adelaide Health Services of Human Research Committee and, where appropriate, the Aboriginal Health Research Ethics Committee. The NWAHS achieved an overall response rate of 49.6 % at baseline (2000–03), and the sample was representative of the population in terms of current smoking status, body mass index (BMI), physical activity, overall health status and proportions with current high blood pressure and cholesterol readings [27].

Study population
The series of baseline data collections that form MAILES Stage 1 include FAMAS from 2002–2005, and NWAHS from 2004–2006. The series of follow-up data collections that form MAILES Stage 2 include FAMAS from 2007–2010, and NWAHS from 2008–2010. The MAILES Stage 1 (baseline) sample was representative of the population in the northern and western regions of Adelaide [22]. A flow of the study population at each stage of the MAILES Study, with the numbers of participants drawn from the respective stages of FAMAS and NWAHS has been published [22]. Of the MAILES participants, 2038 (80.0 %) provided information at both stages of the study. Reasons for loss to follow-up (non-respondents) include death (n = 99), too ill to participate (n = 39), withdrew from the study (n = 141), unable to be tracked due to changes in contact details (n = 77), and refused to take part in MAILES Stage 2 due to work-related and personal reasons (n = 169). Non-respondents in MAILES Stage 2 were more likely than respondents to report diabetes, cardiovascular disease, or depression, or to either not know or to not disclose their chronic-disease status. For self-reported assessments, non-respondents were more likely than respondents to report that they had poorer health and were current smokers or to not to provide information about these factors. For measured assessments, non-respondents were less likely than respondents to be overweight or obese, or to have central adiposity. Finally, there were no statistically significant differences between respondents and non-responders in means for serum testosterone, fasting plasma glucose (FPG), triglycerides, and high density lipoprotein cholesterol (HDL-C).

After excluding participants with diabetes at baseline (n = 317), underweight at baseline (n = 5), unknown BMI at baseline (n = 11), and unknown diabetes status at follow-up (n = 50), the final cohort sample studied consisted of 1655 participants.

Primary outcome
T2D at baseline and follow-up was defined by self-reported medically diagnosed diabetes, or FPG ≥7.0 mmol/L (126.1 mg/dL), or glycated haemoglobin (HbA1c) ≥6.5 % according to the American Diabetes Association criteria [28], or prescriptions for medications used in diabetes based on the Anatomical Therapeutic Chemical classification system codes beginning with letters ‘A10’. Medication use was determined by linkage to the Pharmaceutical Benefits Scheme which includes details and information on medicines subsidised by the Australian Government. Plasma samples were obtained from all subjects (fasting, at clinic visits) and stored at −80 °C. Fasting plasma glucose was measured using an automated chemistry analyser system (Olympus AU5400; Olympus Corp, Tokyo, Japan). Glycated haemoglobin was measured by high-performance liquid chromatography using a spherical cation exchange gel (inter-assay coefficient of variation [CV], 2 % at 6 % of total haemoglobin).

Predictor variables
We selected predictor variables from risk models that could be used in routine clinical practice including the Australian type 2 diabetes risk assessment tool

