

ORIGINAL RESEARCH

Do Androgens and Sex Hormone–Binding Globulin Mediate Sex Differences in the Risk of Stroke and Myocardial Infarction? The KORA Study

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BACKGROUND: Sex differences in cardiovascular disease are well established, but the role of androgens and sex hormone–binding globulin (SHBG) remains unclear. We investigated whether these hormones and SHBG mediate sex differences in the risk of stroke and myocardial infarction (MI).

METHODS: Observational data from the German population-based KORA (Cooperative Health Research in the Region of Augsburg) F4 study (n=1970; mean age, 54 years; 58% men) were used as baseline examination. Incident stroke and incident MI cases were identified through regular follow-ups until the end of 2016. Multivariable linear and Cox regression models were used to explore associations. Mediation analyses were performed to assess direct effects, indirect effects, and the mediating role of androgens (total testosterone, dihydrotestosterone, dehydroepiandrosterone, and dehydroepiandrosterone-sulfate) and SHBG in the association between sex (women versus men) and risk of stroke/MI.

RESULTS: During a median follow-up of 8.6 years, 71 stroke and 63 MI events occurred. An inconsistent mediatory role was observed for total testosterone and dihydrotestosterone on the association between sex and risk of stroke. The direct effects were 0.42 (95% CI, 0.18–0.86) and 0.40 (95% CI, 0.17–0.82) and indirect effects were 2.48 (95% CI, 1.27–6.17) and 2.90 (95% CI, 1.67–6.35) for total testosterone and dihydrotestosterone, respectively. No mediating role was observed for other androgens or SHBG in relation to stroke, and neither androgens nor SHBG mediated the risk of MI.

CONCLUSIONS: Our findings suggest that both total testosterone and dihydrotestosterone may have dimorphic mediating role with stroke risk, and that these associations may vary between men and women.

Key Words: androgens ■ mediation analysis ■ myocardial infarction ■ sex differences ■ stroke

See Editorial by XXX.

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RESEARCH PERSPECTIVE

What Is New?

- This study is among the first to evaluate whether circulating androgens and sex hormone-binding globulin mediate the association between biological sex and incident stroke and myocardial infarction using causal mediation analysis in a population-based cohort study, demonstrating that total testosterone and dihydrotestosterone exhibited inconsistent mediation of stroke risk, but not myocardial infarction, suggesting disease-specific rather than generalized effects of endogenous sex hormones across cardiovascular outcomes.

What Question Should Be Addressed Next?

- Larger prospective studies and mechanistic investigations are needed to validate these findings, clarify the biological pathways linking androgens to stroke, and investigate whether endogenous sex hormones exhibit disease-specific mediating effects across other cardiovascular and chronic disease outcomes.

Nonstandard Abbreviations and Acronyms

DE	direct effect
DHEA	dehydroepiandrosterone
DHEA-S	dehydroepiandrosterone sulfate
DHT	dihydrotestosterone
IE	indirect effect
KORA	Cooperative Health Research in the Region of Augsburg
SHBG	sex hormone-binding globulin
TE	total effect
TT	total testosterone

Cardiovascular diseases (CVDs), especially ischemic heart disease and stroke, are the leading cause of mortality in the world.¹ Substantial sex differences exist in the incidence, presentation, and outcomes of CVD, including myocardial infarction (MI) and stroke.² Although these differences are partly explained by sociocultural factors, biological mechanisms are thought to play a central role.³ Among these, endogenous sex hormones and sex hormone-binding globulin (SHBG) have been proposed as key contributors because of their effects on vascular biology and cardiometabolic regulation.^{4,5} Understanding whether sex hormones mediate sex differences in CVD risk is

essential to identify biologically relevant pathways that may enable sex-specific prevention and treatment strategies.

Epidemiologic evidence suggests that the relationship between sex hormones and CVD risk is sex specific and complex. In men, low testosterone levels have been associated with higher CVD risk,^{6–8} whereas nonlinear associations have been reported for all-cause mortality.^{9,10} In contrast, studies on women have found that high serum total testosterone (TT) and low SHBG concentrations have been associated with higher burden of CVD risk factors and a higher incidence of CVD.^{11–15} The findings from a limited number of studies on dihydrotestosterone (DHT) and CVD risk have primarily focused on men and have reported associations with stroke,¹⁶ incident CVD,¹⁷ and all-cause or CVD-specific mortality.¹⁰

Experimental and clinical evidence suggests that testosterone influences cardiovascular risk through pathways including endothelial function, vascular tone, lipid metabolism, inflammation, and insulin sensitivity.^{18,19} Notably, these effects appear to differ by sex: although lower testosterone in men is associated with increased adiposity, insulin resistance, and proinflammatory states, elevated androgen levels in women have been linked to adverse cardiometabolic risk profiles.^{19,20} In addition, androgens may affect coagulation and atherosclerotic processes, providing biological plausibility for their role in CVD development.²¹ These observations support the hypothesis that sex hormones may contribute differently to CVD risk in women and men.

