ANDROLOGY

ISSN: 2047-2919

ANDROLOGY

ORIGINAL ARTICLE

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Keywords:
diabetes, multi-state modeling, normoglycemia, pre-diabetes, testosterone

Received: 10-Sep-2018
Revised: 21-Oct-2018
Accepted: 2-Nov-2018

doi: 10.1111/andr.12568

The association between serum total testosterone and progression of hyperglycemia: a 15-year prospective cohort study

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ABSTRACT

Background: The association between low testosterone concentration and increased risk of hyperglycemia in men has been demonstrated in observational and interventional studies. However, considering a variety of confounding factors, limited population-based studies have so far been conducted. Also, no information is available regarding the effect of testosterone on progressive development of dysglycemia.

Objective: To examine the effect of total testosterone on development of pre-diabetes/diabetes in normoglycemic middle-aged and older men.

Materials and Methods: Data were obtained from the Tehran Lipid and Glucose Study, a community-based prospective cohort of an Iranian population. Analyses were conducted on 903 normoglycemic eligible men aged 30–70 years. An illness-death model was applied to estimate the probabilities of three transitional phases of normoglycemia→diabetes, normoglycemia→pre-diabetes, and pre-diabetes→diabetes.

Results: Over a median follow-up of 12 years, 0.9% individuals developed diabetes. Per unit increase (ng/mL) in testosterone concentration, the transition rate from normoglycemia to pre-diabetes decreased by 6% [hazard ratios (HRs): 0.94 (95% confidence interval (CI): 0.90, 0.99)]. However, no effect for testosterone on the progression of diabetes from normoglycemia or pre-diabetes was observed [HRs: 0.79 (95% CI: 0.44, 1.41) and 0.98 (95% CI: 0.84, 1.16), respectively]. High body mass index was a strong predictor of hyperglycemia within all transitions.

Discussion: Independent of major confounding factors, low testosterone was associated with normoglycemia progression to pre-diabetes, but not with pre-diabetes to diabetes, which might indirectly highlight the stronger impact of other risk factors after occurrence of pre-diabetes.

Conclusion: Low testosterone concentrations in men are associated with progression from normoglycemia to pre-diabetes, but not from pre-diabetes to diabetes.

INTRODUCTION

Type 2 diabetes mellitus (hereafter referred to as diabetes) is a growing global disease affecting over 382 million people worldwide (Guariguata *et al.*, 2014). Diabetes is typically known as the progression of pre-diabetes, a high-risk precursor for incident diabetes and an intermediate hyperglycemic stage that is defined as impaired fasting glucose (IFG) and/or impaired glucose tolerance (IGT) (Tabák *et al.*, 2012).

Although evidence available in the context of hyperglycemia and hormones in men is controversial, it has been reported that men develop insulin resistance and IFG more often than women, which can partially be attributed to the interrelations of male sex hormones (Cowie *et al.*, 2009); almost all previous studies suggest that decline in testosterone, rather than high testosterone, promotes insulin resistance (Atlantis *et al.*, 2016). Observational studies report that up to 45% of men with type 2 diabetes may

suffer from low total testosterone concentrations (Grossmann *et al.*, 2008). An inverse association has also been suggested in this respect, indicating that diabetes may contribute to low testosterone in aged men (Travison *et al.*, 2007), a finding which complicates the relation as to whether low testosterone is a contributor to diabetes or a result of it or merely a co-existing factor. This bidirectional relationship is also supported by interventional studies demonstrating the efficacy of androgen therapy on reducing insulin resistance (Grossmann *et al.*, 2010) as well as those studies showing improvements in testosterone concentration after treatment with anti-diabetic agents (Wong *et al.*, 2015).

Nevertheless, the relation between androgens and insulin resistance appears to be beyond a bidirectional association as interactions of body mass index (BMI), lipid profile and family history of diabetes, as potent risk factors, confound the situation (Goto *et al.*, 2012). Also, the association becomes more complex considering the impact of confounding factors like sex hormone binding globulin (SHBG). Although an inverse association has been reported between circulating SHBG and insulin resistance (Wallace *et al.*, 2013), and low SHBG has been identified as a predictor of diabetes (Wang *et al.*, 2015), not all the studies are in favor of this association (Mather *et al.*, 2015).