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Demographic information included age, country of birth, and annual gross household income. Ethnicity was classified into two groups using country of birth data (‘Asia, Middle East, North Africa, Southern Europe’ or ‘Other’ [including Australia]). Income was classified into eight ordinal groups. Additional data collected by self-report included family history of diabetes, currently taking high blood pressure medication, doctor diagnosed cardiovascular disease (NWAHS heart attack, stroke, angina, or transient ischaemic attack; FAMAS angina, or other heart conditions), current smoking status, and leisure-time physical activity. Leisure-time physical activity data were derived from self-reported duration and intensity of physical exercise (including walking) for recreation, sport or health/fitness during the past two weeks, at the time of the interview. Total time spent in leisure-time physical activity was multiplied by intensity weights (3.3 for walking, 4.0 for moderate and 8.0 for vigorous intensity exercise) to compute metabolic equivalent-min per week (MET-mins/wk). A MET-mins/wk score of ≥540 is equivalent to 135 min or more per week of at least moderate intensity physical activity. Body weight, height and waist circumference (mean of three measures) were taken using standard anthropometric assessments. BMI was computed as weight in kilograms divided by height in metres squared. Standard international cut-off points were used to define healthy weight (BMI 18.50–24.99 kg/m²), overweight (BMI 25.00–29.99 kg/m²), and obese (BMI ≥30.00 kg/m²) categories. Waist circumference risk categories were classified using ethnic specific cut-off points for Asian (low <90 cm, medium 90–100 cm, high >100 cm), and Other (low ≤102 cm, medium 102–110 cm, and high >110 cm) based on the AUSDRISK criteria [19]. Pre-diabetes was defined by IFG using FPG levels 5.6–6.9 mmol/L (100.9–124.3 mg/dL), or HbA1c levels 5.7–6.4 % according to American Diabetes Association criteria [28]. High blood pressure (≥140/90 mmHg) was determined by the mean of two or three readings after 10 min of seated rest. Serum lipid concentrations were determined enzymatically using a Hitachi 911 chemistry analyser (Boehringer, Germany); (inter-assay CV was 6.7 % for HDL-C, and 3.0 % for triglycerides). Cut-off points were used to classify low HDL-C (<1.0 mmol/L, or 38.6 mg/dL) and high triglycerides (>1.7 mmol/L, or 150.4 mg/dL) based on criteria proposed by the National Cholesterol Education Program, Adult Treatment Panel III [33]. Serum total testosterone was measured by an API-5000 triple-quadrupole mass spectrometer (Applied Biosystems/MDS SCIEX, Toronto, Ontario, Canada). The inter-assay coefficients of variation (CV) for serum total testosterone were 10.1 % at 0.43 nmol/L (12.4 ng/dL), 11.1 % at 1.66 nmol/L (47.8 ng/dL), and 4 % at 8.17 nmol/L (235.4 ng/dL) [34].

Statistical analyses
Predictor variables from previously validated risk models were considered. Missing data on predictor variables were replaced through multiple imputations with five versions of the data set imputed using all predictor variables. Results for each predictor were individually tabulated for men who developed T2D and men who did not and Chi-square tests were used to confirm statistical significance. Multivariable modelling was conducted using logistic regression with developed T2D versus did not as the dichotomous outcome. The goodness of fit of the models was summarised using the Hosmer-Lemeshow (HL) Chi-square statistic; with a statistically significant (p < 0.05) HL statistic indicating poor fit. Akaike and Bayesian information criteria (AIC and BIC) are presented as descriptive indicators of the relative quality of competing statistical models: with smaller AICs and BICs indicating preferred models. The Nagelkerke R² was monitored for sudden movement towards 1 which could indicate overfitting. The overall predictive power of the models was summarised as area under the receiver operating curve (AROC) and associated 95 % confidence intervals. The statistical significance of the difference between AROCs was tested using the method by DeLong et al. (1988) [35]. Competing cut-off points for defining low testosterone levels were visually compared on sensitivity, specificity, positive predictive power and age adjusted odds ratios from logistic regressions. Finally starting with all predictors in the model, a backwards stepwise selection algorithm (likelihood ratio P-value to exclude P > 0.25 and to re-enter P < 0.20) was applied to identify a minimal group of important independent predictors. AROCs were compared using MedCalc software (http://www.medcalc.org/); all other analyses were conducted using SPSS.