A sex-specific Mendelian randomization (MR) study found that genetically predicated higher serum SHBG levels are associated with decreased risk of ischemic stroke in men and women.¹⁴ Findings of another MR study using UK Biobank data indicated that endogenous testosterone was positively associated with thromboembolism, heart failure, and MI, which were stronger in men than women.²² However, another MR study found that both SHBG and TT had no direct causal effect with coronary heart disease (CHD) in men.²³

Evidence on other androgens, such as dehydroepiandrosterone (DHEA) and its sulfate (DHEA-S), remains limited. Findings of a prospective cohort study on older men and women found that low DHEA-S is associated with increased risk for heart failure and mortality but not CHD in both sexes.²⁴

Despite these advances, an important gap remains. Prior studies have largely focused on associations or causal effects of sex hormones within sexes but have not examined whether these hormones/SHBG explain (mediate) the observed differences in CVD risk between women and men. Addressing this question is essential to move beyond descriptive sex differences

toward a mechanistic understanding of CVD. Thus, we aimed to assess the potential mediating role of androgens and SHBG and, if any, to what extent they mediate the association between sex and the incidence of stroke/MI in a population-based setting of middle-aged and elderly adults.

METHODS

Setting and Study Population

The data are subject to national data protection regulations and therefore cannot be made publicly available in an open repository. However, access can be granted on request through an individual project agreement with the KORA (Cooperative Health Research in the Region of Augsburg) study. To obtain permission to use KORA data under such an agreement, please apply via the KORA.PASST digital platform (<https://epi.helmholtz-muenchen.de/>).

This study was conducted among participants of the prospective population-based KORA study, selected from population registries in the city of Augsburg (Germany) and 2 surrounding counties between 1999 and 2001 (baseline visit, KORA S4).

Follow-up examinations were performed between 2006 and 2008 (KORA F4) and 2013 and 2014 (KORA FF4). The KORA F4 study enrolled 3080 participants, which were used as the baseline sample for the current analysis. 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 in detail elsewhere.²⁵

The following participants were excluded from the analysis: participants who withdrew consent (n=3), participants with missing information on fasting status and nonfasting participants (n=21), participants diagnosed with stroke or MI at baseline (n=146), participants taking sex hormones (eg, anti-estrogens, estrogens, progestin, hormone replacement therapy, androgens, antiandrogens, enzyme inhibitors, gonadotropin-releasing hormones, and anabolic steroids) (n=271), women with gynecological surgeries, such as hysterectomy and oophorectomy, or missing information on these surgeries (n=271), participants with missing information on sex hormones (TT, DHT, DHEA, and DHEA-S) and SHBG (n=246), and participants with missing information on incident stroke/MI

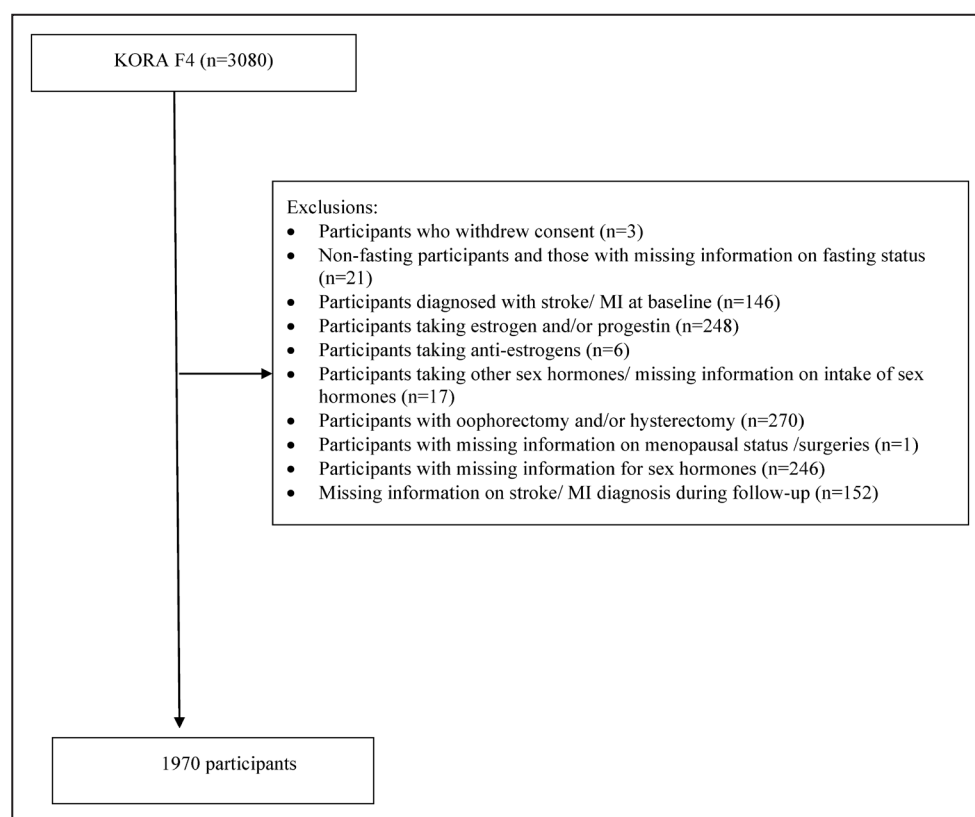


Figure. The study flowchart of included participants.