Despite much attention being given to the precursory effects of testosterone on diabetes development, little is known about its association with pre-diabetic states alone. Diabetes develops through a multi-stage process (Weir & Bonner-Weir, 2004) and over time, an individual may pass through several dysglycemic states before manifesting frank diabetes (Tabak *et al.*, 2009). This raises two questions (i) whether sex hormones, viz. testosterone, equally affect the development of these states? and (ii) whether the effect is independent of or shared with other covariates?

As sex hormones may undergo age-related variations (Orwoll *et al.*, 2010), these questions can ideally be addressed through longitudinal data and by means of an approach which simultaneously analyzes all recorded events together (Touraine *et al.*, 2016). In this regard, we attempted to examine the impact of testosterone on a straightforward progression to pre-/diabetes from normoglycemia and also its indirect effect on diabetes conversion from pre-diabetes, by applying a multi-state modeling approach, adjusted for major risk factors in a prospective cohort of middle-aged and older Iranian men.

MATERIALS AND METHODS

Subjects

Subjects of this study were selected from among participants of the Tehran Lipid and Glucose Study (TLGS), a prospective community-based study investigating the prevalence and incidence of non-communicable diseases and their risk factors among a representative sample of Tehranian residents. Details of TLGS have been published elsewhere (Azizi *et al.*, 2009). Briefly, TLGS, performed on 15,005 people, aged >3 years, includes two major phases: a cross-sectional phase (1999–2001) and a long-term ongoing phase including follow-up visits at 3-year intervals.

A total of 1234 men, aged 30–70 years met the eligibility criteria. Based on illness-death model, we considered all healthy people at the beginning (normoglycemic men) and participants were observed over time to see if individuals experience pre-diabetes and/or diabetes. Participants excluded were those with pre-diabetes (n = 206) or diabetes (n = 99) at baseline. Of the remaining, 26 men were present only at baseline visit (without follow-ups) and were therefore considered as lost to follow-up. Overall, at enrollment, 903 subjects were classified as normoglycemic; these subjects entered into state 1 and were at risk of pre-diabetes (state 2) or diabetes directly (state 3), of whom 415 (45.9%) and 8 (0.9%) developed pre-diabetes and diabetes, respectively. Of a total 415 subjects who reached state 2, 49 (11.8%) progressed to diabetes (state 4), whereas 366 (88.2%) remained pre-diabetic until the end of the study.

Data collection

A standard questionnaire including information on demographics, smoking behavior, physical activity habits, medical history, family history of diabetes, and consumption of anti-diabetic, anti-hypertensive or lipid-lowering drugs was completed via face-to-face interviews. Mean systolic and diastolic blood pressure (SBP and DBP) was used after being measured twice in a sitting position on the right arm, using a standard mercury sphygmomanometer. Weight was measured with individuals minimally clothed, using digital scales (Seca 707: range 0.1–150 kg) and recorded to the nearest 0.1 kg. Height was measured in a standing position, using a tape meter, while shoulders were in normal alignment.

Biochemical assessment

Details of the questionnaires used, anthropometric evaluations and general laboratory measurements have been reported previously (Derakhshan *et al.*, 2014). Testosterone and SHBG concentrations were determined by enzyme immunoassay (DRG Diagnostic, GmbH, Mannheim, Germany) using the Sunrise ELISA reader (Tecan Co., Salzburg, Austria); the intra- and inter-assay CVs were 5.7, 8.4 and 9.6, 8.6% with the detection limit of 0.022 ng/mL and 0.1 ng/mL, respectively. Free testosterone index (FTI) was calculated as a ratio of testosterone (nmol/L)/SHBG (nmol/L) $\times$ 100.

