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The mediatory role of androgens on sex differences in glucose homeostasis and incidence of type 2 diabetes: the KORA study

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Abstract

Background Sex differences exist in type 2 diabetes (T2D), and androgens have been implicated in the etiology of T2D in a sex-specific manner. We therefore aimed to investigate whether androgens play a role in explaining sex differences in glucose homeostasis and incidence of T2D.

Methods We used observational data from the German population-based KORA F4 study ($n = 1975$, mean age: 54 years, 41% women) and its follow-up examination KORA FF4 (median follow-up 6.5 years, $n = 1412$). T2D was determined through self-reporting and confirmed by contacting the physicians and/or reviewing the medical charts. Multivariable linear and logistic regression models were employed to explore associations. Mediation analyses were performed to assess direct effects (DE) and indirect effects (IE), and the mediating role of androgens (total testosterone (TT), dehydroepiandrosterone (DHEA), dehydroepiandrosterone-sulfate (DHEAs)) in the association between sex (women vs. men) and glucose- and insulin-related traits (cross-sectional analysis) and incidence of T2D (longitudinal analysis).

Results After adjustment for confounders, (model 1: adjusted for age; model 2: model 1 + smoking + alcohol consumption + physical activity), women had lower levels of TT, DHEAs, fasting glucose levels, fasting insulin levels, 2 h-glucose levels and HOMA-IR, compared to men. An inverse association was observed for TT and glucose- and insulin-related traits in men, while a positive association was observed for TT and fasting glucose levels in women. We found a mediatory role of TT on the association of sex with fasting glucose levels (IE: $\beta = 3.08$, 95% CI: 2.04, 4.30), fasting insulin levels (IE: $\beta = 0.39$, 95% CI:0.30, 0.47), 2 h-glucose levels (IE: $\beta = 12.77$, 95% CI: 9.01, 16.03) and HOMA-IR

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(IE: β = 0.41, 95% CI: 0.33, 0.50). Also, the inconsistent mediatory role of TT was seen on the association of sex with incidence of T2D (DE: 0.12, 95% CI: 0.06, 0.20 and IE: OR = 7.60, 95% CI: 3.43, 24.54). The opposing DE and IE estimates suggest that the association between sex and either glucose homeostasis or the incidence of T2D may differ when TT is considered as a potential mediator, with higher TT levels being beneficial for glucose metabolism or incidence of T2D in men, while in women, detrimental. No mediatory role was observed for either DHEA or DHEAs on glucose homeostasis or the incidence of T2D.

Conclusions The dimorphic mediatory role of TT highlights its complex role in metabolic health, contributing differently to the glucose dysregulation and risk of T2D in men and women.

Keywords Sex differences, Sex hormones, Androgens, Glycemic control, Type 2 diabetes, Mediation analysis.

Introduction

While the prevalence of type 2 diabetes (T2D) is increasing in both sexes, the diabetes rates are higher in men than women [1]. Sex differences have been also found within diagnosis and prognosis of T2D. For instance, men are usually diagnosed at a younger age with lower body mass index (BMI); on the other hand, women have a higher risk factor burden including excess weight gain and hypertension [2]. Sex differences in T2D complications are more complex. For instance, evidence indicates that women with T2D have a higher relative risk of cardiovascular disease and mortality. However, the absolute risk remains higher in men than in women [3, 4].

Sex hormone-binding globulin and sex hormones have been attributed for sex differences in glucose homeostasis and incidence of T2D [5, 6]. In women, hyperandrogenic conditions such as polycystic ovarian syndrome (PCOS) have been associated with insulin resistance and glucose intolerance and increased risk of T2D [7–9], while hypoandrogenism in men has been linked to obesity and increased glucose and insulin levels [7, 10]. A large systematic review and meta-analysis of prospective observational studies found a lower risk of T2D in men with higher total testosterone (TT) levels; conversely, in women, elevated TT levels were linked to an increased risk of T2D [11]. A recent Mendelian randomization study found a causal role of TT in the pathogenesis of T2D which was more prominent in men than women [12]. Results of another Mendelian randomization study on sex hormones and metabolic traits revealed a beneficial causal effect of bioavailable testosterone on glucose metabolism in men but not women [13]. A large randomized controlled clinical trial involving 1007 men with impaired glucose tolerance or newly diagnosed T2D found that 2 years of testosterone treatment significantly reduced the proportion of individuals with T2D beyond the effects of a lifestyle program [14]. On the other hand, findings from another large controlled clinical trial examining the effects of testosterone replacement therapy in hypogonadal men with prediabetes or diabetes showed no significant differences in the incidence of progression from prediabetes to diabetes or in glycemic control improvements between the treatment and placebo groups [15].