KORA indicates Cooperative Health Research in the Region of Augsburg; and MI, myocardial infarction.

during follow-up (n=152), which resulted in 1970 participants. The study flowchart of included participants is shown in the [Figure](#). The present analysis includes data from the KORA F4 as baseline and follow-up information from various sources until the end of 2016.

Androgens and SHBG Measurements

Blood samples were collected according to standardized protocols during study center visits, typically in the morning after an overnight fast. Sex hormones (TT, DHT, DHEA, and DHEA-S) were measured in plasma or serum samples, which were stored at -80°C until being assayed. The detailed assessment procedures have been described previously.²⁶ Quantification of sex hormones was performed using the AbsoluteIDQ Stero17 Kit in combination with electrospray ionization liquid chromatography–tandem mass spectrometry. The acquisition and quantification protocols of the Stero17 Kit comply with the European Medicines Agency's guideline on bioanalytical method validation (July 21, 2011). Measurements of SHBG in serum were performed using the ARCHITECT SHBG assay, a chemiluminescent microparticle immunoassay for absolute quantification of SHBG (Abbott Diagnostics). Sample preparation and liquid chromatography–tandem mass spectrometry measurements were performed following the manufacturer's manual (UM-STERO17).²⁷ Mass spectrometric analysis was performed using a QTRAP 5500 triple quadrupole mass spectrometer (Sciex Deutschland GmbH, Darmstadt, Germany), coupled with a 1260 Series HPLC system (Agilent Technologies Deutschland GmbH, Böblingen, Germany) and an HTC PAL autosampler (CTC Analytics, Zwingen, Switzerland), operated via Analyst 1.6.2 software. Data evaluation, quantification of hormone concentrations, and quality control were conducted using MultiQuant 3.0.1 and the MetIDQ software package (Biocrates Life Sciences AG, Innsbruck, Austria). Hormone concentrations were normalized; values below the lower limit of detection were imputed, and batch effects were adjusted through sex-specific calibration.

Assessment of Covariates

Baseline data on age, sex, and medication use (antihypertensive medications and lipid-lowering medications [yes/no]) were obtained via standardized interviews conducted by trained medical staff. Participants were asked to bring all medication packages to the study center, and exact preparations were extracted from a medication database and subsequently ATC coded. Additional lifestyle factors, such as smoking status (categorized as never, ex-smoker, irregular smoker, or regular smoker [≥ 1 cigarette/day]), alcohol consumption (g/day), and physical activity (inactive/active), were also collected.²⁸

Anthropometric measurements, including body weight, waist circumference, and body mass index, and blood pressure measurements were taken according to standardized protocols. Baseline type 2 diabetes was initially assessed by self-report and subsequently validated by contacting the treating physician or medical chart review. Participants only reporting current use of glucose-lowering medication were also classified as having type 2 diabetes. High CRP (C-reactive protein) was quantified in plasma using a high-sensitivity latex-enhanced nephelometric assay (BN II Analyzer, Dade Behring). Thyroid-stimulating hormone was measured using electrochemiluminescent methods (Dimension Vista Systems; Siemens, Germany). Total cholesterol was measured in fresh serum by enzymatic methods (CHOL Flex).

Ascertainment of MI and Stroke

Information on self-reported stroke or MI was collected through regular postal follow-up questionnaires or during follow-up examinations involving face-to-face interviews. Vital status was periodically verified across Germany via population registries and, more recently, through, the “Anstalt für Kommunale Datenverarbeitung in Bayern” (<http://www.zemaonline.de/>). Death certificates were obtained from the district health departments for all those who died in Bavaria and were coded for the underlying causes of death according to the *International Classification of Diseases, Ninth Revision (ICD-9)*, and *International Classification of Diseases, Tenth Revision (ICD-10)*.

The following sources were used to validate incidental cases of MI:

First, we used data from the KORA Myocardial Infarction Registry in the Augsburg region of Germany to identify individuals with a first MI. The case definition for both nonfatal and fatal events (coronary death or fatal MI) followed the criteria of the World Health Organization Monitoring Trends and Determinants in Cardiovascular Disease (MONICA) protocol.^{29,30} Additional nonfatal events have been diagnosed according to the European Society of Cardiology and American College of Cardiology criteria since 2001.^{31,32} All cases occurring in individuals aged 25 to 84 years (25–74 years until 2009) with residence and event within the study region were validated through this registry.³³

Second, all self-reported nonfatal MIs obtained from postal follow-up questionnaires or face-to-face interviews that were not covered by the KORA Myocardial Infarction Registry were additionally validated using hospital discharge letters or information provided by the participants' treating physicians. Third, all fatal cases (coronary death or fatal MI) not included in the KORA MI Registry population were validated based on coded death certificates.