Outcome assessment

Participants were classified as having diabetes at baseline or during follow-up if they met at least one of the following criteria: fasting plasma glucose (FPG) ≥7 mmol/L, 2–h post-challenge plasma glucose (PCPG) ≥11.1 mmol/L or taking anti-diabetic medications. People were classified into isolated impaired fasting glucose (i–IFG) (5.55 mmol/L ≤ FPG < 7 mmol/L and 2–h PCPG < 7.77 mmol/L), isolated impaired glucose tolerance (i–IGT) (7.77 mmol/L ≤ 2–h PCPG < 11.1 mmol/L and FGP < 5.55 mmol/L), and the combined IFG/IGT (5.55 mmol/L ≤ FPG < 7 mmol/L and 7.77 mmol/L ≤ 2–h PCPG < 11.1 mmol/L) groups according to the definition of American Diabetes Association (2013). Family history of diabetes was determined based on self-report.

Participants were grouped as ever or passive smokers; ever smoker was defined as a person who has ever been a cigarette smoker, and passive smokers were defined as those who were in the exposure of second-hand smoke inhalation.

The questionnaire was utilized to categorize participants into two groups of low and moderate physical activity. Low activity was defined as participating in vigorous activity <1 day/week; moderate physical activity was defined as participating in a

vigorous activity 1–2 days/week. Those with low physical activity were considered as having a metabolic equivalent task (MET) <300 min/week and those with moderate physical activity as having a 300 ≤ MET <600 min/week (Jeon *et al.*, 2007) (1 MET is equal to 3.5 mL of consumed oxygen per minute for 1 kg of body weight).

Statistics

Results are reported as mean (standard deviation) for numerical variables and number (percentage) for categorical measures. For numerical variables with skewed distribution, median (inter-quartile range) was calculated. In the present study, an 'illness-death' model was applied, which aims at estimating the probabilities of different transitional phases: transition 1: normoglycemia→diabetes, transition 2: normoglycemia→pre-diabetes, and transition 3: pre-diabetes→diabetes. At each transition, different risk factors were evaluated and a unidirectional illness-death model was generated (Fig. 1). Illness-death model is a specific kind of multi-state model, in which individuals are normoglycemic at the initiation and may develop the disease or even die as time progresses; (Hinchliffe *et al.*, 2013) in this model, transition is from state i to j (Tij).

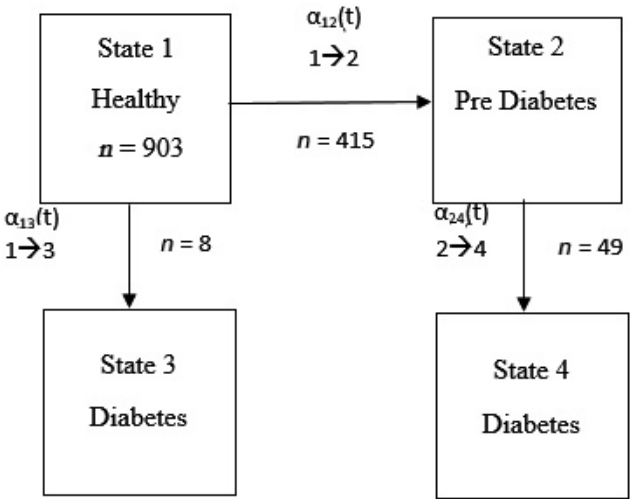
Our intermediate and absorbing states were pre-diabetes and diabetes, respectively. Over time, the normoglycemic participants (state 1) could transit to state 3 (T13: transition 1) (developed diabetes) or to state 2 (T12: transition 2) (developed pre-diabetes). Following diagnosis of pre-diabetes, patients were at risk of incident diabetes, with time T23 (transition 3).

The middle time between the visit at which pre-diabetes or diabetes was detected for the first time and the recent visit preceding the diagnosis defined the event date for patients with pre-diabetes and diabetes. The interval between the first and the last follow-ups described the survival time for our lost to follow-up or censored cases.