The evidence on the sex-specific association of other androgens including dehydroepiandrosterone (DHEA) and dehydroepiandrosterone-sulfate (DHEAs) and risk of T2D is limited. An observational cohort study on 1258 community-dwelling individuals aged > 65 years showed lower risk of T2D with higher DHEAs in men, while no association was observed for women [16]. Other cross-sectional and prospective studies on women have also found no association between DHEA or DHEAs levels and risk of T2D [17–19]. The findings from another prospective cohort study involving 5189 middle-aged and elderly men and women indicated a reduced risk of T2D associated with higher levels of DHEA, but not DHEAs [20].

Thus, we hypothesized that androgens (TT, DHEA and DHEAs) may explain sex differences for glucose homeostasis and incidence of T2D. To test this hypothesis, we aimed to (i) investigate the association of sex with glucose- and insulin-related traits and incidence of T2D; (ii) to investigate the associations of sex with androgens and each sex hormones with glucose- and insulin-related traits and incidence of T2D; and (iii) to assess the potential mediating role of androgens and, if any, to what extent they mediate the association between sex and the incidence of T2D in a population-based setting of middle-aged and elderly adults.

Methods

Data were obtained from the Cooperative Health Research in the Region of Augsburg (KORA) study, with KORA F4 study as baseline (2006–2008) and KORA FF4 study as follow-up (2013–2014), which both are follow-up examinations of the KORA S4 study (1999–2001). All study participants have provided written informed consent. The study was approved by the Ethics Committees of the Bavarian Chamber of Physicians (Ethical Approval Number 06068) adhering to the declaration of Helsinki. The study design has been described previously in detail [21].

The following participants were excluded from the analysis: participants who withdrew consent ($n=3$), participants with missing information on fasting status and non-fasting participants ($n=21$), already known / newly identified diabetes patients ($n=340$), participants with an unclear or missing information on diabetes status ($n=77$), participants taking sex hormones (e.g. anti-estrogens, estrogens, progestin, hormone replacement therapy, androgens, antiandrogens, enzyme inhibitors, gonadotropin releasing hormones, and anabolic steroids) ($n=240$), participants with surgeries (including hysterectomy, oophorectomy) ($n=233$) and finally, participants with missing information on sex hormones (TT, DHEA, and DHEAs) ($n=181$) and glucose- and insulin-related traits (fasting glucose levels, insulin levels, 2 h-glucose levels, homeostatic model assessment for insulin resistance (HOMA-IR)) ($n=10$). Thus, 1975 individuals were included in the cross-sectional analysis. For the longitudinal analysis, we further excluded participants with missing information on OGTT ($n=46$) and who were lost to follow-up in the KORA FF4 ($n=517$) which resulted in 1412 participants. The study flowchart of included participants is shown in Fig. 1.

Androgens, glucose homeostasis and T2D measurements

Sex hormones (TT, DHEA and DHEAs) were measured in the plasma or serum samples which were stored at -80 °C until being assayed. The detailed assessment procedure was described elsewhere [22]. Samples were prepared and sex hormones were quantified by AbsoluteIDQTM Stero17 Kit and electrospray ionization liquid chromatography-mass spectrometry (ESI-LC-MS/MS). The acquisition and quantification methods of AbsoluteIDQTM Stero17 Kit have been proved to follow the European Medicines Agency's Guideline on bioanalytical method validation (July 21st, 2011). Sample preparation and LC-MS/MS measurements were performed as described in the manual UM-STERO17 by the