Incidental strokes were validated analogously to MI, as described above under second and third. Stroke-related deaths were classified using the 3-digit codes

433, 434, and 435 from the German modification of the ICD-9.

Statistical Analysis

Continuous data are presented as mean $\pm$ SD for normally distributed variables and median (interquartile range) for non-normally distributed variables. The distribution of continuous variables and the homogeneity of variances between groups were assessed before group comparisons. Normally distributed variables were compared using the independent-samples *t*-test when the assumption of equal variances was met, and Welch *t*-test was used when variances were unequal. Largely skewed variables were log-transformed before further analysis. Non-normally distributed variables were compared using the Mann–Whitney *U*-test. Categorical variables were compared using the χ^2 test.

Multivariable linear regression models were used to investigate the association of sex with each of androgens/SHBG (exposure-mediator). Multivariable Cox proportional hazards regression models were used to assess the association of sex with the risk of stroke and MI (exposure–outcome) and of androgens/SHBG with the risk of these outcomes (mediator–outcome). All analyses were adjusted for age (model 1) and, additionally, for physical activity, alcohol consumption, and smoking (model 2). These 2 main models were selected based on variables defined as potential confounders derived from previous literature and were considered as main models in the mediation analysis as well. We then performed mediation analyses to investigate whether circulating androgens (TT, DHT, DHEA, and DHEA-S) or SHBG mediate the association between sex (women versus men) and the incidence of stroke/MI. The hypothesized causal pathway is illustrated in Figure S1.

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 using the *cmed()* function from the CMAverse package.³⁴ Within CMAverse, exposure–mediator interaction can be formally assessed by specifying the *EMint=TRUE* option. We evaluated exposure–mediator interaction for all mediators and outcomes included in the study. None of the interaction terms was statistically significant. Consistent with the methodological recommendations of VanderWeele and colleagues, when there is no evidence of meaningful exposure–mediator interaction, the mediation effect can be adequately characterized using a 2-way decomposition consisting of the natural direct and natural indirect effects. Cox proportional hazards regression models were used to estimate hazard ratios (HRs) and 95% CIs for the effects. The DE represents the effect of the exposure (sex) on the outcome (risk of stroke/MI) at a specific level of the mediator variable (sex hormones/

SHBG), differing from the TE, which signifies the overall effect of the exposure (sex) on the outcome (risk of stroke/MI). The IE represents the effect on the outcome (risk of stroke/MI) attributable to variations in the exposure (sex) resulting from different levels of the mediator (TT/DHT/DHEA/DHEA-S and SHBG). DE and IE should operate in the same direction for the proportion mediated to yield a meaningful result.³⁵ The counterfactual framework relies on several key identifiability assumptions, including consistency, positivity, no unmeasured exposure–outcome confounding, no unmeasured exposure–mediator confounding, no unmeasured mediator–outcome confounding, and the absence of mediator–outcome confounders affected by the exposure. Potential confounders were identified a priori based on previous literature and subject-matter knowledge, incorporated into the mediation models, and represented in the directed acyclic graphs provided in Figure S1. The mediator was modeled continuously, and the outcome was time to incident stroke/MI, accounting for censoring. We applied nonparametric bootstrapping with 5000 resamples to obtain robust estimates and 95% CIs for DE, IE, and TE. To assess the plausibility of the missing at random assumption underlying the imputation procedure, we compared the characteristics of participants with missing data with those with complete data. No substantial differences were observed between the 2 groups, supporting the plausibility of the missing at random assumption. Nevertheless, as with all multiple imputation approaches, the missing at random assumption cannot be directly verified. Missing values in confounders were handled using multiple imputation within the mediation analysis framework. Specifically, the multiple imputation option (*multimp=TRUE*) was implemented in the *cmed()* function, allowing missing data in confounders included in the mediation models to be appropriately accounted for during estimation.

Additionally, to evaluate the robustness of our findings and the potential influence of additional cardiometabolic factors, we performed a sensitivity analysis using model 3, which further adjusted for waist circumference, systolic blood pressure, antihypertensive medication, lipid-lowering medication, total cholesterol, CRP, thyroid-stimulating hormone, and baseline diabetes. Because these cardiometabolic variables may also represent intermediate factors on the pathway from sex and sex hormones/SHBG to cardiovascular outcomes, model 3 was not considered the primary model and was interpreted with caution to avoid potential overadjustment. Therefore, the findings from the third model should be interpreted with caution. As noted by VanderWeele,³⁶ the confounding assumptions in mediation analysis are critical, and violations of these assumptions can lead to misleading results. All statistical analyses were performed in R version 4.2.2

using the CMAverse package.³⁷ All results were considered statistically significant at $P < 0.05$.