To calculate the transition hazards (α_{12}, α_{13}, and α_{23}), a flexible parametric survival model was recruited (Fig. 1). Using restricted cubic spline functions, transition models were fitted to model the hazard for each transition. The post-estimation command stpm2cif was used to estimate cumulative incidence function. Risk factors were analyzed in cubic spline regression, considering constant or variable effects on each transition; however, the model with varying effects was a better fit (results not shown). Risk factors of pre-diabetes and diabetes including age, BMI, waist circumference, waist-to-hip ratio, total testosterone, lipid profiles (triglyceride, HDL, and LDL), smoking status, physical activity, family history of diabetes and history of hypertension were included in the parametric survival models. Data for physical activity were missing in 24.7% of participants, and it was hence imputed using multivariate chain equations (assumed as missing at random). Creating multiple imputations compared to single imputation accounts for the statistical uncertainty in the imputations (Azur *et al.*, 2011). Complete case analysis may be an acceptable approach to addressing missing data in cases when missingness is totally random and <5% and does not depend on observed or unobserved values (Graham, 2009); these cases, however, are not common. Despite convenience of complete case analysis, it can contribute to biased estimates and a reduction in power, as it relies upon stronger missing data assumptions than multiple imputations, and hence not significant to be presented (Graham, 2009).

To predict missing data and their averages, we created linear regression model with 100 imputations. Risk factor was added to the cubic spline regression to conduct univariate analysis. To assess the significance of the main effects or the trend, we recruited a likelihood ratio test. Hazard ratios (HRs) along with 95% confidence interval (CI) were estimated by exponentiating coefficients. All analyses were performed using STATA version 14 (Stata Corp, College Station, Texas, USA). *p*-Values <0.05 were considered statistically significant.

Figure 1 Illness-death model for participants of the study



ETHICS

All procedures performed in this study are in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. Informed consent was obtained from all individual participants included in the study. The ethics committee of the Research Institute for Endocrine Sciences (RIES), Shahid Beheshti University of Medical Sciences approved the design of TLGS.

RESULTS

At baseline, mean age and BMI were 44.1 ± 11.1 years and 25.3 ± 3.7 kg/m^2; 24.5, 6.1, and 37.9% of the subjects had family history of diabetes, history of hypertension, and low physical activity, respectively. Median (IQR) of total testosterone, SHBG, and FTI were 3.5 (2.9–5) ng/mL, 31.7 (21.3–44.2) nmol/L, and 36.7 (24.4–56.4), respectively (Table 1).

The 5-, 7- and 10-year probabilities of transition 1 were 0.28, 0.45, and 0.61%; 5-, 7- and 10-year probabilities of transition 2 were 18.8, 27.8, and 40.4% and the corresponding probabilities of transition 3 were 0.13, 1.3, and 5.02%, respectively (Fig. 2).

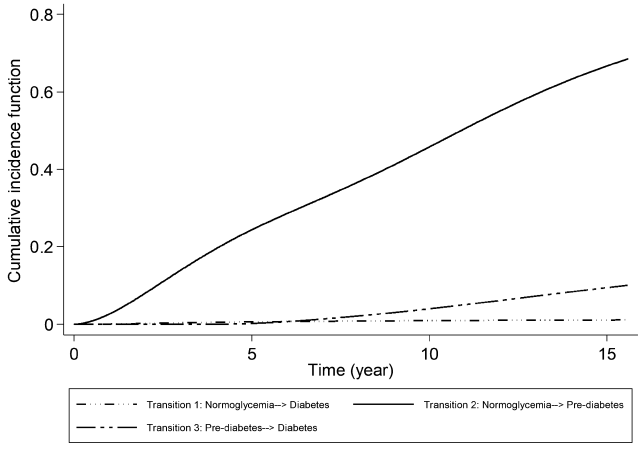
Effects of various covariates on each transition are shown in Table 2. Per unit increase (ng/mL) in testosterone concentration, the transition rate from normoglycemia to pre-diabetes decreased by 6% [HR: 0.94; 95% CI: 0.90, 0.99]. However, testosterone had no effect on diabetes either before or after progressing to pre-diabetes [HR: 0.79; 95% CI: 0.44, 1.41 and HR: 0.98; 95% CI: 0.84, 1.16, respectively]. Per year increase in age, risk of pre-diabetes increased by 2% [HR: 1.02; 95% CI: 1.01, 1.03]. BMI was significantly associated with all three transitions; each unit