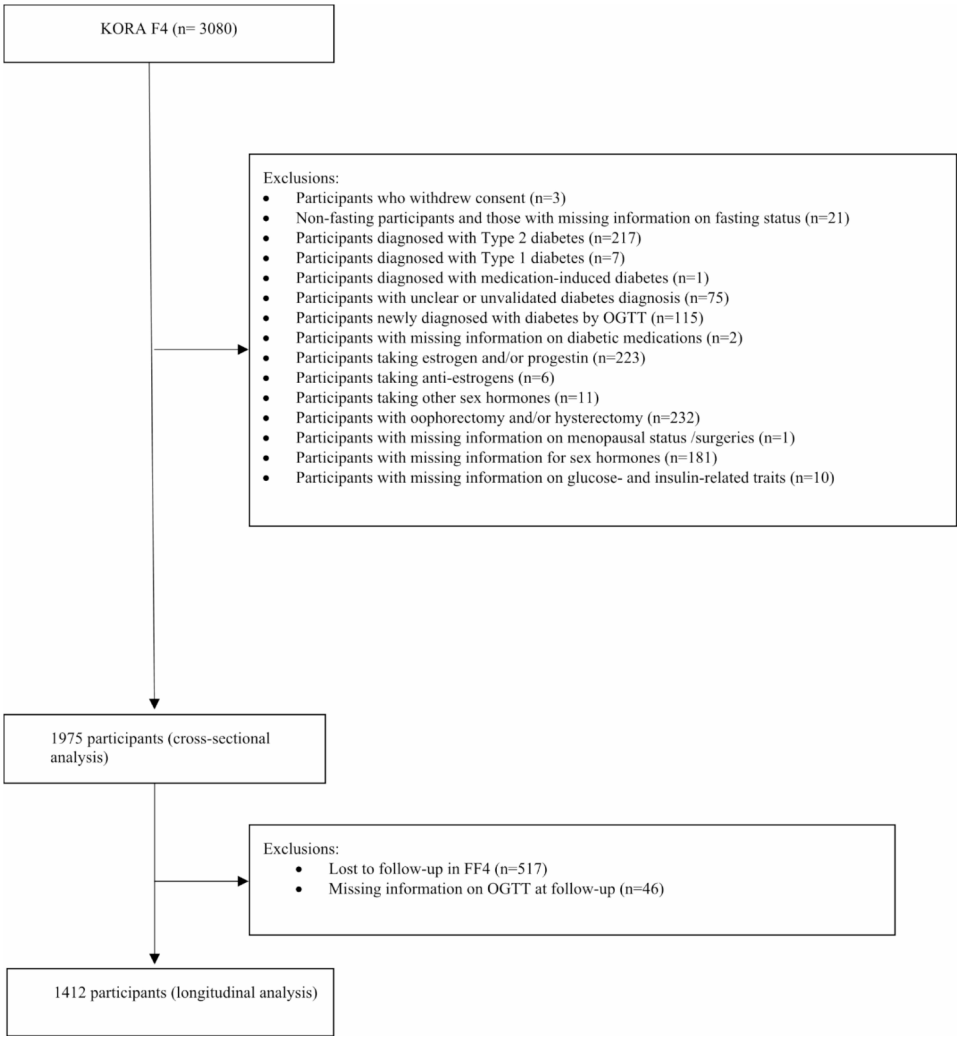

Fig. 1 Flowchart for the selection of study participants

manufacturer [23]. Mass spectrometric analyses were done on a QTRAP 5500 triple quadrupole system (Sciex Deutschland GmbH, Darmstadt, Germany) paired with a 1260 Series HPLC (Agilent Technologies Deutschland GmbH, Böblingen, Germany) and a HTC PAL auto sampler (CTC Analytics, Zwingen, Switzerland) controlled by the software Analyst 1.6.2. The MultiQuant 3.0.1 software (Sciex) and the MetIDQTM software package were used to perform data evaluation for the quantification of sex hormone concentrations and quality assessment. Sex hormones were normalized, the values lower than the lowest limit of detection (LOD) were imputed, and different batches were sex-specifically calibrated.

T2D was self-reported which was validated by a physician or medical chart review, or as self-reported current use of glucose-lowering medication. Oral glucose tolerance test (OGTT) was used for the diagnosis of T2D in participants without known T2D. In participants undergoing an OGTT, newly diagnosed diabetes was defined based on the 1999 World Health Organization diagnostic criteria (i.e., fasting glucose > 6.9 mmol/L and/or 2 h glucose > 11.0 mmol/L) (15). Incident diabetes at follow-up was determined by combining clinically diagnosed diabetes during the follow-up period with newly diagnosed diabetes based on OGTT data at FF4 among those who did not have diabetes at baseline. Fasting glucose levels were measured in fresh serum using hexokinase-G6PD (GLUFlex; Dade Behring, USA). Fasting insulin levels were measured in thawed serum using an electrochemiluminescence immunoassay (Cobas e602 Immunoassay Analyzer; Roche Diagnostics GmbH, Germany).

Assessment of covariates at baseline

Data on baseline characteristics, including age, sex, medication use (lipid-lowering medications (yes/ no)), smoking status (never smokers/ ex-smokers/ irregular smokers/ regular smokers (smoking ≥ 1 cigarette a day), alcohol consumption (gr/day), and physical activity (inactive/ active), were collected by trained medical staff using a standardized interview [24]. Body weight and waist circumference were measured according to the standard guidelines. Total cholesterol was measured in serum samples by enzymatic methods (CHOL Flex).

Statistical analysis

Continuous data are presented as mean ± SD for normally distributed variables and median (IQR) for non-normally distributed variables. Largely skewed variables (fasting insulin levels and HOMA-IR) were log-transformed prior to further analysis. Independent-sample t-test or Mann-Whitney U-test for continuous variables, and the chi-squared test for categorical variables were used to perform sex-stratified analysis for baseline characteristics.

Multivariable linear regression models were used to investigate the association of sex with each of androgens (exposure-mediator) and glucose- and insulin-related traits (fasting glucose levels, 2 h-glucose levels, fasting insulin levels, and HOMA-IR) (exposure-outcome) as well as androgens with glucose- and insulin-related traits (mediator-outcome). In addition, the association of sex and androgens with the incidence of T2D were assessed using logistic regression analysis. All analyses were adjusted for age (model 1) and additionally, for physical activity, alcohol consumption, and smoking (model 2). These two models were selected as potential confounders based on previous literature and were considered as main models in the mediation analysis. Lifestyle factors like physical activity, smoking and alcohol consumption could act as potential mediators in our analyses. However, we considered them as confounders since gender construct may influence these associations [25, 26]. These should be taken into account when interpreting the results of model 2.