RESULTS

Baseline characteristics of the whole population and the stratified analysis based on sex are provided in Table 1. Overall, 1970 individuals (1140 men, 830 women) with a mean age of 54.7 ± 13 years and mean body mass index of 27.4 ± 4.6 kg/m² were included. The median (interquartile range) values of TT, DHT, DHEA, DHEA-S, and SHBG were 14.8 (11.5–18.8), 1.3 (0.90–1.70), 9.2 (5.7–14.7), 3330.0 (1832.1–5309.0), and 47.9 (34.7–65.3) nmol/L for men and 0.7 (0.5–1.0), 0.2 (0.1–0.4), 9.6 (6.3–14.9), 2239.4 (1240.1–3461.1), and 75.4 (53.8–100.4) nmol/L for women. No significant differences were observed for prevalent diabetes, thyroid-stimulating hormone, CRP, total cholesterol, lipid-lowering medications, and physical activity levels between men and women. Alcohol consumption, the proportion of smokers, and systolic blood pressure levels were higher in men than in women. During a median follow-up of 8.6 years, 71 strokes occurred over

16332 person-years (47 in men and 24 in women) and 63 MIs occurred over 16626 person-years (54 in men and 9 in women).

Sex, Androgens, SHBG, and Risk of Stroke/MI

In line with our previous publications,^{38,39} Table S1 presents the association of sex (women versus men) with sex hormones and SHBG. TT, DHT, and DHEA-S levels were significantly lower in women than in men, whereas no significant sex differences were observed for SHBG and DHEA in both models 1 and 2. The associations of sex hormones and SHBG (per unit increase) with risk of stroke among male and female participants are presented in Table S2. An inverse association was found between TT and DHT with risk of stroke in both models. No significant association was observed for SHBG, DHEA, or DHEA-S with risk of stroke in men. No significant associations were observed for sex hormones and SHBG with risk of stroke in women. No sex differences were observed for risk of stroke (Table S3).

The association of sex hormones and SHBG with risk of MI among male and female participants is

Table 1. Baseline Characteristics of Study Participants of KORA F4 in the Overall Population and Stratified by Sex

Variable	Overall (n=1970)	Men (n=1140)	Women (n=830)	P value
Age, y	54.7±13.0	55.5±13.0	53.6±12.8	0.001*
Weight, kg	79.6±15.5	85.8±13.8	71.3±13.9	<0.001*
BMI, kg/m ²	27.4±4.6	27.7±4.1	26.9±5.2	<0.001*
WC, cm	93.5 (84.7–102.5)	97.8 (91.2–105.7)	85.1 (77.0–94.9)	<0.001*
Physically active, n, %	1111 (56.4)	639 (56.1)	472 (56.9)	0.787
Smoking status, n, %				
Regular smoker	320 (16.3)	204 (17.9)	116 (14.0)	<0.001*
Irregular smoker	53 (2.7)	29 (2.5)	24 (2.9)	
Ex-smoker	812 (41.3)	553 (48.6)	259 (31.2)	
Never smoker	782 (39.8)	352 (30.9)	430 (51.9)	
Alcohol consumption, g/d	16.1 (20.7)	22.0 (23.7)	8.1 (11.6)	<0.001*
TT, nmol/L	9.9 (0.8–15.6)	14.8 (11.5–18.8)	0.7 (0.5–1.0)	<0.001*
DHT, nmol/L	0.8 (0.3–1.4)	1.3 (0.9–1.7)	0.2 (0.1–0.4)	<0.001*
DHEA, nmol/L	9.4 (6.0–14.8)	9.2 (5.7–14.7)	9.6 (6.3–14.9)	0.256
DHEA-S, nmol/	2734.9 (1522.5–4565.6)	3330.0 (1832.1–5309.0)	2239.4 (1240.1–3461.1)	<0.001*
SHBG, nmol/L	57.9 (39.7–81.0)	47.9 (34.7–65.3)	75.4 (53.8–100.4)	<0.001*
Without prevalent diabetes, n, %	1438 (73.0)	800 (70.2)	638 (76.9)	0.001*
Lipid-lowering medications, n, %	190 (9.6)	118 (10.4)	72 (8.7)	0.1
Antihypertensive medications, n, %	519 (26.3)	320 (28.1)	199 (24.0%)	0.047*
Total cholesterol, mg/dL	214.0 (188.0–239.0)	214.0 (189.0–238.0)	214.0 (187.2–241.0)	0.617
SBP, mmHg	121.0 (110.0–133.5)	125.5 (116.1–137.0)	112.5 (102.5–125.0)	<0.001*
TSH, mIU/L	1.3 (0.8–1.9)	1.3 (0.9–1.9)	1.3 (0.8–1.9)	0.277*
CRP, mg/L	1.0 (0.5–2.2)	1.0 (0.5–2.3)	1.0 (0.5–2.2)	0.281*