Results
The incidence rate of T2D was 8.9 % (147/1655) over a median follow-up of 4.95 years (IQR 4.35–5.00). Table 1 shows baseline characteristics of participants in the MAILES Stage 1 cohort by T2D status at follow-up, including crude incidence and corresponding age-adjusted odds ratios. The relative incidence of T2D was significantly highest for older age and (after age-adjustment), lowest income, family history of diabetes, pre-diabetes, IFG, currently taking blood pressure medication, high blood pressure, high triglycerides, low HDL-C, obese, and high-risk waist circumference groups.
### Table 1 Baseline characteristics of participants in the MAILES cohort by T2D status at 5 years follow-up, crude incidence and corresponding age-adjusted odds ratios

|                        | Raw data | With multiple imputation of missing data |
|------------------------|----------|-----------------------------------------|
|                        | Incident T2D (n = 147) | No T2D (n = 1508) | P | % with T2D | Age-adjusted odds ratio (95 % CI) |
| Age (years)            |           |                                        |   |         |                                    |
| 35–44                  | 20        | 377                                     | 5.0 | 1        |
| 45–54                  | 34        | 449                                     | 7.0 | 1.43 (0.80,2.52) |
| 55–64                  | 47        | 374                                     | 11.2 | 2.37 (1.38,4.08) |
| ≥65                    | 46        | 308                                     | <0.001 | 13.0 | 2.82 (1.63,4.86) |
| Missing                | 0 (0.0 %) | 0 (0.0 %)                               |        |         |                                    |
| Ethnicity/Country of birth |                  |                                        |   |         |                                    |
| Asia, Middle East, North Africa, Southern Europe | 15 | 119 | 11.1 | 0.83 (0.47,1.47) |
| Other (North West Europe, Americas, Oceania/Australia) | 132 | 1387 | 0.329 | 8.7 | 1 |
| Missing                | 0 (0.0 %) | 2 (0.1 %)                               |        |         |                                    |
| Income (AUD)           |           |                                        |   |         |                                    |
| Up to $12,000          | 15        | 72                                      | 17.1 | 2.89 (1.30,6.38) |
| $12,001 – $20,000      | 23        | 148                                     | 13.7 | 2.13 (1.03,4.39) |
| $20,001 – $30,000      | 24        | 193                                     | 11.5 | 1.82 (0.91,3.64) |
| $30,001 – $40,000      | 16        | 181                                     | 8.8  | 1.52 (0.74,3.13) |
| $40,001 – $50,000      | 13        | 188                                     | 6.5  | 1.16 (0.54,2.47) |
| $50,001 – $60,000      | 15        | 181                                     | 7.6  | 1.41 (0.68,2.92) |
| $60,001 – $80,000      | 18        | 225                                     | 8.0  | 1.54 (0.77,3.11) |
| More than $80,000      | 16        | 294                                     | 0.003 | 5.2 | 1 |
| Missing                | 7 (4.8 %) | 26 (1.7 %)                              |        |         |                                    |
| Family history of diabetes |                  |                                        |   |         |                                    |
| Yes                    | 55        | 410                                     | 11.9 | 1.80 (1.25,2.58) |
| No                     | 91        | 1096                                    | 0.007 | 7.7 | 1 |
| Missing                | 1 (0.6 %) | 3 (0.2 %)                               |        |         |                                    |
| FPG (mean, mmol/L)     |           |                                        |   |         |                                    |
| Mean (sd)              | 5.32 (0.71) | 4.80 (0.60) | <0.001  |
| Missing                | 0 (0.0 %) | 10 (0.7 %)                              |        |         |                                    |
| HbA1c (mean, %)        |           |                                        |   |         |                                    |
| Mean (sd)              | 5.87 (0.34) | 5.49 (0.36) | <0.001  |
| Missing                | 0 (0.0 %) | 8 (0.5 %)                               |        |         |                                    |
| Pre-diabetes (FPG 5.6–6.9 mmol/L or HbA1c 5.7–6.4 %) | | | | | |
| Yes                    | 126       | 562                                     | 18.2 | 9.23 (5.72,14.90) |
| No                     | 21        | 936                                     | <0.001 | 2.2 | 1 |
| Missing                | 0 (0.0 %) | 10 (0.7 %)                              |        |         |                                    |
| Impaired fasting glucose (FPG 5.6–6.9 mmol/L) | | | | | |
| Yes                    | 59        | 142                                     | 28.7 | 5.63 (3.87,8.19) |
| No                     | 88        | 1356                                    | <0.001 | 6.1 | 1 |
| Missing                | 0 (0.0 %) | 10 (0.7 %)                              |        |         |                                    |