We focused on measured androgens (TT, DHEA, DHEAs) as potential mediators and excluded calculated variables such as free testosterone (FT). This decision was due to the lack of data on free testosterone in KORA and the inaccuracy of various equations for calculating FT (e.g., the free androgen index), particularly in women [27, 28]. We performed the mediation analysis to determine whether each of the androgens (TT, DHEA, and DHEAs) could be a potential mediator of the association of sex (women vs. men) and glucose homeostasis and incidence of T2D. The hypothesized causal structure of the association between sex (the exposure) and the outcome (glucose control and incidence of T2D), with androgens as a mediator, are shown as a Directed Acyclic Graph (DAG) in **Supplementary Figs. 1–3**. The regression-based approach in a counterfactual framework was used to assess the direct effect (DE), indirect effect (IE), total effect (TE), and proportion mediated (PM) [29]. Non-parametric bootstrapping (200 times) was used to estimate 95% CI and P values. The proportion mediated (%) was estimated as $OR^{Direct} \times (OR^{Indirect} - 1)/(OR^{Direct} \times OR^{Indirect} - 1) \times 100$ in the case of a binary outcome, or as $(\beta^{Indirect}/\beta^{Total}) \times 100$ in the case of a continuous outcome [30]. The DE represents the effect of the exposure on the outcome at a specific level of the mediator variable, differing from the TE, which signifies the overall effect of the exposure on the outcome. The IE represents the effect on the outcome due to variations in the exposure resulting from different levels of the mediator (TT/ DHEA/ DHEAs). It is important to note that DE and IE should operate in the same direction for the PM to yield a meaningful summary [31]. PM is not valid when DE and mediated effects (IE) have opposite signs which is sometimes referred as inconsistent mediation [31].

Additionally, to test the robustness of our findings, we performed several sensitivity analyses. (i) First, we defined another model with additional adjustments for total cholesterol and lipid-lowering mediations (model 3). We did not consider Model 3 as one of our primary models because the covariates in this model are more likely to serve as intermediaries in the pathways of the investigated associations rather than as confounders. Therefore, the findings from the third model should be interpreted with caution. As noted by VanderWeele [32], the confounding assumptions in mediation analysis are critical, and violations of these assumptions can lead to misleading results. (ii) Second, to investigate the role of age, we divided the participants into two groups based on the median age of 53 and repeated the analyses. 3) Third, to account for the effect of weight, we repeated the mediation analysis for a subset of individuals with normal weight (BMI < 25) and with overweight and obesity (BMI ≥ 25). The second and third sensitivity analyses were run only for glucose- and insulin-related traits as an outcome, and we were not able to rerun the analyses for the incidence of T2D due to the limited number of cases.

Missing values on confounders were treated using multiple imputations. Statistical analyses were performed using R statistical software, version 4.2.2 with CMAverse package [33]. All results were considered statistically significant at a p value < 0.05.

Table 1 Baseline characteristics of study participants of KORA F4 in the overall population and stratified by sex

Characteristic	Total sample (*n* = 1975)	Men (*n* = 1155)	Women (*n* = 820)	*p*-value
Age	54.1 ± 12.9	54.8 ± 12.9	53.1 ± 12.8	**0.004**
Body weight (kg)	79.4 ± 15.5	85.4 ± 13.9	70.8 ± 13.5	**< 0.001**
BMI (kg/m^2)	26.6 (24.2, 29.6)	27.00 (24.9, 29.6)	25.5 (23, 29.5)	**< 0.001**
WC (cm)	93.2 (84.4, 102.1)	97.2 (90.7, 105.2)	84.5 (76.7, 94.5)	**< 0.001**
Fasting glucose levels (mg/dl)	94.2 ± 9.42	96.6 ± 8.98	90.9 ± 9.04	**< 0.001**
2 h-glucose levels (mg/dl)	107 ± 30.1	108 ± 30.3	104 ± 29.7	**0.003**
Fasting insulin levels (µU/ml)	8.5 (6.1, 12)	8.9 (6.5, 12)	7.8 (5.7, 11)	**< 0.001**
HOMA-IR	1.9 (1.3, 2.8)	2.11 (1.5, 3)	1.7 (1.2, 2.5)	**< 0.001**
Testosterone (nmol/l)	10.1 (0.7, 15.9)	14.9 (11.8, 18.9)	0.7 (0.5, 0.9)	**< 0.001**
DHEA (nmol/l)	9.6 (6.2, 15)	9.6 (5.9, 14.8)	9.6 (6.4, 15)	0.44
DHEAs (nmol/l)	2800.2 (1600.6, 4602.5)	3368.1 (1927.7, 5305.)	2280 (1303.5, 3462.5)	**< 0.001**
Alcohol consumption (g/day)	8.5 (0, 22.8)	15.4 (2.8, 31.4)	2.86 (0, 12.3)	**< 0.001**
Physically active n, (%)	1112 (56.3)	647 (56%)	465 (56.7%)	0.82
Smoking				
Regular smoker	334 (16.9%)	209 (18.1%)	125 (15.2%)	**< 0.001**
Irregular smoker	53 (2.7%)	30 (2.6%)	23 (2.8%)	
Ex-smoker	799 (40.5%)	546 (47.3%)	253 (30.9%)	
Never-smoker	786 (39.8%)	368 (31.9%)	418 (51%)	
Total cholesterol (mg/dl)	216 ± 38.6	215 ± 37.9	216 ± 39.5	0.58
Use of lipid lowering medications	199 (10.1%)	134 (11.6%)	65 (7.9%)	**0.009**