Data are presented as mean±SD for normally distributed continuous variables, median (interquartile range) for nonnormally 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 χ^2 test for categorical variables. BMI indicates body mass index; CRP, C-reactive protein; DHEA, dehydroepiandrosterone; DHEA-S, dehydroepiandrosterone-sulfate; DHT, dihydrotestosterone; KORA, Cooperative Health Research in the Region of Augsburg; SBP, systolic blood pressure; SHBG, sex hormone-binding globulin; TSH, thyroid-stimulating hormone; TT, total testosterone; and WC, waist circumference.

* $P < 0.05$.

presented is Table S4. No significant associations were observed for either SHBG or sex hormones with risk of MI in both men and women, whereas sex differences were observed for risk of MI (Table S5).

Mediation Analysis

Based on the above-mentioned results, mediation analyses were performed for TT and DHT to assess whether, and to what extent, the sex differences (women versus men) in risk of stroke mediated by TT or DHT. The results of the mediation analysis of TT and DHT on the association between sex and risk of stroke 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 risk of stroke, highlighting the complex role of TT, suggesting that TT's effects are not uniform among men and women and can lead to differing outcomes in metabolic health across sexes. The DE was HR 0.31 (95% CI, 0.13–0.66) and 0.42 (95% CI, 0.18–0.97), whereas the IE was 2.63 (95% CI, 1.39–5.72) and 2.48 (95% CI, 1.30–5.54) in models 1 and 2, respectively. To understand the decomposition of the effect of sex on stroke risk through TT, the forest plot of the TE, DE, and IE is provided in Figure S2A, indicating that DE and IE act in opposite directions (inconsistent mediation). The mediation analysis on DHT on the association between sex and risk of stroke showed the same pattern (Table 2). The DE was HR 0.30 (95% CI, 0.14–0.57) and 0.39 (95% CI, 0.17–0.80), whereas the IE was 2.87 (95% CI, 1.68–5.84) and 2.90 (95% CI, 1.67–6.12) in models 1 and 2, respectively. The forest plot of the decomposition of the effect of sex on stroke risk through DHT is provided in Figure S2B. The findings of the sensitivity analysis (model 3) were comparable to the main model

findings; the DE was HR 0.59 (95% CI, 0.22–1.37) and 0.51 (95% CI, 0.22–1.06), and the IE was 2.02 (95% CI, 1.03–5.23) and 2.50 (95% CI, 1.38–5.99) for TT and DHT, respectively (Table S6).

DISCUSSION

To our best knowledge, the current study is the first one investigating the mediatory role of androgens and SHBG on explaining sex differences for risk of stroke/MI. Although we found no association between TT and DHT with stroke risk in women, our mediation analysis findings revealed an inconsistent mediating role of TT and DHT in explaining sex differences in stroke risk, suggesting that TT and DHT impact stroke risk differently in men and women. Specifically, higher TT and DHT levels were associated with a lower risk of stroke in men, whereas in women, elevated TT and DHT levels may have adverse effects. No mediating role was observed for other androgens or SHBG in relation to stroke, and neither androgens nor SHBG mediated the risk of MI.

In line with our findings, results of a prospective cohort study including 3690 community-dwelling men aged 70 to 89 years with a mean follow-up of 6.6 years found that higher plasma TT or DHT is a biomarker for reduced risk of stroke, but not MI.¹⁶ Similar findings were reported in another analysis from a comparable cohort of older men (n=3443; mean follow-up, 3.5 years), in which lower TT levels were associated with an increased incidence of stroke or transient ischemic attack.⁴⁰ Another recent prospective cohort study using UK Biobank data including 479 797 participants (55.1% women) with median follow-up of >12.5 years also found no association between TT and risk of MI in either men or women.⁴¹ Additionally, in the population-based FINRISK97 study of 7671 individuals with a median follow-up of 13.8 years, low testosterone levels were not found to be predictive of CHD in either men or women.⁴² Findings of another prospective cohort study on 1032 older men with a median follow-up of 10 years indicated that DHT, but not TT, is associated with risk of stroke.⁴³ This is consistent with our results, as we observed stronger effect estimates for DHT.