| Currently taking blood pressure medication | | | | | |
| Yes                    | 57        | 294                                     | 16.2 | 2.11 (1.43,3.11) |
| No                     | 90        | 1209                                    | <0.001 | 6.9 | 1 |
| Missing                | 0 (0.0 %) | 5 (0.3 %)                               |        |         |                                    |
| Characteristic                                      | Yes     | No     | p-value | Odds Ratio (95% CI) |
|---------------------------------------------------|---------|--------|---------|---------------------|
| **Baseline characteristics of participants in the MAILES cohort by T2D status at 5 years follow-up, crude incidence and corresponding age-adjusted odds ratios (Continued)** |         |        |         |                     |
| **High blood pressure (≥140/90 mmHg)**             | 88      | 629    | 0.001   | 1.77 (1.24,2.53)    |
| Yes                                               | 59      | 874    |         | 6.3                 |
| No                                                | 0 (0.0 %) | 5 (0.3 %) |         |                     |
| **Triglycerides (mean, mmol/L)**                   |         |        |         |                     |
| Mean (sd)                                         | 1.99 (1.36) | 1.70 (1.32) | 0.013   |                     |
| Missing                                           | 0 (0.0 %) | 7 (0.5 %) |         |                     |
| **High triglycerides (>1.7 mmol/L)**               |         |        |         |                     |
| Yes                                               | 68      | 505    | 0.011   | 1.79 (1.27,2.54)    |
| No                                                | 79      | 996    | 0.002   | 7.3                 |
| Missing                                           | 0 (0.0 %) | 7 (0.5 %) |         |                     |
| **HDL-C (mean, mmol/L)**                          |         |        |         |                     |
| Mean (sd)                                         | 1.20 (0.31) | 1.27 (0.30) | 0.011   |                     |
| Missing                                           | 0 (0.0 %) | 7 (0.5 %) |         |                     |
| **Low HDL-C (<1.0 mmol/L)**                        |         |        |         |                     |
| Yes                                               | 30      | 193    | 0.011   | 1.84 (1.19,2.84)    |
| No                                                | 117     | 1308   | 0.008   | 8.2                 |
| Missing                                           | 0 (0.0 %) | 7 (0.5 %) |         |                     |
| **Diagnosed cardiovascular disease**               |         |        |         |                     |
| Yes                                               | 18      | 104    | 0.018   | 1.41 (0.81,2.44)    |
| No                                                | 129     | 1402   | 0.136   | 8.4                 |
| Missing                                           | 0 (0.0 %) | 2 (0.1 %) |         |                     |
| **Total testosterone (mean, nmol/L)**              |         |        |         |                     |
| Mean (sd)                                         | 15.0 (5.9) | 17.7 (5.9) | <0.001  |                     |
| Missing                                           | 10 (6.8 %) | 146 (9.7 %) |         |                     |
| **Currently smoking**                              |         |        |         |                     |
| Yes                                               | 17      | 248    | 0.78    | 0.46 (1.32)         |
| No                                                | 128     | 1257   | 0.136   | 9.4                 |
| Missing                                           | 2 (1.4 %) | 3 (0.2 %) |         |                     |
| **Physically inactivity (<540 METs)**              |         |        |         |                     |
| <540 METs                                         | 77      | 781    | 0.001   | 1.14 (0.81,1.61)    |
| ≥540 METs                                         | 62      | 665    | 0.754   | 8.5                 |
| missing                                           | 8 (5.4 %) | 62 (4.1 %) |         |                     |
| **Body mass index (kg/m2)**                        |         |        |         |                     |
| Healthy weight 18.50–24.99                        | 19      | 341    | 0.001   | 1.42 (0.93,2.15)    |
| Overweight 25.00–29.99                            | 69      | 768    | 0.001   | 1.57 (0.93,2.65)    |
| Obese ≥30.00                                      | 59      | 399    |         | 2.65 (1.54,4.56)    |
| Missing                                           | 0 (0.0 %) | 0 (0.0 %) |         |                     |
| **Waist circumference category**                   |         |        |         |                     |
| Low risk                                          | 65      | 925    | 0.001   | 1.66                 |
| Medium risk                                       | 39      | 361    | 0.001   | 1.42 (0.93,2.15)    |
| High risk                                         | 42      | 215    |         | 2.65 (1.74,4.02)    |
| Missing                                           | 1 (0.7 %) | 7 (0.5 %) |         |                     |