Results

Baseline characteristics of the whole population and the stratified analysis based on sex are provided in Table 1. In the cross-sectional analysis, 1975 individuals (1155 men, 820 women) with a mean age of 54.1 ± 12.9 and median BMI of 26.6 kg/m^2 (IQR; 24.2, 29.6) were included. The median (IQR) of TT, DHEA and DHEAs were (14.9 (11.8, 18.9), 9.6 (5.9, 14.8), 3368.1 (1927.7, 5305.00) nmol/l for men and 0.7 (0.5, 0.9), 9.6 (6.4, 15), 2280 (1303.5, 3462.5) nmol/l for women. Fasting glucose levels, 2 h-glucose levels, fasting insulin levels, and HOMA-IR were significantly higher in women compared to men ($p < 0.05$). No significant differences were observed for total cholesterol and physical activity levels in men and women. Alcohol consumption, the proportion of smokers, and the use of lipid-lowering drugs were higher in men than in women. For the longitudinal analysis, over a median follow-up of 6.5 years, 100 incident T2D cases (70 men, 30 women) were recorded.

Sex, androgens, glucose- and insulin-related traits and T2D

Supplementary Table 1 presents the association of sex (women vs. men) with sex hormones and glucose- and insulin-related traits. TT and DHEAs levels were significantly lower in women than in men, while no significant sex differences was observed for DHEA. Women had lower fasting glucose, 2 h-glucose, fasting insulin and HOMA-IR levels in both model 1 and 2. The association of sex hormones and glucose- and insulin-related traits among male participants are presented is **Supplementary Table 2**. An inverse association was found between TT and fasting glucose, 2 h-glucose, fasting insulin and HOMA-IR levels in both models. No significant association was observed between DHEA or DHEAs and glucose- and insulin-related traits in men. **Supplementary Table 3** shows the association of sex hormones and glucose- and insulin-related traits among females. Only a positive association was found between TT and fasting glucose levels in women. No significant associations were found between either DHEA or DHEAs and glucose- and insulin-related traits in women.

Table 2 Mediation analysis of testosterone on the association between sex (women vs. men [Reference]) and glucose- and insulin-related traits among participants of KORA F4

Effects	ß (95% CI) model 1	*p*-value	ß (95% CI) model 2	*p*-value
Fasting glucose levels				
DE	-8.43 (-10.01, -7.11)	**< 0.001**	-8.05 (-9.62, -6.50)	**< 0.001**
IE	3.16 (2.20, 4.36)	**< 0.001**	3.08 (2.04, 4.30)	**< 0.001**
TE	-5.27 (-6.08, -4.4)	**< 0.001**	-4.95 (-5.63, -4.11)	**< 0.001**
PM	-	-	-	-
Fasting insulin levels				
DE	-0.50 (-0.60, -0.42)	**< 0.001**	-0.52 (-0.62, -0.42)	**< 0.001**
IE	0.40 (0.32, 0.47)	**< 0.001**	0.39 (0.30, 0.47)	**< 0.001**
TE	-0.11 (-0.15, -0.05)	**< 0.001**	-0.13 (-0.18, -0.08)	**< 0.001**
PM	-	-	-	-
2 h-glucose levels				
DE	-16.15 (-20.95, -10.76)	**< 0.001**	-15.67 (-20.63, -11.15)	**< 0.001**
IE	13.57 (9.50, 17.40)	**< 0.001**	12.77 (9.01, 16.03)	**< 0.001**
TE	-2.57 (-5.31, -0.01)	**< 0.001**	-2.90 (-5.64, 0.03)	0.06
PM	-	-		
HOMA-IR				
DE	-0.59 (-0.69, -0.52)	**< 0.001**	-0.60 (-0.71, -0.51)	**< 0.001**
IE	0.43 (0.36, 0.52)	**< 0.001**	0.41 (0.33, 0.50)	**< 0.001**
TE	-0.16 (-0.21, -0.12)	**< 0.001**	-0.18 (-0.24, -0.13)	**< 0.001**
PM	-	-	-	-

Model 1: Age

Model 2: Model 1 + smoking + alcohol consumption + physical activity

fasting insulin levels and HOMA-IR are log-transformed

Abbreviations: DE, direct effect; IE, indirect effect; TE, total effect; PM, proportion mediated

HOMA-IR, homeostatic model assessment for insulin resistance

No sex differences were observed for the incidence of T2D (**Supplementary Table 4).** While an inverse association was seen between TT and T2D in both models, no associations were observed between DHEA or DHEAs and T2D incidence (**Supplementary Table 4**).