We found that TT and DHT were associated with stroke risk in men, but not in women. Although our study focused on the mediating role of sex hormones in sex differences in stroke risk, a recent mediation analysis of 6175 adults investigated blood pressure as a potential mediator of the association between testosterone and stroke. That study reported that blood pressure partially mediated the testosterone–stroke association in men, but not in women,²⁹ highlighting differences in mediation pathways compared with our approach.⁴⁴ The results of a meta-analysis study accompanied by a large prospective cohort study

Table 2. Mediation Analysis of TT and DHT on the Association Between Sex (Women Versus Men [Reference]) and Risk of Stroke

Effects	HR (95% CI) model 1	P value	HR (95% CI) model 2	P value
TT				
DE	0.31 (0.13–0.66)	0.004	0.42 (0.18–0.97)	0.04
IE	2.63 (1.39–5.72)	0.004	2.48 (1.30–5.54)	0.004
TE	0.81 (0.48–1.34)	0.42	1.06 (0.60–1.84)	0.81
PM	...	...	...	...
DHT				
DE	0.30 (0.14–0.57)	0.004	0.39 (0.17–0.80)	0.009
IE	2.87 (1.68–5.84)	<0.001	2.90 (1.67–6.12)	P<0.001
TE	0.85 (0.50–1.46)	0.6	1.13 (0.62–2.02)	0.68
PM	...	...	...	...

Model 1: adjusted for age. Model 2: model 1+smoking+alcohol consumption+physical activity. DE indicates direct effect; DHT, dihydrotestosterone; HR, hazard ratio; IE, indirect effect; PM, proportion mediated; TE, total effect; and TT, total testosterone.

including 4615 adult men and 4724 women with a follow-up of 29 years found that extremely low endogenous testosterone levels are associated with higher risk of ischemic stroke in men, whereas no association was observed for women.⁴⁵ They also found that the risk of ischemic stroke is mediated by body mass index and hypertension.⁴⁵ The results of our sensitivity analysis, which additionally adjusted for other potential confounders (model 3), were consistent with the main findings and indicated inconsistent mediation of TT and DHT. However, these results should be interpreted with caution, as this sensitivity analysis also adjusted for variables that may lie on the causal pathway from sex to the risk of stroke.

The sex-specific associations between testosterone and stroke risk may be driven by distinct physiological and pathophysiological mechanisms in men and women. In men, endogenous testosterone appears to have neuroprotective and vasoprotective effects that may lower the risk of stroke.⁴⁶ Experimental studies suggest that testosterone promotes vasodilation by enhancing nitric oxide synthesis in cerebral arteries, which improves cerebral blood flow and reduces the risk of ischemic events.⁴⁷ Additionally, testosterone has been shown to attenuate oxidative stress and exert anti-inflammatory effects in the central nervous system,^{48,49} while also enhancing antioxidant defense mechanisms, increasing brain-derived neurotrophic factor levels, and promoting neuronal cell proliferation,⁵⁰ factors that may collectively mitigate vascular damage and neuronal injury following an ischemic insult. In contrast, the role of testosterone in cardiovascular health of women is still debatable.⁵¹ Higher androgen levels in women have been associated with adverse cardiometabolic profiles, including increased insulin resistance, dyslipidemia, and hypertension, which are established risk factors for CVD.²⁰ Research has indicated that low testosterone level might be protective for risk of atrial fibrillation or ischemic stroke in women.⁵² These sex-specific differences suggest that testosterone may influence stroke risk through distinct and potentially opposing pathways in men and women, which may help explain the contrasting associations observed in our study.

There are limited sex-specific MR studies on the causal role of sex hormones on risk of CVD. A large MR study investigated the association of genetic predictors of increased testosterone variants in 2 gene regions (SHBG and *JMJD1C*) with cardiovascular risk factors and risk of coronary artery disease and ischemic stroke using CARDIoGRAMplusC4D consortium and UK Biobank data. They found that none of these 2 gene variants were associated with risk of coronary artery disease in either men or women. However, one of these variants was associated with risk of ischemic stroke in men but not in women.⁵³ On the contrary to

our findings, the results of a sex-specific MR study reported that genetically predicted high SHBG is causally associated with risk of ischemic stroke in both men and women.¹⁴ The results of a prospective cohort study including 13 192 postmenopausal women with a median follow-up of 11.6 years found that SHBG levels are inversely associated with risk of ischemic stroke.⁵⁴ Another large prospective cohort study using UK Biobank data on 479 797 participants reported that higher SHBG is associated with a lower risk of MI in men but not women.⁴¹

There is limited evidence on the association of DHEA or DHEA-S and risk of stroke/MI in men or women, and most of the evidence focuses on other forms of CVDs. A nested case-control study of women (461 cases and 461 controls) found that lower DHEA-S is associated with greater risk of ischemic stroke.⁵⁵ Another nested case-control study using the NHS (Nurses' Health Study) cohort found a positive association between plasma concentrations of DHEA and DHEA-S with the risk of subsequent MI among predominantly postmenopausal women.⁵⁶ The findings of a meta-analysis of 14 case-control studies found that DHEA-S might be associated with CHD risk but not with arteriosclerosis.⁵⁷ Additionally, no association was found between DHEA levels and the risk of CHD.⁵⁷ Also, genetic studies in general show no causal impact of DHEA on CVD or major risk factors such as diabetes, suggesting that DHEA could be a marker rather than a causal factor.