Notes: FPG fasting plasma glucose, IFG impaired fasting glucose, HbA1c glycated haemoglobin %, HDL-C high density lipoprotein cholesterol, METs metabolic equivalents of leisure-time physical activity in the past week; Waist circumference risk categories were classified using ethnic-specific cut-off points for Asian and Aboriginal (low ≤90 cm, medium 90–100 cm, high >100 cm), and Other (low ≤102 cm, medium 102–110 cm, and high >110 cm); * not age-adjusted; † independent samples t-test
Table 2 Performance of risk models for predicting 5 year risk of T2D in men

| Risk prediction models: variables | Incidence (n/VN) | AROC (95 % CI) | HL Chi-SQ statistic | P for HL | AIC | BIC |
|---------------------------------|-----------------|----------------|--------------------|---------|-----|-----|
| Model 1: Variables from AUSDRISK a | 147/1655 | 0.76 (0.72,0.80) | 5.29 | 0.726 | 895 | 960 |
| Model 2: Model 1 with variables from other risk models b | 147/1655 | 0.82 (0.79,0.86) | 4.84 | 0.775 | 847 | 987 |
| Model 3: Model 2 with total testosterone (continuous variable) | 147/1655 | 0.82 (0.79,0.86) | 4.45 | 0.815 | 844 | 990 |
| Model 4: Model 2 with total testosterone (<16 vs ≥16 nmol/L) | 147/1655 | 0.83 (0.79,0.86) | 3.97 | 0.860 | 846 | 992 |
| Model 5: Backwards selection model c | 147/1655 | 0.82 (0.78,0.85) | 5.43 | 0.711 | 825 | 885 |

Sensitivity analyses

| Model 6: Model 4 without imputation (15.5 % missing) | 126/1399 | 0.82 (0.78,0.86) | NA | NA | NA | NA |
| Model 7: Model 4 for NWAHS cohort | 62/820 | 0.79 (0.74,0.85) | NA | NA | NA | NA |
| Model 8: Model 4 for FAMAS cohort | 85/835 | 0.84 (0.79,0.88) | NA | NA | NA | NA |

Notes: AROC area under receiver operating characteristic curve (imputation with median AROC reported), HL Hosmer-Lemeshow, AIC Akaike information criterion; Bayesian information criterion, NA not applicable because we are not fitting a new model - just testing the existing model; a Age, ethnicity/country of birth, family history of diabetes, IFG instead of self reported high blood glucose, currently taking blood pressure medications, current smoking status, physical inactivity, waist circumference category; b Pre-diabetes, BMI category, diagnosed cardiovascular disease, high blood pressure, high triglycerides, low HDL-C, income category; c Backwards selection model started with all available variables included in Model 4, dropped if p > 0.25, re-entered if p < 0.20 (retained variables were family history of diabetes, currently taking blood pressure medications, current smoking, waist circumference category, pre-diabetes, high blood pressure, low HDL-C low serum testosterone <16 nmol/L); Net changes in AROC were: Model 5 vs Model 2, 0.0093 (95 % CI: -0.0032,0.0218, P = 0.1455)