Mediation analysis

Based on the above-mentioned results, mediation analysis was performed only for TT, to assess whether, and to what extent, the sex differences (women vs. men) in glucose- and insulin-related traits and incidence of T2D were mediated by TT levels. The results of the mediation analysis of TT on the association between sex and glucose- and insulin-related traits are shown in Table 2. The mediation analysis showed an inconsistent mediatory role of TT (opposing DE and IE) on the association between sex and all glucose- and insulin-related traits, highlighting the complex role of TT, suggesting that TT's effects are not uniform and can lead to differing outcomes in metabolic health across sexes. The Integration of DE and IE within the DAGs, illustrating the association between sex and all glucose- and insulin-related traits through TT as a potential mediator, is provided in **Supplementary Fig. 4A-4D.**

Table 3 Mediation analysis of testosterone on the association between sex (women vs. men [Reference]) and incidence of T2D between KORA F4 and FF4

Effects	OR (95% CI) model 1	*p*-value	OR (95% CI) model 2	*p*-value
DE	0.14 (0.07, 0.28)	**< 0.001**	0.12 (0.06, 0.20)	**< 0.001**
IE	7.75 (3.72, 17.87)	**< 0.001**	7.60 (3.43, 24.54)	**< 0.001**
TE	0.88 (0.56, 1.38)	0.54	0.75 (0.45, 1.22)	0.3
PM	-	-	-	-

Model 1: adjusted for age

Model 2: Model 1 + smoking + alcohol consumption + physical activity

Abbreviations: OR, odds ratio; T2D, type 2 diabetes; DE, direct effect; IE, indirect effect; TE, total effect; PM, proportion mediated

Based on model 2, the DE for fasting glucose, fasting insulin, 2 h-glucose, and HOMA-IR were (β= -8.05, 95% CI: -9.62, -6.50), (β= -0.52, 95% CI: -0.62, -0.42), (β= -15.67, 95% CI: -20.63, -11.15) and (β= -0.60, 95% CI: -0.71, -0.51), while the IE were (β=3.08, 95% CI: 2.04, 4.30), (β=0.39, 95% CI: 0.30, 0.47), (β=12.77, 95% CI: 9.01, 16.03) and, (β=0.41, 95% CI: 0.33, 0.50), respectively.

The mediation analysis of TT on the association between sex and incidence of T2D is presented in Table 3. The DE and IE were in opposite directions (**Supplementary Fig. 4E**). The DE was (OR: 0.14, 95% CI: 0.07, 0.28) and (OR: 0.12, 95% CI: 0.06, 0.20), while the IE was (OR: 7.75, 95% CI: 3.72, 17.87) and (OR: 7.60, 95% CI: 3.43, 24.54) in models 1 and 2, respectively.

The findings were robust in the sensitivity analysis adding total cholesterol and lipid-lowering medications to model 2 (model 3) (**Supplementary Tables 5 and 6**). The DE and IE were in opposite directions for fasting glucose, fasting insulin, 2 h-glucose, HOMA-IR (**Supplementary Table 5)** and incidence of T2D **(Supplementary Table 6)**. The results of sensitivity analysis based on median age were also robust with larger effect estimates in individuals younger than 53 years **(Supplementary Table 7)**. Another sensitivity analysis based on BMI categories (BMI>=25 kg/m^2 and BMI<25 kg/m^2) supported the same results with larger effect estimates in overweight and obese individuals **(Supplementary Table 8)**.

Discussion

To our best knowledge, the current study is the first investigation examining the mediatory role of androgens in explaining sex differences in glucose metabolism and the incidence of T2D. While previous studies have independently examined the sex-specific association of androgens with glucose homeostasis and the incidence of T2D in men and women, we utilized mediation analysis to consider the complex combined effects of sex, sex hormones, and glucose homeostasis/ incidence of T2D. Our results indicated an inconsistent mediatory role

of TT on sex differences in glucose- and insulin-related traits and incidence of T2D which shows that TT affects glucose metabolism and T2D risk differently in men and women. In men, higher TT levels are generally beneficial for glucose metabolism and lower the risk of T2D, while in women, higher TT levels can be detrimental. In addition, no mediatory role was observed for DHEA and DHEAs on the association of sex with glucose- and insulin-related traits or incidence of T2D.