Table 1 Baseline characteristics of participants

Characteristics	*N* = 903
Age (year)[a]	44.1 (11.1)
Survival time for transition 1 (normoglycemia→diabetes)[b]	5.25 (4.09–9.93)
Survival time for transition 2 (normoglycemia→pre-diabetes)[b]	7.38 (3.3–9.83)
Survival time for transition 3 (pre-diabetes→diabetes)[b]	10.96 (8.28–11.9)
Follow-up time (year)[b]	12.7 (11.8–13.9)
Body mass index (kg/m^2)[a]	25.3 (3.7)
Systolic blood pressure (mm/Hg)[a]	116 (16.12)
Diastolic blood pressure (mm/Hg)[a]	76.3 (10.2)
Waist circumference (cm)[a]	87.1 (10.1)
Waist-to-hip ratio[a]	0.9 (0.1)
Smoking, *n* (%)	
Passive smokers	127 (14.1)
Ever smokers	268 (29.7)
Family history of diabetes, *n* (%)	221 (24.5)
History of hypertension, *n* (%)	55 (6.1)
Physical activity (MET), *n* (%)	
Low	342 (37.9)
Moderate	356 (39.4)
Total testosterone (ng/mL)[b]	3.5 (2.9–5)
Sex hormone binding globulin (nmol/L)[b]	31.7 (21.3–44.2)
Free testosterone index[b]	36.7 (24.4–56.4)
Total cholesterol[a]	204.85 (38.3)
High density lipoprotein (mg/dL)[a]	39.2 (9.7)
Low density lipoprotein (mg/dL)[a]	132.6 (33.4)
Triglyceride (mg/dL)[b]	146 (106–204)

MET, metabolic equivalent. [a]Mean (standard deviation); [b]Median (inter-quartile range).

Figure 2 Cumulative incidence functions for different transitions.



increase in BMI raised the risk of progressing to diabetes, pre-diabetes, and diabetes after pre-diabetes by 32% [HR: 1.32; 95% CI: 1.1, 1.6], 6% [HR: 1.06; 95% CI: 1.04, 1.09], and 14% [HR: 1.14; 95% CI: 1.06, 1.23], respectively.

Our models suggest that subjects who have a family history of diabetes are at increased risk of diabetes and pre-diabetes, when compared to participants without this history [HR: 3.2; 95% CI: 1.9, 5.7, and HR: 1.6; 95% CI: 1.3, 1.9, respectively]. High triglyceride levels increased risk of developing pre-diabetes and subsequent diabetes by 1% [HR: 1.01; 95% CI: 1.001, 1.02] and 4% [HR: 1.04; 95% CI: 1.01, 1.06], respectively.

As the only significant effect of testosterone was observed in the second transition (normoglycemia→pre-diabetes), we estimated the cumulative hazard function for this transition based on different age ranges of 30–40, 40–50, 50–60, and 60–70. Cumulative hazard function measures the total amount of risk that has been accumulated up to time *t*. This value for participants aged 30–40 was 16.3, 23.1 and 45% up to 5-, 7-, and 10-year follow-up, respectively. For those in their 60s, these values increased over time and reached 26, 36.1, and 73.4%, respectively.

Based on the first and the fourth quartiles of testosterone, these values for participants in the first quartile aged 60–70 was 27.21, 33.64, and 61.71% up to 5-, 7-, and 10-year follow-up, respectively. For those aged 60–70 who were in the last quartile of the testosterone, the corresponding values were 29.52, 42.88, and 76.55, which were not significantly higher compared to the first quartile (*p*-value = 0.83, 0.45, and 0.19, respectively) (Fig. 3).

DISCUSSION

This study provides the first population-based data regarding the effect of testosterone on the progressive development of dysglycemia by means of a multi-state model. Despite data investigating testosterone's role in relation to incident pre-diabetes and/or diabetes (Atlantis *et al.*, 2016), we found no study examining its progressive effect over time, which however, in part, is owing to the difficulty of distinction between pre-diabetes and diabetes at early stages through cross-sectional or even longitudinal studies with long intervals.