Discussion

The results of this study confirmed that serum testosterone predicts 5 year risk of developing T2D in men (Model 3), independent of all risk factors from T2D risk assessment models or tools applicable for use in routine clinical practice, including the AUSDRISK [19–21, 29–32]. We found that an age-adjusted serum testosterone of <16 nmol/L, which was highly prevalent in the MAILES cohort (43 %), was optimal for equalising sensitivity and specificity in predicting incident T2D and has a 12.9 % PPV, which is comparable to the AUSDRISK (12.7 %) [19] and FINDRISC (13 %)

Table 3 Sensitivity, specificity and positive predictive values for serum total testosterone cut-off points for predicting 5 year risk of T2D in men

| Serum total testosterone cut-off points | Raw data Sensitivity, % (95 % CI) | Raw data Specificity, % (95 % CI) | Raw data Positive predictive value, % (95 % CI) |
|---------------------------------------|----------------------------------|----------------------------------|---------------------------------------------|
| <10 vs ≥10                            | 16.1 (10.5,23.5)                 | 93.4 (91.9,94.6)                 | 19.6 (13.0,28.4)                            |
| <11 vs ≥11                            | 24.1 (17.4,32.3)                 | 89.0 (87.2,90.6)                 | 18.0 (12.9,24.5)                            |
| <12 vs ≥12                            | 28.3 (21.3,36.9)                 | 83.8 (81.8,85.7)                 | 15.1 (11.0,20.1)                            |
| <13 vs ≥13                            | 37.2 (29.2,45.9)                 | 77.9 (75.6,80.1)                 | 14.4 (11.1,18.7)                            |
| <14 vs ≥14                            | 44.5 (36.1,53.2)                 | 71.4 (68.9,73.8)                 | 13.6 (10.6,17.1)                            |
| <15 vs ≥15                            | 54.7 (46.0,63.2)                 | 65.6 (63.0,68.1)                 | 13.8 (11.0,17.0)                            |
| <16 vs ≥16                            | 61.3 (52.6,69.4)                 | 58.3 (55.6,60.9)                 | 12.9 (10.4,15.8)                            |
| <17 vs ≥17                            | 68.6 (60.0,76.1)                 | 51.2 (48.6,53.9)                 | 12.4 (10.2,15.0)                            |
| <18 vs ≥18                            | 75.9 (67.7,82.6)                 | 43.5 (40.9,46.2)                 | 11.9 (9.9,14.3)                             |
| <19 vs ≥19                            | 80.3 (72.4,86.4)                 | 37.2 (34.7,39.9)                 | 11.4 (9.5,13.6)                             |
| <20 vs ≥20                            | 82.5 (74.9,88.2)                 | 31.3 (28.8,33.8)                 | 10.8 (9.0,12.8)                             |
increased the risk of a cardiovascular-related event in mainly older men (pooled odds ratio was 1.54 [95 % CI: 1.09, 2.18]) [57]. However, a more recent systematic review of RCTs in mostly older men found there was an increased cardiovascular risk associated with oral testosterone therapy (pooled relative risk was 2.20 [95 % CI: 1.45, 3.55]), but not with intramuscular (pooled relative risk was 0.66 [95 % CI: 0.28, 1.56) or transdermal (gel or patch) testosterone therapy (pooled relative risk was 1.27 [95 % CI: 0.62, 2.62]) [58]. Further research is needed to establish the safety of specific types of testosterone therapies in specific populations.

Currently, we are undertaking a Phase IIIb multicentre randomized controlled trial (double-blinded and placebo-controlled) to determine whether testosterone therapy (1000 mg testosterone undecanote) combined with lifestyle intervention will reduce the rate of T2D in men with both low testosterone and pre-diabetes or newly diagnosed T2D more than lifestyle intervention alone over two years (http://www.t4dm.org.au/). Testosterone undecanote is registered for use in Australia for the treatment of male hypogonadism (Australian Registration Number AUST R 106946). If testosterone undecanote is shown to be safe and effective pharmacotherapy for preventing T2D in men, then screening for low serum testosterone additional to current T2D risk assessment models (like the AUSDRISK in Australia) would identify a large subgroup of distinct men who might benefit from both targeted pharmacotherapy and lifestyle preventive interventions.