Our findings on sex differences for androgens and glucose homeostasis were in line with previous research, reporting lower levels of TT [8], DHEA [34], and DHEAs [34] and also, lower levels of fasting glucose [4, 35], insulin [4], and HOMA-IR [36] in women compared to men. While some studies reported sex differences for T2D risk factors, incidence and related complications [2, 37], we found no association of sex differences for incidence of T2D.

We found that TT levels may have an inconsistent mediatory role in sex differences for glucose homeostasis and the development of T2D. Specifically, higher levels of TT in men were associated with decreased levels of fasting glucose, fasting insulin, and HOMA-IR along with a lower odds of T2D, while in women, only an inverse association was observed for TT and fasting glucose levels. In accordance with these findings, O'Reilly et al. [38] found some evidence of a sexually dimorphic feature for TT levels in mediating the incidence of T2D; hence, testosterone deficiency and excess may be independent risk factors for T2D in men and women, respectively. Moreover, Goodman-Gruen et al. [39] reported sex differences in the link between TT levels and glucose tolerance in adults, in which men with higher fasting glucose, high glucose tolerance, and T2D had lower levels of TT, and in contrast, women with T2D had higher bioavailable testosterone levels. Similarly, Oh et al. showed that low TT levels in men and high levels of bioavailable testosterone in women are associated with the incidence of T2D [8]. A randomized follow-up study involving 38 women with PCOS reported that higher TT levels were associated with a higher insulin resistance index [40].

A systematic review and meta-analysis of observational studies previously demonstrated that men with higher TT levels (15.6–21.0 nmol/L) had a 42% lower risk of T2D, while higher TT increased T2D risk in women [11]. Gyawali et al. [41] reported similar findings, showing that TT levels were inversely associated with T2D incidence in middle-aged to elderly men, independent of established risk factors. Yao et al. [42], in a meta-analysis of cohort studies, suggested that higher TT levels were significantly associated with lower T2D risk in men. Our findings are also supported by a Mendelian randomization study, which demonstrated that higher bioavailable testosterone in men was associated with lower fasting glucose and favorable effects on glucose metabolism [13]. Another Mendelian randomization study reported evidence of the beneficial impacts of higher testosterone levels on T2D incidence and related traits in men [43].

Moreover, there are some clinical trials, supporting our findings. Canguven et al. [44] found that testosterone therapy may reduce glycated hemoglobin levels in elderly hypogonadal men, which might lower the risk of T2D in those participants. A recent systematic review and meta-analysis reported that testosterone replacement therapy can improve glycemic control and hormone levels in hypogonadal men with T2D [45]. Additionally, Jenkins et al. [46] showed that the highest adherence to testosterone replacement therapy is associated with improved HbA1c and glycemic homeostasis over time in men with hypogonadism and T2D.

In our study, we also performed the subset analysis based on median age, showing that the mediatory role of TT in all glucose- and insulin-related traits was stronger in participants < 53 years of age compared to those aged 53 and over. These findings are consistent with a meta-analysis of cohort studies, primarily involving postmenopausal women, which indicated an increased risk of T2D associated with higher levels of TT and FT, but the results were not statistically significant [47]. This finding might be due to a reduction in both adrenal and testicular function with aging, leading to a decrease in TT levels in men [48]. In women, there is a decline in TT levels with age which is steepest in the early reproductive years. This decline then stabilizes in midlife and tends to slightly increase in the later years [49]. Thus, the variation between men and women becomes narrower in older ages. Furthermore, stratification of mediation analysis based on BMI categories revealed that the mediatory role of TT remains stronger in participants with overweight and obesity. These findings are in line with previous evidence showing that androgen balance is different in people with obesity based on their sex. While in men TT decreases with increasing body weight, women with abdominal obesity may experience functional hyperandrogenism [50].

The specific biological mechanisms underlying the link between TT levels and glucose homeostasis, and the development of T2D in a sex-specific manner remain unclear. Evidence suggests that testosterone regulates the expression of key regulatory enzymes involved in glucose and lipid metabolism in major insulin-responsive tissues, including the liver, adipose tissue, and skeletal muscle [51]. Additionally, some sex differences in inflammatory cytokine production may predict the risk of T2D [52]. The differences in how testosterone affects glucose metabolism and insulin sensitivity may be due to varying interactions with other hormones and differences in body composition between men and women [53]. One

possible hypothesis is that testosterone deficiency in men leads to abdominal obesity, contributing to T2D [54]. Fan et al. [55] found that androgen receptors (AR) significantly affect fat accumulation in male mice. AR knockout reduces the effect of testosterone, leading to obesity, which could contribute to the development of T2D. Testosterone may affect glucose control by increasing lean body mass in men [56, 57]. In women, however, higher testosterone levels can lead to increased body fat, insulin resistance, and higher blood glucose concentrations [58]. Additionally, evidence has shown that testosterone treatment in men might reduce the levels of tumor necrosis factor (TNF-a) [59, 60]. TNF-a is an inflammatory cytokine that can induce insulin resistance in both muscle and adipose tissues [61–63] and is associated with a higher T2D risk [64, 65]. It also has been demonstrated that men with low levels of testosterone, including participants with reduced glucose tolerance and T2D, have manifested lower oxidative phosphorylation in muscle mitochondria, contributing to the increase of insulin resistance [66]. On the other hand, a growing body of evidence has shown that testosterone increases lipogenesis in visceral fat in women, whereas, in men, it stimulates adipose tissue lipolysis [67].