Over an approximate median follow-up of 12 years, we found that each unit increase in testosterone level (ng/mL) prevents progression to pre-diabetes from normoglycemia by 0.6%, independent of major confounding factors. This is, comparable to the overall concept of an inverse association between testosterone and hyperglycemia (Mather *et al.*, 2015); however, in particular, we observed that except for transition 2 (normoglycemia to pre-diabetes), the preventive effect of testosterone was insignificant for other transitions.

In contrast with most, but not all studies (Vandenput *et al.*, 2007; Mather *et al.*, 2015; Joyce *et al.*, 2016), we were unable to show an association between testosterone concentrations and risk of diabetes; however, it should be noted that it was difficult to distinguish between pre-diabetes and diabetes in most of those previous studies; possibly, many of them missed evaluating pre-diabetes separately, and instead, considered hyperglycemia as the sum of pre-diabetes and diabetes together.

Despite the positive impact of testosterone on pre-diabetes, we found that this effect disappeared upon the occurrence of pre-diabetes; this insignificant effect may indirectly signify the additive role of other co-existing factors minimizing the effect of testosterone. Patients with pre-diabetes are typically older, have higher BMI, central adiposity, waist-to-hip ratio, and tend to be more dyslipidemic and hypertensive (Ferrannini, 2014) which altogether place them at higher risk of diabetes and may mask the testosterone effect in a statistical model.

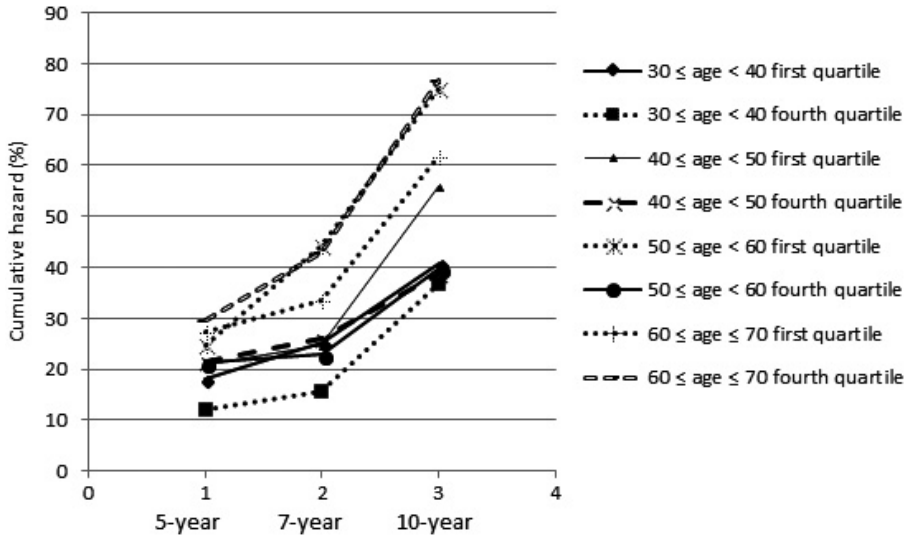
In addition, the underlying factors leading to pre-diabetes or diabetes are somewhat different. Development of pre-diabetes seems to be a steady process, associated mostly with environmental factors (Anjana *et al.*, 2015); this is in agreement with our findings which confirmed that higher BMI and triglyceride levels

Table 2 Independent effect of hyperglycemia risk factors in the three transitions of illness-death model

Transitions	Factors	Hazard ratio	95% CI	*p*-Value
Normoglycemia→diabetes	Total testosterone	0.79	(0.44, 1.41)	0.43
	SHBG	1.01	(0.98, 1.04)	0.33
	FTI	1.004	(0.98, 1.03)	0.65
	Age	0.99	(0.92, 1.08)	0.96
	BMI	1.32	(1.1, 1.6)	0.002
	Family history of diabetes	3.2	(1.9, 5.7)	0.001
	History of hypertension	1.31	(0.93, 1.85)	0.12
	Triglycerides	0.99	(0.99, 1.01)	0.94
Normoglycemia→pre-diabetes	Total testosterone	0.94	(0.90, 0.99)	0.04
	SHBG	1	(0.99, 1.004)	0.94
	FTI	1.001	(0.99, 1.004)	0.30
	Age	1.02	(1.01, 1.03)	<0.001
	BMI	1.06	(1.04, 1.09)	<0.001
	Family history of diabetes	1.6	(1.3, 1.9)	<0.001
	History of hypertension	1.3	(0.89, 1.9)	0.17
	Triglycerides	1.01	(1.001, 1.02)	0.03
Pre-diabetes→diabetes	Total testosterone	0.98	(0.84, 1.16)	0.86
	SHBG	0.99	(0.97, 1.01)	0.48
	FTI	1.006	(1.001, 1.01)	0.01
	Age	1.01	(0.98, 1.04)	0.35
	BMI	1.14	(1.06, 1.23)	<0.001
	Family history of diabetes	1.5	(0.84, 2.63)	0.17
	History of hypertension	1.6	(0.62, 3.9)	0.33
	Triglycerides	1.04	(1.01, 1.06)	0.001