However, screening for low serum testosterone in community-based patients should be applied only to men suggestive of clinical presentations, otherwise additional blood testing would potentially cause a blowout in healthcare costs since serum testosterone level of <16 nmol/L is highly prevalent in men aged 35 years and over [59]. Furthermore, treatment decisions following confirmed screening positives will need to consider not only the optimal cut-off point for low testosterone, but also on the cost-effectiveness of adjunctive testosterone therapy, which is currently being investigated (http://www.t4dm.org.au/), as well as treatment availability.

Important quality items of this study include the large regionally representative sample of Australian men, precision of clinical measures, and the sufficient description of dropouts and non-respondents [22]. Study limitations include the reliance on a small number of self-report measures, respondent compliance, residual confounding, and misclassification of diseases and other factors potentially resulting in bias. While there were only 147 incident cases, the ratio of 100 observations per predictor variable, the relative stability of the AIC and BIC and the
fact that the Nagelkerke $R^2$ is much lower than 1 provides no evidence of over-fitting. Further evidence from prospective cohort studies is needed to confirm the generalizability of these findings and the applicability of screening for low serum testosterone in other male populations and specific healthcare settings.

**Conclusion**

In conclusion, low serum testosterone predicts an increased risk of developing T2D in men over 5 years independent of current T2D risk models applicable for use in routine clinical practice. Screening for low serum testosterone in addition to risk factors from current T2D risk assessment models or tools, including the AUSDRISK, would identify a large subgroup of distinct men who might benefit from targeted preventive interventions.

**Abbreviations**

AIC, alkaline information criteria; ARIC, atherosclerosis risk in communities; AROC, area under the receiver operating characteristic; AUSDRISK, Australian type 2 diabetes risk assessment tool; BIC, bayesian information criteria; BMI, body mass index; CV, coefficient of variation; FAMAS, Florey Adelaide male ageing study cohort; FINDRISC, finnish risk model; FPG, fasting plasma glucose; HbA1c, glycated haemoglobin; HDL, high density lipoprotein; HL, homser-lemeshow; IFG, impaired fasting glucose; IGT, impaired glucose tolerance; MAILES, Men androgen inflammation lifestyle environment and stress cohort; MET, metabolic equivalent; NWAHS, North West Adelaide health study cohort; PPV, positive predictive values; RCT, randomised controlled trial; T2D, type 2 diabetes.

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**Availability of data and materials**

Not applicable.

**Authors’ contributions**

EA is guarantor of the paper, taking responsibility for the integrity of the work as a whole, from inception to published article. EA conceived of the study, interpreted data, drafted the manuscript, and contributed to the acquisition of funding. PF analysed and interpreted data, and revised the manuscript. SM interpreted data, and revised the manuscript. ZS interpreted data, and revised the manuscript. RJA interpreted data, revised the manuscript, and contributed to the acquisition of funding. PO interpreted data, revised the manuscript, and contributed to the acquisition of funding. ZS interpreted data, and revised the manuscript. GW interpreted data, revised the manuscript, and contributed to the acquisition of funding. All authors read and approved the final manuscript.

**Competing interests**

GW is currently conducting research with sponsorship from Bayer Schering, Eli Lilly, and Lawley Pharmaceuticals. GW does not hold stock in these companies. GW has given talks for Bayer, Novo Nordisk, Pfizer, Astra Zeneca, Lilly, Roche, Novartis and Inova.

**Consent for publication**

Not applicable.

**Ethics approval and consent to participate**

Prior to the study commencing, approval for the research was obtained from the Royal Adelaide Hospital Research Ethics Committee, the North West Adelaide Health Service Ethics of Human Research Committee and, where appropriate, the Aboriginal Health Research Ethics Committee. Written informed consent for participation in the study was obtained from all participants prior to the baseline data collection.

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