In contrast to the sexually dimorphic role of TT, we did not find any significant mediatory role for DHEA and DHEAs in glucose- and insulin-related traits and T2D risk. There have been conflicting findings concerning the link between DHEA and DHEAs and glucose metabolism in the general population. Similar to our findings, a prospective study on postmenopausal women found no association between DHEA and the risk of T2D [68]. Additionally, Ding et al. [17] showed that DHEAs was not associated with T2D risk in postmenopausal women. However, another prospective cohort study found that serum levels of DHEA were inversely associated with T2D risk, with no observed sex differences in this association [69]. Moreover, a study by Veronese et al. [16] reported no link between DHEAs and T2D whereas, after stratifying by sex, higher levels of DHEAs were inversely associated with T2D risk in men, but not in women.

The current study was performed, both cross-sectionally and longitudinally, on a well-characterized population-based study and adjustments for a wide range of potential confounding factors. The availability of OGTT data at both baseline and follow-up enabled us to assess not only the development of clinically diagnosed T2D but also of early disorders in glucose metabolism and newly OGTT-diagnosed T2D. Similar to prior research on KORA study focusing on sex hormones [70–72], our study benefits from comprehensive data regarding menopausal surgeries (such as oophorectomy or hysterectomy) and various hormonal treatments (including anti-estrogens, estrogens, progestins, hormone replacement therapy, androgens, antiandrogens, enzyme inhibitors, gonadotropin-releasing hormones, and anabolic steroids). This wealth of information allowed us to account for several significant variables in our eligibility criteria. Due to the observational design, the present study could not establish a causal association. Moreover, data on women with PCOS was not available in the dataset. Even in postmenopausal women, PCOS symptoms can still lead to changes in sex hormone levels and, consequently, in metabolic processes. The lack of precise time-to-event information does not allow us to perform more advanced modelling such as Cox regression analysis. The study's sample size limited our ability to conduct additional sensitivity analyses based on different menopausal status groups. Additionally, we could not stratify our sensitivity analyses for incidence of T2D by categories of age and BMI due to the limited number of incident cases. Lastly, as most of the participants in our study were Europeans, the findings cannot be generalized to other ethnic groups. Thus, we recommend that future mediation analyses on sex hormones and metabolic diseases address these limitations by using larger, more diverse sample sizes and taking menopausal status in women into account.

These findings emphasize the need for a comprehensive and personalized approach to assessing and managing T2D risk that considers both direct and indirect effects of sex and hormones like TT. A simple focus on hormone levels without considering their broader metabolic context might be insufficient. Therefore, understanding the sex-specific effects of testosterone is crucial for developing targeted treatments for diabetes. For men with low testosterone, testosterone replacement therapy might be beneficial in improving metabolic health and reducing diabetes risk. For women, especially those with conditions like PCOS, managing elevated testosterone levels through lifestyle changes or medication might help in mitigating the risk of diabetes. Additionally, the lack of a mediatory role for DHEA and DHEAs suggests that these hormones do not significantly influence the sex differences observed in glucose metabolism and T2D risk. More research is needed to fully understand the mechanisms by which TT influences metabolic health differently in men and women and to explore other potential mediators of sex differences in T2D risk.

Data are presented as mean ± SD for normally distributed continuous variables, median (IQR) for non-normally distributed variables or numbers (percentage) for categorical variables. P-values were generated by independent-sample t-test or Mann-Whitney U-test for continuous variables and chi-square test for categorical variables. P-values < 0.05 are shown in bold.

Abbreviations

BMI	Body mass index
WC	Waist circumference

HOMA-IR	Homeostatic model assessment for insulin resistance
DHEA	Dehydroepiandrosterone
DHEAs	Dehydroepiandrosterone sulfate

Supplementary Information

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Supplementary Material 1

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Author contributions

HR-D, TM and JN contributed to the study design and conception. HR-D performed the statistical analysis. HR-D and SB wrote the first draft of the manuscript. All authors helped to interpret the data and critically revised the manuscript for important intellectual content. JN had full access to all the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis. All authors have read and approved the final manuscript and the authorship list.

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Data availability

All available data are provided within the manuscript and supplementary files.

Declarations

Competing interests

The authors declare no competing interests.

Ethics approval

All study participants have provided written informed consent. The study was approved by the Ethics Committees of the Bavarian Chamber of Physicians (Ethical Approval Number 06068) adhering to the declaration of Helsinki.

Informed consent

Informed consent was obtained from all individual participants included in the study.

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