BMI, body mass index; FTI, free testosterone index; SHBG, sex hormone binding globulin.

Figure 3 Cumulative hazard percentage of progressing pre-diabetes, based on age and testosterone quartile.



are potent risk factors in pre-diabetes progression to diabetes. Progression to diabetes is a result of the combination of both genetic and environmental components (Murea *et al.*, 2012). Genome-wide association studies have so far identified multiple susceptibility loci which affect beta-cell function and interact with lifestyle factors leading to diabetes (McCarthy, 2010). As such, in our cohort, the probability of genetic predisposition for direct progression from normoglycemia to diabetes should not be ignored, which however is beyond the scope of the present study.

Diabetes is a progressive disease, the development of which takes place more than a decade (Harris & Eastman, 2000); therefore, we believe that had our subjects been followed up for a longer time, the significant effects of testosterone on preventing diabetes in normoglycemic men could also have been strong. Majority of diabetes cases occur at older ages reaching a plateau phase at the age of 65 years (Kirkman *et al.*, 2012). Therefore, the mean baseline age of 44 years in our cohort needs longer follow-ups to exhibit diabetes manifestations. In addition, the neutral effect of testosterone on progression to diabetes in our normoglycemic cohort could, in part, be explained by the number of cases who developed diabetes. Of a total of 903 normoglycemic men, eight developed diabetes straightforwardly and out of 415 men with pre-diabetes, 49 cases converted to diabetes, which seem fairly small to cause significant results in transitions 1 and 3, respectively.

Despite the progressive nature of diabetes, we did find a significantly different survival time between normoglycemic

and pre-diabetic subjects. Median survival time to diabetes was 5.25 years for progressors from normoglycemia, whereas, it was 18.3 years for individuals who first developed pre-diabetes and then shifted into diabetes. In other words, the time it took to develop diabetes was about twofold higher in pre-diabetic patients than that of their normoglycemic counterparts (mean age of onset of 62.76 and 49.35 years, respectively); this finding is also in line with the previous notion that the mode and the progression rate of diabetes differ in subjects who develop it at a younger age compared to older subjects (Ferrannini *et al.*, 2004), as younger ones are more likely to be insulin deficient, while older patients tend to be more insulin resistant (Chang & Halter, 2003). However, there is a possibility that those who rapidly progress to diabetes may have gone through a brief IFG or IGT state which was not detectable within our 3-year interval periods.

Despite the evident causal association between testosterone and hyperglycemia, the relationship is far more like a vicious circle in which the precedence of each is rather controversial (Haring *et al.*, 2009), because on the one hand, observational studies have shown testosterone deficiency in men with diabetes (Grossmann *et al.*, 2008), and on the other hand, reports from longitudinal studies have pointed out that testosterone-deficient men are more likely to develop diabetes (Oh *et al.*, 2002) and that testosterone supplementation improves glucose homeostasis and alleviates insulin resistance (Simon *et al.*, 2001). Also, reports from the European Male Aging Study supports the association between severe testosterone deficiency and visceral fat excess (β: 1.93 cm; 0.04–3.81) and insulin resistance (β: 2.81; 1.39–4.23) (Tajar *et al.*, 2012).