Our study has several notable strengths. Although previous research has examined the associations of sex, androgens, and SHBG with the risk of stroke/MI independently, our use of mediation analysis allowed us to investigate the more complex interrelationships between sex, sex hormones, and risk of stroke/MI in combination. Consistent with prior findings from the KORA study on sex hormones,^{38,39,58} our study also benefits from rich data on menopausal surgeries (eg, oophorectomy and hysterectomy) and a wide range of hormonal treatments, including anti-estrogens, estrogens, progestins, hormone replacement therapy, androgens, anti-androgens, enzyme inhibitors, gonadotropin-releasing hormones, and anabolic steroids. This extensive information enabled us to tailor our eligibility criteria to align with our research question. However, because of its observational design, our study cannot establish causal relationships.

The time-to-event analysis enabled us to appropriately account for censored observations using Cox regression, which was further integrated into the mediation analysis to provide more robust evidence. This study has several limitations that should be acknowledged. First, the overall sample size and, in particular, the relatively low number of outcome events may have limited the statistical power of our analyses, especially for mediation effects, resulting in reduced

precision of the estimates and potentially leading to underestimation or nondetection of some associations. Consequently, the findings should be interpreted with caution, as the limited number of events may affect the stability and strength of the observed associations. Although sensitivity analyses and bootstrap-based mediation analyses yielded consistent results, these approaches cannot fully overcome the limitations imposed by a small number of events. In addition, the sample size constrained our ability to perform further sensitivity analyses across different menopausal status groups, and we were unable to stratify analyses by stroke subtype to explore the potential mediating role of TT and DHT in relation to other types of strokes. Finally, as most participants in our study were of European descent, the generalizability of our findings to other ethnic groups may be limited. Future studies with larger sample sizes and a higher number of events are warranted to provide more robust and precise estimates and to confirm the potential mediating role of sex hormones and SHBG in explaining sex differences in CVD.

Our findings may provide complementary insights to the recently published Testosterone Replacement Therapy for Assessment of Long-term Vascular Events and Efficacy Response in Hypogonadal Men (TRAVERSE) trial, which enrolled 5246 men aged 45 to 80 years with preexisting or high cardiovascular risk and hypogonadal symptoms.⁵⁹ The primary cardiovascular safety end point was the first occurrence of death from cardiovascular causes, nonfatal MI, or nonfatal stroke. TRAVERSE demonstrated that testosterone-replacement therapy was noninferior to placebo for this composite end point,⁵⁹ providing reassurance about the overall cardiovascular safety of testosterone therapy in men.

Our mediation findings mirror earlier evidence showing that TT and DHT influence cardiovascular risk in a sex-specific manner but are established here within a different statistical framework that enables a direct comparison between women and men. Whether these differences are attributable to different biological effects or different levels of hormones in men and women needs further exploration. These findings highlight the need to address such discrepancies (eg, through the application of sex-stratified genetic instruments and analyses in future MR studies). Critically, current genome-wide association studies of sex hormones are limited by their predominant use of mixed-sex cohorts, which mask sex-specific signals and yield limited power in female subgroups.⁶⁰ Recent genetic studies have confirmed these limitations, showing that the overlap between variants associated with sex hormone traits in men and women is minimal, except for SHBG, and that even shared loci often exert divergent effects across sexes.⁶¹ This low cross-sex replication rate not only undermines the reliability of genetic instruments for causal inference but also perpetuates a

male-biased understanding of hormone-metabolism interactions, as evidenced by underpowered female strata in landmark studies.⁶¹

In addition, we observed inconsistent mediating roles of TT and DHT in relation to stroke risk, but not MI risk. These findings indicate that endogenous sex hormones may have disease-specific effects rather than acting as general mediators across all CVD outcomes. Moreover, the role of specific hormones may differ by sex; for example, TT and DHT may exert divergent or even opposite effects on stroke risk. Taken together, these findings emphasize the importance of sex-specific MR studies of endogenous sex hormones to clarify their causal and potentially divergent roles in cardiometabolic disease, both overall and in relation to specific condition.

Finally, a better understanding of the sex-specific effects of endogenous hormones may have important clinical implications. If confirmed, these findings could inform the development of sex-specific prevention strategies or therapeutic targets for CVD.

ARTICLE INFORMATION

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Author contributions: H.R.-D., T.M., and J.N. contributed to the study design and conception. H.R.-D. and M.A. performed the statistical analysis. H.R.-D. wrote the first draft of the manuscript. All authors helped to interpret the data and critically revised the manuscript for important intellectual content. J.N. had full access to all the data in the study and takes 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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Disclosures

T.Z. is coinventor of an international patent on the use of a computing device to estimate the probability of myocardial infarction (international publication No. WO2022043229A1) and is a shareholder in ART.EMIS GmbH Hamburg. The remaining authors have no disclosures to report.

Supplemental Material

Tables S1–S6

Figures S1–S2

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