In our study, neither SHBG nor FTI was associated with diabetes in our full model; however, the influence of other covariates such as age and obesity should not be ignored in this regard. Insulin resistance can inhibit SHBG production and obesity can further decrease its concentration, suggesting that the association may vary according to the BMI status (Brand *et al.*, 2014). Similar reports from the Third National Health and Nutrition Survey indicated an increased likelihood of diabetes among men in the lowest free testosterone tertile compared with upper tertiles, after adjustment for age and obesity (Selvin *et al.*, 2007).

The interrelation between testosterone and insulin levels is not always age-dependent (Simon *et al.*, 1992). Although age-related decline in testosterone is thought to be a common phenomenon, there are geographical and racial exceptions in this context (Orwoll *et al.*, 2010)—as among the Iranian population (Ramezani Tehrani *et al.*, 2017). As expected, our subgroup analysis revealed higher cumulative HRs of progressing to pre-diabetes in older men compared to those of their younger counterparts; however, as testosterone does not follow an age-specific decline among healthy Iranian men (Ramezani Tehrani *et al.*, 2017), its preventive effect indicated no superiority at different ages. Likewise, owing to the stable levels of testosterone over time, this beneficial effect on pre-diabetes was found to be irrespective of hormone concentrations in different quartiles.

It is noteworthy that the effect of testosterone on pre-diabetes prevention is of clinical importance, as patients with pre-diabetes have the chance of recovering from IFG/IGT before onset of diabetes (Knowler *et al.*, 2009). Testosterone may regress pre-diabetes to normal glucose regulation or at least defer its complications through inhibition of adipocyte proliferation (Singh *et al.*, 2003) and lipolysis acceleration (Blouin *et al.*, 2008). Even a transient reversion to normoglycemia is associated with significant risk reduction of diabetes (Perreault *et al.*, 2012). Therefore, subnormal testosterone level as a reversible predictor of pre-diabetes, if treated, has the potential to revert hyperglycemia back to normoglycemia, whereas, this will not be possible following characterization of overt diabetes.

Strengths of this study lie in its population-based design, long-term follow-up duration with short-term intervals, repeated measurements of a variety of risk factors and exclusive focus on testosterone effect on progressive development of diabetes. However, several limitations of the present study should be acknowledged. First, the associations found in the present study do not infer the direction of causality between testosterone and pre-diabetes. Second, because the reference laboratory method (equilibrium dialysis) is laborious and costly, we were unable to measure free testosterone and used FTI, instead. It is probable that had we measured free hormone, more significant results could have been obtained in terms of diabetes development. Third, the small number of subjects in transition 1 is another limitation that needs to be further explored in studies with higher sample size for this transition. Fourth, evidence supports the role of other sex hormones such as dihydrotestosterone on dysglycemia (Joyce *et al.*, 2016); however, our study lacks these data, and finally, we assessed men aged 30–70 years, because of which these findings may not be generalized to younger or older populations.

CONCLUSION

Our findings show that testosterone's effect on development of dysglycemia is basically reflected through prevention of pre-diabetes in normoglycemic men. Following occurrence of pre-diabetes, testosterone's effect is insignificant and stronger risk factors other than testosterone seem to influence diabetes development. These findings may help to diagnose men at risk of pre-/diabetes and provide rationale for replacement therapy in older men.

ACKNOWLEDGMENTS

We are indebted to each of the study participants for the substantial time and effort contributed to this study. The authors wish to express their gratitude to Ms. Niloofar Shiva for critical editing of English grammar and syntax of the manuscript.

DISCLOSURE

Authors declare that they have no conflict of interest.

FUNDING

This research did not receive any specific grant from any funding agency in the public, commercial, or not-for-profit sector.

AUTHORS' CONTRIBUTIONS

S.M. wrote the initial draft, contributed to discussion, and revised the final version of the manuscript. F.R.T. contributed to the conception of the study, researched data, wrote the manuscript, and reviewed the final version of the manuscript. M.R. analyzed data, wrote/edited the manuscript. S.A.S. analyzed data, wrote/edited the manuscript. M.T. researched data and wrote the manuscript. Z.S. researched and analyzed data. F.A.

contributed to the conception and design of the study. All authors drafted the manuscript and approved the final version.

DATA ACCESSIBILITY

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

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